Devendra Kumar Yadava
Harsh Kumar Dikshit
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Fundamentals of Field Crop Breeding

Springer

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ISBN 978-981-16-9256-7 ISBN 978-981-16-9257-4 (eBook)
https://doi.org/10.1007/978-981-16-9257-4

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword

The book "Fundamentals of Field Crop Breeding" describes fundamental concepts and also the advances in plant-breeding practices including developments in crop genomics. The book covers origin, reproduction biology, genetic principles, plant-breeding procedures and techniques and tools used in the improvement of major field crops. The chapters are structured well to describe breeding procedures starting from conventional breeding methods to modern molecular tools including several new developments. In addition, the information on status of crop improvement and conservation has added value to the publication. The book bridges the knowledge gap with regard to modern breeding tools for developing high yielding, climate-resilient and micronutrient-dense varieties of different field crops.

I congratulate the editors Drs. Devendra Kumar Yadava, Harsh Kumar Dikshit, Gyan Prakash Mishra and Shailesh Tripathi along with all the contributors of different chapters for bringing out this publication. I hope that the book would greatly benefit students, research scholars, scientists and others having interest in crop breeding.

Department of Agricultural Research and Education
Indian Council of Agricultural Research
New Delhi, Delhi, India
26 November 2021

T. Mohapatra

Preface

The current global population of 7.5 billion is expected to reach 9.3 billion by the year 2050. To meet the food requirements of this burgeoning population, food production has to be enhanced by nearly 60%. Such a huge enhancement in global food production needs an increase in the productivity of nearly all the crops. In addition, more nutritious crops are required to tackle the micronutrient deficiency problem in changing climate. In the present scenario, climate change, biofortification, mitigation of biotic and abiotic stresses are the major challenges before the plant breeders in achieving the required food production in times to come.

The book entitled "Fundamentals of Field Crop Breeding" compiles crop-based breeding procedures for the major crop plants and is an advanced textbook and a reference book for the post-graduate plant-breeding students and also for the plant breeders. Twenty-six chapters covering various aspects of field crop breeding have been included in this book. This book covers details of cereals, commercial crops, oilseeds and pulse crops. Besides, overall progress made in various field crops, efficient breeding in the crops using modern tools and the maintenance breeding in different crops are also suitably covered. In addition, this book consolidates the fundamental concepts and the latest advances in plant breeding of different crop plants. The latest developments in the breeding of crops using both conventional and molecular approaches including genomics are also included. Sincere efforts have been made by the authors to include most of the relevant latest developments about breeding of field crops. The book is expected to expose the students and researchers to modern breeding tools and advanced concepts in the breeding of field crops.

The editors are hopeful that compiled information will serve as a basic resource material for students, teachers and researchers which will be of help in improving the production and productivity of various field crops. The crop-wise information has been compiled by most experienced plant breeders. The editors are extremely thankful to all the authors for their cooperation and contribution.

Delhi, India

Devendra Kumar Yadava
Harsh Kumar Dikshit
Gyan Prakash Mishra
Shailesh Tripathi

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About the Editors

Devendra Kumar Yadava is serving as ADG Seeds, Indian Council of Agricultural Research, New Delhi. Breeder for the last 25 years including 19 years at Indian Agricultural Research Institute (IARI), contributed in the development and release of 21 varieties (mustard 18; pulses 3) including early maturing mustard varieties, viz. Pusa Mustard 25, 27, 28, 26 and RGN 145 which have provided greater choice to farmers under changing climatic scenario. Bred six low erucic acid varieties, viz. Pusa Mustard 21, 22, 24, 29, 30 and 32 and two Canola quality varieties Pusa Double Zero Mustard 31 and 33. He is Fellow of National Academy of Agricultural Sciences (NAAS) since 2015 and recipient of many awards like Rafi Ahmed Kidwai Award 2017, ICAR, New Delhi; Dr. B.P. Pal Memorial Award, 2012, IARI, New Delhi; NAAS Recognition Award 2018; Dr. P.R. Kumar Brassica Outstanding Scientist Award 2017, SRMR, Directorate of Rapeseed Mustard Research, Bharatpur. He has guided three M.Sc. and five Ph.D. students as Chairman and presently guiding four Ph.D. students and published 90 research papers in high impact factor journals.

Harsh Kumar Dikshit is serving as Principal Scientist at ICAR-Indian Agricultural Research Institute, New Delhi (India). He is working on genetic improvement of grain legumes through conventional and molecular approaches. He has developed 15 varieties of different grain legumes (lentil, mungbean and dry beans) for cultivation in India. He is involved in the development of leading mungbean varieties IPM 99-125, IPM 02-3, IPM 02-17 and Pusa 1431 and lentil varieties L 4717 and L 4727. His present focus is on basic and applied research on early maturity, biofortification and biotic stresses of lentil and mungbean. He is faculty of Division of Genetics and is involved in teaching different courses and thesis research guidance to post-graduate students. He has done his Master's and PhD in Plant Breeding from G.B. P.U.A. & T. Pantnagar (India). He is Fellow of ISGPB and ISRPD and recipient of several awards including ICAR-Dr. Rajendra Prasad Award 2020.

Gyan Prakash Mishra is currently working as Principal Scientist at ICAR-Indian Agricultural Research Institute, New Delhi (India). Before that he has worked at DRDO-Defence Institute of High Altitude Research, Leh (India) as Scientist "C",

ICAR-Directorate of Groundnut Research, Junagadh (India) and ICAR-Indian Institute of Vegetable Research, Varanasi (India) as Senior Scientist. His major research interest includes crop improvement through conventional, molecular and transgenics approaches. He has done his Master's and PhD in Genetics from Indian Agricultural Research Institute, New Delhi (India) and postdoc from University of California, Riverside and Purdue University, USA as BOYSCAST-Fellow. As a faculty of Division of Genetics, he is involved in teaching of different courses and also guiding the post-graduate students. He is the recipient of various prestigious awards including NAAS Associate 2018, NASI-Membership 2018, IARI-Merit Medal and Best Student Award 2001, ISCA-Pran Vohra Award 2012, DRDO-National Science Day Award 2009 and ICAR-Dr. Rajendra Prasad Award 2020.

Shailesh Tripathi is currently working as Principal Scientist at ICAR-Indian Agricultural Research Institute, New Delhi (India). Prior to joining IARI, he served as Scientist at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad. He obtained his Ph.D. in Plant Breeding and Genetics in 2004 from G. B. Pant University of Agriculture and Technology, Pantnagar. He was involved in transfer of "QTL hotspot" containing quantitative trait loci for several root and drought tolerance traits into popular chickpea varieties of IARI resulting in the release of drought-tolerant chickpea varieties. He also developed chickpea suitable for machine harvesting and extra-large seeded kabuli chickpea. He has published over 45 research papers in peer-reviewed journals. He is actively involved in teaching post-graduate students as Faculty of Genetics at IARI.

Breeding Field Crops: History, Current Status and Introspections

1

K. K. Vinod, S. Gopala Krishnan, Manoranjan Senapati, and Ashok Kumar Singh

Abstract

Agriculture is the fundamental basis of human evolution and has evolved itself as the civilisations progressed. Crop breeding is a process in which the most chosen plant is selected for further cultivation. The science behind the genetics has paved the way to trait-based breeding, wherein desirable traits were selected over the undesired ones. The breeding work in India began mainly with the widespread evaluation of wheat genotypes for the improvement of grain, straw and rust resistance. This was followed by breeding for a number of crops including tobacco, sisal hemp, barley, flax and a few fruit tree species. In the year 1955, All India Coordinated Research Project (AICRP) was launched which has transformed the cultivar evaluation system and release in India. Even at the international level, there was an upsurge in the establishment of a number of crop-based non-profit research institutions. International Rice Research Institute (IRRI) was the first such institute which was opened in the year 1960 at Philippines. Breeding efforts in India is yet to venture deep into the genomics-assisted breeding including genomic selection and gene editing in majority of the field crops, although the research in this direction is progressing. Recent efforts towards the integration of technologies to translate breeding success into genetic gain are also discussed. With this backdrop, this chapter gives a brief about the key developments in the breeding history of field crops, particularly in India, during the last few decades. Also discussed are the future perspectives.

Crop improvement by breeding is a continuous process. Those who are engaged in this vital task have a hand in crop plant evolution and enjoy the pride and privilege of fighting the war against hunger - Anonymous.

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_1

Keywords

Breeding methods · Green revolution · Marker-assisted selection · Genomic selection · Genetic gain

1.1 Introduction

Agriculture is a tradition as old as human civilisation. Since time immemorial, agriculture was a gatherers' pursuit wherein the grains, roots, fruits and nuts were collected by the early hunters. Evidence for the use of cereal seeds such as sorghum grasses dating back to 0.11 million years in the Late Pleistocene was seen from the starch granule assembly on the surface of middel stone age tools (Mercader 2009). Humans started organised agriculture much later in the Holocene, about 10,000 years ago, with the collective domestication of several grain species such as wheat, rice, barley and chickpea (Eckardt 2010; Willcox et al. 2009; Zuo et al. 2017). However, the crop improvement by breeding began only in the late nineteenth century with the rediscovery of Mendel's research findings. In breeding, selection is the crucial step that requires a multitude of factors and methods to achieve the breeding objective. Starting from intuitive selection to genomic selection, the process requires diversity to choose from. Therefore, generating new varibility is crucial in crop breeding.

All the methods, be it for generating variability and/or for deciding to select, are fundamentally anchored to the science of genetics. Breeding crop varieties revolves around two elemental phenotypic dimensions, namely, quantitative and qualitative. Quantitatively, the need 'for producing more to feed more', is the primary dimension that safeguards the food security for the growing world population; while the second defines how well the crop produce serves as the food and feed. All other breeding objectives such as adaptation, tolerance to stresses, and resilience can be mapped onto these basic dimensions.

Unlike, during the early Holocene when agriculture was mainly in the subsistence mode, in the Anthropecene era, it has metamorphosised into an industry. Thereby another dimension of 'commercial' has been sandwiched between the two basic dimensions of crop breeding. This had brought in a dramatic evolution in agricultural research globally, particularly in crop breeding. The most historical outcome of this evolutionary transformation was the 'green revolution', which brought in a paradigm shift in the way modern cultivars are bred.

Since the onset of the twentieth century, there have been consistent efforts to control and understand the pattern of plant evolution. The contemporary birth of the new sceince of genetics has spearheaded artificial hybridisations resulting in identifying a better variant on a continuous scale facilitated by combining more than one desirable trait at a time. This has led to the refinement of the selection process in breeding. Besides, a weightage was attached to some traits such as yield

over the other, during the selection process. The breeding process has become more systematic, thanks to the laws of inheritance by Gregor J. Mendel, who demonstrated the possibility of predicting the occurrence of variation in a breeding population. Later into the century, heredity has seen its molecular definition through the discovery of deoxyribonucleic acid (DNA) structure by Watson and Crick in 1953. A parallel development in mathematical and statistical predictions, invented by R.A. Fisher, in combination with landmark genetic discoveries of T.H. Morgan, A. H. Sturtvent, W. Bateson, G.W. Beadle, E.L. Tautum, O.T. Avery, C.M. MacLeod, M. McArty, S. Benzer, J.B.S. Haldane, J. Lush, etc., laid out the foundation for the architecture of modern plant breeding.

Humans use only 0.03% of the total flowering species for food. Among the 347,298 known species of vascular plants worldwide (WCVP 2020; Cheek et al. 2020), 50,000 are edible, but only ~120 are cultivated for food (FAO 1996). Among these, just three crops, namely, rice, wheat and corn, supply more than 60% of the world's dietary energy. In addition, oilseeds, legumes and millets contribute more than 15% of the calories (Loftas 1995). These crops, collectively known as field crops form the backbone of global food security. Majority of the field crops are annuals that produce staple grains in human food. They are cultivated in vast areas and occupy lands all around the world. Among the various species humans have domesticated, no other group of crops have undergone rigorous breeding efforts like that of field crops leading to significant changes in the trait forms making them amenable for intensive cultivation.

1.2 Chronicling Breeding for Improvement of Field Crops in India

The breeding efforts in food staples have been tremendous during the last century. Current estimates indicate that food demand will go up by 36–56% from the period between 2010 to 2050 and, meanwhile the people at risk of hunger will shift sharply from −91% to 8% (van Dijk et al. 2021). Crop breeding needs to be continuously impressive as it was, in the coming decades too to meet such a formidable challenge. This is highly relevant to India, which at present harbours 17.7% of the world human population with a meagre 2.4% land share (Kumar 2011), and having an alarming annual population growth rate of 1.0%. At the current rate, one per cent of the Indian population is about 14 million, more than half the size of the population in Australia, which means India adds more than one Australia every two years. Historically, India has always been at the forefront of agricultural development worldwide. A subcontinent that has been struggling to sustain agriculture since the beginning of the twentieth century with continuous famines and crop failures, India was almost absent in the world agricultural map at the beginning of the twentieth century.

Follwing the recommendations of Famine Commission of 1980, the Department of Agriculture under the Goverment of India was revived, alongwith setting up of several provincial agricultural departments. In 1903, the British Indian Government decided to establish a Central Agricultural Research Institute under the Department

of Agriculture to introduce scientific agriculture in India. Consequent inauguration of the Agricultural Research Institute and College in 1905 at Pusa in Bihar marked the beginning of organised agricultural research in India (Howard and Howard 1929). Breeding research at the Agricultural Research Institute began with the extensive evaluation of wheat cultures, with a main emphasis on hybridisation to improve grain, straw and rust resistance. Breeding for other crops such as tobacco, sisal hemp, barley, flax and a few fruit trees was also taken up (ARIC 1909). Agricultural Research Institute and College was later renamed as Imperial Agricultural Research Institute in 1919, and then as Indian Agricultural Research Institute (IARI).

Contemporarily, rice breeding research was initiated in Bengal and Madras provinces with the setting up of several research stations. The Royal Commission of Agriculture in 1928 proposed to scale up and coordinate the agricultural research pan India, by establishing an Imperial Council of Agricultural Research (GoI 1928). The Imperial Council of Agricultural Research established in 1929 was rechristened as Indian Council of Agricultural Research (ICAR), and provided with extensive mandate on different crops. Understanding the immediacy of independent research, ICAR had established several crop-specific research institutes at different locations at different time (Table 1.1).

Among the crops, rice pioneered in setting up of research establishments in India. Although the rice research had begun during the 1911–12 period, only Bengal and Madras provinces were endowed with research, until the establishment of ICAR. Further, several research stations were established, and by 1950, there were 82 research stations spread across 14 states (Ghose et al. 1960). The launch of the All India Coordinated Research Project (AICRP) in 1955 reformed the cultivar evaluation system and release in India. The major mandate of AICRP was to coordinate applied research on national and regional issues and to develop location-specific varieties and technologies in various crops. The first crop-based project was started for maize in 1957, and was named All India Co-ordinated Maize Improvement Project (AICMIP). Subsequently, the All India Coordinated Rice Improvement Project (AICRIP) was established in 1965. Later on, several crop-based coordination projects were initiated by ICAR at different crop research institutes. These institutes were provided with regional research stations covering all major production zones of the respective crops. Currently, every field crop has an independent AICRP system, that links several agricultural universities, institutes and private research organisations, who are involved in varietal/ technological development, evaluation and release. As a whole, AICRP has grown to be the single largest varietal evaluation and release system in the world. Additionally, to make agriculture support countrywide, state governments have opened several agricultural universities and research stations to cater for the need of local farmers and to provide agricultural education. Crop improvement by breeding is one of the major mandates of all crop-based research institutions.

Table 1.1 Establishment of major field crop-specific institutes in India under ICAR

Year	Institute	Location	Mandate Crop(s)
1905	Agricultural Research Institute and College[a]	Pusa	Wheat, barley, tobacco etc.
1937	Indian Agricultural Research Institute[b]	New Delhi	Wheat, barley, rice, legumes, Brassica, horticultural crops
1946	Central Rice Research Institute (presently National Rice Research Institute)	Cuttack	Rice
1976	Central Institute for Cotton Research	Nagpur	Cotton
1977	Directorate of Oilseeds Research[c] (presently Indian Institute of Oilseed Research)	Hyderabad	Oil crops
1978	Directorate of Wheat Research[c] (presently Indian Institute of Wheat and Barley Research)	New Delhi	Wheat
1979	Directorate of Groundnut Research[c]	Junagadh	Groundnut
1983	Directorate of Rice Research[c] (presently Indian Institute of Rice Research)	Hyderabad	Rice
1984	Directorate of Pulses Research[c] (presently Indian Institute of Pulses Research)	Kanpur	Grain legumes
1987	National Research Centre for Sorghum[c] (presently Indian Institute of Millets Research)	Hyderabad	Sorghum
1993	National Research Centre on Rapeseed and Mustard (presently Directorate of Rapeseed-Mustard Research)	Bharatpur	Brassica
1994	Directorate of Maize Research[c] (presently Indian Institute of Maize Research, Ludhiana)	New Delhi	Maize

[a]Renamed as Imperial Agricultural Research Institute in 1919
[b]Shifted to New Delhi following the destruction of IARI at Pusa in Bihar earthquake of 1935
[c]Instituted as AICRP, and later given independent research status

1.3 Role of International Centres in Strengthening the Breeding Efforts

Contemporary to the inception of agricultural research institutions in India, globally, there was a rise in installations of crop-based non-profit international research institutions. The first among these, the International Rice Research Institute (IRRI) was opened in 1960 at Los Baños, Laguna, in the Philippines. Subsequently, International Maize and Wheat Improvement Centre (CIMMYT) came into being in 1966, as a scientific and educational institution exclusively involved in wheat and maize research. The year 1967 saw the opening of two more institutions, the International Institute for Tropical Agriculture (IITA) in Nigeria and the International Centre for Tropical Agriculture (CIAT) in Columbia. Instituted in 1971 at

Montpellier in France, the Consultative Group of International Agricultural Research (CGIAR) brought the international institutions under its governance. Currently, there are 15 research centres under CGIAR, of which seven works on tropical field crops. The major activity of these centres is breeding, besides other allied technological development.

As per the 2020 performance report of the CGIAR, 78% of its innovations were genetic with 66% impact directed to alleviate poverty and 15% directed to nutritional security (CGIAR 2020). Sixty years into existence, international research organisations could transform agricultural research worldwide sowing the foundations of the green revolution in major cereals such as wheat and rice. The noble efforts of Dr. Norman E. Borlaug in developing high yielding, semi-dwarf and disease-resistant wheat varieties, which ensured food security in countries like India, Mexico and Pakistan have earned him the popular title of 'father of green revolution' as well as a Nobel peace prize in 1970 (Swaminathan 2009), besides several others. Aided by Dr. Norman Borlaug in wheat and IRRI in rice, Dr. M. S. Swaminathan spearheaded transformative research in India from the fields of IARI in 1966. The results were remarkable, a quantum jump of 42% increase in wheat production could be achieved in one year. Three genes governing plant height, namely, *rht1* and *rht2* from Norin 10 in wheat and *sd1* from Dee-Geo-Woo-Gen (DGWG) in rice, were responsible for this phenomenal achievement in the history of crop evolution. This gave an impetus to the breeding research in India and breeders geared up this momentum to deliver scores of improved cultivars in the ensuing decades in all the field crops.

1.4 Breeding Research on Field Crops

Until the beginning of the twentieth century, no organised breeding efforts had taken place in field crops, anywhere in the world. The breeding research in India also evolved simultaneously with similar efforts across the globe. Drawing parallel to the developments in the science of genetics, different breeding methods dominated the landscape of field crop improvement from time to time in India. In Fig. 1.1**,** we have taken rice as the model crop to demonstrate the evolutionary pattern of breeding methods, because rice was not only genetically rich in India but also had undergone all the breeding methods developed so far. We would, therefore, be focussing our discussions in this chapter based on Indian experience on breeding methodologies during the past century and their evolution, establishment and impact made on improving different field crops. Also, elaborate details of individual crops will not be covered except for the key landmarks, keeping in mind that such details would be following in subsequent chapters dealing with different field crops.

In the early twentieth century, the indigenous cultivars in India were of genetically admixed populations. Admixed populations had extreme variability within, because of the significant proportion of wild alleles they carried. Since these populations did not undergo serious selection for yield, the wild alleles were more aligned to adaptation, rather than productivity. Because of these, the cultivars were

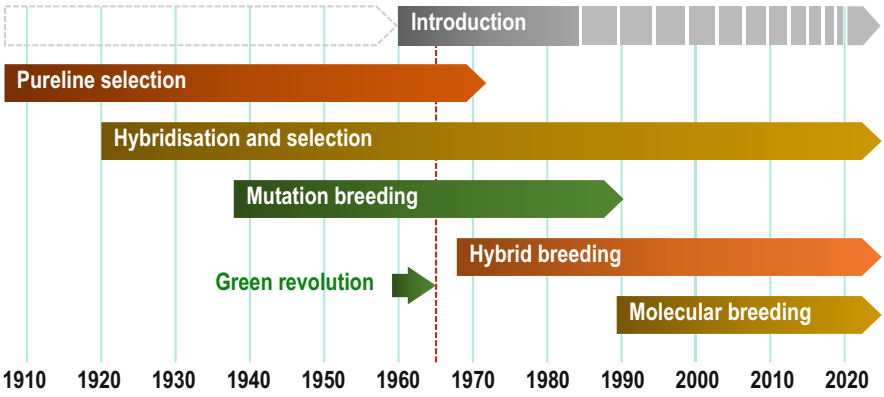

Fig. 1.1 Evolution of breeding methodologies in rice improvement. Unlike in rice, introduction of elite genetic materials had begun in other field crops much earlier (denoted by the dotted lines). Introduction in rice has been comparatively intermittent

tall, prone to lodging, hardy, less yielding, photosensitive and took longer time to yield. Although selections were carried out among these populations in the early years, only marginal yield improvement could be realised initially. However, beginning from the 1960s breeding research has started to become more organised, with the plans of pan India integration of varietal release systems and institutional research. This movement in research organisation paid off with the development and release of newer cultivars and invigorated research systems sowing the seeds of the green revolution in the coutry. A glance at the productivity spectrum of major field crops (Fig. 1.2) reveals that all the crops have experienced a yield gain of at least by a minimum of 107% as in red gram to 539% in maize in the next 70 years. The next highest yield increase was in wheat (516%), followed by cotton (513%), pearl millet (475%) and rice (405%).

1.4.1 Introduction

The initial part of the organisational plant breeding efforts in India was laden with scores of introductions of crop varieties from abroad. Owing to its rich indigenous diversity, introductions in rice were intermittent, but in other field crops such as wheat and maize, a considerable number of genotypes were introduced, particularly through international centres. Several direct introductions happened during the pre-green revolution period. The activity was so intense and programmed, a separate Division of Plant Introduction for coordinating the imports from abroad was started at IARI in 1961 (Pal 1962). The Division later became the current National Bureau of Plant Genetic Resources (NBPGR) in 1976. Among the most notable field crop introductions in IARI were Ridley from Australia; Lerma Rojo 64, Sonora 64 and PV18 from Mexico in wheat; LSB2 and Dolma from the USA; Clipper from

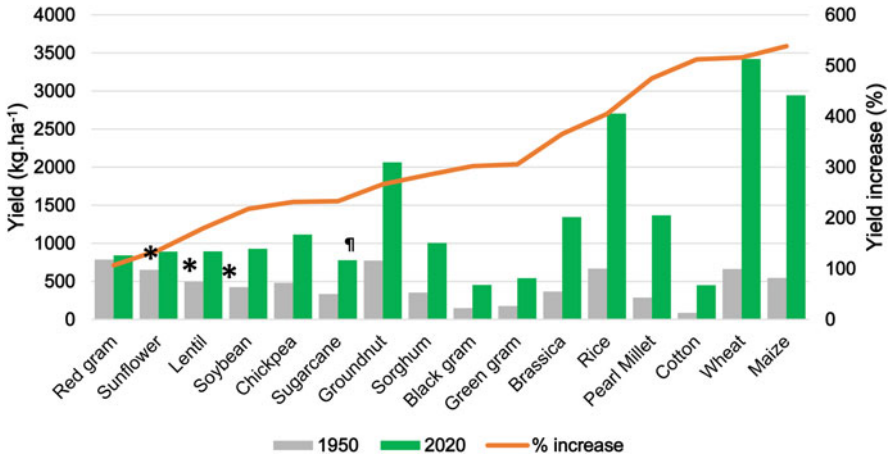

Fig. 1.2 Productivity of major field crops in India, between 1950 and 2020. Except for three crops, productivity increase was more than 200% during this period. *Crops such as sunflower, lentil and soybean were later introductions and their base data is of 1970. ¶Sugarcane yield is given in quintals and not in kilograms. (Data source: GoI 2020)

Australia in barley; IR8, IR20, IR36, IR50 and IR64 from the Philippines in rice; Peredovik and Aramvirikij from USSR in sunflower; Asiriya Mwitunde from Tanganyika (a territory in present Tanzania); Rehovot 33–1 from Israel; M13 from the USA in groundnut; Bragg and Lee, and Improved Pelican from the USA in soybean; and Improved Ghana from Ghana in pearl millet (Singh 1991). Several of these introductions were directly absorbed into the breeding pipelines, while exceptional widely adapted genotypes were released as varieties. One of the most noted introductions was the first rice variety officially released worldwide by IRRI in 1966, IR 8 (Peng et al. 1999).

1.4.2 Pureline Selection

Besides the introduction, initial efforts were also focussed on selection in crops, with or without hybridisation. Before the green revolution in the mid-1960s, pureline selection was the common way of cultivar development in self-pollinated species like rice. Mostly involving local landraces and exotic introductions, there were hundreds of pureline rice varieties released across India. Among the success of pureline selection in India, GEB24 was a milestone rice cultivar. A selection from Konamani, also known as Athur Kichli Samba, a local landrace, GEB24 was considered as a spontaneous mutant. With its fine grain and high quality, GEB24 was officially released for cultivation in 1921 and is considered one of the first officially released crop varieties in India. GEB24 also served as one of the major parental lines in generating the initial breeding materials in IRRI, after its

establishment in 1960. By the end of 1965, there were 66 pureline selected rice varieties released in Tamil Nadu alone, out of 445 rice varieties released across India. Among these, outstanding releases were Dular, Latisail, Manoharsali, MTU 15 and Nagina 22, to name a few (Mishra 2002). There were also noteworthy advancements in quality rice breeding, such as Type 3 (selection for Dehradooni Basmati), Basmati 370 and N105 (selection for Hansraj) and Type 9 (selection from Duniapat).

Unlike in rice, pureline selection in wheat did not sustain for a longer time in India, instead, hybridisation followed by pedigree selection was adopted as early as 1907 in Pusa (Howard 1909). The first 20 years of Imperial Agricultural Research Institute witnessed the release of a few pureline selected wheat varieties such as NP4, NP6, NP12, Pb8, K13, K53, AO13, AO85, Motia, Bansi, Gulab, Arnej 206, etc. (Nagarajan and Singh 1997). Although several of these lines were tested and grown in other countries, NP4 remained one of the most outstanding wheat varieties across the world for its grain quality. NP4 was selected from Mundia, an awnless landrace that had shown remarkable adaptability to varying environments in addition to its exceptional quality (Tomar et al. 2004). Within no time NP4 became the landmark contribution of IARI in its early history.

1.4.3 Recombination Breeding

Hybridisation and selection remained the most adopted breeding strategy among the field crops around the world. Among the early success of hybridisation in India, the most remarkable achievement was the 'nobilization of canes'. In sugarcane, interspecific hybridisation between *Saccharum officinarum* with *S. spontaneum* followed by backcrossing and selection could lead to the development of superior canes called 'noble canes' with high yield and sugar content (Barber 1915). Subsequently, the improved canes coud revolutionize the sugar industry in India. In 1949, a most ambitious intersubspecific hybridisation programme was launched in rice under the aegis of the Food and Agricultural Organization (FAO), to combine the fertiliser responsiveness and hardiness of *japonica* with quality and adaptation of *indica*. Although the programme was not a great success, two popular varieties were evolved, namely, Mahsuri in Malaysia and ADT27 in India. Additionally, two more varieties, Malinja in Malaysia and Circna in Australia, were also released for cultivation under this programme.

The most important landmark in rice breeding came through the development of semi-dwarf varieties aided by the identification and introgression of *sd1* gene. In 1961, Peter Jennings, a young agronomist from the USA was recruited to IRRI by the Rockefeller Foundation to investigate the dwarf rice. He came across a Taiwanese rice variety, Taichung Native 1 (TN1), that was widely grown. Semi-dwarf in nature, TN1 resembled its parent, Dee-geo-woo-gen (DGWG) for the plant height. Jennings made the first 38 crosses at IRRI, with 11 of them using DGWG or TN1 as one of the parents (Hargrove and Coffman 2006). The inheritance of semi-dwarfism was identified as single gene controlled. Later in 1963, Dr. Henry Beachell

made a selection of a line, IR8–288-3 from the F_4 generation of the Jennings' eighth cross, between Peta and DGWG. IR8–288-3, later became the 'miracle rice', IR 8. Introduced into India in the same year, IR8 was released for cultivation in the same name. IR8 could help boost the rice productivity by a whopping 150–200% in its first year of introduction, beginning to transform the face of agriculture itself from poverty-ridden to self-sufficiency.

The development of IR8 remains the most remarkable contribution of IRRI to the world. IR8 could cast the same magic spell worldwide, saving millions from poverty all across the major rice consuming countries like Bangladesh, Vietnam and India. Almost at the same time, a similar story was unfolding in wheat breeding too, wherein the dwarfing genes from a Japanese variety, Norin10 were utilised to develop semi-dwarf varieties. In the early 1960s, Dr. Norman E Borlaug in CIMMYT was striving to improve wheat yields in Mexico by using the semi-dwarfing trait. A series of crosses followed, and Dr. Borlaug could get exceptional success in his 8156th cross between Penjamo 62 and Gabo 55. Named as Ciete Cerros, 8156 was remarkably high yielding and could transform the wheat production scenario in Mexico by 1962. By the mid-1960s, Mexico became self-sufficient in wheat production. Introduced into Pakistan as Mexipak and Kalyan Sona in India, this variety scripted another chapter in the history of green revolution.

Although there were few hybridisation-based varieties released in rice before the 1960s, majority of the post-green revolution era varieties, both in India and elsewhere in the world were developed through hybridisation and selection. Among the nine hybridisation-based varieties released in Tamil Nadu before 1960, CO14 was the first variety released in 1940. As per the compiled information from the Directorate of Rice Development in India, there were 814 rice varieties released between 1969 to 2012, of which 89% came from pedigree breeding. Despite the huge number of varietal releases in India, only a handful went onto become 'mega varieties', having been grown in larger areas and for long time. The first mega variety released in India was Jaya, developed by crossing TN1 with Type 141.

Released in November 1968, Jaya soon replaced IR8 and TN1 that were ruling the rice production in the country. There was another variety, Padma (CR 28–25) released along with Jaya, and both became the torchbearers of the indigenously bred Indian rice under the AICRIP system (Hopper and Freeman 1969). Later on, other megavarieties were released, Swarna (MTU7029) in 1980, Savitri (CR1009) in 1983, Samba Mahsuri (BPT5204) and Pusa Basmati 1 in 1989 (Fig. 1.3a), Pusa 44 in 1994, Cotton Dora Sannalu (MTU1010) in 2000, Pusa Basmati 1121 (Fig. 1.3b) in 2005 and Pusa Basmati 1509 in 2013. The AICRIP system in India steadfastly increased the rice varietal output with an average upward trend (Fig. 1.4). Although there were intermittent years with several releases such as 1978 and 2008, the average trend increased from 1–2 varieties per year during 1969 to 26 per year in 2012.

In the case of wheat, the initial focus of the erstwhile Imperial Agricultural Research Institute was to improve Indian varieties, which were of excellent grain quality. India majorly grows spring wheat and not winter wheat. One of the major problems with the local cultivars was the tall stature of the plants, which made them

Fig. 1.3 Some of the landmark varieties released from ICAR-IARI, New Delhi. (**a**) to (**c**): Pusa Basmati 1, the first semi-dwarf Basmati cultivar; Pusa Basmati 1121, a mega Basmati rice variety with maximum cultivation extent; Pusa RH 10, the first aromatic rice hybrid; (**d**) to (**e**): Two wheat mega varieties, HD2967 and HD3086 with remarkable scale of adoption; (**f**) Vivek QPM 9 Improved, the first QPM hybrid enriched with Pro-vitamin A; (**g**) Pusa 23, a pearl millet hybrid with A1 cytoplasm; (**h**) Pusa 10216, MAS-derived chickpea variety with drought tolerance

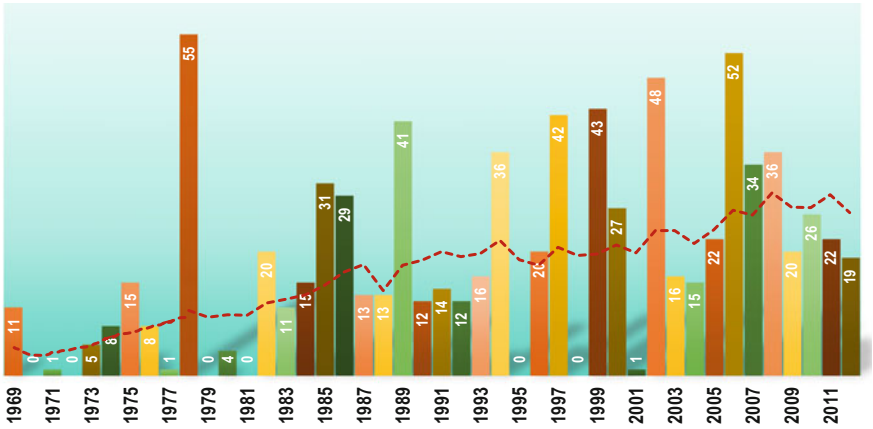

Fig. 1.4 Number of rice varieties released per year between 1969 and 2012 in India, mostly channelled through the AICRIP system. Dotted line indicates 10 years moving average. (Data source: Directorate of Rice Development, Patna)

prone to lodging. An additional problem was the rust disease. Therefore, the initial breeding emphasis was to improve rust resistance, and due to this, the grain yield of Indian wheat varieties relatively remained below 2.0 tons/ha until the beginning of the 1960s. Although the wheat hybridisation at Pusa had started as early as 1907, it did not make the expected success. Contrary to the expectations, an earlier attempt of direct introduction was also met a dead end. Pal and Ramanujam (1944) observed that during the first 40 years of IARI, the 40% increase in wheat area had come with a reduction in productivity. Until the import of semi-dwarf wheat from Mexico having Norin 10 lineage, the situation did not improve.

The inception of the All India Coordinated Wheat Improvement Project (AICWIP) in 1965 was a gamechanger in the wheat history of India. The first set of varietal introductions from CIMMYT included Sonora 63, Sonora 64, Mayo 64 and Lerma Rojo 64. Introduced into cultivation, these varieties helped to double the wheat production within two years, ending the 60 years' drought for high-yielding varieties. It was so coincidental that the green revolution in the major staple crops, rice and wheat, happened at the same time, orchestrated by three genes, *Sd1* in rice and *Rht1* and *Rht2* in wheat, all controlling the same trait, semi-dwarfism. Borlaug's 8156 became the first wheat megavariety in India, in the name of Kalyan Sona. Kalyan Sona had both the *Rht* genes. The post-green revolution era in wheat witnessed a cascade of new varieties as happened in rice, with IARI playing a flagship role. Starting from UP301 released in 1969, varieties were evolved continuously, all with Mexican lines as one of the parents. Cultivars such as Hira, Moti, Janak etc., followed, finding niches in the timely sown, high fertility conditions. As the years advanced, newer wheat varieties sharing complex pedigrees began to show up. Further, high yielding varieties such as HD2189, HD2204 etc. were also got released in neighbouring countries, Nepal, Bangladesh and Pakistan. Since then,

with the latest editions of high yielding cultivars such as HD2967, HD3086, HD3226 and HD3249, wheat varieties have often helped to touch the surplus production marks in India. Released in 2014, HD2967 and HD3086 (Fig. 1.3d, e) together currently occupy more than 50% of the wheat area in India (Mishra et al. 2020). The role of CIMMYT derived wheat lines in cultivar release in India is immense, and several high yielding varieties such as the recently released variety, DBW222 are direct introductions.

Introduced into India in the seventeenth century, maize was not the crop of choice of the Indian population as its staple cereal, unlike that of rice and wheat. At the beginning of the twentieth century, maize was in cultivation as a primitive cereal, in the north-eastern and sub-Himalayan India. Besides, there were other landraces too, but very limited in number and acreage. By the turn of the twenty-first century, however, there was a marked shift in the priority of maize in India's staple food spectrum, by becoming the third major food staple after rice and wheat (Yadav et al. 2014).

Elsewhere in the world too, maize cultivation was not as advanced as today during the beginning of the last century. In the USA for instance, yield lingered around 1.5 tons/ha during the 1900s to around 2 tons/ha until 1950, with the world average touching 1.9 tones/ha by 1960 (Duvick 2005). Maize yield began to rise sharply since the 1950s, however, there was no single cause that can be attributed to the yield boom, as that in the case of rice and wheat. However, the availability of nitrogenous fertilisers and concerted efforts in hybrid breeding could be considered as the prominent reasons for improved maize yields. Introduced during the 1930s in the USA, inbred-based hybrids made a dramatic shift in maize production, with the yield levels climbing from 2 tons/ha to 4 tons/ha by 1945, while the hybrid area expanded from 1 to 99% by 1960. Yield continued to climb with the introduction of lines from other countries, deriving new combinations. Population improvement became a routine breeding practice this time around, having the name 'recurrent selection' introduced by George Frederick Sprague in 1952, to distinguish it from pedigree breeding (Hallauer 2000).

In India, maize breeding got an impetus with the launch of the All India Co-ordinated Maize Improvement Project (AICMIP) in 1957, the first of its kind in the country. In the 1950s, average maize yield was around 600 kg/ha, predominated with tall flint type low yielding varieties and primitive lines. Realising the potential of maize as an alternate cereal with wide uses as food and feed, ICAR launched its first coordinated research programme in maize ahead of the staple cereals, rice and wheat. By the time AICMIP was launched, several introductions of exotic lines were facilitated by Rockefeller Foundation. AICMIP took the further initiative to bolster exotic genetic base subsequently with the help of CIMMYT, starting from the year of its inception in 1966. The initial launch of hybrids from AICMIP included Ganga 1, Ganga 101, Ranjit and Deccan by 1961 (Yadav et al. 2015). Presently, all the high yielding maize cultivars in the country share the lineage with the introduced germplasm, which had both yield potential and adaptability in varying degrees under Indian conditions.

Population improvement played a significant role in maize improvement in India until the 1980s, beyond which hybrids have taken over the horizon. Currently, maize breeding has taken a major reorientation towards grain quality improvement and product diversification. With the introduction of quality protein maize (QPM) from CIMMYT, another milestone laid by Dr. Surinder K Vasal and Dr. Evangelina Villegas, India released its own QPM hybrid 'Sakthiman 1' in 1981. The role of IARI in recent maize improvement in India is noteworthy, with the release of several improved hybrids such as Pusa HQPM 5 Improved, Pusa HQPM 7 Improved, Pusa Vivek QPM 9 Improved (Fig. 1.3f) combining high pro-vitamin A together with increased lysine and tryptophan fractions. Pusa Vivek QPM 9 Improved is the world's first QPM hybrid combining pro-vitamin A.

Population improvement in other major cereals such as sorghum and pearl millet also drew parallels to the success in major cereals. Established in 1972, the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) had a mission to *reduce poverty, hunger, malnutrition and environmental degradation in the dryland tropics*. All the five mandate crops of ICRISAT were field crops, two cereals—sorghum and pearl millet, and three legumes—chickpea, pigeon pea and groundnut, marked to provide a balanced nutrition of carbohydrates, protein and fat. Prior to this, ICAR launched a coordinated programme 'All India Coordinated Millet Improvement Project' in 1965 with IARI as its headquarters. Pearl millet was included in the project initially, and in 1969 sorghum was added. Later, with the substantial development, both the crops were separated into independent co-ordinating units, pearl millet in 1985 and sorghum in 1987.

With all the research systems in place, the breeding outturn in these crops was tremendous, with ICRISAT playing a major role in bringing in exotic germplasm. The results were commendable, pearl millet yields went up from 300 kg/ha to 1250 kg/ha under minimal input and harsh tropical conditions, within 70 years. Currently, there are about 175 hybrids released in pearl millet through the coordinated varietal evaluation system. Unlike that in maize, sorghum variability in India was tremendous, and the population improvement had begun as early as the 1930s. Sorghum improvement in India, as a whole, is not much celebrated in the later years of the twentieth century. Although yield potential improved, the area declined drastically since the 1990s, bringing down the total production along with.

1.4.4 Mutation Breeding

Another major method employed in the field crop breeding was induced mutagenesis, which became very popular after the 1960s. Established as a method for barley breeding (Freisleben and Lein 1942), mutation breeding soon became very popular in all the crops, particularly in field crops, due to the ease of mutation process. Although physical mutagens like X-rays were used in the early stages, chemical mutagenesis soon became the most popular choice. By the 1960s, use of gamma rays was introduced as a follow-up of peaceful use of ionising radiation after world war II (Micke et al. 1990). A gamma garden was established in IARI in the year 1960, the

second in India, after the first one was established at the Bose Institute, Calcutta in 1959 (Pal 1962). By 1990, several mutant varieties were released around the world totalling 519 among the field crops such as rice, wheat, maize, sorghum, pearl millet, chickpea, pigeon pea, soybean, Brassica and cotton (Micke et al. 1990). Among these, 251 varieties (48.4%) were from rice, followed by 104 (20.0%) from wheat.

The early introductions of Mexican wheat such as Sonora 64 and Lerma Rojo 64A had red grains not preferred by Indian people. Mutation works on these varieties at IARI, resulted in the release of Sharbati Sonora and Pusa Lerma both with amber coloured grains. Although several mutants were developed in wheat through mutation breeding, only a few traits such as grain colour, disease resistance and plant height had notable improvement. Comparing to other methods of breeding, induced mutations were random and often produced relatively less success when compared to the number of mutagenic attempts. Further, the research accomplishments published on mutation breeding across crops were more academic and described similar mutagenic effects, camouflaging the actual success stories. Notwithstanding, the recent experience in identifying a novel herbicide-tolerant *AHAS* mutant of rice, 'Robin', reinforces the faith in mutation breeding (Shoba et al. 2017).

1.4.5 Hybrid Breeding

Hybrid vigour, or the superiority of progenies over their parents, has been observed by several naturalists much before Mendel, including him, but with marginal attention (Mather 1955). Darwin was convinced of the phenomenon and reported it in 1876 (Darwin 1876). A century later, the phenomenon has become one of the most accomplished breeding methods in crop plants, ensuring food security to millions of humans and livestock. Hybrid development in each of the major field crops has a story to narrate, except in wheat which is still being explored to strike a convincing advantage for yield. The conviction that hybrid vigour can be used for crop improvement came much earlier, which saw the birth of the term 'heterosis' by G H Shull in 1914 (Shull 1914) and after his extensive work on maize (Shull 1946). But a major bottleneck remained – controlled pollination. Especially in crops like maize, every grain in a cob used to be a different hybrid due to open pollination. Shull could initially create several inbreds by inbreeding but could not control the deterioration in certain lines (Shull 1908).

Nevertheless, mechanical emasculation in maize was possible through detasselling, a laborious task, but hybrids could be produced. By the 1930s, commercial-scale maize hybrids were available in the USA. At this time around, the Texas Agricultural Experiment Station (TAES) at College Station was bustling with research activities on male sterility systems and hybrid vigour in crop plants. In the 1950s, came the phenomenal discovery of cytoplasmic genetic male sterility (CMS) in maize with the identification of Texas cytoplasm (*cms-T*). The *cms-T* was first described in the line 'Golden June' in 1952 (Rogers and Edwardson 1952) and became one of the most studied CMS systems in crop plants. Since *cms-T* was proved to be a perfect CMS system, soon several hybrids started appearing at

commercial scale. It was strikingly popular that by 1970, 85% of the US maize hybrids possessed *cms-T* cytoplasm. Within the two years spanning 1969 and 1970, 15% of the hybrids were wiped out by the outbreak of southern corn leaf blight incited by *Bipolaris maydis* race T. The T toxin produced by the pathogen had a specific binding site on the T-urf13 protein produced by the *cms-T*. The devastation was so severe that by 1972, most of the hybrids with *cms-T* were withdrawn (Levings III 1990).

There were other cytoplasms available in maize (Beckett 1971), that can be broadly grouped into C (Charrua) and S (USDA), that saved the hybrid maize industry (Weider et al. 2009). Use of these CMS systems has since been moved to other countries. In India, use of CMS systems for hybrid production is not commonly practised, because the process of detasselling is not as expensive as elsewhere. Further, the manual detasselling does not warrant any specific maintenance breeding as that in the case of male sterility systems. One of the major problems for CMS systems is the maintenance of parental lines. Maintenance breeding of three lines, A, B and R, particularly A-line is an arduous task with isolation distances and controlled pollination, which is expensive than detasselling. Moreover, contaminated A-lines are difficult to purify. Therefore, almost all of the commercial maize hybrids in India has normal cytoplasm. Nevertheless, most recently in IARI, a baby corn hybrid has been developed using *cms-T* which is currently in the advanced stages of variety release. Compared to other CMS, *cms-T* remains most stable in Indian conditions.

Although there were various reports of CMS discovery in rice, the breaking news of commercial hybrids came from China (Virmani and Edwards 1983). In the autumn of 1970, Li Bi Hu of the Hunan Academy of Agricultural Sciences (HAAS) identified a natural male sterile line among the weedy rice (*Oryza sativa* f. *spontanea*) populations in Hainan island. Named as 'wild abortive (WA)', Dr. Yuan Long Ping of HAAS made several crosses with this WA line to produce several MS lines. One of the first MS lines developed was Er-jiu-nan 1A, which was later used extensively in hybrid rice production (Lin and Yuan 1980). Er-jiu-nan 1A was developed from the early maturing variety 6044, by four backcrosses. Other male sterile lines were also developed subsequently such as Zhen Shan 97A, V20A and several others. The first set of WA-based hybrids was released soon, and by 1978, about five million ha was under hybrid rice in China. The first set of hybrids were named after their female parents. Those derived from Er-jiu-nan 1A were named as 'Nan-You', hybrids, those from Zhen Shan 97A were known as 'Shan-You' and those from V20A were named with the prefix 'Wei You'. In the initial years of development, there were two other male-sterile systems used in China, Boro and Hong-Lien, which showed practical prominence.

Boro cytoplasm was initially described by Shinjyo (1969) when the cross between Chinsurah Boro 2 was made with Taichung 65. However, efficient transfer of Boro type met with difficulties in *indica* rice, leaving WA as the only source of viable CMS in rice. The restorer genes, *Rf1* and *Rf2* were identified for Boro type, while *Rf3* and *Rf4* restored fertility in WA CMS system. Obtained from China through IRRI, WA cytoplasm and its derived lines spread throughout the world

with a new hope. By 1980, IRRI began full-fledged research of hybrid rice with WA cytoplasm as the pivot. Several, hybrids were developed, however, the resulting heterosis was not as prominent as reported in China. This slowed down the initial thrust a bit, but still, the works continued in several countries. In India, hybrid rice research had begun in 1980 with the collaboration of IRRI as the major knowledge and resource partner (Jachuck et al. 1986).

By 1981, Er-jiu-nan 1A, Zhen Shan 97 A and V20A were introduced into India along with their maintainers. Initial hybrid development was done using the introduced lines directly, however, there was a significant issue of grain quality among the hybrids developed under Indian conditions. Moreover, they showed increased susceptibility to pests and diseases. Conversion of Indian lines into male sterile lines was felt indispensable. By 1989, a national programme on hybrid rice was launched. The efforts bore fruit, the first set of hybrid rice was released in India in 1994, comprising four of them, APRH1, APRH2, MGR1 and KRH1. At the initial stage, all the successful hybrids were developed using only one A-line, IR58025A. This line, IR58025A, was developed by Dr. Sant Singh Virmani, the hybrid rice breeder at IRRI in 1988. When the conversion of Indian lines took momentum, in IARI, the same was replicated using lines under Basmati lineage. Two lines were identified as maintainers of WA cytoplasm, Pusa 167–120–3–2 and Pusa 150–21–1–1, both with exceptional grain quality, long slender grains and agronomic features. Among these, Pusa 167–12–3–2 was derived from the cross of Type 3/Ratna. IR48483A, a male sterile line developed at IRRI by crossing Zhen Shan 97A/MR365, was backcrossed to Pusa 167–12–3–2, and after six backcrosses IR58025A was developed. As a result, this line possessed long slender grains with aroma. IR58025A was further crossed to Pusa 150–21–1–1 to develop Pusa 6A after six backcrosses. By 2001, Pusa 6A became the parent of the first superfine grain aromatic rice hybrid in the world, Pusa RH 10 (Fig. 1.3c). Before the development of indigenously derived male sterile lines with better combining ability and grain quality, IR58025A was the major A-line used in hybrid rice development in India. Among the 127 rice hybrids released as of today, at least 15% have been developed using IR58025A as female parent.

Besides the involvement in hybrid development, IR58025A stands as a major contributor to the male sterile line development in India. One of the major drawbacks of using IR58025A was the aroma it imparted in the hybrids, which was an undesired feature in the non-aromatic grain sector. However, this problem has now been overcome in the latest array of male-sterile lines. At the beginning of the 1990s, hybrid rice in India was restricted to the public sector institutions, as there were few private sector companies involoved. This sitiation soon changed, and during the 10 years between 2000 and 2009, 44% of the rice hybrids released were from the private sector. After 2010, the scenario completely changed with private sector hybrid contribution going up to 91%. Only seven public sector hybrids were released between 2010 to 2021.

In wheat, unlike in maize and rice, hybrid development has always been a dubious challenge. The existence of male sterility driven by cytoplasmic factors in wheat was known before that in rice, when Kihara (1951) reported it for the first

time. Ten years later, a usable version was identified when *Triticum timopheevi* cytoplasm was found to induce sterility by interaction with *T. aestivum* nucleus (Wilson and Ross 1962). By this time, several reports of male sterility manifestation were pouring out of laboratories worldwide. From India, Rana and Swaminathan (1968) reported *T. zhukovskyi* as a source of MS cytoplasm. But there was a common problem. The availability of restorer genes was scarce. For *timopheevi* cytoplasm, *Rf* genes were sourced from *T. timpheevi* itself. It was the only system that looked viable. The first male sterile line 'Bison' was developed (Wilson and Ross 1962), and it could be successfully used to generate fertile hybrids (Schmidt et al. 1962).

Other than the restorer gene scarcity, another major bottleneck existed in wheat for diversifying cytoplasm in the form of undesirable nuclear-cytoplasmic interactions. Here again, *timopheevi* cytoplasm showed an exception, as no such interaction was reported (Virmani and Edwards 1983). Additionally, hybrids based on male sterility systems often showed less yield heterosis than the hand-pollinated hybrids. In the meantime, some commercial hybrids were released, but with little impact. All over the world, enthusiasm for hybrid wheat was dying down, until in the 1990s the efforts took another turn with the advent of chemical hybridisation agents (CHA). CHAs are growth-regulating chemicals that can selectively interfere with pollen production (McRae 1985; Duvick 1999). With renewed hope, the global area under hybrid wheat has begun to rise, but at a slow pace. Altogether, in Europe and America more than 60 hybrids were released (Gupta et al. 2019). In India, no wheat hybrids are released for commercial cultivation, except for two CMS-based hybrids, Pratham 7070 and Pratham 7272. However, they could not compete with the high yielding pure line cultivars.

A significant level of hybrid developments had happened in two other field crops, pearl millet and sorghum. Hybrid production in the early years of pearl millet improvement used the protogynous flowering behaviour as a mode of effecting cross-pollination. However, this method was not failproof, as any human error could result in reduced hybrid seed set. With the discovery of the CMS system 'Tift 23A' (Burton 1965a), the hybrid production scenario was set for a change in pearl millet. Tift 23A was developed by Glenn W. Burton in 1965 at Tifton, Georgia, in the USA after several years of research on male sterility systems. Tift23A carried A1 cytoplasm, which was stable enough to promote hybrid seed production. Along with Tift23A, there was another line, Tift18A developed by Burton (Burton 1965b). Introduced into India, two parallel programmes progressed, one for diversification and the other for hybrid development. IARI and Punjab Agricultural University (PAU) were the pioneers of pearl millet breeding in India (Srivastava et al. 2020). The first hybrid, Hybrid Bajra 1 (HB1) was released by PAU in 1965. HB1 was developed from Tift23A by crossing to BilB3 (Athwal 1966). Subsequently, several other cytoplasms were reported, but A1 cytoplasm remained the major source of female lines for hybrid production in India (Kumar and Andrews 1984; Srivastava et al. 2020). One of the IARI developed hybrids, Pusa 23 using A1 cytoplasm, has become widely adopted in the northern plains (Fig. 1.3g). The major issue with hybrid pearl millet was increased susceptibility to diseases, particularly downey mildew. Diversification of parental base hence has become a major strategy for

developing newer hybrids in pearl millet, an effort continuing in different institutions of India.

In the case of Sorghum too, hybrid breeding research before the pre-green revolution period was negligible. The preliminary report of the heterosis in sorghum came from the Lubbock substation of TAES in 1927 (Conner and Karper 1927). Twenty-seven years later, Stephens and Holland (1954) reported a novel CMS cytoplasm 'Milo', from a cross between Double Dwarf Yellow sooner Milo and Texas Blackhull *kafir* at TAES. They identified the possibility of using the system for commercial hybrid production and developed Combine Kafir (CK) lines since *kafir* nuclear genes could restore the sterility induced by milo. Some of these lines were introduced to India, after the inception of AICMIP. Coordinated research efforts under the project on sorghum resulted in the release of the first hybrid, CSH1 or Coordinated Sorghum Hybrid 1 in 1964. Its parents were CK60A and IS84 (IS84-SA7529-55-1-1-1-1 from the Texas Durra Caudatum race) were both introduced from Texas. The second hybrid, CSH2 followed next year from the cross, CK60A/IS 3691. Initially, at least six hybrids were released with the directly introduced parents or from the lines selected within them. In the meantime, launch of ICRISAT has accelerated the hybrid breeding activities in sorghum, with an increasing number of conversions taking place using local germplasm. Starting with CSH1, as many as 26 *Kharif* sorghum hybrids have been released in India, until 2016.

Hybrid breeding in other field crops has not been much accomplished as that in cereals, particularly using male sterility systems. Notwithstanding, several commercial hybrids using conventional methods such as hand pollination has been released in sunflower, safflower, Brassica and groundnut claiming different levels of heterosis realisation. Notable exceptions are pigeon pea and Brassica, wherein extensive research on male sterility was done. First reported male sterility in pigeon pea was genetic male sterility (GMS) in 1978 (Reddy et al. 1978). Although few other GMS systems were identified, maintenance of male sterility was the major bottleneck in the commercialisation of this technology. Search for alternate systems such as CMS was begun, with the particular objective of wide hybridisation. First report came in 1995, with Ariyanayagam et al. (1995) identifying *Cajanun sericeus* cytoplasm induced male sterility in *C. cajan*. The *sericeus* system was denoted as A_1 cytoplasm, followed by the discovery of A_2 cytoplasm from *C. scarabaeoidus* (Chauhan et al. 2004).

Dalvi et al. (2010) provided a comprehensive review on the male sterility systems in pigeon pea. So far, two hybrids are released, ICPH 3762 in 2010 and ICPH 2740 in 2015, but the breakthrough in yield and other desirable traits are yet to be realised. In Brassica also, the CMS systems such as *tour*, *trachy* and *mori* were used for hybrid development. The first CMS-based hybrid in India was released in Punjab, PGSH 51, based on *tour* cytoplasm. Later, CMS-based hybrids such as NRCHB 506, DMH 1 and Coral 432 (PAC 432) were released (Chauhan et al. 2011). Hybrid breeding in Brassica has another accomplishment. In 2008, a transgenic hybrid, DMH 11 was developed by Delhi University which became India's first transgenic hybrid (Jagannath et al. 2002). Recent developments in hybrid Brassica have

attracted private sector researchers too (Yadava et al. 2012). There were also attempts to develop male sterility systems in chickpea and groundnut which remain unresolved at the commercial level.

1.4.6 Genomics-Assisted Breeding

By the end of the 1980s, genomics-based breeding began to take shape in field crops, pioneering from the development of DNA based markers in rice. Contemporarily, tissue culture techniques were also found extensive development, but the initial enthusiasm soon died as the expected returns eluded the researchers. The promise offered by genetic engineering for targeted crop improvement has faced hurdles in commercial deployment due to anti-genetically modified organisms (GMO) activism. Interest in this field was so enormous, several laboratories for agricultural biotechnology research have come up all over the world. Notwithstanding the setback in transgenic plants, techniques such as the development of linkage maps and mapping of loci, targeting both qualitative and quantitative traits, were invigorating the scientists with a ray of hope of success. During the 1990s, there was a quantum leap in molecular techniques, thanks to the Human Genome Project (HGP), a worldwide consortium. Began in 1990 and concluded in 2003 (www.genome.gov), HGP provided several cutting-edge technologies that has parallelly been translated to crop genomes. The first attempt to sequence a cultivated crop genome began in 1998 with the inception of the International Rice Genome Sequencing Project (IRGSP) in the same mode as that of HGP.

The genome of the *japonica* cultivar, Nipponbare, was completely decoded by 2005 (IRGSP and Sasaki 2005). The availability of the genome information was soon made public. Parallelly, with the help of several bioinformatic tools supported by the modern computing platforms, the whole rice genome was annotated under a different project, Rice Genome Annotation Project (RGAP) beginning from 2004. Several databases have been created and made publically available. All these developments have completely reinvented the way trait-based breeding was done. Similar developments in other field crops had seen the unfolding of many crop genomes. Almost at the same period, the publications of the first linkage map of rice (McCouch 1990; McCouch et al. 1988) based on restriction fragment length polymorphism (RFLP) and microsatellite markers (Temnykh et al. 2001; McCouch et al. 2002) were made. Microsatellites, also known as simple sequence repeats (SSR) found abundantly in the genome particularly dispersed within the non-coding regions and widely distributed, could generate high density linkage maps due to their enormity in the rice genome.

All this was possible by the Nobel winning invention of Kary Banks Mullis, who described a lab-based method to amplify DNA in vitro using an enzymatic reaction (Mullis et al. 1986) known as polymerase chain reaction (PCR). This method was simple and very effective in the targeted amplification of DNA. PCR spurred a series of discoveries of different molecular markers, but none prevailed as that of SSRs. By the beginning of the twenty-first century, SSRs have been widely recruited as the

molecular tool targeting various genetic studies such as diversity, linkage and quantitative trait locus (QTL) mapping. Most of the QTLs, mapped earlier using other marker systems such as RFLP were remapped using SSRs. Since SSR profiles were highly reproducible across genotypes, they found their way into the breeder's kit – as an indispensable selection tool.

To use any marker for selection, it must establish a close linkage with the gene of interest. Stronger the linkage more efficient the marker becomes in selection. Several QTLs have also been reported at the same time, flanked between two adjacent SSRs. The abundance of SSRs helped the researchers to narrow down to the gene by recruiting SSRs within the flanks. This way, most of the major QTLs have been fine mapped. Integrating the sequencing techniques of the amplified fragments, the target gene could be easily identified using an annotation database constructed from model species such as *Arabidopsis* and rice. Now gene-based/functional markers are also available for targeted selection.

1.4.7 Marker-Assisted Selection in Breeding

Marker assisted selection (MAS) denotes employing molecular markers in the selection process. Since they are based on the DNA itself, their usefulness becomes definitive in the selection process. Moreover, the whole process allows several progenies to be looked into, even when they are young, which adds to the throughputness of the MAS. Although MAS can be integrated into several breeding methods, it has been particularly successfull in backcross breeding for rectifying specific defects in already popular crop varieties. Currently, marker-assisted backcross breeding (MABB) is being widely used in field crops in India. MABB targets to augment the selection process to reduce the turnaround time for varietal development, most economically. After identifying the target gene/QTL, MABB allows them to be transferred to an elite/popular varietal background where specific traits need improvement. Additionally, integration of desired traits by pyramiding the target genes/QTLs can also be undertaken under MABB programmes.

During the last 10 years, MABB has undergone several refinements in the protocols targeting precision and economy. One of the major changes was the integration of a rigorous phenotypic selection along with foreground and background selection, particularly in the early generations (Singh et al. 2011). This has not only helped recovery of the recurrent parent phenome to its near totality but also aided in accelerating the breeding process. Integration of phenotypic selection in MAB has been a crucial factor in Basmati breeding, because of the exclusive grain quality of this group of rice. Often when a non-Basmati source is used as the gene/QTL donor, severe impairment of Basmati quality is experienced requiring further refinements (Babu et al. 2017). In the preliminary protocols of MABB, only foreground and background selection were included (Liu et al. 2003) with the idea that recovery of maximum recurrent parent genome (RPG) would recover the phenome also. However, in practice, this does not seem to occur due to several undetected regions of the donor genome lying latently in the progenies. While this can be

attributed to the limited number of background markers, scaling them to a high-density coverage can make the entire selection process expensive and time-consuming (Ellur et al. 2016), jeopardising the fundamental objective of accelerated breeding. However, the early generation phenotypic selection could address this issue effortlessly. Yet another improvement was the reductive screening in background selection, in which the completely recovered background markers were progressively eliminated from the selection process (Sagar et al. 2020). This could not only economise the selection by reducing the time but also could aid in conserving the resources.

Recently, postponing the entire background selection to a later generation was also attempted, relying initially on phenotypic selection along with foreground selection. The results were dramatic, as the final selections could accumulate as much RPG recovery as possible along with the phenome recovery, ultimately saving a lot of time and money, along with aiding the initial screening of a large number of progenies (Oo et al. 2021). Cultivar releases using MAS in India has taken off in 2007, with the release of Improved Pusa Basamti 1 and immediately followed by Improved Samba Mashsuri. Since then, several improved varieties have been released for commercial cultivation (Table 1.2). Among the institutions, IARI has the maximum share of 36% among the releases, covering three crops, rice, maize and chickpea. All of the rice cultivars released using MABB, targets bacterial blight and/or blast resistance, while in maize hybrids focus was on developing QPM hybrids, with or without pro-vitamin A enrichment. In chickpea, two MAS derived cultivars were released, Super Annigeri 1 and Pusa Chickpea 10,216 (Fig. 1.3g) having fusarium wilt resistance and drought tolerance, respectively. There are four Indian rice cultivars improved by IRRI, directly released for cultivation in India such as Swarna Sub1, Samba Sub1, CR1009 Sub1 and IR64 Drt1 (DRR Dhan 42).

1.4.8 Genomic Selection (GS)

GS is the contemporary buzzword in genomic assisted breeding. If MAS is used for individualistic improvement, especially targeting elite cultivars, GS envisions population improvement even for self-pollinated crops. Similar to MAS, GS also weighs on the availability of desired alleles and amasses them into a set of individuals through a series of breeding steps. However, there are fundamental differences in the approaches that are followed. MAS requires mapping of the target alleles before their use in the introgression or pyramiding programmes, whereas GS does not require mapping individual target alleles. While MAS requires donor and recipient (recurrent) parents, GS typically needs training and testing (breeding) populations. Fundamentally, GS operates on a closed breeding system, which means it starts with a set of diverse founders that are known to harbour different allelic combinations of target loci. Thus, founders in the GS programme are elite genotypes with high breeding values, that are interbred to develop a large number of biparental populations. Bred to near homozygosity, the progenies of these crosses are divided into two, a training set and a testing set. Training set undergoes a low- or mid-density SNP genotyping

Table 1.2 MAS derived varieties of field crops released and notified in India

Sl. No.	Crop	Improved Variety	Trait	Genes incorporated	Markers used	Year of release	Developer
1	Rice	Improved Pusa Basmati 1	Bacterial blight	*xa13*, *Xa21*	CAPS, STS	2007	IARI
2	Rice	Improved Samba Mahsuri	Bacterial blight	*xa5*, *xa13*, *Xa21*	SSR	2008	IARI
3	Rice	Swarna Sub1	Submergence tolerance	*Sub1*	InDel	2009	IRRI
4	Rice	Samba Sub1	Submergence tolerance	*Sub1*	InDel	2011	IRRI
5	Rice	Improved Lalat	Bacterial blight	*Xa4*, *xa5*, *xa13*, *Xa21*	STS	2012	NRRI
6	Rice	Improved Tapaswini	Bacterial blight	*Xa4*, *xa5*, *xa13*, *Xa21*	STS	2012	NRRI
7	Rice	PR122	Bacterial blight	*Xa4*, *xa13*, *Xa21*	SSR	2013	PAU
8	Rice	PR121	Bacterial blight	*Xa4*, *xa13*, *Xa21*	SSR	2013	PAU
9	Rice	Pusa 6 (Pusa 1612)	Blast	*Pi2*, *Pi54*	SSR	2013	IARI
10	Rice	CR1009 Sub1	Submergence tolerance	*Sub1*	InDel	2013	IRRI
11	Rice	PR123	Bacterial blight	*Xa4*, *xa13*, *Xa21*	SSR	2014	PAU
12	Rice	IR64 Drt1 (DRR Dhan 42)	Drought tolerance	*qDTY2.2*, *qDTY4.1*	SSR	2014	IRRI
13	Rice	Pusa 1592	Bacterial blight	*xa13*, *Xa21*	SSR, STS	2015	IARI
14	Rice	PR124	Bacterial blight	*Xa4*, *xa13*	SSR	2015	PAU
15	Rice	Pusa Basmati 1609	Blast	*Pi2*, *Pi54*	SSR	2015	IARI
16	Rice	Pusa Basmati 1728	Bacterial blight	*xa13*, *Xa21*	SSR, STS	2016	IARI
17	Rice	Punjab Basmati 3	Bacterial blight	*xa13*, *Xa21*	SSR	2016	PAU
18	Rice	CR Dhan 800	Bacterial blight	*xa5*, *xa13*, *Xa21*	STS	2016	CRRI
19	Rice	Pusa Basmati 1637	Blast	*Pi9*	SSR	2016	IARI
20	Rice	Ranjit Sub1	Submergence tolerance	*Sub1*	InDel	2016	AAU
21	Rice	Bahadur Sub1	Submergence tolerance	*Sub1*	InDel	2016	AAU
22	Rice	Pusa Basmati 1718	Bacterial blight	*xa13*, *Xa21*	SSR, STS	2017	IARI
23	Rice	Punjab Basmati 4	Bacterial blight	*xa13*, *Xa21*	SSR	2017	PAU

(continued)

Table 1.2 (continued)

Sl. No.	Crop	Improved Variety	Trait	Genes incorporated	Markers used	Year of release	Developer
24	Rice	Punjab Basmati 5	Bacterial blight	*xa13*, *Xa21*	SSR	2017	PAU
25	Rice	PR127	Bacterial blight	*Xa45*(t)	STS	2018	PAU
26	Rice	DRR Dhan 51	Blast	*Pi2*	SSR	2018	IIRR
27	Rice	Pusa Samba 1850	Blast	*Pi1*, *Pi54*, Pita	SSR, STS	2018	IARI
28	Rice	CO43 Sub1	Submergence tolerance	*Sub1*	InDel	2018	TNAU
29	Rice	DRR Dhan 50	Submergence, drought tolerance	*qSub1*, *qDTY2.1*, *qDTY3.1*	SSR	2018	IIRR
30	Rice	CR Dhan 801	Submergence, drought tolerance	*qSub1*, *qDTY1.1*, *qDTY2.1*, *qDTY3.1*	SSR	2018	NRRI
31	Rice	CR Dhan 802 (subhash)	Submergence, drought tolerance	*qSub1*, *qDTY1.1*, *qDTY2.1*	SSR	2018	NRRI
32	Wheat	PBW723 (Unnat PBW343)	Stripe and leaf rust resistance	*Yr17*, *Yr40*, *Lr37*, *Lr57*	SSR, CAPS	2017	PAU
33	Wheat	PBW761 (Unnat PBW550)	Stripe rust resistance	*Yr15*	SSR	2019	PAU
34	Wheat	PBW752	Stripe rust resistance	*Yr10*	SSR	2019	PAU
35	Wheat	PBW757	Stripe rust resistance	*Yr15*	SSR	2019	PAU
36	Wheat	PBW771	Stripe and leaf rust resistance	*Yr40*, *Lr57*	CAPS, SSR	2020	PAU
37	Maize	Vivek QPM9	Lysine and tryptophan	*opaque2*	SSR	2008	VPKS
38	Maize	Pusa HM4 improved	Lysine and tryptophan	*opaque2*	SSR	2017	IARI
39	Maize	Pusa HM8 improved	Lysine and tryptophan	*opaque2*	SSR	2017	IARI
40	Maize	Pusa HM9 improved	Lysine and tryptophan	*opaque2*	SSR	2017	IARI
41	Maize	Pusa Vivek QPM9 improved	Provitamin-A	*crtRB1*	InDel	2017	IARI
42	Maize		Provitamin-A	*crtRB1*	InDel	2020	IARI

		Pusa Vivek hybrid 27 improved					
43	Maize	Pusa HQPM5 improved	Provitamin-A	*crtRB1*	InDel	2020	IARI
44	Maize	Pusa HQPM7 improved	Provitamin-A	*crtRB1*	InDel	2020	IARI
45	Chickpea	Super Annigeri 1	Fusarium wilt resistance	*Foc4*	SSR	2019	IARI
46	Chickpea	Pusa chickpea 10216	Drought tolerance	QTL hotspot on LG4	SSR	2019	IARI
47	Pearl millet	HHB67 improved	Downy mildew resistance	*QRsg1*, *QRsg4*	RFLP	2005	ICRISAT
48	Soybean	NRC127	KTI free	Null allele of *KTi3*	SSR	2018	IISR
49	Soybean	NRC142	KTI + lipoxygenase free	Null allele of *KTi3*	SSR	2019	IISR

IARI ICAR-Indian Agricultural Research Institute, New Delhi, *ICRISAT* International Crops Research Institute for Semi-Arid Tropics, Patancheru, *IIRR* ICAR-Indian Institute of Rice Research, Hyderabad, *IISR* ICAR-Indian Institute of Soybean Research, Indore, *IRRI* International Rice Research Institute, Los Banos, *NRRI* ICAR-National Rice Research Institute, Cuttack, *PAU* Punjab Agricultural University, Ludhiana, *TNAU* Tamil Nadu Agricultural University, Coimbatore, *VPKAS* ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora

covering the whole genome, as well as a multi-location evaluation within the target population of environment (TPE). The data is used for generating a valid model that connects genotypic and phenotypic data. This model is then used for predicting the genomic estimated breeding values (GEBVs) of the testing panel. Since the training data involves multi-location data, the element of genotype-by-environment interaction is inbuilt in the GS model.

A minimum of three locations is required, and not more than two replications to improve heritability. There are several statistical approaches to modelling, however, two methods, genomic best linear unbiased predictor (gBLUP) and ridge regression BLUP (rrBLUP) are adjudged to be the best methods in model building (Meuwissen et al. 2001). Further, a large population is desirable because the GS models attempt to capture total additive genetic variance for predicting GEBVs. A larger population requires robust experimental designs such as augmented, spatial, partially replicated (p-rep) or sparse testing. The selected lines based on the GEBVs form the Stage 1 cohort, which can either be recycled for the next breeding cycle or be advanced for varietal development. Selection intensity needs to be high to capture maximum number of allelic combinations. Further, accelerated breeding cycle improves the effectiveness of GS in crop breeding programmes.

1.5 An Overview of Breeding Research in the Last Decade

In the decades past 1960s, the green revolution has cast its magical spell to a significant extent on the field crops. All the crops experienced an emulated version of the yield increase as that happened in the three prime cereals. Strong scientific intervention on varietal development, along with improved cultural practices and calculated fertilisation, has all contributed to increased productivity. In the last 10 years, two remarkable shifts have occurred; for the first time, use of marker-assisted breeding has resulted in the release of 46 cultivars, and a quantum increase in the release of cultivars bred by the private sector. In rice alone, 29 cultivars were released by marker-assisted breeding, with notable improvement in disease resistance targeting both bacterial blight and blast, either singly or in combination (Table 1.2). In rice, about 90% (68 out of 75) of the hybrids released during the last decade has come from the private sector (Fig. 1.5). This is a welcome change, as several private producers are coming forward in seed production and marketing, making the seed availability to farmers unlimited. Furthermore, private-sector research organisations are looking for higher yield and grain quality besides stress resilience as the major breeding targets to sustain competition among themselves as well as with the public sector institutions. This major shift towards commercial agriculture is not for rice alone. Currently, the hybrid sector in field crops is dominated by private organisations and are giving stiff competition to public sector organisations. The exception is wheat, wherein private seed suppliers produce and market high yielding pure line varieties to a considerable extent.

Another noteworthy development in the breeding research in India comes from the varietal turnover, particularly from the non-hybrid sector. So far, this sector

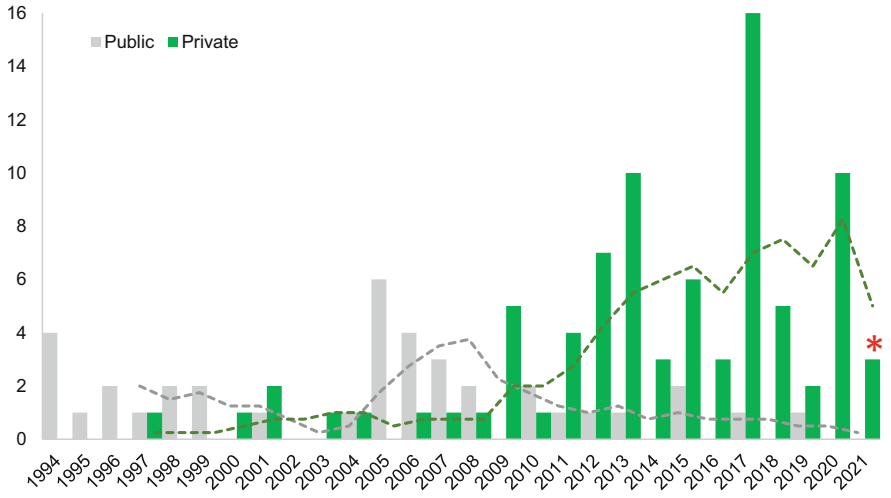

Fig. 1.5 Pattern of release of rice hybrids in India between 1994 and 2021. There is a marked shift in private sector hybrids during the last ten years. The dotted lines indicate three year moving average. *Data for 2021 is incomplete

remains the mainstay for the public sector institutions. The varietal release system in India takes two routes, national varietal release and provincial varietal release. National release follows rigorous nationwide testing and widely adapted cultivars are only passed through this system, ultimately released and notified by the Central Sub-committee on Crop Standards, Notification and Release of Varieties for Agricultural Crops (CVRC). Most of the specifically adapted cultivars are approved through provincial bodies such as State Varietal Release Committees (SVRCs). Examining the varietal release during the last 10 years, in 14 field crops, which included both hybrid and non-hybrid cultivars, one could see a marked difference in the pattern of release. The most striking feature is the number of releases, 743 under SVRC, a 34% increase over the CVRC releases totalling 554 cultivars (Fig. 1.6). However, CVRC releases looked relatively more balanced than the SVRC releases. Although rice dominated the number of releases, an equally good number of varietal releases happened in maize, wheat and cotton under CVRC, while a predominance of rice cultivar release was seen under SVRC.

In rice, SVRC release was three times more than that in CVRC. This pattern raises more concerns than comfort because indiscriminate release of varieties that are specifically adapted would render them less adopted, leaving the varieties mostly to the breeder than the ultimate stakeholder, the farmer. This also would slow down the dissemination of widely adapted cultivars, which need to find its adoption against the flash flood of narrowly adapted varieties. Another dimension to this problem is the performance evaluation system of the breeder's service under public funded organisations. Often, the number of cultivars released than the number adopted is the

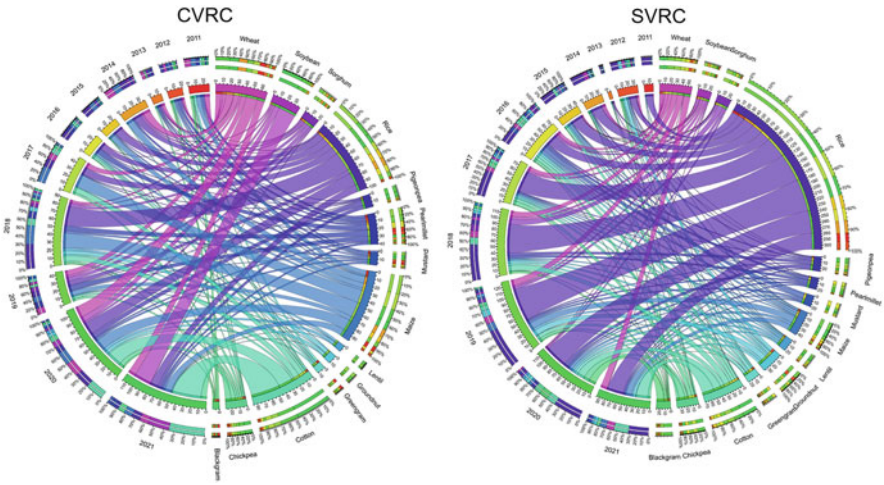

Fig. 1.6 Varietal release pattern among 14 field crops between 2011 and 2021. The CVRC releases were relatively more balanced among the major crops, while SVRC releases were mostly dominated by rice cultivars

criteria followed for assessing a breeder. This leads to unhealthy practices and competitions, thwarting the very basic objective of crop improvement.

Notwithstanding, the breeding efforts during the first two decades of the twenty-first century has been eventful. Several high yielding varieties have seen remarkable adoption within a short period of time from release. Varieties such as HD2967 and HD3086 in wheat, Pusa Basmati 1121 and Pusa Basmati 1509 in rice, etc. have grown into megavarietal proportions. Another important dimension is the focus shift towards grain quality, as well as climate resilience. With the technological advancements, breeding efforts in India is yet to venture into genomics assisted breeding. Modern tools such as genomic selection and gene editing have not been used in improving field crops in India. Although the research in this direction is progressing, the looming threat of anti-GMO campaigns shadows the future of gene-edited breeding lines, although all of them do not fall under the category of GMOs. Recently, efforts are also underway to integrate technologies to translate breeding success into genetic gain.

1.6 Towards Improving Genetic Gain

Genetic gain, the advantage accrued every generation within a unit of time, by genetic improvement of crops has been a pivot of discussion for a long time. Hazel and Lush (1942) defined genetic gain as the average improvement in genotypic/ phenotypic value within a population as a consequence of selection. In the practical sense, this means a perpetual increase in productivity, described more comprehensively as an ‘evergreen revolution’ by Prof. M. S. Swaminathan

(Swaminathan 1996). The dimensions of the evergreen revolution are multifaceted and subsume ecological, economical and sustainable components (Swaminathan 2006). Genetic gain is an ultimate translation of trait advantage as a result of the accrual of beneficial alleles. Therefore, the gain can happen in any trait individually or in combination resulting in an overall advantage to the selected population. The combining of multiple traits for selection can be realised through the use of appropriate selection indices. One of the primary requirements to achieve gain is heritability. We know that heritability increases in several ways, the most common route is through the accumulation of favourable alleles which cumulatively improves the trait expressivity. Breeding interventions such as selecting a large set of genotypes increases the probability of accumulating more variants, and more variants provide the opportunity of generating several allelic combinations. Several allelic combinations allow the steady accumulation of them, a perpetual increase leading to the evergreen revolution.

Encapsulating all these components into a mathematical expression, Jay Laurence Lush (1937) made the famous breeder's equation, which would help to predict the expected genetic gain in generations under selection. Breeder's equation primarily has three components, the selection intensity (i), heritability (h^2) and additive variance (σ^2_A), the product of which will help us to predict the gain. As discussed above, i allows drawing the maximum number possible from a spectrum of alleles (σ^2_A), with h^2 translating the effect into the gain. Later a fourth component was added as a denominator to the equation, the cycle time (L), which is the average time per generation (Eberhart 1970), which has more relevance today than during the time of Lush. Probably, Lush could not have imagined having this component added as the original equation was framed for animal breeding, and it was not relevant to animal breeding as reducing the gestation period in animals was impractical. Having several generations squeezed within a time frame allows more opportunities for accrual of alleles, therefore lower the cycle time increases the gain phenomenally. For instance, keeping all the genetic factors constant, but having two generations a year than the regular one, alone can double the gain. But directly applying the equation to the public sector plant breeding has a catch. Mostly, the public sector plant breeding has been random, which means parents are selected at random and progenies are also selected at random which leads to the breeding success also becoming random. Breeder's equation does not work with this randomness but requires a closed system rather than an open one. A closed system means the founders of the population should breed and the progenies must interbreed, recycled through the selection process, accumulating the gain. The conundrum of random breeding therefore cannot provide an actual assessment of genetic gain in the true sense. However, assuming the whole breeding population within a species as the breeding population, we can make a rough estimate of gain through era trials.

Era trials, evaluation of representative cultivars of different breeding eras, can help us in indirectly estimating the realised genetic gain approximately (Rutkoski 2019a, b). Era trials have been used to estimate the genetic gain in maize hybrids (Meghji et al. 1984), which was later extended to later eras as well (Duvick 2005). There are other cautions too, era trials cannot compare the effect of breeding

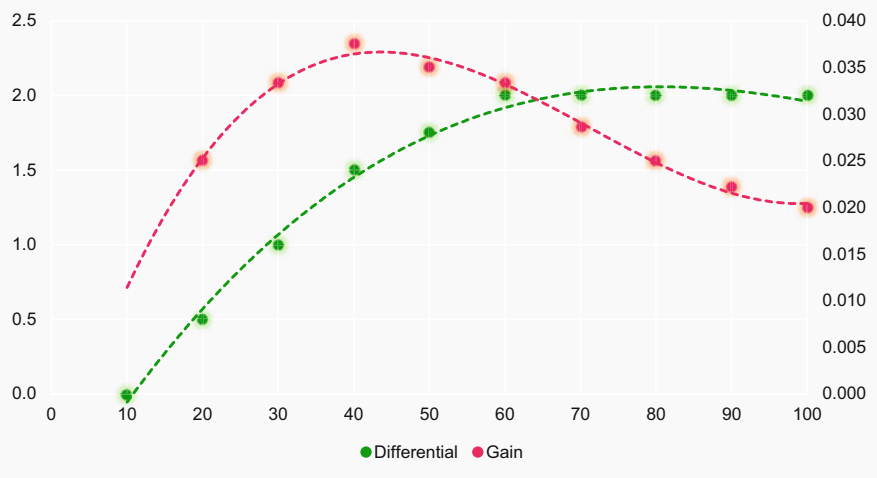

Fig. 1.7 A hypothetical relationship between realised yield and genetic gain in a crop for 100 years. The yield differential is calculated with respect to the base value at the beginning of the breeding. Progressive genetic gain is calculated per year. Once the yield plateaus gain starts to decay significantly

programmes, such as genetic gains by hybrids vs pure line varieties, because the breeding itself is an outcome of a random set of parents and the pattern of yield increase is not linear between eras. Further, the random representative(s) of each era may not be truly representative. However, era trials can depict an overall picture of where the breeding system stands currently. Another factor that decays the genetic gain is the yield plateauing. In a hypothetical system as given in Fig. 1.7, the yield plateauing can result in decay in genetic gain significantly if no breeding intervention is made to uplift the yield levels, a situation currently being faced by several field crops.

Realised genetic gains in field crops in India, show varying trends. It is not surprising because, the quantum genetic gain for the evergreen revolution in different crops should differ, based on their importance in the food chain as well as on the base yield potential. It is proposed that a genetic gain of 1.3% per annum is required to sustain food production in wheat (Rosegrant and Agcaoili 2010). Currently, data are being generated to quantify realised genetic gains through era trials in major field crops. In a recent exploration of yield and related traits in wheat, Yadav et al. (2020) examined wheat yield from 1905 to 2016 by mining the historical data and found that average genetic gain ranged from 0.54% per year to 0.82% per year (over the first released variety, NP4). This estimate comes closer to the estimates made by Lopes et al. (2012) using CIMMYT lines within a window of 30 years, in which a gain of 0.9% among high yielding, 0.7% among intermediate and 0.5% among low yielding cultivars have been reported. However, considering the wide window used by Yadav et al. (2020), (no methods were found to explain how the estimates were

made) the yield gain in India could have been much larger, if we consider the decay in genetic gain over the periods of intermittent yield stagnation.

Throwing light into this assumption, the preliminary trend from the era trials in wheat indicates improved gain (unpublished data). The wheat yield improvement in India could be attributed to parallel genetic gain in biomass, grain number per spike and reduced duration. Examining the genetic gain in rice, with a specific focus on rainfed environments, Kumar et al. (2021) report rather a short-term genetic gain in rice, for a 10-year window between 2005 and 2014. They found an annual genetic gain of 0.68% among irrigated checks, which increased to 0.87% under moderate reproductive stage drought stress, while 1.9% gain could be achieved under severe drought conditions. Although these figures cannot be scaled to rice breeding in general, but indicates that trait targeted breeding still offers opportunities to improve gain in crops like rice. In another such study in pearl millet, Yadav et al. (2021) report achievement of 4% genetic gain per year during the last 30 years, primarily attributable to hybrids. There was a marked gain in ear length and ear diameter, and they are rated as the major components of yield gain. In chickpea too, the preliminary reports from era trials indicate significant genetic gain for the last 60 years (unpublished data). Presently, efforts are underway to accelerate genetic gain in crop breeding systems involving field crops.

One of the global movements towards accelerating the genetic gain is to employ GS in a strict sense that oversees all the components of genetic gain. In India, recently a pilot programme has been launched with selected field crops and involving several national and international institutions to start GS-based crop improvement with the help of organisations such as Bill and Melinda Gates Foundation (BMGF), Excellence in Breeding (EiB) and Breeding programme assessment tool (BPAT). The major objective of this project is to generate crop product profiles for TPEs, breeding programme optimisation, implementing GS and data digitalisation through breeding management system (BMS).

1.7 Threats and Opportunities

In this section, we concisely present the prospects of crop breeding in India. This is not particular to field crops alone. For several parts of the last 120 years, owing to various factors, crop breeding in India has progressed through leaps and bounds. Except for few milestones, public sector plant breeding has not been much eventful before the 1950s. Restructuring of breeding research was the main highlight of the pre-green revolution phase, which could successfully prepare the grounds for green revolution. Yadav et al. (2019), while examining the production and productivity pattern of major cereals, reveal that the phase immediately following the green revolution was more productive than the green revolution itself. Although the threat of yield stagnation is lingering, as seen occurring in wheat yield in Europe (Brisson et al. 2010), the current situation in India shows a more comfortable scenario. However, with unpredictable phenomena such as climate change on the horizon, time is ripe to have another reorientation of breeding research in India to sustain an

evergreen revolution. With the caveats of climate change around, it is estimated that as many as 30% of the world population would be at risk of hunger by 2050.

Events related to climate change are currently being reported around the world, and in India, a tropical country, the threat is more formidable. Major challenges are drought, flood, soil salinisation, submergence, temperature fluctuations, low nutrients besides biological threats from pests, diseases and weeds. Besides, physiological disorders can also emerge under shifting environments. Therefore, future breeding should reorient towards precision agriculture with added resilience in crops to face unexpected adverse events. This means the future belongs to widely adapted cultivars than specifically adapted ones. It is time to recycle specifically adapted cultivars through breeding to evolve wide adapted ones.

Agriculture is the largest industry in the world with the lowest capital input. This is particularly relevant to countries like India, where crop breeding happens unorganised. Comparing to other nations, Indian agriculture has a long way to go in harnessing crop productivity in key crops. The most single reason for this is the lack of adequate support. Despite having human and genetic resources, crop breeding development in India has been heavily dependent on international inputs. Several genes that are being used today are discovered in Indian landraces but the discoveries have been made abroad. Unless increased focus is given with sufficient institutional and financial support, future of plant breeding in India will be challenging. The reorientation of agricultural research that happened in the post-1950s came with futuristic investments such as AICRP and research institutions. Since its inception, the AICRP system has not undergone serious restructuring, and still follows outdated protocols. A total reorientation of breeding with product profile and TPE oriented system attached with state of the art infrastructure, standard operating protocols, a modernised AICRP system and a renewed futuristic plan is the need of the hour from the policy intervention front.

The technologic front in crop breeding has been transformative in the last three decades. Particularly, developments in genome biology have changed the landscape of trait-based breeding. With accurate interventions such as MABB, now we have the capability to transfer a target allele to an elite background, where a particular allele is lacking. Besides, techniques for large scale mining of alleles are also available. With the high-end computational capabilities and high throughput genotyping and phenotyping platforms breeding time is set to reduce considerably in the future. Accurate predictions are taking over phenology-based selections. Besides, accelerated generation turnover technologies such as doubled haploids, rapid generation advancement and speed breeding are going to augment genomics assisted breeding for better realisation of genetic gain. Additionally, genome editing techniques are also getting ready for manipulation of individual genes to generate novel traits that are hitherto lacking. In the twenty-first century, technological options are unlimited for breeding modernisation. However, rational use of these techniques is warranted for which appropriate human resource development is essential.

The natural reserves of enormous genetic diversity and the availability of the best human resources in the world are the primary opportunities we are bestowed with.

Compared to the global scenario, several genetic resources in India remain underutilised. Currently, a megaproject is in operation to characterise and utilise 15,000 Indian rice landraces that are conserved in the National Gene Bank, for allele mining, gene and trait discovery targeting several biotic and abiotic stress tolerance, physiological and quality parameters. This project is expected to offer various novel solutions hitherto not utilised in rice breeding. One of the major problems concerning human resources has been the insufficient number of highly trained personnel in research. Brain drain in agriculture research needs to be plugged, particularly in crop improvement, for which strategic integration of genetic resources, institutions, infrastructure, human resources, technology and market is to be made.

We need to build a globally competitive research and education system in India. Further, the research should be strictly oriented to meet the challenge of future food needs for our growing population. With his futuristic vision, Prof. Swaminathan wrote while introducing the concept of the evergreen revolution, 'Countries like India, China, and Bangladesh have to produce more and more food and other farm commodities from diminishing per capita arable land and irrigation water resources. Therefore, productivity enhancement is the only pathway available to us to produce more to feed the growing population. This is why an Evergreen Revolution approach is exceedingly important. An Evergreen Revolution needs the integration of frontier technologies like biotechnology and information communication technology with traditional ecological prudence' (Swaminathan 2006).

1.8 Conclusion

In the present chapter, we have chronicled the breeding research in India, particularly keeping field crops in focus, while introspecting the achievements made. More discussions happened around the major field crops, but without critical details as there are specific chapters to follow for those crops. However, we have tried to narrate the milestones in the history of breeding wherever appropriate, while keeping the future course in mind. No specific dealing of stress tolerance and quality improvement has been made in this chapter, as that would be redundant. From the modest beginning with the inception of IARI to commence the organised research in agriculture, then through ICAR, modern breeding has transformed India from a 'ship to mouth' economy to a self-reliant economy. We have also discussed the opportunities of utilising modern technologies in fast-forwarding breeding and thereby genetic gain.

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Wheat Breeding

2

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Abstract

Wheat (*Triticum* spp.) is a major staple rabi cereal contributing about 20% calories to the human diet and important cereal for ensuring food and nutritional security in many parts of the world. Burgeoning population and diversification of food habits involving more of wheat-based products have increased the demand for wheat. Therefore, integration of modern crop tools with conventional approaches is necessary to develop the future genotypes that not only will be climate resilient and input responsive but also have higher yield per unit time and space so as to meet the growing demand. It deals with the wheat crop in a holistic manner focussing on a wide range of topics including origin, evolution, taxonomy, genetics, breeding, quality, stresses, high throughput phenotyping, genomic selection, genome editing, speed breeding and new plant breeding techniques (NPBT). This chapter also covers Indian approaches of wheat varietal development and testing under co-ordinated system as a case study, and thus would be a valuable reading material for students, researchers, academicians and policy makers.

Keywords

Wheat · Genetics · Quality · Stresses · Genome editing · New breeding techniques

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_2

2.1 Introduction

Wheat (*Triticum* spp.) is a major cereal crop contributing about 20% calories to the diet and is a staple crop of many countries including India. Wheat-based multiple products for the end users have increased the demand for wheat. Apart from major source of starch and energy, it also provides variable amounts of a different components which are essential or beneficial for health including protein, vitamins, minerals, and phytochemicals. Wheat being a major source of cereal dietary fibre, its consumption reduces the risk of cardio-vascular disease, type 2 diabetes, and certain forms of cancer. Compound annual growth rate in production was much higher at national level with 2.07% as compared to 1.35% at global level; attributed to India's positive growth in productivity (1.23%), followed by crop acreage (0.84%). The level of global and national wheat productivity is broadly similar, although global trends are slightly higher side on year-to-year basis, with the exception of 2000, 2002, 2012 and 2020 production years. It is important to state that in India, we cultivate only spring wheat genotypes with maturity duration of ~140 days as compared to the global scenario that includes a major portion of winter wheats (close to 300 plus days for maturity) and this clearly indicates that our per day productivity is much higher.

2.1.1 Importance of Crop and Progress Made in the Past 25 Years

The last 25 years of global and national trends on wheat area, production and productivity are analysed and presented in Table 2.1 and Fig. 2.1 (FAOSTAT 2020). In India, wheat area under cultivation increased by 6.35 million hectares (25.38%), i.e. from 25.01 million hectares during 1996 to 31.36 million hectares during 2020. Contrary to the national scenario, global wheat area reduced by 2.73 million hectares (−1.21%), i.e. from 224.58 million hectares to 221.85 million hectares during the same period. India's wheat production increased from 62.10

Table 2.1 Dynamics in wheat area, production and productivity for India vis-à-vis in the world

Parameter	CAGR (%)	CV (%)	Change	
			Quantum	%
Period: 1996–2020				
A. India				
Area (mha)	0.84	6.99	6.35	25.38
Production (mt)	2.07	16.41	45.76	73.69
Productivity (kg/ha)	1.23	10.52	1015.51	40.90
B. World				
Area (mha)	0.01	2.12	−2.73	−1.21
Production (mt)	1.35	10.69	197.48	34.13
Productivity (kg/ha)	1.32	10.11	862.91	33.49

CAGR is compound annual growth rate and CV is coefficient of variation

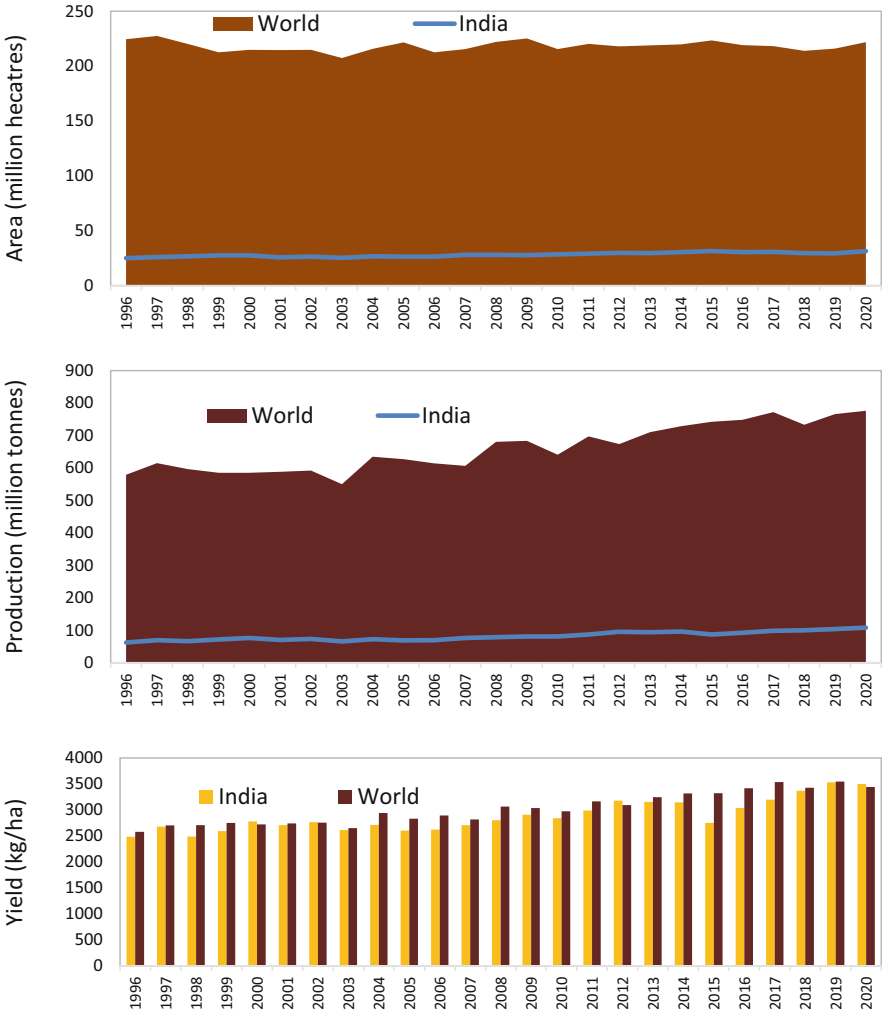

Fig. 2.1 Trend in wheat area, production and productivity for India vis-à-vis the world

million tons during 1996 to a record production of 107.86 million tons during 2020 with a quantum jump of 45.76 million tons. Similar trends at global level were also observed for the wheat production. However, India's performance with respect to percentage change (73.69%) for production was more than double as compared to the world's wheat production (34.13%). Our national wheat productivity increased from 2483 kg/ha during 1996 to 3498 kg/ha during 2020. A similar trend was observed for global wheat yield; however, again percentage change was much higher at national level (40.90%) compared to global (33.49%). Overall, during the past 25 years, wheat area increased at national level but it has marginally decreased at global level.

2.2 Origin, Evolution and Distribution of Species and Forms: Wild Relatives

Wheat is a classic example for understanding the evolutionary theory of allopolyploid, speciation, adaptation and domestication in plants (Gustafson et al. 2009). Origin and evolutionary pattern of wheat are presented in Fig. 2.2. The cytogenetic and genomic studies indicated that the mutation, polyploidy in the form of amphiploidy and inter-generic hybridisations are the major factors responsible for the evolution of present-day wheat. The wheat species can be broadly classified into three groups, namely diploid, tetraploid and hexaploid, based on the number of chromosome in the reproductive cell, i.e. $n = 7, 14, 21$, respectively. Cytogenetic studies revealed that four different genomes (ABD and G) representing four different sets of seven chromosome contributed to the origin of tetraploid and hexaploid wheats. The hexaploid wheat consists of two evolutionary lineages. The most known and grown *T. aestivum* (AABBDD) comprises one lineage while *T. zhukovskyi* (genome AAAAGG) comprises another lineage.

The earliest cultivated forms were diploid (genome AA) and believed to be domesticated in south-eastern Turkey (Heun et al. 1997). During the process of

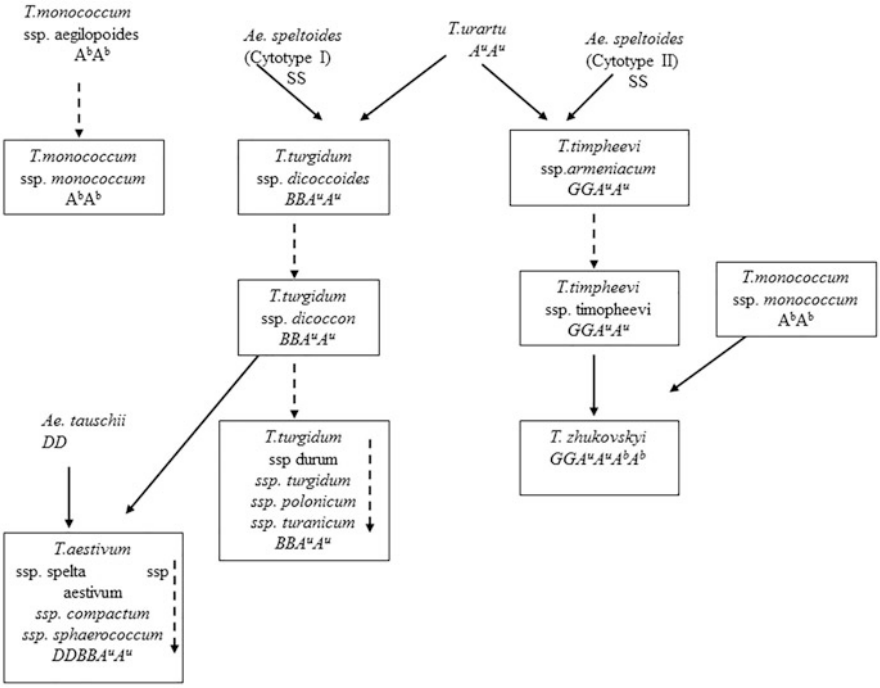

Fig. 2.2 Origin and evolutionary pattern of wheat. Solid arrows indicate hybridisation followed by chromosome doubling. Dashed arrows indicate domestication or direct selection within a species. Boxes indicate cultivated taxa

domestication major emphasis was given on two traits, i.e. non-brittle rachis or tough rachis and the hull-less grains or free threshing grains. The source of A genome of tetraploid and hexaploid wheats was related to two diploid species, i.e. *T. monococcum* and *T. urartu*. Earlier studies by Kihara (1944) indicate that AA genomes were contributed by *T. monococcum*. However, on the basis of repeated nucleotide sequences in the phylogeny of polyploidy species of wheat, Dvorak et al. (1993) concluded that AA genomes in the tetraploid and hexaploid wheats are more related to AA genomes of *T. urartu*. The BB genomes of the tetraploid and hexaploid wheats are closely related to *Sitopsis* section of *Aegilops* with *Ae. speltoides* being the closest species. The SS genomes of *Ae. speltoides* is also closest to the GG genomes of *T. timopheevi*. The AABBDD genomes are thought to be arisen through hybridisation of *T. turgidum* with *Aegilops tauschii*. Petersen et al. (2006) re-sequenced two single copy nuclear genes *DMC1* and *EF-G* isolated from each of the three genomes in hexaploid wheat (BBAADD) and two genomes of the tetraploid wheat (BBAA) using a sophisticated extension of PCR technique, and on the basis of phylogenetic analysis with diploid species, they suggested that the DD genomes of wheat was derived from *Ae. taushcii*. The D genome contributed significantly to the wheat flour properties that make bread wheat so valuable in bread making (Morris and Sears 1967).

The diploid wheats include two species, viz., *T. monococcum* and *T. urartu*. The sterility in the hybrids of *monococcum* and *urartu* (Johnson and Dhaliwal 1976) indicate that they are valid species. Reproductive barrier is not essential but one of the important criteria for recognising the species and natural hybridisation between diploid species is a rare phenomenon. The cultivated einkorn *T. monococcum* ssp. *monococcum* is the domesticated form of wild einkorn *T. monococcum* ssp. *aegilopoides*. The cultivated ssp. of *monococcum* differs from ssp. *aegilopoides* in having slightly large kernel and less brittle rachis. It is believed to be domesticated in 'Fertile Crescent' of Near East, which encompasses the eastern Mediterranean, south-eastern Turkey, northern and western Iraq and its neighbouring regions of Transcaucasia (Matsuoka 2011). The wild diploid species *T. urartu* also found in Fertile Crescent though it has never been domesticated. The tetraploid wheat ($2n = 4x = 28$) originated as a result of natural crossing between *T. urartu* and *Aegilops speltoides*. This resulted in origin of both the tetraploid species, namely *T. turgidum* (AABB genome) and *T. timopheevi* (AAGG genome).

The wild form of *T. turgidum* ssp. *dicoccoides* with brittle spike is reported from the Fertile Crescent. The non-brittle spikes with tough rachis among the wild form were selected by farmers. From there it spread to the other parts of world. Durum wheat is said to be derived from *T. dicoccum* (Damania 1998). The emmer wheat underwent further diversification in response to the agro-ecological conditions and gave rise to free threshing tetraploid wheats, namely rivet wheat (*T. turgidum* ssp. turgidum), polish wheat (*T. turgidum* ssp. *polonicum)* and khorasan wheat (*T. turgidum* ssp. turanicum). Interploidy introgression in hybrid swarms is thought to be contributed to further diversification of the *turgidum* wheat by giving rise to two sub-species *T. turgidum* ssp. *paleocolchicum* and *T. turgidum* ssp. *carthlicum.* McFadden and Sears (1946) created an artificial spelta (hexaploid wheat) from a

hybrid of *T. dicoccum* and *Ae. Tauschii*, followed by chromosome doubling with colchicine. Therefore it was inferred that spontaneous hybridisation between emmer wheat (*T. dicoccum*) and goatgrass produce an early spelta (*T. spelta* $2n = 6x = 42$ genome AABBDD). Later more easily free threshing bread wheat was evolved through natural mutation and selection. Hexaploid *T. zhukovskyi* originated recently by interspecific hybridisation of cultivated *T. timopheevii* with cultivated *T. monococcum* (Dvorak and Luo 2001).

2.3 Taxonomic Position of Genus *Triticum*

In botanical classification, wheat is a member of the grass family Poaceae (also called Graminae), the subfamily Pooideae and the tribe Triticeae. Beside wheat, many agricultural important crop species like barley, rye, and several forage species belongs to the tribe triticeae. The tribe is mainly distributed in the east Mediterranean and central Asiatic regions. Linnaeus (1753) describes five genera, namely *Secale*, *Triticum*, *Hordeum*, *Aegilops* and *Elymus*, in this tribe, whereas Clayton and Renvoize (1986) recognised 18 genera in the tribe triticeae. Later on van Slageren (1994) added *Ambylopyrum* genus in the tribe. All these genera were classified into two sub-tribes, namely Hordeineae (barley lineage with nine genera (*Elymus*, *Hystrix*, *Sitanion*, *Leymus*, *Psathyrostachys*, *Hordelymus*, *Hordeum*, *Taeniatherum*, *Crithopsis*)) and Triticineae (wheat lineage with nine genera (*Agropyron*, *Eremopyrum*, *Heteranthellium*, *Secale*, *Dasypyrum*, *Triticum*, *Ambylopyrum*, *Aegilops*, *Henrardia*)) (Feldman and Levy 2015). The genus *Brachypodium* was earlier included in this tribe but later on excluded from this tribe (Hasterok et al. 2004). Bowden (1959) advocated that there is no need to treat *Aegilops* as a separate genus from *Triticum*, however van Slageren (1994) considered *Aegilops* and *Triticum* are closely related but separate genera.

Several species of genus *Secale* and *Aegilops* serve as primary genepool based on crossability with *Triticum* species, while several species of genera *Elymus*, *Leymus*, *Psathyrostachys*, *Dasypyrum* are capable of being hybridised with wheat (Wang 2011), and considered as secondary and tertiary genepool of wheat. The taxonomy of genus *Triticum* is always debatable. In recent years, Mac Key (1966 and 1977) and Dorofeev et al. (1979) illustrated the system of classification of genus *Triticum*, while van Slageren (1994) revised the classification provided by Mac Key (1966). Goncharov (2011) reviewed the taxonomic history of genus *Triticum* and differences between various classifications of genus *Triticum*. van Slageren (1994) classified the genus *Triticum* into 3 sections and 6 species. These 6 species were further divided into 4 autonym and 13 non-typical subspecies, while Dorofeev et al. (1979) recognised 27 species in the genus *Triticum* (Table 2.2). van Slageren (1994) considered *T. sinskajae* a free threshing mutant of *T. monococcum*, *T. jakubzineri* a form of *T. turgidum*, *T. militinae* a free threshing mutant selected from single specimen of cultivated *T. timopheevi*, *T. petropavlovskyi* mutated form of *T. polonicum*, *T. aethiopicum* free threshing emmer selected from *T. dicoccon*, *T. isphanicum* as a form of *T. polonicum* due to more standard glume morphology.

Table 2.2 Overview of *Triticum* species

Van Slageren (1994)		Dorofeev et al. (1979)		Chr. No.	Common name	Genomic constitution
Sect	Species and Sub-species	Sect	Species			
Monococcon	*T. monococcum* L	Monococcon	*T. monococcum* L	14	Cultivated Einkorn	A^bA^b
	ssp. *monococcum* L		*T. sinskajae* A. F ilatet Kurk	14	Naked einkorn	A^bA^b
	ssp. *aegilopoides* (Link) Thell		*T. boeoticum* (Boiss)	14	Wild einkorn	A^bA^b
	T. urartu Thumanian ex Gandilyan	Urartu	*T. urartu* Thumanian ex Gandilyan	14	Wild form	A^uA^u
Dicoccoidea	*T. turgidum* L	Dicoccoidea				
	ssp. *dicoccoides* (Korn. Ex Asch. & Graebn.) Thell		*T. dicoccoides* (Korn. Ex Asch. & Graebn.) Thell	28	Wild emmer wehat	A^uA^u BB
	ssp. *dicoccon* (Schrank) Thell		*T. dicoccum* (Schrank) Schuebl	28	Emmer wheat	A^uA^u BB
	ssp. *paleocolchicum* (Menabde) A love and D. Love		*T. karamyschevii* Nevski	28	Georgian wheat	A^uA^u BB
			T. ispahanicum Heslot	28	Emmer wheat	A^uA^u BB
	ssp. *turgidum* L	Triticum	*T. turgidum* L	28	Rivet, cone or pollard wheat	A^uA^u BB
	ssp. *durum* (Desf) Husn		*T. durum* Desf	28	Macaroni wheat	A^uA^u BB
	ssp. *carthlicum* (Nevski) A. Love & D. Love		*T. carthlicum* Nevski (syn. T. persicum Vav.)	28	Persian wheat	A^uA^u BB
	ssp. *turanicum* (Jakubz.) A love and D. Love		*T. turanicum* Jakubz	28	Khorassan wheat	A^uA^u BB
	ssp. *polonicum* (L.) Thell		*T. polonicum* L	28	Polish wheat	A^uA^u BB
			T. jakubzineri Udacz. et Schachm	28	Naked tetraploid	A^uA^u BB
			T. aethiopicum Jakubz	28	Ethiopian wheat	A^uA^u BB
	T. timopheevi (Zhuk.) Zhuk					
	ssp. *timopheevi* (Zhuk) Zhuk	Timopheevii	*T. timopheevii* (Zhuk.) Zhuk	28	Zanduri wheat	A^bA^b GG
	ssp. *armeniacum* (Jakubz.) Zhuk syn *T. araticum*		*T. araraticum* Jakubz	28	Wild Zanduri	A^bA^b GG

(continued)

Table 2.2 (continued)

Van Slageren (1994)		Dorofeev et al. (1979)		Chr. No.	Common name	Genomic constitution
Sect	Species and Sub-species	Sect	Species			
			T. militinae Zhuk. et Migusch	28	Naked tetraploid	A^bA^b
Triticum	*T. aestivum* L.	Triticum				
	ssp *aestivum* L		*T. aestivum* L	42	Bread wheat	A^uA^uBBDD
	ssp. *compactum* (Host) Mackey		*T. compactum* Host	42	Club wheat	A^uA^uBBDD
	ssp. *sphaerococcum* (Percival) Mackey		*T.sphaerococcum* Perciv	42	Indian dwarf wheat	A^uA^uBBDD
			T. petropavlovskyi Udacz. et Migusch	42	Rice-head wheat	A^uA^uBBDD
	ssp *macha* (Dekapr and Menabde) Mackey	Spelt	*T. macha* Dekapr. et Menabde	42	Macha wheat	A^uA^uBBDD
	ssp. *spelta* (L.) Thell		*T. spelta* L	42	Dinkel wheat	A^uA^uBBDD
			T. vavilovii (Thum.) Jakubz	42	Yunanense Wheat	A^uA^uBBGG
		Kiharae	*T. kiharae* Dorof. et Migusch	42		A^bA^bGGDD
	T. zhukovskyi Menabde & Ericz	Timopheevii	*T. zhukovskyi* Menabde et Erizjan	42	Zhukovsky's wheat	$A^bA^bA^uA^uGG$

These were not considered as separate taxa at species or sub-species level by van Slageren (1994).

2.4 Spike Morphology and Pollination System

The wheat inflorescence commonly known as ear and in botany called as terminal distichous compound spike. In spike, the spikelets are arranged on the alternate side of the zig-zag rachis (Fig. 2.3). In a majority of tetraploid and hexaploid species of *Triticum*, the rachis is tough and resists disarticulation. However, *T. dicoccoides* and *T. dicoccon* disarticulate easily when mature. Each spikelet is subtended by two sterile or empty glumes. These glumes are arranged alternately on opposite side of the short central axis called rachilla. In a majority of *Triticum* species the glumes are shorter than the spikelet. The glumes are glabrous or pubescent. In some of the *Triticum* species, the glumes are keeled throughout, while in some species lower portion of glume is rounded. The shape of glume apex differs significantly in different species of *Triticum*. In *T. aestivum*, each spikelet consists of 3–6 florets attached alternately on rachilla. Each floret has its own lemma and two nerved palea. The tip of lemma may or may not be extended to awn. Based on the length of awn, the wheat is classified as long medium, short or awnless. The wheat flower is hypogynous and simple in structure consisting of whorl of three stamens and a single carpel. The uppermost floret of spikelet is imperfect (Percival 1921).

Wheat is usually self-pollinated species and anthesis starts from the middle third of the spike and proceeds upwards rapidly and downwards little slower. In spikelet, the basal flower is first to open then the secondary florets (de Vries 1971). The receptivity of stigma depends on weather conditions. The anther dehiscence begins with elongation of filament and is completed after the anther is pushed out of floret due to lodicules swelling. Parts of the pollen fall on their own stigma and fertilise the ovary. The wheat pollen is short lived and the viability of pollen depends on the weather conditions. Generally, pollen remains viable upto 30 to 40 min after pollen shedding. The florets close again due to collapse of lodicules. In case ovary remains

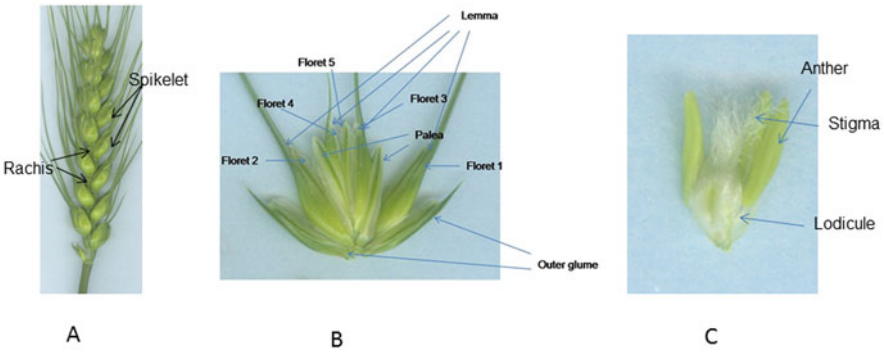

Fig. 2.3 Wheat inflorescence. (**a**) Spike of wheat. (**b**) Spikelet of wheat ×4. (**c**) Floret of wheat ×8

unfertilised, Okada et al. (2018) reported the second opening of flowers in wheat within a few days of post-anthesis. In the second opening there is a significant enlargement of ovary and forces the lemma end to be apart. Despite cleistogamous flower, less than 1% out crossing is reported in the wheat.

2.5 Gene Banks and Conservation of Genetic Resources

The key to success in any crop breeding programme depends on tapping the genetic variability of existing plant genetic resources in hybridisation programme. The extent of diversity present in the gene bank still remain untapped and may contain many useful traits that can be used in accelerating the rate of genetic gain in wheat breeding. The wheat genetic resources conserved in various gene banks also stored much useful information on passport and evaluation data. The gene bank serves as a focal point for providing information on plant genetic resources. The basic purpose of collating such information is to enable the plant breeders to make more thorough use of plant genetic resources. Significant progress has been made in various modern biotechnology tools such as recombinant DNA technology, cell biology and allied disciplines in recent years. Such tools should be targeted to mine genetic diversity as part of pre-breeding programmes for efficient integration with conventional breeding programme so as to achieve better and faster breeding outcomes.

Conservation of wheat genetic resources is a global concern. The in-situ conservation is one of the way of conservation of biodiversity, especially crop wild relatives. Damania (1996) advocated the need to safeguard the natural ecosystem as it is highly dynamic and understanding it's components in short notice is unrealistic. Various countries, like Armenia, Russia, Syria, and Turkey, are involved in in-situ conservation of wild relatives of wheat crop (Meilleur and Hodgkin 2004). However, ex-situ conservation of wheat genetic resources had contributed a lot to the improvement of wheat yield, disease resistance and nutritional quality. Around 856,168 germplasm accessions are being conserved in various national and international organisations. Our global partner, CIMMYT has made significant contribution in conserving, improving and distributing wheat germplasm particularly to developing countries. CIMMYT's wheat programme has a focus on development of new germplasm and their distribution in the form of various international trials and nurseries. In the gene bank of CIMMYT, around 110,281 accessions of wheat are conserved (Table 2.3). These are nearly 13% of total wheat germplasm conserved in various gene banks. The introduction of Norin 10 genes in the CIMMYT programme revolutionised the wheat production in many parts of the world. Pavon, Veery, Bobwhite, Attila and Kauz are some of the CIMMYT lines, which have been used extensively in the breeding programme. The National Small Grain Collection at USA also safeguards the important global collection. Over the years, India and China have also developed sound scientific management for ex situ conservation of wheat genetic resources.

Table 2.3 Germplasm holding of wheat in various gene banks

Name of the institute	Accessions
CIMMYT, Mexico	110,281
National Small Grain Collection, USDA	57,348
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences, China	43,039
National Bureau of Plant Genetic Resources, India	35,889
International Center for Agricultural Research in the Dry Areas (ICARDA), Syria	34,951
National Institute of Agrobiological Sciences, Japan	34,652
N.I. Vavilov Research Institute of Plant Industry, Russia	34,253
Instituto di Genetica Vegetale (IGV), Bari, Italy	32,751
Leibniz Institute of Plant Genetics and Crops Plant Research (IPK), Gatersleben, Germany	26,842
Australian Winter Cereals Collection, Agricultural Research Centre, Tamworth, Australia	23,811
National Plant Gene Bank of Iran, Iran	18,442
Kazakh Research Institute of Agriculture and Plant Growing	18,000
Others	385,909
Total	8,56,168

Source: FAO (2010)

2.6 Molecular Cytogenetics and Breeding

Wheat cytogenetics started with the discovery of chromosome number of durum wheat (2n = 28) by Karl Sax in 1918, followed by the reporting of chromosome number of eight wheat species, viz. *T. monococcum* ($2n = 14$), *T. dicoccum* ($2n = 28$), *T. durum* ($2n = 28$), *T. turgidum* ($2n = 28$), *T. polonicum* ($2n = 28$), *T. spelta* ($2n = 42$), *T. compactum* ($2n = 42$) and *T. vulgare* ($2n = 42$), with the basic chromosome number of $x = 7$ by Sakamura in Japan (Sakamura 1918). These chromosomes numbers of identified species were later confirmed by Kihara and Sax in 1919 and 1922. Kihara used genomic analysis to study the evolutionary relationship among different *Triticum* species as earlier successfully demonstrated in genus *Drosera* by Rosenberg (1909). Genomic analysis involves the crossing of allopolyploids with their presumed diploid ancestral species to study pairing pattern of their triploid hybrids. In the hybrids, if there is occurrence of basic number of bivalents, it is taken as a sign of genomic homology of the diploid parent with one of the genomes present in allopolyploid species.

Genomic homology becomes confusing when there is presence of higher order of pairing like trivalents, tetravalents and pentavalents, indicating some degree of differentiation of otherwise identical genomes. Kihara (1919) analysed chromosome pairing behaviour in the crosses between tetraploids and hexaploids, and observed 14^{II} and 7^{I} in the pollen mother cells. Similarly, Sax in 1922 observed 7^{II} and 7^{I} in the pollen mother cells of hybrids generated from tetraploid and diploid crosses. Whereas, in crosses of *T. vulagre* and *Ae. cylindrica* (CCDD) giving F1 hybrid

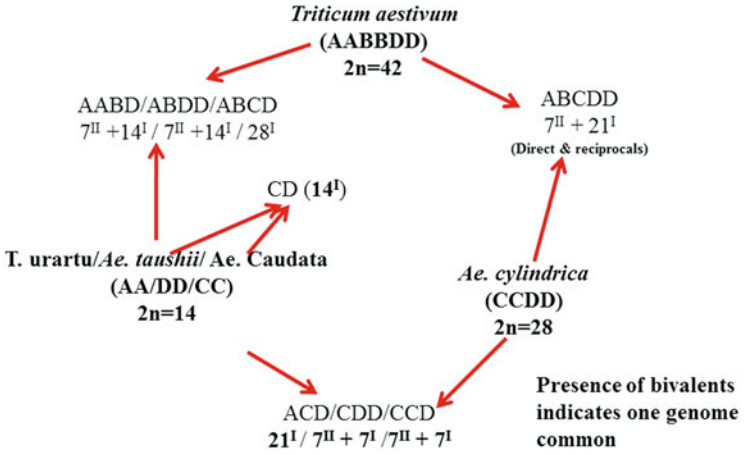

Fig. 2.4 Genomic analysis based on pairing behaviour among diploid and tetra and allohexaploids as described by Kihara (1919)

having $7^{II} + 21^{I}$ (ABCDD) as well as in reciprocal crosses in the pollen mother cells as earlier reported by Kihara. Based on these studies as well as other studies as depicted in Fig. 2.4, it was concluded that the genus *Triticum* had three different genomes having 7 chromosomes each and these genomes were designated by Kihara as A, B, C and D. It has been concluded that the hexaploid wheat genomes A and D have been contributed from *T. uratru* and *Ae. Taushii* (not from *Ae. Caudata* and designated as D).

The exact donor of B genome of present-day hexaploid and diploid bread wheat is still lacking, although many related diploid species exhibited partial homology and chromosome pairing but not to the extent observed for the A and D genome donors. Currently, *Ae. speltoides* ($2n = 14$, BB) species considered as the probable B genome donor (Dvorak et al. 1973). However, there are studies against the general acceptance including Kimber (1974), the study suggest that *T. speltoides* is probably homologous to the G genome of *T. timopheevii* and B genome donor to *T. turgidum* or *T. aestivum* is unrecognised. The C-banding technique helps in the identification of heterochromatin (dark-staining regions) and euchromatin (light-staining regions) on the chromosome axis. Within a homoeologous group, there was no similarity for the C-banding of chromosomes except for fifth group. C-banding comparisons of the diploid species *T. monococcum*, *T. speltoides*, and *T. tauschii* with that of the A, B, and D genomes, respectively, in hexaploid wheat confirmed that *T. speltoides* could not be the donor of the B genome to wheat and that *T. monococcum* and *T. tauschii* are the probable donors of the A and D genomes, respectively (for details see Gill and Kimber 1974).

2.6.1 Structure of Wheat Chromosomes

There are 21 pair of chromosomes identified cytologically having a primary constriction/centromere with secondary constrictions on two pairs of chromosomes indicating sites for ribosomal RNA genes. According to the observations of Sears (1954), the length of chromosomes at metaphase is on average 5.6 μm enclosed in the nucleus having a total volume of 1700 μm^3 in which about 36.2 pg of DNA is accommodated. The total DNA length is about 11.2 m with a volume of 35 μm^3 and is divided into 42 chromosomes. The DNA is coiled extensively and the first level of compaction is the nucleosome as revealed under electron microscope and by partially digestion of DNA with micrococcal nucleases. A haploid nuclear genome of hexaploid bread wheat has 16.72 × 109 bp (17.325 pg) of DNA, distributed on 42 chromosome 0.682 pg on 1DL to 2.475 pg on 3BL. The DNA contents in the A, B and D sub-genomes are found in the ratio 1.16:1.2:1.0 (Gupta 1991). It has been shown through reassociation kinetics that F75% of this DNA is repetitive (Mitra and Bhatia 1973).

Another 20% of DNA constitutes non-coding unique sequences. In addition, 30% of cytosine residues in this DNA are highly methylated regulating the expression of genes (Moore et al. 1993). Only 1% of this DNA is known to take part in protein synthesis; in this 1% DNA, F1, 000 genes have already been recognised, some of them representing multigene families (Lagudah et al. 2001; McIntosh et al. 1998). With the advancements in banding techniques, Sears (1954) identified individual wheat chromosomes through monatomic analysis and made some morphological observations, although cytologically most chromosomes were indistinguishable. Furthermore, Sears and Sears (1978) expedite the chromosome identification through isolation of marker telocentric chromosomes. Gill and Kimber (1974) identified individual wheat chromosomes, and wheat ideogram was constructed using C bands, which was facilitated by the telocentric chromosomes to recognise individual chromosomes.

2.6.1.1 Cytogenetic Stocks in Wheat

Wheat Aneuploids

Aneuploids in wheat have been developed by ER Sears, using cytogenetics techniques in the Chinese spring cultivars. These stocks are possible because wheat genome is polyploidy and can tolerate aneuploidy (Sears 1954, 1966a). During the 1930s, ER Sears was able to identify a few haploid plants, resulting from the cross between Chinese Spring with rye, which formed the basis of development of aneuploid stocks. In total, 220 lines were isolated, which comprised the stocks: 21 Monosomics (20″ + 1″); 7 Monoisosomic (20″ + i"); 21 Trisomics (20"+1'"); 38 Nullisomic–tetrasomic (20"+1″'"); 4 Monosomic–tetrasomic (20"+1'+1'"'); 8 Nullisomic (20"); 20 Double monosomic (19"+1'+1'); Ditelosomic (20"+t"), 21; 20 Double-ditelosomic (20"+tS" + tL") and 41 Ditelomonotelosomic (20″ + t" + t'). But the maintenance of these stocks is very difficult as

it is necessary to characterise the progeny of each aneuploid for ascertaining the chromosome number.

These aneuploidy stocks have a great potential for localisation of genes to specific chromosomes/chromosomal arms (McIntosh 1988). A unique genetic system for production of deletion stocks systematically with variable sized terminal deletions in individual chromosome arms was reported by Endo (1988). He suggested that presence of certain chromosome from *Aegilops cylindrica* in Chinese spring in the monosomic condition, it induces chromosomal breaks in the gametes lacking *A. Cylindrica* chromosome and results in generation of various chromosomal aberrations, including deletions. The broken chromosome ends, if not fused to other broken ends, are stabilised by the rapid gain of telomere structure (Werner et al. 1992). Such deletions in plants without the *A. cylindrica* chromosome are transmitted regularly to the offspring, and they identified 436 deletions by C-banding.

Alien Addition and Substitution Lines

Development of alien addition and substitution lines started during the late 1950s by producing amphiploids through crosses between hexaploid wheat and any diploid alien species, whose chromosomes were to be added or substituted. An amphiploid having $2n = 56$ was first crossed with hexaploid wheat, giving a heptaploid ($2n = 49$), which on selfing would produce progenies having different chromosome numbers including monosomic addition lines, which could then be selfed to obtain disomic alien addition lines. Such alien addition lines (including wheat-rye addition lines) were developed in the USA and Canada. These alien addition lines can be crossed with monosomics, and on selfing will give progenies having disomic substitution lines.

The aneuploid stocks offer great benefit as the cytogenetic markers identified on each of the 21 chromosomes will help in identification of each chromosome and chromosome arm. Because of the nulli-tetrasomic lines, Sears (1966b) was able to place the 21 wheat chromosomes into 3 genomes and 7 homeologous groups. For locating genes on to individual chromosomes, monosomic and telosomic have been used (McIntosh et al. 1995; Sears 1966c). These deletion stocks have been used to place the EST markers onto specific deletion bins for precisely map genes of interest (Qi et al. 2004). In addition, the deletion stocks were crucial in relating genetic maps to physical maps of chromosomes, map-based cloning of genes (Simons et al. 2006; Yan et al. 2003), and studying the distribution of genes (Gill et al. 1996) and recombination frequency along the chromosomes (Akhunov et al. 2003). The whole genome sequencing project of wheat has been made possible by the use of the ditelosomic stocks for isolating individual chromosome arm through flow sorting for the construction of arm-specific BAC libraries (Safar et al. 2004).

Diploidising System

Bread wheat contains three closely related genomes therefore; loss of any chromosome of a homeologous group can be compensated by the homeologous group chromosomes because of which monosomics have been possibly developed. Despite

closely related chromosomes, homeologous group chromosomes strictly follow diploid type behaviour and form 21 bivalents. The genetic control of chromosome pairing in wheat is dependent on a series of promoters and suppressing pairing homologues genes (Sears 1976). The strongest effect on pairing is associated with a gene on chromosome 5BL known as *Ph1* locus, which prevents homoeologous chromosomes to pair at meiosis. Apart from *Ph1 locus*, two more suppressors have been identified *Ph2* on 3DS and another suppressor on chromosome 3AS. This feature of *Ph1* locus was utilised for transfer of desirable genes from alien species to wheat, because in wheat plants deficient for 5B or those carrying a mutation for *Ph1* locus (Sears 1977) pairing between wheat chromosomes and the corresponding homoeologous alien chromosomes would occur. This will allow transfer of alien genes on to wheat chromosomes through recombination.

Alien Introgressions

Development of new plant varieties requires genetic variability, which can be created by crossing the available germplasm in the primary gene pool. Modern plant breeding, although increased crop productivity world-wide, however, it also eroded the genetic variability of the crops (Gill et al. 2011; Hoisington et al. 1999). Alien introgression of different gene(s) for tolerance to biotic/abiotic stress tolerance in wheat is presented in Table 2.4. To introduce new variability, use of secondary and tertiary gene pools is required and is generally referred as 'wide hybridisation'. Many wheat varieties have been released around the world carrying alien

Table 2.4 Alien introgression of different gene(s) for tolerance to biotic/abiotic stress tolerance in wheat

Source	Gene/trait	Reference
T. monococcum	*Sr21*, *Sr22*, *Sr35*	The (1973), Kerber and Dyck (1973), McIntosh et al. (1984), Rouse and Jin (2011)
T. dicoccum	*Sr9*	Knott and Anderson (1956)
T. turgidum var. *dicoccoides*	*Sr2*	McFadden (1930)
T. turgidum var. *durum*	*Sr11*, *Sr12*	Knott and Anderson (1956), Sheen and Snyder (1964)
T. dicoccoides	*Lr64*, *Pm16*, *Pm 26*, *Pm 30*, *Pm31*	Kolmer et al. (2010), Reader and Miller (1991)
T. timopheevii	Sr36, Sr 37	McIntosh (1988), McIntosh and Gyarfas (1971)
Th. bessarabicum and *Th. elongatum*	Salt tolerance	Colmer et al. (2006)
Ae. ventricosa	2 ns blast resistance, nematode resistance, *Rkn3*	Maia (1967), Helguera et al. (2003), Williamson et al. (2013)

chromosomal introgression from related wild species. About 110 leaf rust (Lr), 86 stem rust (Sr), and 83 stripe rust (Yr) resistance genes have been identified in wheat or wild relatives, most conferring race-specific resistance to these rust pathogens (Bhatta et al. 2019; Cox et al. 1992; Huerta-Espino et al. 2011; Ram et al. 2005; Singh et al. 2015).

Using ionising radiation, Sears (1956) transferred a leaf rust resistance gene from *Aegilops umbellulata* to chromosome 6B of bread wheat. The classical example of alien introgression T1BL·1RS resulted from the breakage of wheat chromosome 1BL·1BS at the centromere, and the 1BS arm of wheat was replaced by the 1RS arm of rye. This introgressed chromosomal segment carried group of resistance genes to leaf rust (Lr26), stem rust (Sr31), stripe rust (Yr9), powdery mildew (Pm8) (Friebe et al. 1996), along with robust drought-tolerant root system (Sharma et al. 2011). Similarly, powdery mildew resistance gene Pm 21 (T6AL·6VS) was introgressed from Dasyprum villosum (L.) Candargy had other important genes for resistance to wheat curl mite, stripe rust and Fusarium head scab (De Pace et al. 2011). Transfer of Ae. Umbellulata-derived leaf and stripe rust resistance to hexaploid wheat has been demonstrated by Chhuneja et al. (2008) and Bansal et al. (2017). Direct cross between wheat and Ae. kotschyi have been used to develop amphiploids with high grain iron and zinc and flag leaf iron and zinc concentrations as that of the *Ae. kotschyi* parent (Rawat et al. 2009). CIMMYT had developed about 1300 primary SHW, and have been confirmed to possess valuable traits for better performance under biotic and abiotic stresses, along with yield potential (Mujeeb-Kazi et al. 2008; Yumurtaci 2015). Dyck and Kerber (1970) transferred first leaf rust resistance gene, Lr21, from *Ae. squarrosa* var. *strangulata* 'R.L.5271' to Canthatch and Thatcher wheat cultivars through direct hybridisation. Lr42 has been one of the most effective Lr genes introduced from *Ae. tauschii* accession TA2450 for further utilisation in hexaploid wheat breeding (Gill et al. 2019).

2.7 Genetic Studies of Qualitative and Quantitative Traits

Improvement in any crop requires the presence of genetic variation and also the exploitation/manipulation of that variation for developing improved genotypes. Selection is generally practised for selecting desirable plants within the population, which is based only on identification phenotypically superior plants. Phenotype is composed of both heritable and non-heritable variation. The heritable component is governed by the gene(s) present in the genome of the plant, whereas the unexplained variation (environmental influence) affecting the expression of trait is non-heritable. In order to select the plants, understanding of fundamental laws of inheritance and genetics of different traits is necessary. Some characters are governed by one or few genes and are also less influenced by environmental variation and are referred as oligogenic/qualitative traits. These traits produce specific effect, and population can be classified into distinct classes. Some traits are controlled by several genes having small effect are referred to as polygenic/quantitative traits. These traits are largely affected by environmental factors, and population shows continuous variation and

cannot be classified into distinct classes as in case of oligogenic traits. The earliest examples of genetics studies in wheat for presence of awns and glume hairiness were reported to be governed by genes, which were monogenically inherited.

However later studies indicated more complex inheritance. Based on chromosome substitution lines genes conditioning earliness were reported to be present on seven chromosomes (Kuspira and Unrau 1957). Similarly, genetics have been worked out by different workers for different wheat traits, like biotic stresses (rusts, powdery mildew, Karnal bunt, Spot blotch, Tan spot, etc.), abiotic stresses (heat, drought, waterlogging, salinity, PHST, etc.), agro-morphological traits, viz. earliness, tillering, lodging, plant height, spike density, 1000-kernel weight, grain number and yield using conventional inheritance studies as well as using cytogenetic stocks to identify chromosomal regions governing these traits. The qualitative inheritance have been reported mainly for biotic stresses, particularly rust and powdery mildew resistance, whereas the quantitative inheritance have been reported for spot blotch, Karnal bunt, Septoria, head scab, heat and drought tolerance and most of the yield contributing traits. Grain yield being a complex trait generally associated with different yield contributing traits, which are also polygenically inherited. Selection for oligogenic traits can be practised early in generation as they are having high heritability. The quantitative traits like grain yield are having low heritability and are also influenced by environmental factors, so selection will be better if practised later in generations as it gives opportunity for combing several component traits to be selected upon. Therefore knowledge of trait genetics is of utmost importance for wheat breeding.

2.8 Breeding Objectives

2.8.1 Grain Yield: The Ultimate Aim

Breeding for higher yield is one of the primary objectives of wheat improvement to meet the ever increasing demand for wheat-based diets across the globe. Breeders of autogenous crops aim to develop inbred varieties through selection and accumulation of desirable alleles with additive effects on desirable traits, including yield. During the initial 3 decades of green revolution, the global wheat yield was around 3% per annum, whereas, the following two decades witnessed the growth of merely 1.4% (FAO 2020). The quantum jump in wheat yields across the globe started in the 1960s, with the continuous effort of Nobel Laureate Norman E. Borlaug with his crossing programme including Norin-10/Brevor cross that introduced the *Rht-B1* and *Rht-D1* dwarfing alleles, which led to the development and release of input responsive semi-dwarf varieties led to Green Revolution.

Historically, chromosomal translocation of 1RS-1BL between wheat and rye was one of the most landmark introgression till date in wheat improvement, which increased the wheat yield potential and tolerance to biotic and abiotic stresses.

This landmark segment is still present in many of the important wheat cultivars, currently cultivated across the globe (Schlegel 1997). The contribution of E.R. Sears deserves a special mention for his great contribution to this field. Development of synthetic wheat is another important strategy by repeating the interspecific crosses that occurred in nature that led to the formation of hexaploid wheat. Different accessions of the hexaploid wheat progenitors *T. monococcum*, *T. turgidum*, and *Ae. tauschii* used for the formation of new genetic constitutions of wheat, greatly increasing the genetic variability of the primary gene pool (Mujeeb-Kazi et al. 2008). Numerous synthetic wheat germplasm pools have been developed by CIMMYT, Mexico.

2.8.1.1 Trait-based Breeding to Increase the Yield Gain

Selection of wheat genotypes based on yield components will help in the cultivar improvement along with yield per se selection, since yield is a complex trait and influenced by several component traits. Introgression of multiple traits for better agronomic and physiological performance into a single variety could help in the genetic progress of grain yield. Some of the yield components that have been successfully utilised for cultivar development with improved grain yield in different wheat breeding programmes are discussed below.

Early Flowering and Maturity

Development of new high yielding wheat cultivars having early flowering and maturity is one of the important breeding objectives. The main focus of breeding programmes is to develop genotypes, which mature early as an adaptive mechanism for terminal heat and drought stress experiencing environments. Most of the present wheat cultivars incorporated vernalisation and photoperiod insensitive genes to promote early flowering and maturity (Chen et al. 2016). Vernalisation genes *Vrn-A1*, *Vrn-B1*, and *Vrn-D1* control flowering and maturity in wheat. Wheat workers developed cultivars combining vernalisation to promote early maturity and improve grain yield potential. Photoperiod sensitive genes including *Ppd-D1a*, *Ppd-B1*, and *Ppd-A1* control photoperiod sensitivity, regulating flowering and maturation times in wheat (Gomez et al. 2014). High yielding with early maturing genotypes suitable for diverse growing conditions have been developed in different breeding programmes across the globe by incorporating the vernalisation, photoperiod, and dwarfing genes (Royo et al. 2018). By articulating wheat phenology, breeding for high yielding and early-maturing wheat genotypes can be achieved due to negative correlations between flowering and yield (Mondal et al. 2016). Such cultivars should have faster growth rates to accumulate sufficient biomass in shorter duration to increase grain yield potential.

Plant Height

Global wheat productivity has significantly increased due to the development of semi-dwarf wheat cultivars. Dwarfing/height reduction genes like *Rht1* (*Rht-B1b*), *Rht2* (*Rht-D1b*), *Rht-D1c*, and *Rht8* have been extensively utilised for cultivar development by many breeding programmes across the globe (Zhang et al. 2016).

Dwarfing genes reduce the coleoptile and internode length resulting in the reduction of plant height, thereby increasing assimilate partitioning to the ear, which results in higher HI and lodging resistance. Although numerous dwarfing genes are available, only a few genes have been extensively utilised for wheat yield improvement (Chen and Hao 2015). Therefore, integration of less explored dwarfing genes, such as *Rht4*, *Rht5*, *Rht11*, *Rht12*, and *Rht24* with the commonly used dwarfing genes (i.e. *Rht1*, *Rht2*, *Rht8*), would further help in improving the yield and lodging resistance (Rebetzke et al. 2012a,b). Some studies including Shearman et al. (2005) suggested that the plant height has reached its theoretical limit of around 70–80 cm in wheat, indicating that little progress will be achieved through further reduction in plant height. Further reduction of plant height will have effect on biomass and yield (Berry et al. 2015). Therefore, strategic breeding that combines both plant height and grain yield to maximise yield potential and lodging resistance has been suggested (Gao et al. 2017).

Harvest Index

Harvest index (HI) is one of the important physiological traits; it has considerable effect on grain yield. Despite significant improvement in HI, the trait has not been exploited to its full potential, and the trait has remained at approximately 0.55, which is below a theoretical limit of 0.62 (Gaju et al. 2009). Positive and linear relationship was observed between HI with grain yield over time suggesting that HI can improve yield gains even further (Zheng et al. 2011).

Biomass

Total biomass has a definitive role in wheat grain yield improvement. Physiologically, increase in biomass has been largely attributed to higher photosynthetic rate, stomatal conductance, leaf chlorophyll content and improved radiation-use efficiency (Bustos et al. 2013). It has been suggested that further improvements in grain yield can be achieved by increasing photosynthetic capacity by optimising biomass production, while maintaining lodging resistance (Beche et al. 2014). They suggested that by increasing photosynthetic capacity through optimisation of biomass production, while maintaining lodging resistance will further improve the grain yield.

Kernel Weight

Grain yield improvement has been associated with increased thousand kernel weight (TKW). TKW is reported nearly linear with moderate to high correlation with grain yield (Gao et al. 2017), suggesting selection of bolder grains could be highly effective for improving wheat yield. As a result, increasing grain weight potential at specific positions within the spikelet has been suggested (Calderini and Reynolds 2000), rather than breeding for higher TKW.

2.8.2 Quality

There are about 46 technological parameters that can be used to judge the quality of wheat grain and its physical and chemical constituents. Among the physical characters, virtuousness, kernel hardness and hectolitre or test weight are the most studied. Among the chemical parameters, starch and proteins are most predominant constituents of wheat grain, which play a vital role in determining its quality. Mainly two approaches are being followed in various breeding programmes across the globe to improve the wheat quality, i.e. testing of fixed lines at F_6/F_7 generations or testing from F_2 onwards. In most of the breeding programmes, testing of fixed lines for various quality parameters is the common practice due to various practical challenges. But for maximum genetic gain selection, easily measurable quality traits like sedimentation value, protein content, iron and zinc are preferable at early generation breeding material. Since age old, the main focus of breeding programmes across the globe is to enhance the productivity per se to feed the increasing the population. After reaching the self-sufficiency of food grains, now breeding for better quality is one of the prime research areas across the globe. Breeding for quality is a tedious, cost intensive and time consuming process, which makes breeding for quality a slow and protracted. Wheat quality has historically been the last to utilise new technology for breeding.

2.8.2.1 Genetic Resources: A Valuable Donor for Quality Improvement

Genetic resources including wild relatives, synthetic wheats and landraces have been reported to contribute to enhance wheat grain quality (Ogbonnaya et al. 2013). Enormous diversity associated with landraces makes them a good source of bread-making related alleles such as *wbm* (Sanchez-Garcia et al. 2015). Landraces are one of the most important sources of wheat biofortification, as a collection of Sicilian landraces found to contain high levels of micronutrients (Rasheed et al. 2019). Conventional breeding approaches have been successfully used to incorporate several novel alleles for grain zinc content into elite breeding material by crossing high yielding elite wheat lines with *Ae. tauschii*-based synthetic hexaploid wheats or *T. spelta* accessions (Velu et al. 2018). Pre-breeding is an important approach in plant breeding and is practiced when desired variations are exhausted in the routine germplasm lines and cultivars. It is an important step wherein one harnesses diversity arising from wild relatives/wild germplasm/unexploited or other unimproved materials. It refers to all activities designed to identify desirable traits and/or genes from unadapted germplasm or materials that cannot be used directly in breeding programme. These traits can be bred to an intermediate set of materials that can be further utilised for producing new varieties for farmers.

Promising sources of high Zn and Fe in wheat are wild emmer (*T. dicoccoides*), einkorn (*T. monococcum*), diploid progenitors of hexaploid wheat (such as *Aegilops tauschii*), *T. spelta*, *T. polonicum*, and landraces of *T. aestivum*. Genetic variability in cultivated hexaploid and tetraploid wheat is low, but wild and primitive wheats are promising genetic resources. Fe and Zn content in wild relatives were 50% higher

than in the modern cultivated wheat, and the highest concentrations were upto twice than that of modern cultivars. Among wild wheats, the collections of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, showed greater genetic variation with as high as 14 to 190 mg/kg of grain Zn concentrations (Cakmak et al. 2004). In a study consisting of different recombinant substitution lines derived from *Triticum dicoccoides*, a *Gpc-B1* locus has been identified on the short arm of 6B chromosome and that locus affects both wheat grain protein and Zn concentrations (Distelfeld et al. 2007). The grain Fe and Zn concentration of *Aegilops kotschyi* and *A. tauschii* showed that the S and D genome species accumulate significantly higher Fe and Zn than the cultivated wheats.

2.8.2.2 Genetic Improvement of Grain Industrial Quality

The definition of wheat quality varies across stakeholders in the wheat chain. The grading system to classify wheat grain into different classes varies considerably among countries. End product appeal and organoleptic properties are the most important attribute of wheat quality for small and marginal farmers with subsistence farming practices as they generally mill and process the wheat grain to feed their families. On the other hand, commercial farmers consider wheat quality characteristics that allow the sale of their grain at the best possible price. Miller's primary interest is the higher flour/semolina recovery of suitable quality with low milling cost. Therefore, grain size, density, hardness, and roundness are the most important traits for miller's quality (Edwards et al. 2010). Millers are also interested in the production of suitable flour for processing industry (the ability of a flour or semolina to be processed at minimum cost and to give a uniform product). To produce the flour with the desired characteristics for processing industry, very often millers blend and combine different types of grain differing in quality traits.

Grain hardness, gluten extensibility and strength are the most important for processing industry. Grain hardness and dough visco-elastic properties to be considered while breeding wheat genotypes for industrial grain quality, which includes milling, processing and end-use quality. At global market, generally wheat grains are classified as soft, hard or very hard (durum wheat), sometimes locally as medium hard wheat. Genetically, presence of two small proteins called puroindolines encoded by the *Pina-D1* and Pinb-D1genes determines the grain hardness. Presence of both the puroindolines makes the grain texture soft, but if either one is mutated or altered then it makes the grain texture hard. Durum wheat is hard as both the puroindolines are absent due to the lack of the D genome durum wheat species. Grain hardness affects the water absorption, flour particle size, and milling process and partly defines end-use quality like hard grain flour for bread and soft grain flour for cookies and pastries. In contrast, gluten content and composition defines the dough visco-elastic properties including gluten extensibility and elasticity.

Gluten is mainly formed by the glutenins and gliadines, further glutenins are divided into high and low molecular weight. Generally, glutenins are associated with gluten strength or elasticity and gliadins with viscosity and extensibility. The most important genes controlling these proteins are *Glu-1*, *Glu-3* and *Gli-2*, and it is well known that their different alleles have been associated to gluten quality

characteristics. Other less explored but important quality traits such as starch properties and enzymatic activities are in their infancy to utilise them in breeding programmes. Therefore, breeding programmes need to develop a holistic breeding approach, to ensure suitable gluten quality with diverse levels of gluten strength combined with the required extensibility along with medium protein content in hard to semi-hard grain texture genotypes. On the other hand, the overall strategy for durum wheat has been to develop genotypes with suitable quality to be accepted by the pasta-making industry across the globe. Durum wheat genotypes with large kernel size, medium to low gluten strength, and with high yellow pigment content are preferred.

2.8.2.3 Genetic Improvement of Nutritional Quality

Nutritional quality can be defined as the ability of a food to provide enough nutrients for a correct physical and mental development for a healthy life of human beings (Guzman et al. 2019). Recently, development of nutri-dense crops is one of the important research priorities and breeding objectives, particularly for the staple crops including wheat that represent a major proportion of the food and calories in developing and under developed countries. Wheat is a potential source of different micronutrients and other bioactive components, but the levels of some of these are not high enough to meet the daily requirements of people in countries where wheat represents the main source of calories.

2.8.2.4 Biofortification: A Promising Strategy to Contain Malnutrition

Nutrition (protein, vitamins and minerals) deficiency is one of the most important public health issues across the globe, particularly in developing and underdeveloped countries. Biofortification is a process to improve the nutritional value of crop plants through plant breeding, agronomic and transgenic approaches. Consumption of biofortified staple crops will help in the alleviation of malnutrition, thereby it improves the human health condition. Presently, development of nutrient dense staple food crops is one of the prime research areas for the scientific community. Biofortification has been recognised as an economical and sustainable strategy that can be useful as a complementary solution to the problem of malnutrition. Staple crops often exhibit genetic variation in essential nutrient contents, which enable breeders to develop nutrient dense high yielding genotypes through conventional or molecular breeding approaches. Development of nutrient dense crops through conventional/molecular breeding approaches is both economical and does not have any effect on consumer acceptance, unlike transgenic approaches.

With the aim of developing bread wheat cultivars with 40% higher Zn concentration over the current commercial cultivars in the target regions of South Asia, CIMMYT is leading the partnership-based global effort within the HarvestPlus project (Velu et al. 2011). Millions of resource-poor wheat consumers in South Asia and Africa are prone to Zn deficiency, making Zinc to be the primary target nutrient for wheat under the HarvestPlus project. Large genetic variability has been identified for zinc in the wheat genetic resources, which enables to develop high yielding wheat cultivars with elevated levels of zinc through various breeding

approaches. Currently, CIMMYT breeding programme mainly focused on transferring genes governing higher Fe and Zn from *Triticum dicoccon* and *Triticum spelta*-based synthetics, landraces, and others reported high Zn and Fe sources in to the high yielding elite wheat backgrounds.

Synthetic hexaploids and other donor parents with significantly higher Fe and Zn concentrations were used as donor parents for a limited-backcross breeding approach onto adapted CIMMYT wheat parents (Velu et al. 2011). Energy-dispersive X-ray fluorescence spectrometry is used for rapid estimation of grain iron and zinc centration in biofortification programmes (Paltridge et al. 2012). Five biofortified wheat varieties have been released in different countries with elevated levels of Zn (Bari Gom 33 in Bangladesh, Zinc Shakti (Chitra), WB02 and HPBW-01 in India, and Zincol 2016 in Pakistan (Velu et al. 2015). In India, a project entitled Consortia for Research Platform (CRP) in 'Biofortification in wheat for nutritional security' is undergoing under the umbrella of ICAR.

2.8.3 Biotic Stresses

2.8.3.1 Rusts

Historically, the rust diseases have been one of the major biotic production constraints for wheat production. Globally, yellow rust (*Puccinia striiformis* f. sp. *tritici*), stem rust (*Puccinia graminis* f. sp. *tritici*), and leaf rust (*Puccinia triticina*) are the most damaging diseases of wheat (Roelfs et al. 1992). Historically, yellow rust has caused and is presently causing significant and severe losses on susceptible wheat cultivars worldwide. Moreover, detection of the widely virulent race *Ug99* in Uganda in 1998 challenged the misconception that stem rust was a conquered disease. Now, upto 90% of world's wheat cultivars are considered stem rust susceptible (Singh et al. 2006), and the disease is threatening 20% of the world's wheat in Central and North Africa, the Middle East and Asia.

Leaf rust of wheat, caused by the fungus *Puccini tritici*, can cause heavy yield losses in wheat. Symptoms are small, round-to-oval, raised, orange-red, dusty pustules that are scattered mostly on the upper surface of the leaves and leaf sheaths of infected plants. Leaf rust frequently starts on the lower leaves and gradually progresses up the plant to the flag leaf. As the season progresses, the pustules become more and more numerous until more of the total leaf area is destroyed. Such severely infected leaves usually shrivel and die prematurely. Leaf rust decreases the yield, grain quality and forage value, and in most wheat producing regions the use of susceptible cultivars has resulted in yield losses of 10%–70%.

Stripe rust caused by *Puccinia striiformis* f. sp. *Tritici* continues to be a dominant factor, limiting yield potential in wheat. Pustules coalesce to produce long yellow stripes between veins of the leaf and sheath and so are also known as yellow rust. Small yellow, linear lesions occur on floral bracts. These pustules are filled with spores of the fungus. The stem rust disease caused by fungus *Puccinia graminis* f. sp. *Tritici*, appears as elongate blister like pustules, or uredinia, most frequently on the leaf sheaths of the wheat plant, but also on stem tissues, leaves, glumes and awns.

Stem rust pustules on leaves develop mostly on the lower side, but may penetrate and make limited sporulation on the upper side. As infected plants mature, uredinia change into telia, altering colour from red into dark brown to black, thus the disease is also called black rust. Severe infection of stems interrupts nutrient flow to the developing heads, resulting in shrivelled grains and stems weakened by rust infection are prone to lodging (Roelfs et al. 1992). Severe wheat yield losses due to stem rust ranged from 9 to 33% in Scandinavia in 1951 and 5–20% in eastern and central Europe in 1932 (Zadoks 1961).

2.8.3.2 Powdery Mildew

Powdery mildew of wheat is caused by an obligate, biotrophic ascomycetous fungus *Blumeria graminis* sp. *Tritici* during the late winter and early spring. Powdery mildew is a wind-borne disease favoured by the presence of disease in the preceding season. Disease infection can start during early crop growth when conditions are cool and wet. As the temperature rises and the humidity falls, the incidence and severity tend to diminish. The disease is preferred by mild temperatures (10–22 °C), and 100% relative humidity (RH) favours the conidium germination. Prolonged cloudy weather fastens the disease development. During winter, spores survive in the host tissue after infection and may come from earlier infections within the field or from fields farther away. The disease is most common in dense early sown crops with high nitrogen fertility and rapid plant growth. Cultivation of disease-resistant cultivars/varieties is an efficient method for commercial breeding and disease control by the introgression of resistance genes which enhance the durability of the variety. Host resistance is more likely to be durable when two or more resistance genes are pyramided in a single wheat variety. Information about the genetic diversity and distribution of *Pm* genes in a set of wheat varieties is required for the pyramiding of resistance genes. Until now, nearly 73 *Pm* genes/alleles have been identified in 60 loci from common wheat and its wild relatives.

2.8.3.3 Loose Smut, Karnal Bunt, Common Bunt and Tan Spot

Loose smut (*Ustilago tritici*) is a disease that affects wheat all over the world. This seed borne fungus survives from crop to crop in the embryo and is disseminated with the grain, making it spread easily but difficult to control. The fungus is protected within the seed and grows with the growing point of the wheat plant, therefore not only is it protected from being cleaned from the seed, it is also protected from contact (protectant) fungicides. Karnal bunt (KB) is a fungal disease caused by *Tilletia indica* (Mitra). The incidence of KB varies considerably from year to year, depending on the variability in favourable weather during heading stage of wheat crop. The importance of KB lies in the fact that the disease is prevalent only in a few countries around the world, and the pathogen being seed, soil and airborne is difficult to manage once introduced in an area. Therefore, wheat importing countries have imposed strict quarantine measures and insist on zero tolerance limits on shipment of wheat from KB prone regions.

Common bunt (CB) caused by *Tilletia caries*, is the world's most destructive wheat disease. Instead of spikes filled with healthy wheat seeds present at crop

maturity, the fungus produces kernels filled with bunt balls of spores that possess a fishy odour. The bunt spores then adhere to healthy wheat seeds during harvest. Tan spot is a foliar spotting disease caused by the fungus *Pyrenophora tritici repentis* also known as yellow spot or yellow leaf blotch and occurs in all the major wheat-growing areas worldwide. Intensified wheat production, changes in cultural practices involving adoption of conservation agriculture practices, including shifts from conventional tillage and stubble burning to reduced or zero tillage practices with residue retention, and shorter crop rotations contributed to the development of tan spot in epidemic proportions worldwide.

2.8.3.4 Head Blight/Head Scab

Fusarium head blight (FHB), also called ear blight or head scab, is caused by *Fusarium* spp. and is one of the most destructive diseases of wheat (*Triticum aestivum*). FHB occurs primarily in warm and humid climatic conditions during the flowering stage. The most common species causing FHB is *Fusarium graminearum* (sexual stage *Gibberella zeae*). This fungus is also associated with stalk rot of corn. Another *Fusarium* species that causes FHB is *Fusarium culmorum*.

2.8.3.5 Septoria Blotch

Septoria of wheat is a disease complex caused by three pathogens: *Mycosphaerella graminicola*, *Phaeosphaeria nodorum* and *P. avenaria triticae*. Nowadays the name for the disease caused by *Mycosphaerella graminicola* usually is referred to as *Septoria tritici* blotch (STB) or simply Septoria blotch, while that caused by *P. avenaria* and *P. nodorum* is called Stagonospora blotch. Losses to Stagonospora blotch caused by *S. nodorum* typically are much lower, but can reach 15% or more (King et al. 1983). Both pathogens can reduce grain test weight in addition to yield, and also reduce the quality of the grain produced. Losses to STB can range from 30 to 50% during severe epidemics (Eyal and Levy 1987) but typically are much lower. Epidemics of STB are most severe in areas with extended periods of cool, wet weather, particularly northern North America, northern Europe and areas with a Mediterranean climate such as North Africa, South Africa, parts of South America and western North America.

2.8.3.6 Viral Diseases

The economically most important wheat infecting viruses are the soil-borne viruses either belonging to the genus *Furovirus* (family *Potyviridae*), i.e. soil-borne wheat mosaic virus (SBWMV) and soil-borne cereal mosaic virus (SBCMV), or belonging to the genus *Bymovirus*, i.e. wheat spindle streak mosaic virus (WSSMV) and wheat yellow mosaic virus (WYMV). All these viruses are transmitted by the soil-borne plasmodiophorid *Polymyxa graminis* Ledingham.

2.8.3.7 Nematode Disease

Plant parasitic nematodes are recognised as one of the major constraints in wheat production with nearly 17 important nematode species, the majority of them belonging to three genera, namely *Heterodera*, *Pratylenchus* and *Meloidogyne*.

2.8.3.8 Wheat Blast: A New Threat

Wheat blast, caused by *Magnaporthe oryzae* pathotype *triticum* (MoT), was discovered in Parana state of Brazil in 1985, and since then spreading to an area of about 3.0 mha, causing losses of 10–100% depending on years, genotypes, planting date and environment. Later the wheat blast disease reported in central and south low-lying areas of Santa Cruz region of Bolivia, south and south-east Paraguay, and north east Argentina. Resistance genes for wheat blast disease are presented in Table 2.5. Most recently it was observed in Bangladesh in 2016, eight districts with upto 25–30% yield loss. Wheat blast is a seed and airborne disease, till date resistance sources are identified but there was limited knowledge on its genetics available. MoT population is very diverse and exhibits many pathotypes that could cross-infect different hosts and overcome resistance. Fungicide schemes are partially effective under low to medium WB pressure. Pathogen has the ability to develop fungicide resistance. The wheat blast pathogen an attack any aerial parts of wheat plant but disease is seen mainly on spikes. The pathogen spread is favoured by warm and humid weather due to rainy days and temperature of 18–25 °C, during flowering followed by hot, sunny and humid days. Minimum temperature for the infection is 10 °C and maximum is 32 °C with optimum between 25 and 30 °C.

India has proactively been involved in stopping the spread of wheat blast further from the neighbouring country Bangladesh. After the Bangladesh outbreak, India, the world's second largest wheat producer, all of sudden became vulnerable to WB although as of the cropping season pertaining to the year 2020, this deadly disease has been successfully averted to gain an entry into the country (Goddard et al. 2020). The major wheat growing regions of North Eastern Plain Zone (NEPZ) and the Central Zone (CZ) could probably be vulnerable based on pathogen climate requirements. Even the country's main wheat-producing region, i.e. North Western Plain Zone (NWPZ), is also vulnerable to blast winters and becomes humid and warm (Cardoso et al. 2008). The Government of India (GOI) enforced wheat holidays in Nadia and Murshidabad districts of West Bengal and created wheat-free zones of 5 kilometres from the Bangladesh border. ICAR-Indian Institute of Wheat and Barley Research conducts regular survey and surveillance for monitoring

Table 2.5 Resistance genes for wheat blast disease

Resistance Genes	Reference
Rmg1 (Rwt4)	Takabayashi et al. (2002)
Rmg2 and *Rmg3* (7A and 6B)	Zhan et al. (2008)
Rmg4 and *Rmg5* (4A and 6D)	Nga et al. (2009)
Rmg6 (1D)	Vy et al. (2014)
RmgTd (t)	Cumagun et al. (2014)
Rmg7 (2A in *T. dicoccum*)	Tagle et al. (2015)
Rmg8 (2B)	Anh et al. (2015)
RmgGR119	Wang et al. (2019)
2NS translocation (*Ae. ventricosa*)	Cruz et al. (2016)

and mitigating the blast threat. Blast-resistant varieties like DBW 187, HD 3249 and DBW 352 were recommended to grow in the disease-prone areas of NEPZ.

2.8.4 Abiotic Stress

Abiotic factors are one of the major yield-limiting factors for crop plants including wheat. Temperature extremes, drought, flooding, salinity and heavy metal stress, among others, affect the growth and yield of crop plants. Abiotic stresses at a given growth phase are likely to affect the organs development and so yield components set at that phase, leading to a reduction in yield potential. Drought and heat are the two major abiotic factors limiting the wheat crop productivity; the problem is further complexed by climate change. Wheat is particularly vulnerable to high temperatures and every single degree raise in average temperature during reproductive period may lead to significant yield losses (Yu et al. 2014).

Rising of every 1–2 °C temperature reduces the time taken for grain filling and also affects the survivability of the productive tillers around 15.38%, which ultimately affects the grain yield (53.57%) (Nahar et al. 2010). Even a short spell of heat waves during grain filling may result in substantial grain yield loss (Mason et al. 2010). Although frost injury to wheat is relatively less compared to heat stress situations, mountainous and sub-mountainous region are experiencing cold or frost injury due to sudden temperature fall. Sodicity or salinity is another abiotic stress which significantly affects crop growth and yield. Globally one-fifth of the irrigated land and 2% of the dry land agriculture is affected by salt stress. In India, about 6.73 mha land is salt affected, out of which a 3.77 mha is sodic while the remaining 2.96 mha is under salinity affected (Mythili and Goedecke 2016).

Another major and highly unpredictable abiotic stress is lodging. Inadequate root anchorage, poor stem structure and strength, and adverse weather disturbances like excess wind velocity, rain, hailstorm along with topography, soil type, crop management practices and disease collectively may result in lodging (Mulsanti et al. 2018). It is a global problem as many parts of the world have been recorded with significant percent of yield reduction caused due to crop lodging. Along with these, other abiotic stresses like pre-harvest sprouting and water logging are further exacerbating the yield loss in wheat (Abhinandan et al. 2018).

Breeding for abiotic stress prone environments has been the major focus area from decades, but the advancements are far below the expectation due to its complex nature. Advancement in developing stress tolerant germplasm relies heavily on the efficient breeding programmes and phenotyping approaches. Phenotyping includes identification, induction and categorisation of desired target environment, stress management and complete characterisation of experimental material. Phenotyping is mainly required to understand the complexity of genotype-phenotype interaction and to accelerate plant breeding through deeper understanding of plant phenology and physiology. Recent scenario of the agricultural research strongly favours the adoption of a 'trait-based' crop improvement approach for increasing productivity under changing climatic conditions. Identification and selection of right traits allow

the plants to uptake more resources under stress condition and also to use them more efficiently. Physiological traits linked to abiotic stress adaptation are the best available opportunities for genetic improvement of wheat, as they involve a combination of favourable alleles (Reynolds et al. 2009).

2.8.4.1 Role of Phytohormones Under Abiotic Stress

Hormones play an important role in plants adaptation to adverse environmental conditions. Cross-talk in hormone signalling reflects plant ability to integrate different inputs and respond appropriately. There are six main groups of hormones, namely auxin, cytokinin(CK), gibberellic acid (GA), abscisic acid(ABA), ethylene and brassinosteroids. Among all plant hormones ABA is most critical and hence termed as 'stress hormone'. Stress-induced senescence and abscission are the characteristic features of ABA. Under water-deficit conditions, ABA-modified root architecture contributes for the development of deeper root system along with enhancing hydraulic conductivity of plant and maintenance of cell turgor, which will finally contribute towards desiccation tolerance. Other hormones such as auxin, ethylene and cytokinins (CKs), may alter the effect and biosynthesis of ABA.

Under water and temperature stress, ethylene can regulate root growth and development by limiting organ expansion. A significant positive correlation was observed between rate of grain filling and ABA content. The higher grain ABA concentration might result from autosynthesis within the grain and partly by the translocation from leaves and roots during soil drying. ABA increased the endogenous content of proline under drought conditions. CK plays a supportive role during water deficit conditions by stimulating osmotic adjustment. Brassinosteroids increase the tolerance to high temperature in wheat leaves and brome grass. The tolerance in plants to high temperature due to application of brassinosteroids is associated with induction of de novo polypeptide (heat shock protein) synthesis. In a dwarf wheat variety, high temperature-induced decrease in cytokinin content was found to be responsible for reduced kernel filling and its dry weight.

2.8.4.2 Wheat Improvement for Waterlogging Tolerance

Frequent occurrence of climatic extremes such as heavy rainfalls, reduction in freshwater availability and saline water intrusion close to the coastal area adversely affect agricultural production worldwide. Waterlogging adversely affects bread wheat production in about 4.5 million hectares in irrigated soils of the Indo-Gangetic Plains of Northern India and some other parts of the country. Under high soil salinity, leaf area index, leaf area, maturity duration and dry matter accumulation in spikes are also reduced. The combined salt and waterlogging stresses significantly reduce wheat yield by reduction in effective tiller number grain weight, length of spike and spikelets number, and also show more adverse effect than salt alone stress in case of compacted soils. The soil where water stands on the soil surface for a prolonged period of time or the available water fraction in the soil surface layer is at least 20% higher than the field water capacity, is defined as waterlogged soil. Tolerance to waterlogging by plants defined as the capability to maintain high rates of growth coupled with greater source to sink accumulation and eventually

higher grain yield under adverse climatic conditions. Due to the increased frequency of extreme climate events, waterlogging has become an important constraint to crop production globally.

2.8.4.3 Pre-harvest Sprouting Tolerance in Wheat

Among abiotic stresses, pre-harvest sprouting (PHS) is a major concern for wheat cultivation in eastern and far-eastern parts of the country due to untimely rains around maturity time. The PHS in wheat is characterised by premature germination of kernels in a mature spike prior to harvest (usually under wet and humid conditions) due to early breakage of seed dormancy usually the result of moist weather conditions that persist after physiological maturity. It causes yield loss due to decrease in thousand grain weight and also affects end product quality.

Besides, wheat flour made from the sprouted wheat loses its thickening power and bread baked from sprouted wheat grain shows smaller volume and a compact interior. This decrease in quality is mainly due to early α-amylase activity, which can be characterised by Hagberg falling number. Either too low or high seed dormancy is undesirable, allowing pre-harvest sprouting after seed maturity or delaying germination after seed sowing. Therefore, development of wheat genotypes with a balanced degree of seed dormancy is needed to grow wheat in such areas where temperature and moisture during grain development adversely affects the expression of dormancy and pre-harvest sprouting resistance. PHS tolerance is a complex trait and its genetics need to be dissected using modern methods of QTL analysis.

2.8.5 Heterosis and Hybrid Development

Hybrid wheat is considered to be one of the possibilities for increasing wheat yield potential. Despite over 45 years of research primarily on development of hybrid wheat through cytoplasmic genetic male sterility-fertility restoration system, only limited success could be achieved. The reasons for its limited success are limited heterotic advantage; their lack of agronomic quality or diverse resistance advantage, high cost of seed production and fixing in polyploid with the result no advantage over pure lines was observed. Few reports indicate that hybrid cultivars have also been released through the use of chemical hybridising agents (CHA) in some countries. Though the prospects of exploiting of hybrid vigour in wheat were recognised as early as 1962 and work on hybrid wheat started the world over including India, due to the advent of high-yielding varieties utilising *Rht* gene (s) the emphasis was shifted. In the post Green Revolution era, it was felt that the northwestern plains of the country comprising Punjab, Haryana, western Uttar Pradesh, plains of Jammu and Uttarakhand and northern Rajasthan, which were considered as the seat of the Green revolution and contributed most to the wheat basket, have reached a sort of saturation level. To keep the productivity growth rate in tune to the future demand, it is needed to explore new innovative approaches to break the yield barriers and make wheat cultivation more remunerative. In this context, exploiting hybrid vigour at commercial level through development of

hybrid wheat is considered promising that offer a significant means of overcoming food shortages because of yield heterosis.

Although the heterosis was reported in wheat in the early periods of the twentieth century, the discovery of an effective cytoplasmic male sterility (CMS) and pollen fertility restoration systems in wheat in 1951 by Kihara opened up new avenues for commercial hybrid seed production. After search of the male sterility system in wheat, the efforts were made in India to explore the basic facts for development of hybrid wheat. The levels of heterosis were explored through hand pollination, and reports have provided ample evidence of significant and positive heterobeltiosis (heterosis over better parent) for yield ranging from 0 to 100% in wheat, but most of these results are based on space planted and small plot trials. Harvest index was noticed as an important indicator of source to sink relationship, and therefore, the possibility of increasing yield potential through better harvest index was advocated during the 1990s. Under the field conditions, the minimum accepted standard heterosis for yield was established at 20% level for commercial exploitation of a hybrid and studies indicated significant standard heterosis of more than 20% for yield and yield traits under drill sown conditions.

As floral biology of wheat is key factor in order to understand the outcrossing behaviour, the extent of natural outcrossing up to 1.82% in cultivated varieties of wheat was observed. It was reported that wheat has mixed chasmogamous/cleistogamous type of flowering and autogamous/allogamous mode of pollination. Important floral traits that influence outcrossing in wheat are stigma size, anther size, anther extrusion, pollen number and pollen viability. The stigma length of wheat genotypes has been noted to be 1.84–3.75 mm, whereas anther length was observed from 2.87 mm to 5.07 mm. Anther extrusion has been observed from 8.7 to 87.4%. Phenotypic differences among wheat cultivars for days to heading, anthesis, anther size, pollen grain size, pollen viability, duration of floral opening and openness of florets were also observed, and it was suggested that the selection for long anthers, high rate of anther extrusion and more openness of florets may be effective in promoting natural cross pollination.

The pollen viability in wheat ranged from 83.4 to 98.4%. The largest separation angle between the glumes of the first two florets of spikelet was found to be 11.5^0–35.8^0, and it was observed that wheat florets get closed within 8.7–40.3 min of floral opening. All the viable pollen grains that have access to the stigma surface germinate as soon as they come in contact with the stigma. There is a positive association between anther size and the quantity of pollen produced /anther. A significant association of anther length with stigma length and anther extrusion with duration of floral opening was observed, and it was suggested that the selection for the traits that promote outcrossing may result in the genotypes with more open pollination ability and these may be utilised as parents to improve yielding ability through enhanced heterozygosity.

2.8.5.1 Male Sterility and Fertility Restoration

Male sterility and fertility restoration are two most important components in cytoplasmic genetic male sterility system. A number of studies have been conducted for

utilisation of cytoplasmic male sterility system for hybrid production in wheat. In the investigations on basic aspects of male sterility system, the effect of *timopheevii* cytoplasm was reported on level of hybrid vigour, and it was observed that restoration of male sterility in the F_1 was genetically complex, incomplete and affected by genetic background. Investigations on genetic male sterility were also carried out, and a specific type of male sterility 'S738' caused by the interaction of three recessive genes was reported for the first time that had an additive effect occurring in F_2 population of a composite cross received from Mexico. In this system, degree of male sterility was dependent upon *ms* gene number (the lesser the number, the lesser is the expression of male sterility). Thus, the genotypes having one, two and three *ms* genes exhibited 36–49, 47–68 and 73–97% male sterility, respectively. This expression was found to be influenced by environment and by modifying genes. The application of genetic male sterility in recurrent selection schemes was also advocated.

2.8.5.2 The CMS System

Systematic investigations revealed that *T. timopheevii* and *T. araraticum* cytoplasm (G type) induced complete male sterility and there are genes restoring fertility for G type cytoplasm. At Indian Agricultural Research Institute (IARI), New Delhi, ten CMS lines, namely Lok-1KMS 9A, 2009KMS 9A, 2038 A, 2046KMS 9A, 2041 KMS 9A, 2022 A, 2042 A, 2046 KMS A, 2019KMS 9A and 2160A having cytoplasm from *T. timopheevii*, *T. araraticum*, *T. zhukovskyi*, *Aegilops speltoides and Ae. caudata*, have been developed. Three cytoplasmic male sterile lines, namely 2041 KMS 9A, 2046 KMS 9A and 2338 KMS 20A carrying *T. araraticum* cytoplasm, were characterised. Cytoplasmic male sterile lines carrying cytoplasm from *Aegilops umbellulata*, *Ae. comosa*, *Ae. caudata and Ae. speltoides* have also been produced.

However gene(s) restoring fertility in these cytoplasms have not yet been identified. No apparent adverse effects on morphology by *T. araraticum and Ae. speltoides* have been noticed. About eighty CMS lines including those of *T. araraticum*, *T. timopheevii*, *Ae. speltoides*, *Ae. kotschyii* and *Ae. Variabilis* were backcrossed with respective maintainers for diversification. Two exotic genetic stocks registered as PWR 4099 and PWR 4101 indicated complete fertility restoration in *T. timopheevii*-based CMS lines. Genetic studies revealed that there are duplicate dominant genes involved in restoring complete fertility in *T. araraticum* cytoplasm and that one out of two genes restored fertility with greater degree than the other. In order to enhance variability among alloplasmic lines, agronomically superior genotypes maintaining male sterility have been utilised. Although there is no significant result for heterosis for yield in totality, few hybrids showed heterosis for yield components, viz. spikelet number, spike length and tillers/plant.

2.8.5.3 Genetic Male Sterility

Although genetic male sterility has been reported in wheat, there is no report available showing development of any hybrid wheat. The reason could be the non-availability of appropriate kind of genetic male sterility system. A number of

male sterile plants were isolated from crosses involving Selection 212 and other hexaploid wheats including Selection 82. These were isolated from F_3 progenies derived from a cross involving Selection 212 and HD 2009. In Selection 82, male sterility is caused by the modification of anthers into fully fertile ovaries. This modification of anther into ovaries takes place in 85 to 90% of the florets per spike. Breeding behaviour of genetic male sterile plants was studied for 8 generations, which showed consistency of *hpg-mst* trait. During this period, selfed *hpg-mst* spikes produced 6.06 seeds per spike, which was much lower than Selection 212 (40.4 seeds per spike). In terms of sterility, *hpg-mst* plants exhibited 85 to 100% male sterility and 100% female fertility. The inheritance of *hpg-mst* traits was studied by crossing with cultivar Kundan. All the F_1 hybrids produced 45 seeds per spike amounting to full fertility, indicating the dominance of fertility trait over the sterility.

A segregation ratio of 3 fertile: 1 sterile plants in the F_2 generation clearly revealed the monogenic recessive control of *hpg-mst* trait. The *hpg-mst* system has few advantages as the *hpg-mst* plants produce 10–12% seeds (selfed or left open); therefore, it is most economical to maintain it in homozygous condition and it enhances the chances of out crossing (female fertility) because of the presence of multiple ovaries in the florets of *hpg-mst* plants. Selection 82 was used for developing wheat hybrids involving ten different genotypes. In one combination where pollinator was Agra Local, 53 hybrid plants produced 3240 grams of grain yield in 3 m^2 area. On the other hand, it has disadvantages also as *hpg-mst* seeds in crossing block may give rise to 10–12% male sterile plants that lower the production per unit area. The genotype possessing *hpg-mst* are slightly tall (about 110 cm), and therefore, not suited to favourable environments. However, diversification of genetic male sterility system to dwarf genotypes is needed to exploit the system to its fullest extent.

2.8.5.4 Chemical Hybridising Agents

The chemicals were used as a tool to create male sterility in wheat; and more than 40 chemicals have been patented as potential chemical hybridising agents (CHAs) world over. In the 1970s, the use of CHAs as pollen suppressant began to get serious thought, and maleic hydrazide (MH) was identified as potential chemical in 1960 at IARI, New Delhi. The technique of inducing male gametic sterility looked promising as it does not require restorer parents and large quantity of seed can be produced using CHA. Some of the growth regulators like ethrel and herbicide daltapon, which expectedly have strong phytotoxic response, were also tried. Results indicated that these chemicals induced partial male sterility.

The successful utilisation of anilates as chemical hybridising agents in rice inspired the synthesis and screening of some oxanilates and inalonani lates at premeiotic stage on few varieties during 1997. Systematic investigation helped in identification of most effective fluoro-anilates inducing male sterility without having any adverse effect on different growth and yield parameters including female fertility. In the 1990s, comparative studies were made to study the effect of etherel and MH, two most widely used CHAs, and it was observed that the etherel was more

effective towards reduction in seed set. The appropriate stage for higher efficacy of CHA was reported from spike length of 7–8 mm to early boot stage. Further, the late sown crop was found more responsive to CHA than normal sown crop. Deficiency of copper was also reported to induce male sterility in wheat. In the private sector, the Maharashtra Hybrid Seed Company (MAHYCO) in collaboration with Monsanto of USA is the only company, which has commercialised hybrid wheat based on CMS approach and has released three varieties Pratham 7050, Pratham 7070 and New gold for the Central and Eastern zone of India.

2.8.5.5 Bottlenecks in Research

The insufficient levels of heterosis, low seed multiplication rate and complexity of the hybridisation systems were explored as major limiting factors for hybrid wheat development. The self-pollinated nature of wheat with occasional outcrossing of usually less than 1% makes the selection for floral characters, which enable sufficient cross fertilisation like more open flowering habit, duration of flower opening, improved anther extrusion in the male parent and stigma receptivity in the female parent more crucial for successful development of hybrids. These traits need to be investigated properly to identify the parents that can be put under conversion to male sterility system and fertility restoration.

The discovery of an effective cytoplasmic male sterility and pollen fertility restoration systems in wheat using *Ae. caudata* cytoplasm opened up new avenues for commercial hybrid seed production, but the stability of male sterility across the locations is another bottleneck in the direction of development of hybrids, which restricts the hybrid lines to the location specificity. *T. timopheevii* seems to be the most suitable one for commercial production of hybrid seed. The inclusion of yield potential in the bread wheat is also an important issue. As wheat in natural polyploid (allohexaploid), the transfer of donor traits from related species takes in more negative traits than the positive components. This needs strengthening of the pre-breeding activities for improving parental lines. The economics of hybrid seed production is of major concern for successful hybrid technology. The contributing factors such as the plant population, male and female row ratios, plant spacings and input managements should be optimised for getting maximum hybrid seed at lower costs.

2.9 Breeding Approaches: Conventional and Molecular Including Use of Genomic Tools

Bread wheat is one of the most important food crops improved by mankind over the past 8000 to 10,000 years when the species first arose in the world. The wheat domestication coincides with the beginning of agriculture, and since then it has been constantly under selection by humans sometimes with accidental and also applying intentional but empirical selective pressures. With the advancement in scientific breeding methods, the focus of breeding programmes on yield potential, resistance/tolerance to biotic and abiotic stresses and quality have been prioritised.

Additionally efforts have been made to introgress new variability from alien species which otherwise was not available in the primary gene pool. With the development of molecular genetics, tools like molecular markers, genome sequencing, better understanding of the complex traits are also progressing. New approaches like transgenic, gene editing, speed breeding and high-throughput phenotyping are emerging and have shown promise to improve and enhance the efficiency in crop breeding.

2.9.1 Conventional Breeding Methods

2.9.1.1 Mass Selection

From a variable population, seeds are collected from phenotypically desirable individuals in a population, and next generation is planted from the selected mixed seed. Mass selection has been practised by local conservators/farming communities to improve old varieties/land races. A variant of this method is progeny selection, in which best plants are harvested separately and their progenies are grown separately and compared for their performance. The better performing progenies are selected and the rest are rejected based not only on phenotypic selection but also on progeny performance.

2.9.1.2 Pure-Line Selection

From a genetically variable population, several phenotypically superior plants are selected and individual plant progenies are grown and evaluated over a period of several years. The homozygous progenies are bulk harvested separately, and yield trials are undertaken to ascertain the yield performance of selected progenies. Any progeny performing superior to varieties under cultivation is then released as a new 'pure-line' variety.

2.9.1.3 Hybridisation-Based Methods

Breeding methods employing hybridisation have the objective of combining superior gene/gene combinations from diverse parents to produce homozygous progenies in self-pollinated crops. Genes, however, are always in the company of other genes in a collection called a genotype. Different methods like pedigree, bulk and backcross breeding methods are in practice.

Pedigree Method

Pedigree method involves crossing of two genotypes differing in a few traits but possessing traits, which are absent, in each one of them followed by selfing and selection in successive segregating generations. The unique feature of this method is that the record is maintained for parent-progeny relationship in all the segregating generations until homozygosity is achieved. From every F_2 plant individual, F_3 progeny is planted separately and a few plants are selected based on desirable features to constitute the next generation followed up to $F_{5–6}$. At this stage, each phenotypically superior progeny is harvested in bulk to obtain large seed for further

evaluation in yield trials. These lines are evaluated for yield, disease resistance, quality features, etc.

Bulk Method

Bulk method of breeding does not require the record keeping of parent-progeny as in case of pedigree method and also in handling of segregating generations. In bulk method of breeding, the F_2 onwards seed is harvested in bulk and used for raising the next generation, and both natural selection and artificial selection are practised. After several cycles of selfing, single plants are selected and evaluated just like in the pedigree method.

Back Cross Method

Backcross method is generally applied in situations when an outstanding variety becomes susceptible to major diseases or it lacks some important character. In this method the outstanding variety lacking few traits is crossed with donor having that trait followed by four to six backcrosses with the outstanding variety to recover recurrent parent genome. At the end of backcrossing cycles, selfing is done to recover the homozygous progenies having the target trait in the recurrent parent genome.

Hybrid Breeding

Hybrids are generally referred to a specific cross between two good combiners in a particular cross and the superior performance of hybrids over parents is referred to as hybrid vigour (a separate section in this chapter provides further details).

2.9.2 Non-Conventional Breeding Methods

Till the 1980s, genetic enhancement of crop plants was primarily based on conventional plant breeding approaches. Although conventional breeding has continued to be the breeder's choice, faster genetic gain is hampered particularly for complex traits (Tuberosa 2012). Selection of desirable traits by indirect selection through closely linked molecular markers was conceived as an alternative to solve the limitations of conventional breeding (Collard and Mackill 2008). Since the 1990s, marker assisted selection (MAS) has been used in plant breeding programmes through tagging of the major genes, which enabled in the development of many varieties in different crop plants. Initially, both public and private sector organisations funded large number of marker assisted backcross breeding (MABB) projects across the globe; later the focus was shifted towards only foreground selection due to faster varietal replacement ratio. Later, marker assisted recurrent selection (MARS) being used as an effective tool to accumulate the alleles for the trait of interest (Rai et al. 2018). Nevertheless, MAS (Collard and Mackill 2008; Servin et al. 2004) and MARS (Crossa et al. 2010) are still the breeder's choice to rectify the drawbacks associated with widely adapted cultivars through gene pyramiding and further incorporate novel gene(s) into desirable parents. Recently,

genomic selection (GS) and speed breeding have emerged as the most promising breeding strategies to accelerate genetic gain in crop plants. GS has a clear-cut advantage over pedigree breeding and MAS to enhance genetic gains for complex traits (Crossa et al. 2017). Integrated approach of genomic selection and speed breeding could fast-track gene bank mining for rapid genetic gain (Li et al. 2018).

2.10 Precise and High Throughput Phenotyping Protocols

2.10.1 Physiological Techniques for Abiotic Stresses

2.10.1.1 Normalised Difference Vegetation Index (NDVI)

Canopy health in terms of canopy greenness indicating canopy growth or early vigour is a crucial characteristic of a high yielding cultivar and therefore is largely used as a proxy trait for abiotic stress tolerance. Canopy greenness is measured by green seeker or NDVI (Normalised Difference Vegetation Index), which is also used to estimate the nitrogen levels in many agricultural crops (Lawal et al. 2018). Several studies indicated significant linear relationship between NDVI values and seedling vigour, growth rate and senescence patterns in wheat (Lopes and Reynolds 2012).

2.10.1.2 Canopy Temperature (CT)

The genotypes with low canopy temperature are able to regulate stomatal functioning efficiently along with extraction of water from deeper layer of soil. Plant canopy emit long wave infrared radiation, which are sensed by infrared thermometer and displayed as temperature. Several studies demonstrated CT as a promising trait for screening large population under low moisture with multiple complementary traits like, deep root, stomatal conductance and finally better water use (Balota et al. 2008). However, CT is affected by various factors such as soil moisture, solar radiation, wind speed, temperature and relative humidity. Water stress was imposed between tillering and anthesis stages in wheat (Rashid et al. 1999), and mean canopy temperatures were used as a selection trait. These CT variations among genotypes were significantly correlated with yield.

2.10.1.3 Water Use Efficiency (WUE)

In general, efficient user is expected to be a higher producer, and hence higher water use efficiency is a preferred trait. Schulz et al. (2021) pursued WUE as a crucial factor for determining the final yield as it is directly related to the stomatal evaporative rate and the biomass produced. However, management practices also play an important role for WUE but at plant level, stomata are the key players. WUE is highly dependent on the carboxylation pathway, and compared to C_3 and C_4 plants CAM plants have a higher WUE because of unique carboxylation pattern (Hatfield and Dold 2019). WUE can be used as a key trait to screen large segregating population and significantly correlated with lower stomatal conductance. The importance of selecting crop cultivars with higher WUE which can produce higher or at

par yield under limited or reduced irrigation has been previously reported (Meena et al. 2019).

There is a necessity for designing specialised experiments aimed at identification and development of germplasm with true genetic ability with increased WUE. Physiological trait-based identification of drought adaptive genotypes have been earlier reported by Fletcher et al. (2018) and Nakhforoosh et al. (2016). These studies had a few limitations in the form of screening a very few genotypes initially and that too under pot culture conditions, such results are often difficult to translate to field conditions (Meena et al. 2019). Few planned wheat breeding efforts by screening large set of germplasm lines leading to identification of cultivars with enhanced WUE at global level were reported earlier (Meena et al. 2019).

2.10.1.4 Transpiration Efficiency (TE)

The balance between transpiration (H_2O) and gas exchange (CO_2) helps to maintain optimum leaf temperature and photo-respiratory activity for better productivity, and hence TE used as an alternate trait for WUE (Farooq et al. 2009; Pietragalla and Vega 2012). So, under moisture deficit condition genotypes that reduce water loss through efficient transpiration should be identified and selected. TE is an important representative selection trait for light interception, leaf transpiration, gas exchange and photosynthetic ability (Steduto et al. 2007). However, it is also directly or indirectly dependent on the carboxylation efficiency of genotype (Ludlow and Muchow 1990) and higher for C_4 plants (maize and sorghum), compared to C_3 plants (wheat, oats, cotton). The cereal crops have higher TE than the legumes due to higher energy requirements of N fixation. For wheat, wide variations in TE has been found and correlated well with carbon isotope discrimination. Transpiration efficiency has a significantly negative association with carbon isotope discrimination (CID). The relationship between CID and TE was confirmed in many C_3 crops (Richards et al. 2010). Rebetzke et al. (2002) concluded that low 13C/12C discrimination along with low stomatal conductance helps wheat to improve water use efficiently to improve harvest index.

2.10.1.5 Stomatal Conductance (SC)

For an active soil–plant–atmosphere continuum, stomata are the key controller. Under the variable soil–atmosphere moisture, stomatal alterations help to maintain plant internal water status by restricting the CO_2: H_2O movement between leaf and atmosphere. The significance of SC as an early response to water stress has been documented extensively for drought conditions (Damour et al. 2010; Ennahli and Earl 2005). Many studies have found a significant positive correlation between SC and crop yield, but still for field selection SC is not given much importance in breeding programmes (Roche 2015). Changhai et al. (2010) showed that the transpiration efficiency was more affected than the photosynthetic efficiency in wheat under drought stress. The major role to maintain transpiration efficiency was played by stomatal conductance and more precisely by stomatal pores. In a drought experiment, Liu et al. (2005) highlighted a significant reduction in stomatal conductance in water-stressed potato plants at tuber initiation and tuber bulking stages after 48 h and

24 h of withholding of irrigation, respectively. Studies have shown that partially stomatal closure is an adaptive strategy under drought stress to reduce SC and loss of water (Zait et al. 2019). However, the exact mechanism of stomatal closure is not well understood, but root hydraulic conductance and abscisic acid (a signalling molecule) are reported as the main determinant of SC. Under well irrigated conditions, higher stomatal conductance will maximise photosynthetic rates and hence the yield, but when the water is limiting then sustained SC accompany drought avoidance in plants to maintain adequate yield.

2.10.1.6 Relative Water Content (RWC)

RWC is a regularly used parameter to identify the variation in plant water status among genotypes and to quantify the extent of dehydration under abiotic stress (Guo et al. 2010). The RWC value ranges between 95 and 98% for a tolerant genotype or under normal conditions and ~ 40% under severe stress condition.

2.10.1.7 Leaf Chlorophyll Content

The crop canopy greenness contributed mainly by the photosynthetic pigment; chlorophyll is another trait of importance to screen germplasm for heat tolerance in germplasm lines. The chlorophyll pigment reflects only the green fraction of the light after absorbing all other colour fractions and hence it is green in colour. The canopy greenness is directly related to photosynthetic efficiency of the plants. The chlorophyll content of the leaf can be estimated by a destructive lab-based DMSO: acetone extraction method and by using an instrument called chlorophyll meter, which is non-destructive and optical method. The measurement by optical method using different types of chlorophyll meters is found to be more relevant than DMSO method under field conditions (Dwyer et al. 1991). The chlorophyll content measured through chlorophyll meters is in the form of an index called chlorophyll content index (CCI). The CCI ranges from 0 to 99.9, and with the increase in the level of heat stress the CCI decreases and CCI of healthy plant ranges from 40 to 60. As optical method is based on leaf reflectance, it is influenced by time of day in terms of light (Mamrutha et al. 2017). Care should be taken to measure chlorophyll content at uniform time and in specific leaf across the genotypes under field (Mamrutha et al. 2017).

2.10.1.8 Canopy Greenness/Stay Green Habit

Prolonged maintenance of canopy greenness also referred to as stay-green nature is a physiological adaptation mechanism by plants under heat stress and drought environments. Lim et al. (2007) described stay greenness as 'leaf senescence is characterized initially by structural changes in the chloroplast, followed by a controlled vacuolar collapse, and a final loss of integrity of plasma membrane and disruption of cellular homeostasis'. Stay green trait in tolerant genotypes helps in withstanding chlorophyll loss and maintains photosynthesis levels under high temperature stress. Association of stay green habit with sustained yield levels under heat stress has been earlier reported, and QTL regions regulating this have been identified (Vijayalakshmi et al. 2010). There are mainly two types of stay green types. One is

productive type, where in the stay green plant parts actually contribute for sink/grain filling. Another is cosmetic stay green type, where in greenness in these plants will not contribute for grain filling. Hence, identification of true and productive stay green types are also a challenge and can be done by considering other traits like water soluble carbohydrates in stem, peduncle, etc.

The canopy greenness can be measured by an instrument known as normalized difference vegetation index (NDVI) sensor. Spectral reflectance-based NDVI values are highly correlated with yield under temperature stress (Lopes and Reynolds 2012). NDVI values range from 0 to 1. Zero represents no greenness and one represents maximum greenness (Mamrutha et al. 2017). Stay green habit can also be measured by other instruments such as canopy analyser (Licor) or porometer, which measures leaf area index and green area index (GAI). Many other techniques like the digital photography of the canopy can also be taken from same height from the ground level, and pictures can be analysed with different softwares (Adobe photoshop CS3 extended or later version) to assess the early ground cover (Mullan and Reynolds 2010).

2.10.1.9 Earliness Per Se in Wheat

Earliness (earliness per se) in wheat is an adaptation strategy characterised by early heading, followed by early maturity of genotypes under high temperature stress environments. Earliness helps genotypes to complete the essential plant growth stages, such as seed setting and grain filling under favourable temperatures, thereby avoiding the occurrence of terminal/late heat stress. Mondal et al. (2013) reported that the early heading entries performed well in areas affected from terminal heat stress as earliness helps them to escape high temperatures during grain filling stages. In addition to helping them escape the terminal heat stress, earliness also resulted in achieving $>10\%$ higher yield compared to the local check varieties under high temperature stress environments. High grain filling rate in early maturing genotypes was also reported to be promoting heat stress tolerance in durum wheat (Al-Karaki 2012). Tewolde et al. (2006) reported that earliness helped cultivars adapt to high temperature stress as they had longer post-heading period resulting in longer grain filling duration. Therefore, earliness was also suggested as a key trait in breeding for high temperature stress tolerance (Joshi et al. 2007).

2.10.1.10 Photosynthetic Efficiency

The differential rate of photosynthesis expressed as photosynthetic efficiency is again a very essential component trait contributing to tolerance under high temperature stress. Stable photosynthetic rates over longer duration in heat tolerant genotypes contributed to higher grain weight, higher harvest index under stress showing the positive association of rate of photosynthesis with yield parameters under heat (Al-Khatib and Paulsen 1990). Looking at the major role played by photosynthesis in determining yield under heat stress, it is also pertinent to have phenotyping techniques to help breeders to select for genotypes with higher photosynthetic efficiency. The relative photosynthetic efficiency can be indirectly predicted using the chlorophyll content index, however there are instruments

available which can measure the photosynthesis exactly. Infra-red gas analyser (IRGA) is used to measure the photosynthesis on a real time basis when stress period is available or stress is imposed under experimental conditions. IRGA measures the amount of CO_2 fixed during photosynthesis by estimating the difference in amount of CO_2 pumped in and moving out of closed leaf chamber (Nataraja and Jacob 1999).

2.10.1.11 Cell Membrane Thermal Stability

Under high temperature conditions, the cell membrane becomes weak and tends to rupture, leading to leakage of electrolytes. Membrane thermal stability is being repeatedly used as a measure of electrolyte diffusion resulting from heat induced cell membrane leakage. Increased level of electrolyte leachates diffused from cells is measured here. Heat tolerant genotypes are identified by measuring electrical conductivity as an index to indirectly measure membrane thermal stability (Blum and Ebercon 1981). Greater amount of electrical conductivity said to be indicating better heat-stress tolerance (Saadalla et al. 1990). Presence of high genetic heritability of membrane stability in wheat was seen to be an advantage for its use in breeding for heat tolerance (Fokar et al. 1998).

2.10.1.12 Root Studies

Based on the hypothesis that under water-stress conditions root biomass contributes significantly towards higher yields, Jain et al. (2014) conducted an experiment to identify the most stable wheat variety under water-deficit environment for root dry matter and root volume. They also calculated the stress tolerance index (STI), which indicates the tolerance to moisture stress. Tomar et al. (2016) studied a set of 158 wheat genotypes after screening in polyvinyl chloride (PVC) pipes for root architecture traits. The visible evaluation of root images using WinRhizo root scanner of HW2004 (water stress tolerant) indicated compact root system with longer depth, while HD2877 (water stress sensitive) exhibited higher horizontal root spread and less depth at reproductive stage. The importance of water is much more in the crops requiring higher water input, like rice and semi-irrigated aerobic cultivation of rice is recommended as a water saver strategy.

2.10.2 Other Screening Methods

2.10.2.1 Index-Based Field Screening

For large scale screening in open field conditions, we do not have a standardised methodology as we don't have any hold over the indirect water sources available like rain, dew, etc. Hence, we could only screen the same if there is no rain fall during the crop period. Under such scenario we use physiological indices like Drought Sensitivity Index (DSI). The formula for DSI calculation is as given below:

$$\text{DSI} = (1 - Y_D/Y_i)/(1 - X_D/X_i).$$

where Y_D is the grain yield for each genotype under drought condition, Y_i is the grain yield for each genotype under irrigated condition, X_D is the mean of genotypes grain yield under drought condition, X_i is the mean of genotypes grain yield under irrigated condition, DSI less than 1 is desirable for water moisture stress-tolerant genotypes. The lower value of DSI represents better tolerance under water stress.

2.10.2.2 High Throughput Screening Platforms

Under controlled conditions screening could be done with high-throughput phenotyping (HTP). High-throughput phenotyping is a remote sensing technology, which may meet the requirements for the phenotyping of large number of genotypes grown in plots in less time. Use of active and passive spectral sensing systems for drought and high yield was described by Becker and Schmidhalter (2017) is also a high-throughput technology used to evaluate the drought tolerance of winter wheat. Wheat crop was grown under drought conditions for 2 years, to estimate the moisture stress tolerance of 20 wheat cultivars by using high-throughput measurements. Thermometric measurements showed a strong linear relationship to drought-related parameters (RLWC and CID of leaf and grain) and grain yield under drought stress, and demonstrated a significantly high suitability for high-throughput measurements. Additionally, four spectral reflectance sensors, including a hyperspectral passive sensor, an active flash sensor, the Crop Circle and the GreenSeeker were utilised to evaluate drought stress related destructive and non-destructive morpho-physiological characteristics. The experimental results emphasised that precision phenotyping supported the incorporation of plant traits in breeding programmes for effective phenotyping to screen drought-tolerant genotypes.

In addition to using spectral reflectance measurements, novel facilities such as temperature-controlled phenotyping facility (TCPF) has been employed to precisely phenotype genotypes for abiotic stresses such as heat (Sharma et al. 2018, 2019) (Fig. 2.5). In a study involving 75 genotypes from a recombinant inbred line population, they screened against heat stress using TCPF, the authors reported that

Fig. 2.5 Temperature-controlled phenotyping facility (TCPF) at ICAR-IIWBR, Karnal

greater precision in differentiating high-temperature responses in the TCPF was evident from the repeatability in terms of growth, physiology and productivity.

2.11 Emerging Challenges at National and International Level

Wheat is one of the widely grown staple food crops for feeding the global human population. Suitability of wheat for making infinite and diverse end products has made it a popular cereal over other crops. As per the WHO estimates, the world would need almost 60% higher wheat by 2050 from its current production level of 732 million tons in 2018–19 (FAO 2021); more specifically South Asia and Sub-Saharan Africa are expected to double their wheat production to meet the then population load (Fig. 2.6). The survey report of FAO also estimates that North African, Middle East's, Sub-Saharan Africa's, Indonesia's, Philippines and Brazilian wheat imports would soar by 2050 due to population growth rate and wheat consumption per capita growth rate. The world's significant wheat exporters, viz., USA, Canada, Australia, the Black Sea Region, Europe and Argentina, are expected to see minimal, or even negative, population growth towards 2050. In contrast, population growth will be strongest in the countries of the tropic and subtropical regions where little wheat is grown. It is believed that, even without projecting large imports by China, the world wheat trade will likely double by 2050, to 240 MMT or more.

Thus, realising that wheat already accounts for one-third of all global grain traded. Such a large expansion of trade will have major implications for all segments of the industry, including buyers, shippers, handlers, and especially the producers in those countries that will supply the increased exports, including the USA. To meet

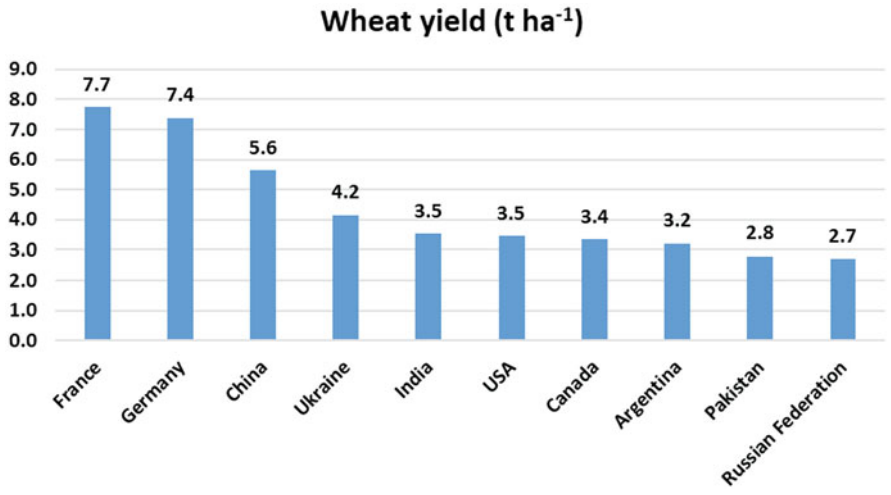

Fig. 2.6 Comparative wheat yield (t/ha)—India vis-à-vis other countries during 2019

this demand, developing countries should increase their wheat production by 77%, and more than 80% of demand should come from vertical expansion. The production target is not very high, however, it has to be achieved when productivity growth (genetic gain per annum) in wheat is hovering around 0.9%. Besides, there is an urgent need for enhancing productivity through agronomic (water, nutrients, weed management, etc.), genetic and physiological interventions along with resource conservation technologies.

Providing an adequate supply of food, however, seems to be achieved at present for the current global population, sustaining this to future will be very challenging in view of steadily increasing population, increasing purchasing power and continuous diminishing of available fertile cultivable land and water for agriculture. The challenge is expected to make even more difficult by the projected changes in climate, particularly higher temperatures and changes in rainfall distribution and amount (Parry et al. 2010). Food supply will need to grow by 2–3% each year to meet the projected demand; but in the past decade the yields of the major cereals, rice, maize and wheat have increased at less than half this rate. Apart from that, a majority of the population in the developing countries is facing the challenge of malnutrition because of the consumption of cereals-based diets for meeting their energy demand. HarvestPlus have initiated bio-fortification programme prioritising different nutrients for different countries in different crops. Zinc is target micronutrient for which bio-fortification programme on wheat is underway in India.

The AICRP on Wheat and Barley is successful in releasing the bio-fortified varieties for cultivation in different zones of the country. WB 02 is the first bio-fortified variety released for cultivation in the country having a fair amount of grain iron and zinc contents (Chatrath et al. 2018). The climate predictions by the Intergovernmental Panel on Climate Change (IPCC) indicated that the mean atmospheric temperatures are expected to increase between 1.8 and 5.8 °C by the end of this century (IPCC 2007). The increase in frequency of hot days and greater variability in temperatures in the future is also predicted as an effect of climate change (Pittock et al. 2003; Team et al. 2014). Important crops like maize and wheat are more prone to be affected as they produce less grain at temperatures above 30 °C. The impacts of climate change on food systems are expected to be widespread, complex, geographically and temporally variable, and profoundly influenced by the socio-economic conditions (Vermeulen et al. 2012). The projected rise in temperature of 0.5 °C to 1.2 °C will be the major cause of grain yield reduction in most areas of South Asia. Higher temperatures are likely to affect around seven million hectares of wheat area in developing countries and around 36 million hectares in temperate wheat production countries. The Asia-Pacific region is likely to face the worst impacts on cereal crop yields. Loss in yields of wheat, rice and maize are estimated in the vicinity of 50, 17 and 6% respectively by 2050 (IFPRI 2009). This yield loss will threaten the food security of at least 1.6 billion people in South Asia. Warmer temperatures resulted in an annual wheat yield reduction to the tune of 19 million tons, amounting to a monetary loss of $2.6 billion between 1981 and 2002 (Lobell and Field 2007).

In India, it has been predicted that with every rise in 1 °C temperature, the wheat production will decline by 4–six million tons (Ramadas et al. 2019). Approximately, three million ha wheat area in north eastern and north western plain zones is exposed to terminal/reproductive heat stress (Gupta et al. 2013). Another report by Joshi et al. (2007) stated that around 13.5 million ha wheat area in India is vulnerable to heat stress. Temperatures above 34 °C in northern Indian plains leading to significant yield loss was reported (Lobell et al. 2012). India is considered to be the second largest producer of wheat. The Northern Indian states such as Uttar Pradesh, Punjab and Haryana are some of the major wheat producing states, where the crop is more vulnerable at a 1 °C rise in temperature resulting in reduction wheat yield. In South Asia, higher night temperatures during February and March impacted subsequent wheat production and would likely to endanger food security (Janjua et al. 2010; Paymard et al. 2019; de Lima et al. 2020). High temperature stress affects wheat crop at germination, early establishment stages, dry matter partitioning, reproductive organ development and reproductive processes leading to decrease germination, poor seedling emergence leading to abnormal seedlings, poor vigour, reduced overall growth of developing seedlings, decreased seed set and low grain number (Kumar et al. 2021; Prasad and Djanaguiraman 2014; Sehgal et al. 2018).

Climate change also affects the cropping patterns and crop rotations and influences the emergence of new biotypes/pathoypes. The outbreak of *Ug*99 stem rust causing major upheaval in *Ug*99, as it has overcome the resistance in most wheat cultivars. An estimated 80–90% of all global wheat cultivars growing in farmer's fields are now susceptible to *Ug*99 or variants (Joshi et al. 2008). Similarly, *Yr*9 virulence first reported in East Africa and then migrated to South Asia through Middle East and West Asia over 10 years and caused heavy yield losses, and this virulence was reported in 1996, in North Western India (Nayar et al. 1996). Now, for the first time, Bangladesh reported wheat blast in early 2016, a deadly disease which otherwise confined to Latin America. Now the wheat blast disease was also reported from Zambia (Tembo et al. 2020). This disease results in complete crop loss in case of severe infection as it attacks the rachis and may completely hamper the grain filling. There are a few reported sources of resistance, which confers moderate level of resistance to the wheat crop (Urashima et al. 2004 and 2005). In China, sharp eye spot disease caused by *Rhizoctonia cerealis* is an emerging problem in high intensity cropping systems, and during 2003, this disease was also reported from Egypt (Hammouda 2003). Similarly, the prevalent pathogens and pests may evolve due to selection pressure posed by resistance gene deployment as well as due to climate change.

With the introduction and adoption of semi-dwarfing high yielding varieties of wheat for cultivation during and after green revolution, the soil fertility status have been depleted. Micronutrient deficiency and organic carbon content have gone down. There is an urgent need to address this issue by following addition of green manuring crops in the cropping system, residue retention and farm yard manure. Further the use of high doses of fertilisers and excess use of irrigation led to the depletion and deterioration of soil health and water. Half of the applied fertilisers are lost in one way or the other. There is an urgent need to breed for efficient genotypes,

which are both efficient in utilisation of fertiliser and also give optimum yield under limited or reduced irrigation. With the adoption of conservation agriculture practices in high intensive cropping systems, the foliar diseases of wheat like septorea, tan spot and Fusarium head blight are more frequently affecting the crops due to the hemi-biotrophic nature of pathogens (Sharma and Duveiller 2003).

In the humid subtropics of South Asia, there is evidence of stress conditions, which favour foliar blight (Dubin and Bimb 1994). Factors such as minimum tillage or surface seeding, irrigation, late planting or low soil fertility may be responsible for higher foliar blight severity in the wheat-based cropping systems of the Indo-Gangetic plains. Keeping in view the present and future challenges, researchers need to look for new variability in cultivated as well as secondary and tertiary pools and keep on continuously churning that into the breeding programmes to develop climate resilient, nutrient rich and efficient genotypes in terms of nutrient, water and radiation use.

2.12 Breeding Progress and Varietal Development

2.12.1 Conventional Breeding

In India three wheat species, namely *T. aestivum* (bread wheat), *T. durum* (*Kathia* or Macaroni wheat) and *T. dicoccum* (*Khapli* or Emmer wheat), are commercially cultivated in different parts of the country. The wheat growing farmers have played a significant role in preserving enormous variability in blend of land races, comprising of variable grain traits and morphological characteristics. The systematic wheat improvement work started with the establishment of the Imperial Agricultural Research Institute at Pusa in Bihar by Howard and Howard in 1905. Land races were subjected to pure-line selection for developing better yielding NP Series of wheat varieties. Among these, special mention can be made of NP 4, which won several national and international awards for its grain quality. In the next phase, the wheat improvement was carried out through recombination breeding involving selected pure lines in hybridisation process at number of government agricultural colleges came up at Kanpur, Lyallpur, Pune and Sabour. Also, the contemporary wheat researchers took up massive hybridisation work at Shimla, Powarkheda, Niphad and some other places, which led to develop large number of improved wheat varieties bearing initials like NP, K, C, Pb, Pbc, AO, Hyb, RS, Niphad, Kenphad, etc.

Later the focus shifted towards resistance breeding and in this pursuit, success was achieved in the development of a classical variety NP 809, the first Indian wheat variety resistant to all the three rust and loose smut diseases. Later on NP 824 was the first variety developed for the good management conditions. Among large number of varieties developed during this period prior to 1965, the notable varieties like Pbc 518, Pbc 591, NP 52, NP 165, K 13, K 46, K 65, K 68, Pbc 228, C 273, C 281, C 519, Niphad 4, AO 113, AO 115, Hyb 23, Hyb 38, RS 31–1, Kenphad 28, etc. played important role in augmenting wheat production to some extent. During 1947,

the wheat production of the country was 5.6 million tons, which was increased to the extent of 12.3 million tons in 1965 but was far below to meet the demand which was mainly due to the tall growing habit as well as proneness to lodging under high fertility conditions. A famine-like situation was predicted by Paddok brothers in India by the year of 1975. In fact it was a do or die situation, and there was no immediate solution in sight to bail out the country from such precarious situation. At this juncture when no other approach proved fruitful, a dwarf Korean wheat land race 'Daruma' showed a ray of hope. The Japanese researchers developed the well-known 'Norin-10' dwarf wheat genotype by crossing with Daruma.

Later on after the Second World War was over, the Norin-10 was picked up by U.S. biologist SD Salmon. In USA., Orville Vogel recombined the dwarfing trait of Norin-10 in the winter wheat background and successfully developed the first high yielding dwarf winter wheat variety known as 'Gains' in the early 1950s. It was Norman Ernest Borlaug who for the first time in the latter half of the 1950s transferred the Norin-10 dwarfing genes (*Rht1*, *Rht2*) in to the spring wheat background, while working in Mexico. The Indian wheat scientists took note of these dwarf Mexican wheat in the international nurseries received from Borlaug and grown at Indian Agricultural Research Institute (IARI), New Delhi, during 1961–62. From this point onwards what had happened in the field of wheat research in India is a history. It was a turning point for achieving a spectacular change in the production and productivity of wheat in the country. A food deficit country not only became self-sufficient in wheat production but also joined the elite group of wheat exporting countries in the world. Due to the coordinated efforts made by the multidisciplinary team of scientists with the progressive support of able administrators and hard work of farmers under the banner of the All India Coordinated Wheat Improvement Project (AICWIP) initiated by the Indian Council of Agricultural Research (ICAR) in 1965.

It could be a sheer coincidence that the advent of dwarf wheats and initiation of AICWIP took place simultaneously in India, in 1965. Considering the encouraging results obtained from the preliminary trials on dwarf wheats, the Indian Scientists visited Mexico to take on the spot stock of large number of dwarf wheat strains being grown there. In the beginning, the seed of four varieties, namely Lerma Rojo 64A, Sonora 63, Sonora 64 and Mayo 64 along with 613 advance generation progenies exhibiting segregation for rust resistance, plant height, maturity duration, grain attributes and phenomenon of grain shattering were introduced. After conducting the multilocational field trials, Lerma Rojo 64A and Sonora 64 were released for the first time as dwarf wheats for commercial cultivation in the country. However, the consumers did not like them because of their red grains and poor *Chapati* making quality.

For seeking answer to this drawback, the segregating generations of 613 progenies introduced from Mexico were subjected to rigorous selection by the breeders working at IARI, New Delhi; Punjab Agricultural University (PAU), Ludhiana; Govind Ballabh Pant University of Agriculture & Technology (GBPUA&T), Pantnagar; Government Agriculture College, Kanpur and Chaudhary Charan Singh Hisar Agricultural University (CCSHAU), Hisar. Number of amber-seeded

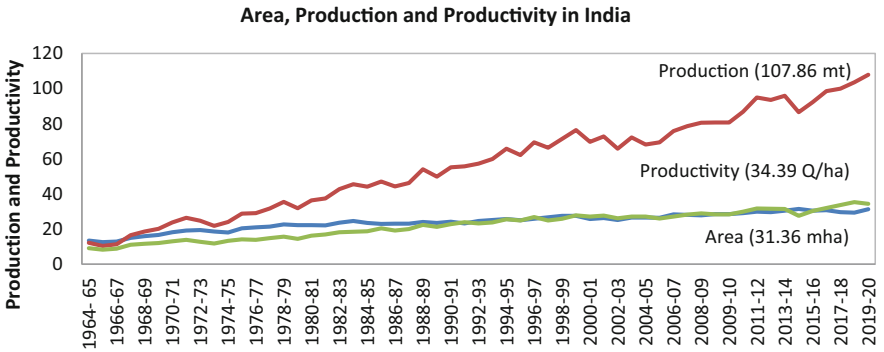

Fig. 2.7 Trend of area, production and productivity of wheat in India

genotypes exhibiting rust resistance, semi-dwarf plant type and appropriate maturity duration were developed at these centres for yield evaluations. Based on their yield performance in multilocational coordinated trials and National demonstrations, four amber-seeded improvement dwarf varieties, namely Kalyansona, Sonalika, Safed Lerma and *Chhoti* Lerma were released in 1967. The commercial success of these varieties acted like a catalyst, which not only brought revolution in the wheat production but also encouraged the breeders to work with more vigour and dedication. Very soon, Kalyansona and Sonalika became most popular varieties among the farmers and both these varieties occupied larger area in the country.

The area under wheat has increased from 12.84 million hectares in 1966–1967 to 20.92 million hectares in 1976–1977, but the production jumped tremendously from 11.4 million tons to a record level of 29 million tons during the corresponding period. In this way, an era of 'Green Revolution' was actually ushered in India. A massive hybridisation programme was initiated by Indian breeders, involving the dwarf wheat germplasm received from the International Centre for Maize and Wheat Research (CIMMYT), Mexico and indigenous cultivars/land races. This strategy of breeding led to develop better wheat varieties year after year to suit to varying production conditions of different wheat growing zones of the country. The wheat production progressively scaled new heights year after year. The phenomenon increase in area, production and productivity established new to newer records (Fig. 2.7).

The AICRP on Wheat and Barley have been successful in releasing more than 480 high yielding wheat varieties, which are notified for the different agro-ecological regions of the country. Of which, 311 wheat varieties have been released and notified by the CVRC, while 169 wheat varieties released by the SVRC. So far, 405 bread wheat varieties, 64 durum wheat, and 7 *dicoccum* wheat besides 4 triticale varieties have been notified. Among these, the land mark varieties like Kalyansona, Sonalika, C 306, WL 711, UP 262, WH 147, HD 2189, HD 2009, Lok 1, HUW 234, HD 2329, VL 616, HD 2285, GW 496, HI 8498, GW 322, WH 542, PBW 343, UP 2338, HD 2733, DBW 17, HD 2967, HD 3086 and DBW 187, etc. dominated and occupied larger area for seasons of wider adaptability, high yield potential, disease resistance,

better grain quality, etc. At present, good choice of improved varieties is available for farmers for growing under different production conditions (Table 2.6).

Two new bread wheat varieties namely, DBW 187 and DBW 303 have been released recently for cultivation in the North Western Plains zones, the country having high yield potential and are adoptable for early sown high productive environments (Table 2.7). The extent of genetic gain in grain yield achieved in the Indo-Gangetic plains can be seen from the yield potential of wheat varieties, which rose from 33.7 quintal per hectare in 1965 to 61.3 quintal per hectare in 2020 (Fig. 2.8). The released varieties have been identified for making various products like bread, biscuit, *chapatis*, pasta products, etc. (Table 2.8).

In addition to improved varieties, 247 wheat genetic stocks were registered by the Plant Germplasm Registration Committee of NBPGR, New Delhi. Of them, 140 genetic stocks are for biotic stresses, 42 for quality components and 23 for abiotic stresses. These genetic stocks are continuously shared with the researchers in the country for use in their breeding programmes. A single genotype HD 2160 has played important role in the development of as many as 18 improved wheat varieties (HD 2987, HD 2967, HD 2501, HD 2428, HD 2402, HD 2327, HD 2307, HD 2281, DL 788-2, PBW 54, PBW 120, PBW 154, PBW 175, PBW 222, K 9465, K 8962, K 8434 and Raj 1972). In the same context, a single cross number 8156 performed in the 1950s at Mexico proved most productive, giving rise to well-known varieties like Kalyansona, Mexi-Pak, Super X, etc., which led the foundation of wheat revolution in the developing countries of Indian sub-continent.

India is producing enough wheat, and now the country is exporting wheat to several countries. Besides production, breeding programmes are focusing to develop nutrient rich wheat for consumption to mitigate wide spread micronutrient malnutrition. WB 02 is the first biofortified bread wheat variety released for cultivation in North India, which is rich in zinc (42.0 ppm) and iron (40.0 ppm) developed by the ICAR-Indian Institute of Wheat and Barley Research, Karnal, Haryana. Besides this a few other wheat varieties like HPBW 01 (iron 40.0 ppm and zinc 40.6 ppm), Pusa Tejas (HI 8759) durum wheat variety (12%protein, 42.1 ppm iron and 42.8 ppm zinc), Pusa Ujala (HI 1605) (high protein 13%, iron 43 ppm and zinc 35 ppm), MACS 4028 (d) (14.7% protein 46.1 ppm iron and 40.3 ppm zinc) have been released during the last 5 years. The Indian wheat breeding programme has made tremendous achievements in terms of developing high yielding varieties and making India not only self-sufficient but also exporter of wheat.

2.12.2 Genomics Assisted Breeding

At national level, a few varieties developed using marker-assisted selection have been released for cultivation. PBW723 (*Unnat* PBW343) is the first variety using modified marker assisted back cross breeding (MABB) and released at the national level having five resistant genes introgressed into it. Based on APR against individual pathotypes, PBW723 possesses resistance against all predominant pathotypes of yellow and brown rusts. PBW723 also has enhanced resistance to Karnal bunt

Table 2.6 Wheat varieties for different zones and production conditions in India

Zone	Production condition	Varieties
Northern Hills Zone (NHZ)	ES-RF-low fertility	HS 542, HPW 251, VL 829
	TS-RF-low fertility	HS 562, HPW 349, HS 507, VL 907, VL 804
	TS-IR-high fertility	HS 562, HPW 349, HS 507, VL 907, VL 804
	LS-RI	VL 892, HS 490, HS 420
	High altitude areas	VL 832, HS 375
North Western Plains Zone (NWPZ)	ES-IR-high fertility	DBW 303, DBW 187, WH 1270
	TS-IR-high fertility	DBW 222, DBW 187, HD 3226, PBW 723, HPBW 01, WB 2, DBW 88, HD 3086, WH 1105, HD 2967, WHD 943(d), PDW 314 (d), PDW 291(d)
	LS-IR-medium fertility	HD 3298, PBW 771, HI 1621(VLS), HD 3271(VLS), PBW 752, PBW 757(VLS), DBW 173, DBW 90, WH 1124, DBW 71, HD 3059, PBW 590, WH 1021, DBW 16, WR 544 (VLS), RAJ 3765
	TS-RF-low fertility/RI	HI 1628, NIAW 3170, HD 3237, HI 1620, PBW 660, WH 1142, PBW 644, WH 1080, HD 3043, PBW 396
North Eastern Plains Zone (NEPZ)	TS-IR-high fertility	HD 3249, DBW 187, NW 5054, K 1006, HD 2967, DBW 39, CBW 38, Raj 4120, K 307, HD 2824, HD 2733, PBW 443, HUW 468, K 9107
	LS-IR-medium fertility	HI 1621(VLS), HD 3271(VLS), DBW 107, HD 3118, HD 2985, HI 1563, NW 2036, DBW 14, NW 1014, HD 2643
	TS-RF/RI	HD 3293, DBW 252, HI 1612, K 1317, HD 3171, HD 2888, K 8027, C 306
Central Zone (CZ)	TS-IR-high fertility	HI 1544, GW 366, GW 322, GW 273, HI 8759(d), HI 8737(d), HD 4728(d), HI 8713 (d), MPO 1215 (d), HI 8498(d)
	LS-IR	HI 1634, CG 1029, MP 3336, MP 1203, HD 2932, HD 2864, MP 4010
	TS-RF-low fertility/RI	Raj 4238, DBW 110, MP 3288, MP 3173, HI 1531, HI 1500, DDW 47(d), UAS 466(d), HD 4672(d), HW 2004 (Amar)
Peninsular Zone (PZ)	TS-IR-high fertility	DBW 168, MACS 6478, UAS 304, MACS 6222, NIAW 917, Raj 4037, GW 322, DDW48, MACS 3949 (d), UAS 428 (d), UAS 415 (d), MACS 2971(dic), DDK 1029 (dic), DDK 1025(dic)
	LS-IR	HI 1633, HD 3090, AKAW 4627, HD 2932, Raj 4083, HD 2833
	TS-RF-low fertility/RI	NIAW 3170, UAS 375, HI 1605, UAS 347, DBW 93, NIDW 1149 (d), MACS 4058(d), GW 1346(d),

(continued)

Table 2.6 (continued)

Zone	Production condition	Varieties
		HI8805(d), HI 8802(d), HI 8777(d), UAS 446 (d), NIAW 1415, HD 2987, HD 2781, AKDW 2997–16(d)
Southern Hills Zone (SHZ)	TS-RI-medium fertility	HW 5216, COW (W) -1, HW 2044, HW 1098 (dic)
Marginal areas	Salinity-alkalinity condition	KRL 210, KRL 213, KRL 19
Resistant to WB	NEPZ	DBW 187, HD 3293, HD 3249, HD 2967, DBW 252, HD 3171
	NWPZ	DBW 303, DBW 222, WB 02, WH 1105, DBW 88, DBW 173, HD 3043

Where *ES, TS, LS, VLS* early, timely, late and very late sown; *IR, RF, RI* irrigated, rainfed, restricted irrigation; *(d), dic. Trit* durum, dicoccum and triticale

Table 2.7 Landmark varieties of wheat in India and their yielding ability

S. No.	Variety	Year of release	Yield potential (q/ha)	S. No.	Variety	Year of release	Yield potential (q/ha)
1	S 227	1965	33.7	14	UP 2338	1990	51.3
2	C 306	1965	36.0	15	WH 542	1992	61.5
3	Sonalika	1967	45.5	16	Raj 3765	1995	48.9
4	Kalyan Sona	1970	46.0	17	PBW 343	1995	63.0
5	WL 711	1975	46.8	18	HD 2687	1999	62.9
6	UP 262	1977	44.0	19	HD 2733	2001	61.5
7	WH 147	1977	45.1	20	GW 322	2002	61.0
8	HD 2189	1979	45.7	21	DBW 17	2006	64.1
9	HD 2009	1980	45.8	22	HD 2967	2011	66.0
10	Lok 1	1981	45.4	23	HD 3086	2014	71.1
11	HUW 234	1984	35.3	24	DBW 187	2019	96.6
12	HD 2285	1985	42.5	25	DBW 222	2020	82.1
13	HD 2329	1985	47.1	26	DBW 303	2021	97.4

Note: DBW 187 and DBW 303 are recommended for early high fertility condition

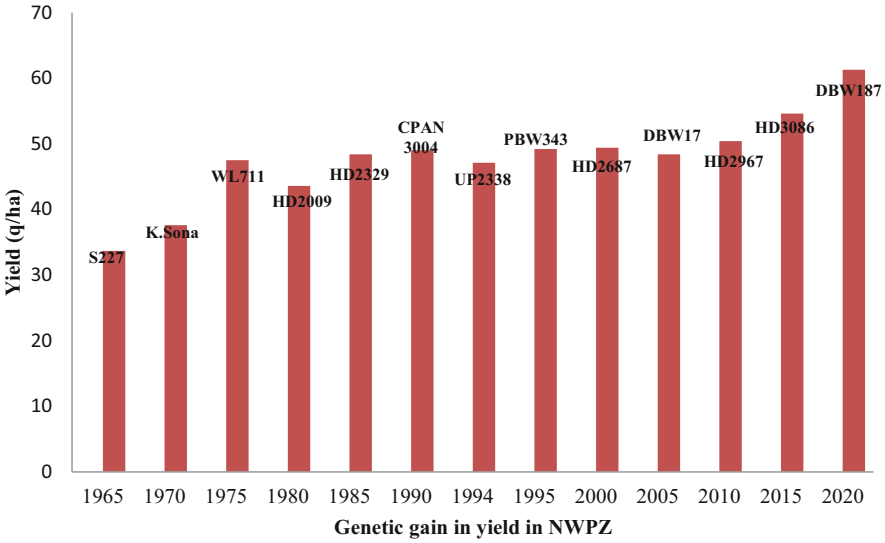

Fig. 2.8 Landmark varieties of wheat in India

Table 2.8 Promising genotypes for specific end product quality

Quality product	Promising genotypes
Chapatti (Score > 8.0/10)	C 306, LOK 1, SUJATA, RAJ 3765, HD 2285, PBW 373, PBW 533, HUW 234, K 9107, MACS 6145, MACS 6164, NW 1014, HI 1500, UP 262, HW 2004, DL 788–2, GW 273, GW 322, HD 2833, GW 173
Bread (>575 ml bread loaf volume)	HI 977, HS 240, VL 738, HD 2285, HD 2733, LOK 1, GW 120, GW 173, GW 190, HD 2189, MACS 2496, NI 5439, K 9107, HD 2733 and NIAW 917
Biscuit (>7.5 biscuit spread factor)	Sonalika, UP 2425, WH 542, HD 2687, Raj 3765, PBW 373, and DBW 16
Pasta	PDW 233, WH 896, HI 8498, HD 4672, RAJ 1555, A-9-30-1, MACS 2846, DDK 1009 and NP 200

compared to recipient variety PBW343. Post release, the variety PBW723 has made its way to farmer's field and is being grown in Punjab at an average yield of 55–60 qtls/ha. More than 11,000 quintals seed have been produced since the last 2 years (Sharma et al. 2021).

Another variety, *Unnat* PBW550 possesses gene *Yr15* in PBW550 background and provides complete foliage resistance to rusts. Gene *Lr57/Yr40* has been introgressed in DBW17 background and the variety PBW771 has been released and recommended for cultivation under late sown conditions of Indo Gangetic Plains. Similarly, another cultivar, PBW752 having *Yr10* gene has been released and recommended for NWPZ for late sown irrigated conditions. PBW757, a short

duration cultivar released by PAU for cultivation under very late sown conditions having *Yr36* gene in PBW550 background. A spectrum of wheat varieties having one or more resistant genes are available for cultivation under almost all target environments of the region and are the outcomes of systematic resistance breeding efforts.

2.13 Modernisation of Crop Improvement Programme

Several new tools and techniques have been constantly invented and are being used to facilitate breeding of new improved crop varieties including wheat. Although the field of New Plant Breeding Techniques (NPBTs) is young, it reveals a great potential and offers several advantages over conventional breeding techniques.

2.13.1 New Plant Breeding Techniques

2.13.1.1 Targeted Mutagenesis: ODM, ZFN, MGN, TALEN

Targeted mutagenesis aims in the creation of small mutations in the plant DNA at the pre-determined specific sites, and sometimes it is also known as 'site-specific mutagenesis'. Conventionally, plant cells are exposed to chemical or physical mutagens to obtain random mutations. Whereas, in the targeted mutagenesis minor mutations occur at pre-decided sites, usually to inactivate a target gene of interest or to restore the function of a mutated gene. However, precise knowledge of the targeted gene is an essential pre-requisite in targeted mutagenesis compared to conventional mutagenesis. Several targeted mutagenesis techniques developed in the past decade can be employed in plants, including ZFN (Zinc Finger Nuclease) techniques, ODM (Oligonucleotide Directed Mutagenesis), MGN (Meganuclease) techniques and TALEN (Transcriptional Activator like Effector—Nuclease) technique.

ODM is mainly based on the use of oligonucleotides for the induction of targeted mutations in the plant genome. Approximately, 20–100 long oligonucleotides chemically synthesised in order to share homology with the target sequence except nucleotides to be modified in the host genome. ZFNs are custom-designed proteins to cut at specific DNA sequences. They consist of a 'zinc finger' domain (recognising specific DNA sequences in the genome of the plant) and a nuclease that cuts double-stranded DNA. ZFN techniques in plant breeding are the novel tools for the introduction of site-specific mutations in the plant genome or the site-specific integration of genes (Osakabe et al. 2010). MGNs are very specific restriction enzymes that recognise 12–30 base pairs of DNA sequences and create a double strand break (DSB) that activates repair mechanisms and DNA recombination. MGNs are a very broad group of proteins expressed by several different organisms. Among them, the family group of LAGLIDAGD MGNs is commonly used for targeted mutagenesis. TALENs are artificial restriction enzymes that are custom-designed to cut at specific DNA sequences like ZFN.

2.13.1.2 Techniques Resulting in 'Negative Segregants': Reverse Breeding, RdDM

This group of techniques also called 'transgenic construct-driven breeding techniques' (Lusser et al. 2012) has a common feature of transgenesis only in an intermediate step of the breeding process. The transgene used is subsequently eliminated by crossing and selection and is therefore not present in the final products, and for this reason it is called 'negative (for the absence of the transgene) segregants'.

2.13.1.3 Variants of Plant Transformation Techniques: Cisgenesis and Intragenesis

As against transgenesis which can be used to insert genes from any organism, both eukaryotic and prokaryotic, into plant genomes, cisgenesis and intragenesis are terms recently created by scientists to describe the restriction of transgenesis to DNA fragments from the species itself or from a cross-compatible species. In the case of cisgenesis, the inserted genes, associated introns and regulatory elements are contiguous and unchanged. In the case of intragenesis, the inserted DNA can be a new combination of DNA fragments from the species itself or from a cross-compatible species (Rommens 2007; Schouten and Jacobsen 2008).

2.13.2 Speed Breeding: Faster and Better Phenotyping

Speed breeding is a recent technique that involves the manipulation of environmental conditions under which crop genotypes are grown, aiming to accelerate flowering and seed set, to advance to the next breeding generation as quickly as possible. Speed breeding (SB) techniques have now been developed for wheat by accelerating plant growth, flowering, seed set and maturation using supplemental lighting under controlled growth conditions. Consequently, the generation time of crop plants is reduced significantly as compared to the field or normal glasshouse conditions. The method saves breeding time and resources through rapid generation advancement. Watson et al. (2018) could get upto 6 generations of spring wheat (*Triticum aestivum*), durum wheat (*T. durum*) in a year with supplemental lighting using LED lights in environment controlled growth chambers and single seed descent (SSD) method, compared to 2–3 under glasshouse conditions and one generation under field conditions. As a result, speed breeding offers opportunities to rapidly develop homozygous and stable genotypes, and to facilitate rapid generation advancement, resulting in accelerated development and release of new cultivars (Watson et al. 2018). Also, speed breeding technology fits well with MAS and high-throughput phenotyping methodologies for multiple trait selection.

2.13.3 Genome Editing

The second generation genome editing tool CRISPR (clustered regularly interspaced short pal-indromic repeats)/associated nuclease Cas9 (CRISPR/Cas9) system is gaining momentum over the ZFN and TALEN genome editing strategies (Upadhyay et al. 2013). Just like other nucleases, CRISPR/Cas9 involves RNA-guided Cas9 nuclease from bacteria or archaea to generate targeted double-stranded breaks that are repaired by NHEJ or HDR for efficient genome editing in eukaryotes (Horvath and Barrangou 2010). Genome editing technologies can accelerate wheat breeding by allowing the introduction of precise and predictable modifications directly in an elite varietal background. Recently, the International Wheat Genome Sequencing Consortium (IWGSC; https://www.wheatgenome.org) released the fully annotated high quality reference genome of bread wheat variety 'Chinese Spring'. This will provide more novel target genes responsive to various biotic, abiotic, quality and agronomical traits improvement through CRISPR/Cas9 system. CRISPR technology is useful both in precise enhancing the activity of positive-regulator genes and in eliminating the negative-regulator genes that affect the trait of interest.

However, there are only few reports available for validation of CRISPR technique in wheat compared to other crops like rice. Most of these genes are targeted by wheat researchers to address the major abiotic and biotic stresses, along with improving agronomic traits in wheat. The first demonstrations of the CRISPR/Cas9 system in wheat were used to knockout TaMLO locus (Shan et al. 2013), TaPDS and TaINOX (Upadhyay et al. 2013). In subsequent research, simultaneous knockout of the three TaMLO homoeoalleles has been established to confer resistance to powdery mildew in bread wheat (Wang et al. 2014). Shan et al. (2014) also validated the CRISPR/Cas9 system by targeting TaLOX2 by expressing the sgRNA under the transcriptional control of TaU6 promoter in wheat. Recently, Wang et al. (2019) showed multiplexed genome editing through CRISPR in hexaploid wheat by targeting three different genes, viz. TaGW2, TaLpx-1 and TaMLO.

2.13.4 Genomic Selection: Rapid Genetic Gain

Recently, GS and speed breeding have emerged as the most promising breeding strategies to accelerate genetic gain in crop plants. An integrated approach of GS and speed breeding could fast-track gene bank mining for rapid genetic gain in crop plants (Li et al. 2018). GS could be a promising strategy to accelerate genetic gain per unit time and cost, especially for traits governed by small and cumulative effect genes. However, the optimal integration of GS in active breeding programmes faces several challenges. Nevertheless, GS has a clear-cut advantage over other breeding techniques to enhance genetic gains for complex traits (Crossa et al. 2017). During the last one decade number of empirical GS studies reported in different crop plants, highest number of GS studies reported in wheat, followed by maize and rice, which together make upto 75% of all the studies (Krishnappa et al. 2021). Various studies suggest that GS is becoming a substantial component of modern crop breeding

programmes due to rapid increase of genetic gain (Gorjanc et al. 2018). But there are very limited reports on the actual impact of GS on realised performance improvement (Voss-Fels et al. 2019) in different breeding programmes. Maize is an exception; drought-tolerant high-yielding commercial maize referred to as 'AQUAmax' hybrids were developed by the private sector through precision phenotyping and crop growth models in genomic prediction frameworks in the USA (Cooper et al. 2014).

2.14 Status Varietal Development and Maintenance Breeding

During the recent decades, India has observed a remarkable advancement in agricultural production and productivity owing to development of high yielding varieties by the National Agricultural Research System. The strides made in varietal development programme would not have been possible without concurrent advancement of institutional system for crop breeding research and quality seed production. Crop improvement research was initiated at various Indian Council of Agricultural Research (ICAR) institutes and State Agricultural Universities (SAU's) and further strengthened with initiation of All India Coordinated Improvement Projects during the 1960s. The system of varietal release, farm verification trials and maintenance breeding are very well developed and standardised in wheat. There are precise guidelines for conducting All-India Coordinated Trials in a uniform way, which ensures quality breeding material/potential entries are promoted further for release. Once the variety is identified, seeds of the variety are to be deposited with the NBPGR for conservation in gene bank. After obtaining the acknowledgment with IC No. from the NBPGR, the release and notification proposal of the variety/hybrid needs to be submitted to the Central Sub-Committee on Crop Standards, Notification and Release of Varieties.

The AICWIP was initiated during 1965 at the Indian Agricultural Research Institute (IARI), New Delhi, and under this project several high-yielding wheat varieties were developed. Varieties developed by AICWIP were quickly adopted by farmers due to their high yield potential and wider adaptability (Singh et al. 2019). During 2017, the erstwhile AICWIP project was reconstituted as the All India Coordinated Research Project (AICRP) on Wheat and Barley under the ICAR-Indian Institute of Wheat and Barley Research (ICAR-IIWBR) at Karnal (Haryana). ICAR-IIWBR through its network of cooperating centres engaged in coordinating the multidisciplinary and multi-location testing of varieties across the different ecosystems for enhancing and sustaining the wheat production.

Breeder seed indents are reflections of demand of variety at national level and extent of adoption of particular variety by the farmers. As breeder seeds are produced by the ICAR institutes/SAUs and further supplied to the various indenting agencies, viz. National Seed Corporation, State Seed Corporations, State Department of Agriculture, Private Seed Companies for multiplication into foundation and certified seed. During the past 5 years, among various wheat varieties, HD- 2967 developed by the ICAR-IARI, New Delhi, remained as a top indented variety; however, during

2021-22, HD-3086 released during 2014 by the ICAR-IARI, New Delhi, took over the top slot. Further, two latest varieties, viz. DBW- 187 (2019) and DBW-222 (2020) developed by the ICAR-IIWBR, Karnal, rapidly adopted by the farmers and demand for breeder seed production has increased within a short span of time to 1617.4 q and 506.3 q during 2021–22, respectively. All the below mentioned varieties (Table 2.9) are outcome of the concentrated efforts of scientist engaged in the varietal development programme through (AICRP) on Wheat and Barley.

The new varieties developed by the plant breeder needs to be genetically pure, uniform and free from any seed borne disease. The purity of basic seed/breeder seed is the most critical aspect, which determines the success of entire seed multiplication chain. Genetically impure seed may lead to high cost of rogueing and eventually may lead to even rejection of foundation and certified seed plots. In order to maintain highest genetic purity, utmost care needs to be taken while production of nucleus and breeder seed under the strict supervision of concerned breeder (Chakarabrty and Sharma 2018).

2.14.1 Maintenance Breeding

2.14.1.1 Selection of Earhead

In a plot of advanced generation seed multiplication at second year of AVT Trial, more than 350 years may be selected randomly based upon diagnostic characters of variety. In case of released varieties, ear heads may be collected from uniform seed multiplication filed. These selected ears are threshed separately and are examined for their colour, shape and size, and ear heads which are not true to type are rejected.

2.14.1.2 Nucleus Seed Multiplication: Stage 1 (Ear to Row Method)

Seeds of selected ear heads are sown in three-meter row (six) for each variety, hence known as ear to row method. The individual rows are periodically examined throughout the growing season. All the segregating and lines containing off types are rejected. Ear rows which show the typical characters of variety and show uniformity are harvested and threshed individually. The harvested seed if bulked and used for planting of breeder seed plot, then called as nucleus seed. Around 300–500 ear to rows are grown, considering the demand of breeder seed of particular variety. Generally, five-meter isolation distance is maintained to avoid any contamination from other source.

2.14.1.3 Nucleus Seed Multiplication: Stage II (Ear to Row Progeny Plot)

In case of varieties having very large area under cultivation, breeder seed requirement is higher. Therefore, in such cases, another cycle of nucleus seed production is followed, which involves sowing of seeds of each selected ear row separately. The plots sown from seed of each selected ear row is known as 'Ear to Row Progeny Plot'. These plots are examined for the essential diagnostic characters of the varieties at different stages as in the nucleus seed multiplication stage. In this method, six-meter length of rows are sown in six rows having spacing of 20 cm each, further

Table 2.9 Year-wise breeder seed production of highest indented varieties of wheat in India (in quintals)

S. No.	2017–18		2018–19		2019–20		2020–21		2021–22	
	Variety	Ind.	Variety	Ind.	Variety	Ind.	Variety	Ind.	Variety	Ind.
1	HD-2967	3079.9	HD- 2967	2893.8	HD-2967	2972.9	HD-2967	2467.3	HD- 3086	1700.6
2	WH 1105	1367.0	HD-3086	1327.6	HD-3086	1936.3	HD- 3086	1731.7	HD-2967	1659.0
3	HD 3086	1347.2	RAJ 4238	1119.5	PBW 723	1569.4	PBW 723	1148.8	DBW 187	1617.4
4	LOK-1	916.0	WH 1105	847.9	RAJ-4238	955.0	WH 1105	583.0	HD-3226	1151.3
5	Raj 4079	887.9	LOK-1	810.4	PBW 725	746.2	RAJ 4238	560.8	HI 8759	846.2
6	GW-366	766.0	Raj-4079	716.0	LOK-1	600.0	PBW-725	439.4	RAJ 4238	676.4
7	RAJ 4238	756.0	GW-322	564.2	HI 8713	462.2	WB-2	358.8	PBW 723	593.0
8	GW-322	745.8	PBW-725	557.2	GW-366	445.0	HI 8759	356.4	DBW 222	506.3
9	DPW 621–50	522.8	HI 1544	549.9	HI 1544	398.8	HD 2851	354.1	HD 2851	352.1
10	HI 1544	480.1	PBW 723	468.0	GW-322	386.6	GW-366	335.2	JW-3382	298.4

Source: Seed net India Portal

these plots are isolated with minimum of five-meter length for any other varieties to avoid contamination in NSS-II.

2.14.1.4 Breeder Seed Production

Breeder seed is produced as per the allocation of indents by the Department of Agriculture Cooperation and Farmers Welfare, Government of India to the concerned institute. The breeder seed is produced from the nucleus seed (stage I or II) under the supervision of a qualified plant breeder. The isolation of three meter is recommended from other wheat variety and needs to ensure that the breeder seed plot is 150 meter isolated from loose smut infected wheat plots. At the time of sowing, care must be taken to keep one row blank after every eight rows for easy inspection and roguing. All plants which are not typical of the variety are considered as offtypes, and it is very essential to rogue out these offtypes from breeder seed plots to maintain purity of foundation and certified seeds. Breeder seed plots are monitored by team of experts consist of breeder of the variety, the concerned Project Director or his/her nominee, representative of the National Seed Corporation or seed certification agency and based on the monitoring report of team (BSP III), breeder seed is harvested and made available to the varied indenting agencies for further multiplication.

Varietal improvement/development programme is the backbone of food security of India. Wheat improvement programme over the years have developed varied high yielding, multiple stress tolerant and bio-fortified varieties catering the needs of farmers. Further, vigorous efforts are being made to make available quality seeds of such varieties at farmer's doorstep, which is reflected in terms of higher varietal replacement rate (74.0% for varieties which are less than 10 years old) and seed replacement rate (40.30%) in wheat in India.

2.15 Coordinated System of Testing

In India, the systematic wheat research started about 100 years ago after joining of Sir Howards as the Imperial Botanist at Pusa (Bihar) in 1905. Later on, after establishment of the Indian Council of Agricultural Research (ICAR) in 1935, it became the main funding agency and promoter of wheat research in India. An important milestone in this process was the establishment of the All India Coordinated Wheat Improvement Project (AICWIP) in the year 1965, by the ICAR. Then AICWIP was elevated to the status of the Directorate of Wheat Research (DWR) in the year 1978, and in 1991, it moved from IARI, New Delhi, to its present location at Karnal, along with two regional stations (Flowerdale, Shimla and Dalang Maidan). In 2014, it became an institute, ICAR-Indian Institute of Wheat and Barley Research. In India, wheat is grown on an area of about 30 million hectares, and the cultivation extends from 9^0N (Palni hills) to above 35^0N (Srinagar valley of J & K), thus the wheat crop is exposed to a wide range of agro-climatic changes such as humidity, temperature, photoperiod during crop season, soil types, altitudes, latitudes and cropping systems. From wheat research and coordination point of view and based

Table 2.10 Five major zones and states covered under each zone in the country

Zone	Area covered
Northern Hills Zone (NHZ)	Western Himalayan regions of J&K (except Jammu and Kathuadistt.); H.P. (except Una and PaontaValley); Uttarakhand (except Tarai area); Sikkim and hills of West Bengal and N.E.States
North Western Plains Zone (NWPZ)	Punjab, Haryana, Delhi, Rajasthan (except Kota and Udaipur divisions) and Western UP (except Jhansi division), parts of J&K (Jammu and Kathua distt.) and parts of HP (Una dist. And Paonta valley) and Uttarakhand (Tarai region)
North Eastern Plains Zone (NEPZ)	Eastern UP, Bihar, Jharkhand, Orissa, West Bengal, Assam and plains of NE States
Central Zone (CZ)	Madhya Pradesh, Chhattisgarh, Gujarat, Kota and Udaipur divisions of Rajasthan and Jhansi division of Uttar Pradesh
Peninsular Zone (PZ)	Maharashtra, Karnataka, Andhra Pradesh, Goa, plains of Tamil Nadu

on land use planning, the country is divided into following five major zones: (1) Northern Hills Zone (NHZ), (2) North Western Plains Zone (NWPZ), (3) North Eastern Plains Zone (NEPZ), (4) Central Zone (CZ) and (5) Peninsular Zone (PZ) as described below Table 2.10.

Through coordinated research efforts, many high yielding wheat varieties suited to different agro-ecological conditions and growing situations have been released. These genotypes were very successfully helped in increasing the wheat production from a mere 12.5 million tons in 1964 to 108.75 million tons during 2020–2021. The wheat crop in India is menaced by a number of diseases. The survey and surveillance activity has helped to monitor the dynamics of important wheat diseases, particularly the three rust diseases. Through this mechanism, the occurrence/evolution of new pathotypes is made known before crossing the threshold limit of disease infestation, in the meantime, the genetic resistance is created against the new virulence in form of resistant varieties. Large number of donors lines carrying 'R-genes' conferring resistance against different rust races have been identified for utilisation in breeding programmes. The coordinated research through the AICRP on Wheat and Barley caters to the needs of cooperating centres by streamlining the research efforts and facilitating the evaluation and screening of the breeding materials. Every year national and international nurseries are supplied to different centres across various wheat growing zones, with an aim of screening the lines at hotspots and assessing the resistances across locations and environments.

Multi-disciplinary approach of variety testing in AICRP on Wheat and Barley: The Crop Improvement Division is primarily involved in coordination activities of the AICRP on Wheat and Barley, wherein the multilocation evaluation of wheat varietal trials and nurseries are undertaken. In addition to breeding work, pathological, agronomical and quality programmes also support in variety of development and testing approach of wheat in India. The wheat coordinated varietal evaluation programme entails a huge multilocation testing programme, which is undertaken

Table 2.11 Different National initial varietal trials (NIVTs) under coordinated system of testing

NIVT	Cultural conditions	Zones
NIVT-1A&1B	Timely sown, irrigated condition (*T. aestivum*)	NWPZ & NEPZ
NIVT-2	Timely sown irrigated condition (*T. aestivum*)	CZ & PZ
NIVT-3A	Late sown irrigated condition (*T. aestivum*)	NWPZ, NEPZ
NIVT-3B	Late sown irrigated condition (*T. aestivum*)	CZ, PZ
NIVT-4	Timely sown irrigated (*T. durum*)	CZ and PZ
NIVT-5A	Timely sown, restricted irrigation (*T. aestivum*)	NWPZ, NEPZ
NIVT-5B	Timely sown, restricted irrigation (*T. aestivum & durum*)	CZ and PZ

with the cooperation of 29 funded and 95 voluntary centres spread across 5 wheat growing zones in the country.

Initially, the system of varietal evaluation was confined to the specific zones including initial varietal trials and advance varietal trials for different production conditions. Under this system flow material from one zone to another zone was not done, and the adaptability of genotypes was limited to specific zones. Realising this problem and widening the testing environments and to have free flow material of material across zones, the system of testing was re-structured with the incorporation of National Initial Varietal Trials (NIVTs) and their details along with production conditions and zones are presented below Table 2.11.

However, in NHZ separate zone specific Initial Variety Trials (IVTs) in place of NIVTs are conducted. This way, the Indian wheat programme is unique regarding the multi-location testing of new genotypes through different trials. The procedure of evaluation system was re-structured in such a way that the materials from different centres are pooled and tested at different levels, namely station trials, national initial varietal trials (NIVT) and advance varietal trials (AVTs) to sort out superior germplasm with respect to yield, disease resistance and quality in the following manner. After 1 year of testing in IVTs, deserving genotypes are promoted to AVTs in NHZ at zonal level. However, in case of remaining four zones, deserving genotypes come from NIVTs for advance testing in each of four AVTs of NWPZ, NEPZ, CZ and PZ (Table 2.12 and Fig. 2.9).

The impact of wheat varieties is immense in Indian agriculture and helped country to increase ten-fold wheat production from 9.5 million tons in 1963–64 to 108.75 million tons in 2021–21. Thus, it has contributed in un-precedential growth in wheat production could have been possible because of continuous replacement of old varieties with high yielding improved varieties (Table 2.4) as a result of concerted efforts of wheat researchers and a systematic and effective seed replacement mechanism. In nut shell, this system is very unique and un-parallel that has been very effective for development and deployment of high yielding, rust resistant and end product specific quality wheat meet food and nutritional security of the country. The importance of this system of evaluation in different trials and nurseries in India's wheat improvement programme is reflected in the effective management of Wheat and Barley rusts in India through the deployment of diverse rust resistant wheat varieties based on the pathotype distribution in different areas.

Table 2.12 Trial series and criterion for promotion of different trials in coordinated system

Materials are evaluated for one year in station trials for yield potential and for disease reactions in IPPSN before entering national testing system under AICRP	
↓	
One year inter zonal test	
Trial series	Criteria of promotion/retention
Respective NIVT (NIVT-1A, NIVT-1B, NIVT-2, NIVT-3A, NIVT-3B, NIVT-4, NIVT-5A, NIVT-5B	Yield potential, disease reactions and quality parameters are taken into account for promoting materials into various zonal level AVTs
↓ AVT-I (first year)	
One year zonal test	
AVT-IR-TS-TAS/TAD/TDM AVT-IR-LS-TAS/TAD AVT-RIR-TS-TAD	Yield potential, disease reactions and quality parameters are taken into account for retaining materials in AVT-II
↓ AVT-II (final year)	
One year zonal test	
AVT-IR-TS-TAS/TAD/TDM AVT-IR-LS-TAS/TAD AVT-RIR-TS-TAD	Yield potential, disease reactions, quality parameters and agronomical evaluations are performed on final year entries

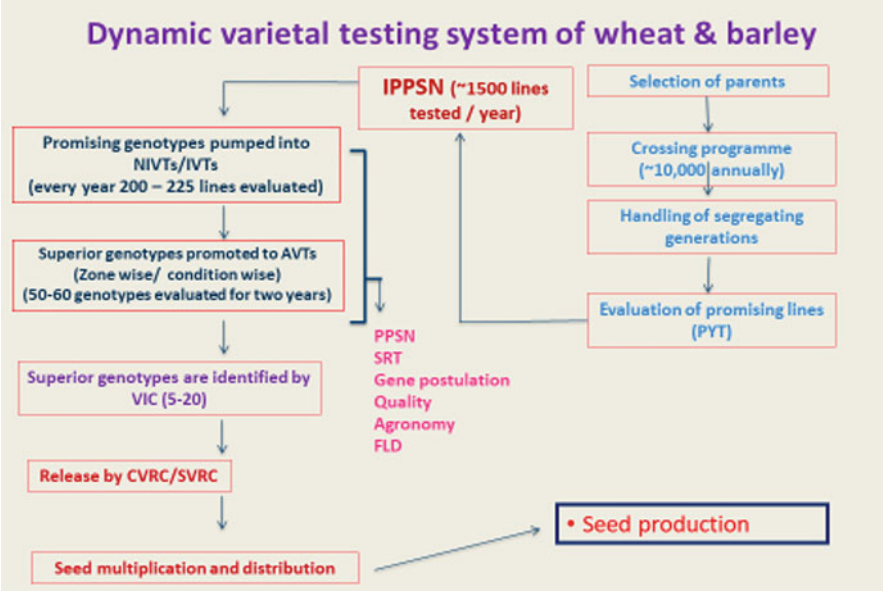

Fig. 2.9 Flow chart showing varietal testing system in AICRP on wheat and Barley

2.16 Conclusions

Breeding programmes in India have made significant progress, and achievements are reflected in terms of record wheat production of 108.75 million tons during 2021 as compared to 12.3 million tons in 1965. Deployment of high yielding, resistant varieties through the use of both cultivated and wild germplasm have led to significant improvement in yield potential, and breeding programme have been able to contain the losses caused by biotic and abiotic stresses. Nutrient rich varieties have been developed and released to minimise the micronutrient malnutrition. Varieties having specific traits which are required for industrial products like *Chapatti*, bread, biscuit, pasta, macaroni, noodles, etc. have been released. The wheat crop is also facing challenges of abiotic stresses particularly heat and drought. Although several heat tolerant varieties have been released but needs further improvements in the traits to breed better climate resilience. The natural resources like land and water are shrinking due to urbanisation and depletion of ground water due to excessive use. Varieties having high nutrient, water and radiation use efficiency, and productivity needs to be targeted so that same or higher production levels can be achieved with minimum use of these resources. The varietal replacement rate with the deployment of new varieties has been improved in the recent decade in comparison to the past. Many high yielding and disease resistant varieties have been released for different zones of the country but the life span of varieties is usually considered to be 3–5 years due to the breakdown of deployed resistance. The recent advances in genomics like GWAS, genomic selection, speed breeding and phenotyping platforms need to be adopted in the breeding programmes for better understanding and dissecting of complex traits. These technologies can assist the conventional breeding approaches to breed high yielding, nutrient rich and climate resilient varieties with a higher precision. Besides record production the country achieved, there is a continuous need to sustain and further improve the productivity in India to feed the growing population.

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https://www.fao.org/news/archive/news-by-date/2021/en/

Rice Breeding

3

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Abstract

Rice (*Oryza sativa* L.) belongs to Poaceae family and serves as food for more than half of the global population. Domesticated from *O. preperennis* about 10,000 years ago, it is cultivated in a wide range of ecosystems right from below the sea level to high mountain regions. Genetic improvement in India has helped in improving average productivity from 0.67 tons/ha in 1950–1951 to 2.74 t/ha during 2020–2021. Development of research infrastructure along with basic research helped in gaining understanding of the genetic nature of economically important traits. The major breakthrough in yield improvement was achieved through utilisation of *sd1* in developing semi-dwarf high yielding varieties with sturdy stem, spurring the green revolution. Hybrid rice further enhanced rice productivity. Deciphering rice genome and advances in genomics helped in functional characterisation of ~2800 economically important genes governing yield, resistance to major biotic and abiotic stresses, grain and nutritional quality, which enabled the integration of molecular markers in breeding. Thirty-six MAS-derived varieties with resistance to biotic and/or abiotic stress and 12 varieties with enhanced Zn and/or protein content have been released in India. Advances in genetics, breeding, genomics and phenomics, have enabled population improvement through genomic selection, while genome-editing has helped in creating novel alleles for useful genes. Aided by an evolving system for multi-location evaluation of elite genotypes under the All India Coordinated Rice Improvement Programme and a robust system of seed supply chain for dissemination of improved rice varieties, higher adoption by farmers has helped in improving and sustaining rice productivity. However, sustaining it in the face

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_3

of climate change is a major challenge. Adoption of advanced tools and techniques can help rapid and precise breeding of improved rice varieties suited to changing human needs.

Keywords

Breeding · Genome-editing · Gene pool · Genomic selection · Heterosis · Phenomics · Rice · Yield · Biotic and abiotic stress · Grain and nutritional quality

3.1 Introduction

Rice (*Oryza sativa* L.) is the most important staple-food crop feeding more than half of the world's population. Currently, rice is cultivated in an area of 192.02 million hectares (mha) with an estimated annual production of 966.88 mt of paddy (644.91 mt of milled rice equivalent) and an average productivity of 4.06 t/ha of paddy across the world. India has the world's largest area under rice and the second highest production in the world (Table 3.1). Only about 31 million tonnes of rice is traded through the international market. Leading rice exporting countries are India, Thailand, Vietnam, the USA and Pakistan. India has surpassed Thailand to become the first among the rice exporting countries in 2012 with the export of more than ten million tonnes and is still the top exporter of rice. Rice export contributes to nearly 25% of total agricultural exports from the country.

Rice is the staple food for ~800 million (65%) population of India. Rice research in India has helped in the production of 121.46 million tonnes of milled rice from an area of 43.78 mha at an average productivity of 2.77 t/ha during 2020–2021 as compared to merely 20.60 million tons of milled rice from an area of 30.8 mha with average rice productivity of 0.67 tons/ha in 1950–1951. The crop is grown in highly

Table 3.1 Area, production and productivity of rice in top ten rice producing countries across the globe

Rank	Country	Area (mha)	Production (mt)	Productivity (t/ha)
1	India	43.78	191.91 (121.46)	4.38
2	China	29.96	211.41 (141.01)	7.06
3	Bangladesh	11.52	54.59 (36.41)	4.74
4	Indonesia	10.68	54.60 (36.42)	5.11
5	Thailand	9.72	28.36 (18.91)	2.92
6	Viet Nam	7.47	43.45 (28.98)	5.82
7	Myanmar	6.92	26.27 (17.52)	3.8
8	Nigeria	5.28	8.44 (5.63)	1.6
9	Philippines	4.65	18.81 (12.55)	4.04
10	Pakistan	3.03	11.12 (7.41)	3.66
World		192.02	966.88 (644.91)	4.06

Figures in parentheses are milled rice equivalent
Source: FAOSTAT, http://www.fao.org/faostat/en/#data/QCL accessed on 29/08/2021

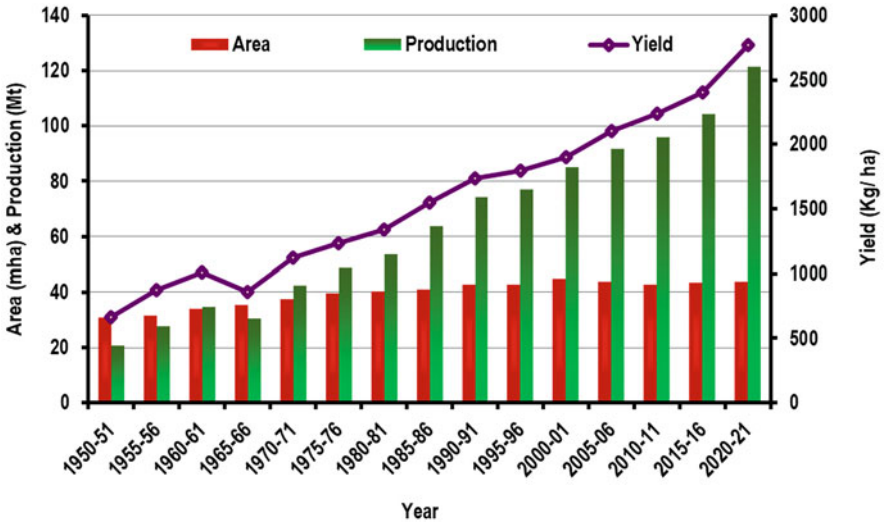

Fig. 3.1 Trends in area, production and productivity of rice in India

diverse conditions ranging from hills to coasts. Primarily a *Kharif* crop, it is cultivated round the year in one or the other parts of the country. The area under rice has remained almost unchanged over the years, but production has increased more than five times (Fig. 3.1).

The average productivity of rice in India is 2.77 t/ha, which is less than the global average rice productivity and is significantly lower as compared to China which produces 141.01 mt from an area of only 29.96 mha with average productivity of 4.71 t/ha of milled rice. India contributes 21% of global rice production. The year 2020–2021 recorded the highest rice production of 121.46 mt. The annual area, production and productivity of rice during the last two and half decades is presented in Table 3.2. Currently, out of the total area of 43.78 mha, 24 mha is under irrigated rice in eight states, namely Punjab, Haryana, Uttar Pradesh, West Bengal, Bihar, Odisha parts of Andhra Pradesh and Tamil Nadu. About 17.2 mha is under the rainfed ecosystem with more than 70% in eastern India. Around 85% of the total rice production is contributed by the favourable ecologies of irrigated and shallow-lowland ecosystems. Rice is one of the most resource intensive crops among all the crops, and annually it uses about 200 km^3 of irrigation water, 6.5 mt of fertilisers, 17% of total pesticides used in Indian agriculture and emits 3.5 mt of methane (Pathak et al. 2018).

Future projections indicate that the current levels of production may not be sufficient to feed the ever-increasing population of our country. To meet the food demands for the future, it is projected that India should produce about 137.3 million tons of rice by the year 2050, i.e. it needs to produce about 0.56 million tons additional rice every year. This increased production has to necessarily come from

Table 3.2 Annual area, production and productivity of rice in India during the last 25 years

Year	Area (mha)	Production (mt)	Productivity (kg/ha)
1995–1996	42.84	76.98	1797
1996–1997	43.43	81.73	1882
1997–1998	43.45	82.54	1900
1998–1999	44.80	86.08	1921
1999–2000	45.16	89.68	1986
2000–2001	44.71	84.98	1901
2001–2002	44.90	93.34	2079
2002–2003	41.18	71.82	1744
2003–2004	42.59	88.53	2077
2004–2005	41.91	85.13	1984
2005–2006	43.66	91.79	2102
2006–2007	43.81	93.35	2131
2007–2008	43.91	96.69	2202
2008–2009	45.54	99.18	2178
2009–2010	41.92	89.09	2125
2010–2011	42.86	95.98	2239
2011–2012	44.01	105.3	2393
2012–2013	42.75	105.24	2462
2013–2014	44.14	106.54	2416
2014–2015	44.11	105.48	2391
2015–2016	43.39	104.41	2404
2016–2017	43.99	109.70	2494
2017–2018	43.77	112.76	2576
2018–2019	43.79	116.42	2658
2019–2020	43.66	118.87	2723
2020–2021	43.79	121.46	2774

increased productivity rather than an increase in area under rice and that too under declining soil, water and other natural resources.

3.2 Origin, Evolution and Distribution of *Oryza* Gene Pool

Cultivated rice belongs to the Poaceae family, subfamily Bambusoideae and tribe Oryzeae. The genus, *Oryza*, is the only cultivated genera among the 11 genera belonging to the tribe Oryzeae with an evolutionary history, spanning approximately 15 million years (Stein et al. 2018). It has two cultivated and 25 wild species representing 11 genomes, namely AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, KKLL and genome size variation of upto 3.6 times (Vaughan 1994; Ge et al. 1999; Jacquemin et al. 2013; Table 3.3). *Oryza* originated ~130 million years ago and spread like wild grass in the erstwhile Gondwanaland and spread

Table 3.3 The different species of genus *Oryza* with their genomes, chromosome number and distribution

Species	Genome	Somatic chromosome number (2*n*)	Distribution
Oryza sativa L.	AA	24	Worldwide
O. glaberrima Steud.	A^gA^g	24	West Africa
O. nivara Sharma et Shastry	AA	24	Tropical and subtropical Asia
O. rufipogon Griff.	AA	24	Tropical and subtropical Asia, tropical Australia
O. breviligulata A. Chev. Et Roehr. (*O. barthii*)	A^gA^g	24	Africa
O. longistaminata A. Chev. et Roehr.	A^lA^l	24	Africa
O. meriodionalis Ng	A^mA^m	24	Tropical Australia
O. glumaepatula Steud.	$A^{gp}A^{gp}$	24	South and Central America
O. punctata Kotschy ex Steud.	BB	24	Africa
O. minuta J.S. Presl. Ex C.B. Presl.	BBCC	48	The Philippines and Papua New Guinea
O. schweinfurthiana Prodoehl	BBCC	48	Africa
O. officinalis Wall ex Watt	CC	24	Tropical Australia
O. rhizomatis Vaughan	CC	24	Sri Lanka
O. eichingeri A. Peter	CC	24	South Asia and East Africa
O. alta Swallen	CCDD	48	South and Central America
O. grandiglumis (Doell) Prod.	CCDD	48	South and Central America
O. latifolia Desv.	CCDD	48	South and Central America
O. australiensis Domin.	EE	24	Tropical Australia
O. brachyantha A. Chev. et Roehr.	FF	24	Africa
O. granulate Nees et Am. Ex Watt	GG	24	South and Southeast Asia
O. meyeriana (Zoll. Et (Mor. Ex Steud.) Baill.	GG	24	Southeast Asia
O. longiglumis Jansen	HHJJ	48	Indonesia and Papua New Guinea
O. ridleyi Hook. F.	HHJJ	48	South Asia
O. schlechteri Pilger	HHKK	48	Papua New Guinea
O. coarctata Roxb.	KKLL	48	South Asia

across different continents, namely Asia, Africa, Australia and America, after the continental drift separated these continents.

The genus *Oryza* has been classified into four species complexes, namely:

(a) *Sativa* complex consisting of eight diploid species ($2n = 24$) belonging to the AA genome. It includes two cultivated species, namely *O. sativa* and *O. glaberrima*; and six wild species, namely *O. nivara*, *O. rufipogon*, *O. breviligulata* (*O. barthii*), *O. longistaminata*, *O. meridionalis* and *O. glumaepatula*. All of them belong to the primary gene pool and are easily crossable with cultivated rice.
(b) *Officinalis* complex consisting of six diploid species, namely *O. punctata*, *O. officinalis*, *O. rhizomatis*, *O. eichengiri*, *O. australiensis* and *O. brachyantha,* and six allotetraploid species, namely *O. schweinfurthiana*, *O. minuta*, *O. malampuzhaensis*, *O. latifolia*, *O. alta* and *O. grandiglumis*. The species in the *officinalis* complex belong to a secondary gene pool as crosses can be achieved through embryo rescue.
(c) *Meyeriana* complex consisting of three G genome diploid species, namely *O. meyeriana*, *O. granulata* and *O. neocaledonica*.
(d) *Ridleyi* complex consisting of two tetraploid species, namely *O. longiglumis* and *O. ridleyi*. Besides these, there are two other tetraploid species, namely *O. schlechteri* and *O. coarctata,* which are under unclassified groups. The species belonging to *meyeriana*, *ridleyi* complexes and the unclassified group constitute the tertiary gene pool (Chang, 1964; Fig. 3.2).

Several of the wild species are naturally distributed in South and Southeast Asia especially in eastern and central India. Among these *O. rufipogon* is widely distributed (Fig. 3.3), followed by *O. nivara* and weedy rice.

3.3 Origin and Evolution of Rice

The genus *Oryza* originated about 130 million years ago in the erstwhile supercontinent Gondwanaland, and different species got distributed into different continents as the supercontinent broke up and drifted apart. Rice was domesticated around 10,000 years ago. The two cultivated species of the *Oryza*, namely *O. sativa*, and *O. glaberrima*, originated from a common A genome ancestor, *O. perennis*, which is possibly extinct (Khush 1997). *O. sativa* is commonly cultivated across Asia and in general referred to as Asian cultivated rice, while *O. glaberrima* is mainly cultivated in western Africa and is known as African rice (Fig. 3.4). The domestication of rice has been a subject of debate with contradictory findings on single or multiple independent domestication events. Kato et al. (1928) proposed independent origin of the *indica* and *japonica* subspecies of *O. sativa*, which have been substantiated by interspersion pattern of short interspersed elements (SINEs) (Cheng et al. 2003); cytoplasmic diversity (Kawakami et al. 2007); estimates of genomic divergence between *indica* and *japonica* (0.4–0.2 Mya), preceding domestication (Vitte et al. 2004; Zhu and Ge 2005) and phylogeographic haplotype association (Londo et al. 2006). On the other hand, identical mutation for non-shattering grains (*Sh4*) (Li et al. 2006), red pericarp (*Rc*) (Sweeney et al. 2006) and severe domestication bottleneck (Zhu et al. 2007) supports single

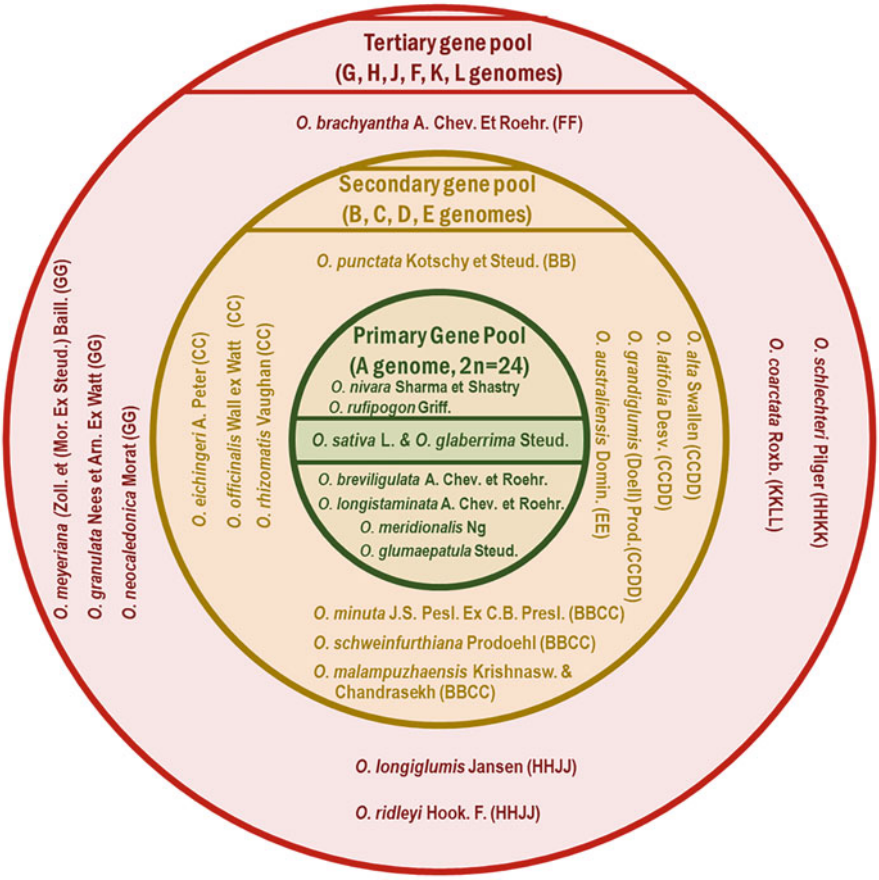

Fig. 3.2 The gene pool of the genus *Oryza*

domestication event (Chang 1976; Vaughan et al. 2008). The domestication of rice in Africa occurred later than the Asian rice. Carbon dating of the archaeobotanical data from the site of Dia, in the middle Niger Delta, and Mali suggests *O. glaberrima* has domesticated 3500 years ago (Murray 2004).

The two key domestication traits in cultivated rice are loss of shattering and secondary dormancy. *Japonica* varieties do not possess an abscission layer at the base of the spikelet making them non-shattering and hard to thresh. The *indica* varieties have a partial abscission layer making them free threshing, while the wild and weedy rice possesses a complete abscission layer rendering them prone to shattering (Vaughan et al. 2008). One of the most universal changes during the domestication of cereal crops is the loss of grain shattering, which enabled efficient harvesting of the crop. The gene governing shattering, *Sh4* is a transcription factor involved in the formation of the abscission layer through the cell wall. A large-effect Quantitative Trait Locus (QTL) on chromosome 12 explaining about 50% of the

Fig. 3.3 *Oryza rufipogon* grows in natural swamps near Goria Karma (Jharkhand state) during the rainy season

phenotypic variance was mapped in a Thai accession (Gu et al. 2005). QTLs/genes associated with other major domestication syndrome traits such as grain shattering, growth habit, panicle and ligule development, grain number, pericarp colour, hull colour, awn length, grain width and grain filling are listed in Table 3.4.

After domestication, *indica* rice spread to Madagascar, East and West Africa, while it also spread eastwards to Southeast Asia and north to China. The *japonica* rice moved north of China to become a temperate *japonica*, which was then introduced into Korea and Japan at the beginning of the first century. In the hilly areas of Southeast Asia, *japonica* rice was cultivated in uplands as well as lowlands. Both *indica* and *japonica* rice were introduced later to Malaysia, Philippines and Indonesia, and from Philippines to Taiwan (Khush 1997).

3.4 Rice Ecosystems

Rice is grown across the world from 39° S in Australia to 50° N latitude in China. Broadly, rice is produced from four different ecosystems, namely irrigated, rainfed lowland, upland and flood-prone ecosystems. Under irrigated ecosystem, rice is grown maintaining flooding of water with adequate water control measures in place. Irrigation is provided from various sources such as canals, reservoirs and wells. The rainfed lowland production ecologies rely on the rainfall for the source of

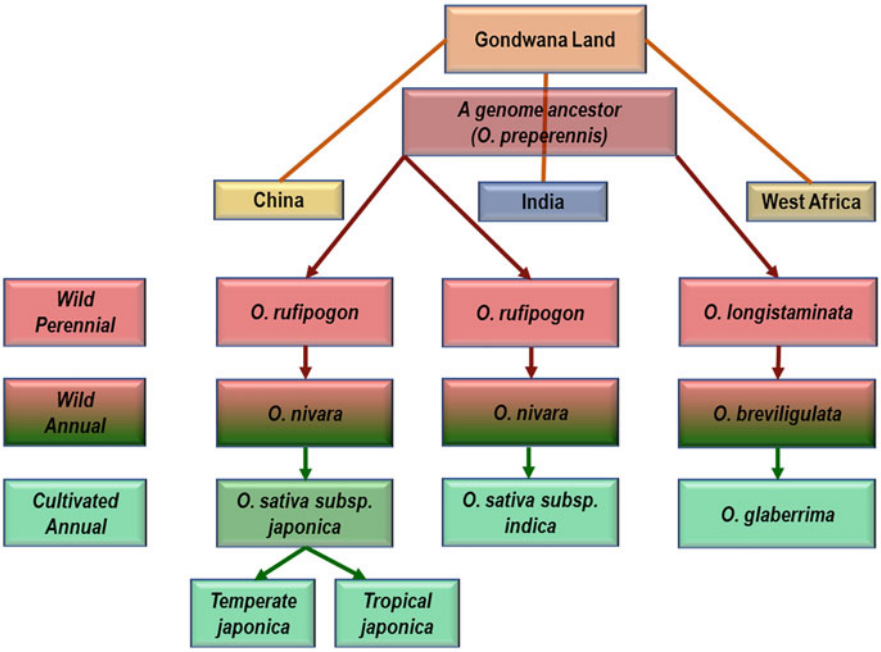

Fig. 3.4 Evolution of two cultivated A genome rice species and their subspecies from a common ancestor

Table 3.4 QTLs/genes associated with other major domestication syndrome traits

Genes	Gene ID	Chromosome	Trait	Reference
Gn1a	Os01g0197700	1	Grain number	Ashikari et al. (2005)
Rd	Os01g0633500	1	Pericarp colour	Furukawa et al. (2006)
qSH1	Os01g0848400	1	Grain Shattering	Konishi et al. (2006)
Bh4	Os04g0460000	4	Hull colour	Zhu et al. (2011)
sh4	Os04g0670900	4	Grain shattering	Li et al. (2006)
LABA1I	Os04g0518800	4	Awn length	Gu et al. (2015)
GIF1	Os04g0413500	4	Grain filling	Wang et al. (2008)
OsLG1	Os04g0656500	4	Panicle shape and ligule development	Ishii et al. (2013)
qSW5	Os05g0187500	5	Grain width	Song et al. (2007)
PROG1	Os07g0153600	7	Growth habit	Tan et al. (2008)
Rc	Os07g0211500	7	Pericarp colour	Sweeney et al. (2006)

water, and the fields are bunded to retain the water from the rainfall. The rainfed lowlands are further sub-classified into:

(a) Rainfed shallow favourable ecosystem with adequate rainfall throughout the crop season so that the crop is free from any moisture stress/even if stress is experienced, its occurrence is limited to shorter periods.
(b) Rainfed shallow drought-prone ecosystem, which experience short rainy period from 90 to 100 days. As they are completely dependent on rainfall, this ecosystem is bound to suffer from mild to severe stress during the crop growth period.
(c) Rainfed shallow submergence-prone ecosystems which experience very heavy rainfall for upto 10 days and rains for longer periods over the growing season.
(d) Rainfed medium-deep ecosystems, where the field are low lying and gets flooded with water stagnating for 2–5 months due to poor drainage. In the rainfed upland ecosystems, rice is solely grown with rainwater without any supplementation with surface water. The flood-prone ecosystems, which are prone to submergence due to inundation from excess rains/overflow of rivers/streams, to a depth exceeding 100 cm for a period ranging from 10 days to 5 months, as there are no structured water control options available in this ecosystem.

In India, rice is grown under diverse conditions from 79° to 90°E longitude and 16° to 28° N latitude under varying agro-ecological zones. Rice is grown in a wide range of environments, varying in water availability (from exclusively rainfed crop completely dependent on monsoon rains to deep waters, where the water level reaches 5 meters or more); regions varying in altitudes, right from below sea level in Kerala to 2000 MSL (6600 ft) in Jammu and Kashmir; at different temperature regimes from 4°C to 45°C and varying rainfall from 25 cm in Rajasthan to 1250 mm in Assam. A wide range of rainfall distribution patterns (drought, submergence and deep water) and distinct differences in soils (coastal and inland salinity, alkalinity and acidity), agro-climatic situations (high humidity, dry) and seasons exist in the country, necessitating the cultivation of a large number of diverse rice varieties. Depending on the patterns of rainfall distribution, it is cultivated as a completely rainfed upland crop in Jharkhand, Chhattisgarh, Western Orissa (Jeypore), parts of Rajasthan, Uttarakhand and West Bengal (Purulia and Bankura regions). On the contrary, it is also grown in shallow (upto 30 cm), semi-deep (30–100 cm) and deep water (>100 cm) ecosystems in eastern Uttar Pradesh, Bihar, West Bengal, Assam and Orissa. The area under various rice ecologies, production and productivity is presented in Table 3.5.

Improved rice varieties with high productivity are preferred in irrigated as rainfed shallow favourable ecosystems, and they are comparatively high in productivity. Drought-tolerant, photoperiod-insensitive short duration varieties are preferred for rainfed shallow drought-prone ecosystems as well as rainfed uplands; while photoperiod-sensitive long duration varieties with high productivity are preferred in rainfed shallow submergence-prone ecosystems; and varieties tolerant to stagnant

Table 3.5 Area, production and productivity of rice in India and world in different ecologies

Parameter	Irrigated		Rainfed lowland		Rainfed upland		Flood prone/ deep water	
	World	India	World	India	World	India	World	India
Area (mha)	79.2 (55.0)	17.8 (42.0)	40.6 (25.0)	15.0 (35.5)	17.2 (12.0)	7.0 (16.8)	11.5 (8.0)	2.4 (5.7)
Production (mt)[a]	484.0 (75.0)	52.0 (63.5)	108.8 (16.9)	22.0 (26.8)	27.6 (4.3)	4.7 (5.7)	24.6 (3.8)	3.3 (4.0)
Productivity (t/ha)[a]	5.00	2.97	2.00	1.47	1.20	0.67	1.60	1.37

Figures within parenthesis represent percentage area/production. *mha* million hectares, *my* million tons

[a] Milled rice in mt

flooding are preferred in rainfed medium deep ecosystems. Deepwater long duration photosensitive varieties with an inherent ability to elongate in tune with the flood water levels with excellent kneeing ability to avoid complete lodging due to an increase in plant height during the vegetative stage.

3.5 Rice Genetic Resources

Genetic resources form the foundation for crop improvement. The collection of indigenous land races started with the establishment of rice research facilities at Dacca (part of erstwhile India) in 1911 and the Paddy Breeding station at Coimbatore in the following year (1912). The Central Rice Research Institute (presently ICAR-National Rice Research Institute, Cuttack) was established in 1946. Concurrent efforts were made for the collection of the rice germplasm at different research stations across India and better performing accessions among the collections were released as varieties through pureline selection. Systematic explorations were made in the Jeypore tract of Odisha and erstwhile Madhya Pradesh from 1955 resulting in 1745 collections. During 1965 to 1967, 900 traditional cultivars from Manipur were collected. A large number of collections were also made from Assam famously known as Assam Rice Collection (ARC) between 1968 and 1973 (Sharma 1982). Another noteworthy collection of 19,116 local rice cultivars from Madhya Pradesh was made by Dr. R.H. Richharia from 1971 to 1976. The National Bureau of Plant Genetic Resources, New Delhi, was established in 1976 with a mandate of undertaking systematic exploration, collection, characterisation, evaluation, conservation and documentation for germplasms of crops including rice. Since then, a large number of rice germplasm collections have been made from different parts of the country.

In rice, ~5,00,000 accessions are conserved in the gene banks across the world, which also includes duplicates in different gene banks. Around 70.0% of the germplasm accessions of rice are conserved in six gene banks located in Asia, with half of the collections maintained in the gene banks at International Rice

Table 3.6 Rice germplasm accessions conserved in the gene banks across the world

No.	Institute/Country	No. of germplasm accessions conserved
1.	International Rice Research Institute, Philippines	1,32,123
2.	ICAR-National Bureau of Plant Genetic Resources, India	1,05,019
3.	China National Rice Research Institute, China	74,184
4.	USDA, United States of America	52,660
5.	NIAS, Japan	28,000
6.	Brazil	36,018
7.	RDA, South Korea	24,673
8.	Africa Rice, Cote d' Ivoire	21,815
9.	Myanmar	7484
10.	Russia	4228
11.	Indonesia	3891
12.	Australia	1732
13.	Kenya	1303
14.	Portugal	1138
15.	Bulgaria	888
16.	Italy	719
17.	Ukraine	700
18.	Romania	669
19.	Malaysia	632
20.	North Macedonia	276
21.	Zambia	258
22.	Hungary	253
23.	Albania	102
24.	Spain	67
25.	United Arab Emirates	55
26.	United Kingdom	22
27.	Ethiopia	17
28.	Israel	11
29.	Azerbaijan	3
30.	Costa Rica	3
	Total accessions	4,98,943

Research Institute (IRRI), Philippines and ICAR-National Bureau of Plant Genetic Resources (NBPGR), India. These gene banks along with those from China, USA, Japan, Brazil, South Korea, Côte d'Ivoire, Russia and Australia house long-term seed storage facilities capable of conserving rice collections for future prosperity. The remaining germplasm of the global holdings is distributed across a large number of national collections widely distributed throughout rice-growing countries of the world (Table 3.6). Among these, the wild rice accessions, which are difficult to conserve as seeds are also conserved ex situ in some field gene banks at IRRI, Philippines and at Chinese Academy of Agricultural Sciences, China. In India, ex

Table 3.7 Safety duplicates of different species of rice conserved at the Svalbard Global Seed Vault

Species	Accessions	Depositors	Country of collection
Oryza sativa	1,60,619	18	136
Oryza glaberrima	5473	3	39
Oryza nivara	1567	2	13
Oryza rufipogon	1075	3	19
Oryza hybrid	454	4	19
Oryza spp.	332	3	27
Oryza officinalis	307	2	14
Oryza spontanea	262	2	11
Oryza barthii	261	2	17
Oryza longistaminata	213	3	23
Oryza latifolia	99	3	15
Oryza punctata	83	1	16
Oryza meridionalis	68	2	2
Oryza minuta	64	1	3
Oryza australiensis	63	2	2
Oryza glumaepatula	59	2	8
Oryza granulata	24	1	11
Oryza eichingeri	22	1	3
Oryza rhizomatis	21	1	1
Oryza brachyantha	17	1	6
Oryza grandiglumis	15	2	2
Oryza alta	12	1	7
Oryza ridleyi	12	1	4
Oryza meyeriana	8	1	2
Oryza longiglumis	6	1	2
Total	1,71,136	63	402

situ field gene banks for conserving wild species of *Oryza* are located in ICAR-National Rice Research Institute, Cuttack, and Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore.

A duplicate set of rice germplasm conserved at many of these gene banks is also stored at the Svalbard Global Seed Vault in Norway as a safety duplicate under permafrost conditions. The seeds of diverse species originating from diffferent countries are stored in the Norwegian Archipelago of Svalbard under black box conditions, as safety duplicate to tide over any eventuality following international laws including the International Treaty for Plant Genetic Resources for Food and Agriculture (Table 3.7). As many as 63 organisations have contributed to the safety duplicate include IRRI, Africa rice, the National Plant Germplasm System from the USA, Taiwan, Brazil, Korea, India, etc.

3.6 Floral Biology

The rice inflorescence is a raceme, commonly known as panicles, because of its conical shape (Itoh et al. 2005). The inflorescence of the rice plant is a modified shoot. The shoot apical meristem under favourable conditions gets converted into an inflorescence meristem. The panicle is borne on the uppermost internode of the culm. The uppermost leaf in the culm is known as flag leaf. The extent to which the panicle and a portion of the uppermost internode extend beyond the flag leaf sheath determines the exsertion of the panicle (Chang and Bardenas 1965). The panicle has two types of meristems, namely rachis meristem and branch meristem. The rachis meristem forms bracts and branches as lateral organs, and finally aborts and remains as a vestige near the uppermost branch. The branch meristem differentiates into spikelets and branches and is ultimately converted into a terminal spikelet. The main axis of the panicle is called the rachis, which bears 10 or more primary rachis branches and the flowers on these branches known as spikelets. The primary branches are arranged in spiral phyllotaxy, which is completely different from that of 1/2 alternate phyllotaxy observed in the vegetative phase. The spikelets on the primary branches are arranged in a biased distichous phyllotaxy. The length of the fourth or fifth primary branch is the largest from where it gradually decreases towards the apex. The primary branches bear secondary branches and spikelets. The number of secondary branches is correlated with the length of the primary branch length. The length of the inflorescence/rachis and the number of primary branches vary widely among cultivars (Ikeda et al. 2004).

The rice spikelet is borne on the pedicel (spikelet axis), which is morphologically a peduncle (Fig. 3.5). The spikelet of *Oryza* comprises of three flowers, two of which were reduced in development resulting in an enlarged, cup-like apex consisting of two very small sterile glumes called the rudimentary glumes. Above the inner

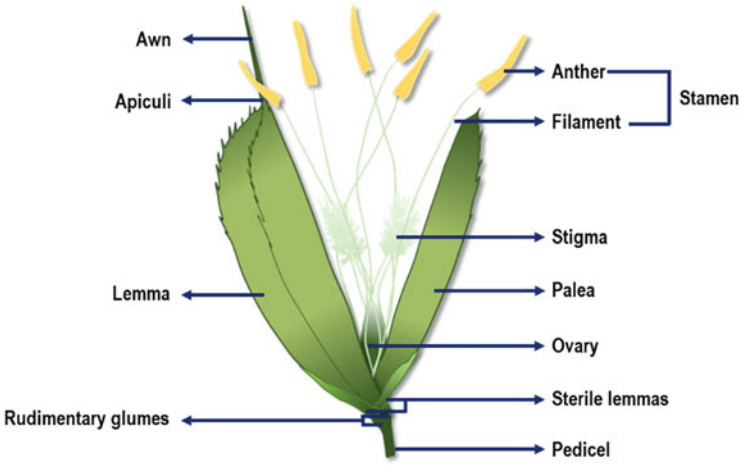

Fig. 3.5 Spikelet of rice

rudimentary glume, there are two more small lower bracts which are always sterile and are comparatively larger than the rudimentary glumes called the sterile lemmas. The upper bracts consist of two interlocking large glumes, the lemma (fertile lemma) and palea. The lemma, palea, and the included flower form the floret. The sterile lemmas are generally shorter than the lemma and palea, never exceed one-third length of the latter. The lemma is larger and partly envelops the palea. It is indurate (hardened) with five vascular bundles/nerves, whereas the palea has three vascular bundles/nerves. The six glumes are arranged in 1/2 alternate phyllotaxy. The rice flowers are devoid of sepals. Inside the palea, two lodicules (corresponding to petals) biased to the lemma side are present in the whorl. Lodicules are small and whitish in structure, which plays a vital role in the opening of the floret. The rice spikelet consists of six stamens positioned inside the lodicules and one pistil at the centre of the whorl. The pistil consists of a single carpel with a bifid hairy stigma at the apex. The extended tips of the lemma and the palea are the apiculi. Some rice genotypes may possess awn in the spikelet, which is a filiform extension of the keel of the lemma (Chang and Bardenas 1965).

Panicle initiation in rice starts almost 30 days before the emergence of the panicle. The flag leaf usually emerges about 18 days before anthesis. The flag leaf sheath thickens 6 days before anthesis due to panicle growth which is referred to as the booting stage. The panicle emergence from the flag leaf sheath is completed within a day. Days to complete flowering from the panicle emergence usually vary from 5–15 days in rice depending on the variety (Yoshida 1981). Anthesis in rice commences immediately after the emergence of the panicle. The spikelet opening begins with the opening of the tip portions of the lemma and palea, following which the filaments elongate leading to exsertion of anthers as well as the tip of the stigma. Each spikelet remains open for about 30 min. Anther dehiscence occurs just before or at the opening of lemma and palea due to which many pollen grains fall into the stigma of the same spikelet leading to self-pollination. Anthesis starts in the spikelets of primary branches at the tip of the panicle and proceeds basipetally towards the bottom of the panicle. It takes 7–10 days to complete the anthesis of all the spikelets within a panicle. The time of anthesis during a given day varies with weather conditions. If there is a time lag between opening of spikelet and the anther dehiscence, it may lead to cross pollination in rice. Under normal weather in the tropical region, anthesis begins at 8:00 AM and continues upto 01:00 PM, attaining the maximum anthesis between 10:00 AM to 11:00 AM. An individual spikelet remains open only for a short period, ranging from 30 to 90 min on a given day (Hector 1913). However, the opening of spikelets and anthesis is dependent on temperature, relative humidity (RH) and light. Temperature ranging from 28°C to 31°C, RH of 70–80% and orange light are the ideal parameters to accelerate spikelet opening and anthesis in rice (Nguchi 1929). Pollen grains are viable only for 5 min, while the stigma remains receptive for 3–7 days. Normally, the pollen grains germinate on the stigma in 3 min after pollination. Fertilisation normally takes place within 5–6 h after anthesis. The fertilised ovary develops into caryopsis about 30 days after fertilisation.

3.6.1 Emasculation and Pollination

Emasculation is done for attempting hybridisation between selected genotypes through controlled pollination. Emasculation is done one day earlier before the beginning of anthesis in the evening hours. Even though it is possible to emasculate early in the morning well ahead of anthesis before 8:00 AM, emasculation in the morning is avoided in general, to avoid inadvertent self-pollination. Since the maximum number of spikelets open on the third or fourth day of anthesis, panicles of that stage are selected for emasculation. As many as seven different methods of emasculation have been described for effecting hybridisation in rice. The methods widely used for hybridisation in rice include:

(a) *Clipping method*, which involves clipping one-third portions of both the top and bottom portions of the panicle of the desired female parent in the previous day evening using scissors leaving the middle spikelets (Torrens 1930). In the middle spikelets, the top one third in each spikelet is clipped-off in a slanting position. Emasculation is done by removing six anthers in each spikelet with the help of the forceps/needles (Emasculation) with appropriate care to not damage the gynoecium. The emasculated spikelets are then covered with a butter/parchment paper bag to avoid contamination with the undesired pollen. The bloomed panicle from the desired male parent is collected the next day morning (at about 9:00 AM) for effecting pollination. The top portion of the butter/parchment paper bag covering the emasculated panicle in the female parent is cut to expose the panicle. The male parent panicle is inserted in an inverted position into the butter paper bag and turned in both ways to disperse the pollen. After ensuring the abundant disbursement of pollen, the opened butter paper bag is closed using a pin. Tagging is done with labels in the pollinated panicles indicating the date of pollination and parentage.

(b) *Hot water method*, which utilises the differential response of pollen grains and ovary to high temperature using hot water treatment (Jordon 1938). The pollens are highly sensitive to high temperatures and lose their viability with hot water at 43°C for 7 h while stigma receptivity is not affected. The desirable panicles are selected from the female parents and an hour or so before anthesis (~ at 7:00 AM), the underdeveloped and opened spikelets are removed. Then, the tiller is bent over carefully (to avoid breaking), and the selected panicle is immersed in hot water at 40–44°C contained in an insulated bottle for 5 to 10 min. This treatment causes the florets to open in a normal manner and avoids injury. Then, emasculation is done by removing the six dead stamens by fine forceps or needles and then pollination is done.

(c) *Dr. Ramiah's method,* wherein the selected panicle is covered with a wet cloth after removing the top and lower spikelets, and the air is blown from the mouth. This increases humidity and facilitates the opening of spikelets. After 2–3 min, the wet cloth is removed and the anthers are removed from the open spikelets.

(d) *Vacuum emasculation method*, which works on the principle of suction pressure (Mulimbayan 1936). The spikelets of the desirable panicles are clipped off

before exposure to vacuum suction. Minute pipettes are pointed towards the anthers in the clipped spikelets, and the anthers are sucked in due to vacuum pressure. Upto six panicles can be emasculated at a time, using a Vacuum emasculator with six nozzles. Vacuum emasculation is a high throughput method, through which six persons can emasculate 3000 to 3600 florets/hour simultaneously as compared to hand emasculation where a person can emasculate a maximum of 100 florets only.

(e) *Cuttack method*, which was developed at erstwhile Central Rice Research Institute (now ICAR-National Rice Research Institute), Cuttack. The panicle to be emasculated is inserted into a hollow piece of bamboo closed at one end and plugged with cotton wool and split cork at the other end. These spikelets in the enclosed panicles will open within 5–10 min after which the anthers are removed.

(f) *Brown paper method*, wherein the panicles are covered in a brown paper cover couple of hours before blooming. The anthers extrude within 15–30 min due to heat inside the brown paper bag but do not dehisce, which makes it easier to remove the anthers (Ramiah 1927). The stigmatic surface is then dusted with pollen grains collected from the desired male parent.

(g) *Rhind's method*, which uses the warmth and humidity in the flask which is filled with hot water and decanted. The panicle to be emasculated is inserted into the flask and leftover for some time. Due to high temperature and humidity, the spikelets open and the anthers protrude out, which makes it easy to remove them with the help of forceps.

3.7 Cytology and Molecular Cytogenetics

Kuwada (1910) carried out the first study of microsporogenesis, megasporogenesis and mitosis in *Oryza sativa* and determined its somatic chromosome number as $2n = 24$. Nandi (1936) reported that the variation in the length of somatic chromosomes ranged from 0.7 μ to 2.8 μ. Nandi (1936) described the morphology of metaphase chromosomes, based on which they were classified into largest pair of chromosomes with medium constrictions, medium sized chromosomes with medial insertion region and small chromosomes. The largest chromosome measured 2.1 μ, while the smallest one was 1.4 μ (Sethi 1937). At meiosis, the chromosomes in both *O. sativa* and *O. glaberrima* form 12 bivalents in pollen mother cells (PMCs). The chromosomes of *O. officinalis* were reported to be slightly larger than that of *O. sativa* (Ramanujam 1938). Sen (1963) reported the only description of a gametophytic karyotype in the first microspore division. Shastry et al. (1960) reported the morphology of the pachytene chromosome complement in rice for the first time. The occurrence of triploids and tetraploids in rice was first reported by Nakamori (1932). Morinaga and Fukushima (1931) along with Ramiah et al. (1933) reported the first occurrence of haploids.

However, rice chromosomes are very small and morphologically similar making it difficult to peruse them using any cytogenetic tools/banding technique before

1960. Hu (1961) first reported the differences in chromosome size among different wild species, namely B, C, D, E, F and G genomes. Dr. Nori Kurata from Kyushu University invented a technique that revealed rice somatic prometaphase chromosomes with clear-cut centromere position and variations in chromosome length. Even though the sizes of chromosomes and genome varied among different wild rice genomes (Kurata and Fukui 2003), the genomes exhibited very little morphological variations (Kurata 1985). Primary trisomics were utilised by Iwata and Omura (1975) for establishing the association between eight primary trisomics with linkage groups. Khush et al. (1984) established the association between the 12 linkage groups with cytologically identifiable chromosomes at the pachytene stage of meiosis for the first time, using the complete set of 12 primary trisomics in rice. However, due to the lack of an objective method, there were several discrepancies in the cytological characterisation and identification of the rice chromosomes (Oka and Wu 1988).

Molecular cytogenetics combining molecular techniques and cytogenetics have contributed to our understanding of chromosome and genome structure, phylogeny and genome evolution in the *Oryza* species. Genome analysis of the *Oryza* genus including 2 cultivated species and 24 wild species has identified 11 genomes. Assessing genome size helps assess its complexity and difficulty in mapping and map-based cloning of genes (Martinez et al. 1994). The nuclear DNA content of different *Oryza* species has been estimated microspectrophotometrically through fuelgen staining of prophase nuclei (Iyengar and Sen 1978) and by flow cytometry (Martinez et al. 1994) and employing flow cytometry and chromosome analysis (Miyabayashi et al. 2007). Even though the genome size of *Oryza* species exhibits good correspondence to the enlargement of chromosomes with more than twofold difference in genome sizes, the chromosome complements of all species show very similar morphology (Kurata 2008). To identify combinations of alleles and regions of the genome responsible for heterosis and transgressive variation and learn how to predict which introgressions from diverse wild or exotic donors are likely to enhance performance in elite genetic backgrounds of interest, several groups have constructed libraries of chromosome segment substitution lines (CSSLs), which greatly facilitate the identification of agronomically valuable genes introduced from wild or unadapted donors (Tian et al. 2006; Ali et al. 2010).

Technological advances in cytology have enabled visualisation of nucleotide sequences localised onto chromosomes, nuclei and tissues. Fukui et al. (1987) produced the first reliable in situ hybridisation to locate the 18S-5.8S-25S ribosomal RNA gene (45S rDNA) onto pair of rice chromosomes using ^{125}iodine labeled ribosomal RNA. Imaging techniques in combination with staining (Fukui and Iijima 1991) and non-radioactive labelling using haptenes such as biotin (Iijima et al. 1991) enabled quantitative estimation of the condensation pattern at the prometaphase stage, thereby helping in the development of the somatic map of rice chromosomes. Although the detection technique was stable and safe, they suffered from low spatial resolution, limitations in probe size and number. Based on further technological advances in enzymatic and fluorescence techniques in conjecture with new imaging methods such as cooled CCD camera to capture even the faint fluorescence signals, a

reproducible and convenient fluorescence in situ hybridisation (FISH) technique was developed in rice (Fukui et al. 1994; Ohmido et al. 1998). With the manifold advantages offered by FISH including good sensitivity and high spatial resolution, the ability to simultaneously detect several probes using different fluorochromes and the versatility of three-dimensional analyses, FISH made it possible for dramatic advances in molecular cytological studies in rice (Ohmido et al. 2010).

The pachytene chromosomes of rice are ideal for FISH mapping. FISH has been very useful in identifying the genome-wide distribution of different types of repetitive sequences. TrsA repeats of 355 bp length were one of the first tandem repeat sequences identified in rice (Ohtsubo et al. 1991). It is detected in the subtelomeric regions of both cultivated and wild species of rice (Ohmido and Fukui 1997). TrsA also contributes to the rice terminal structures and genome sizes of various rice species (Ohmido and Fukui 2004). Fibre DNA technology provided a breakthrough for estimating the distance between TrsA and telomere sequences at a chromosomal end. Extended DNA fibres (EDFs) isolated from rice nuclei, in tandem with FISH provides higher spatial resolution, thereby allowing accurate estimation of the number of copies of the repetitive sequence and the physical length of target nucleotide sequences (Ohmido et al. 2000). Multicolour FISH (McFISH) using telomere- and TrsA-specific probes on rice somatic cells, pachytene chromosomes and EDFs enables determination of the size of telomeres in rice. The improved sensitivity of FISH is also used in detecting DNA-protein interactions with the use of Chromatin immunoprecipitation (ChIP) and immunostaining.

Genomic in situ hybridisation (GISH) which uses total genomic DNA as the probe is a versatile tool for cytology and molecular cytogenetics in plants (Heslop-Harrison et al. 1990). GISH enables the characterisation of polyploids, F_1s, and Recombinant lines through the comparison of chromosomes and genomes of different species. Fukui et al. (1997) demonstrated that GISH is effective in discriminating the B, C and D genomes of rice with very small chromosomes. Multicolour GISH (McGISH) with two genomic probes one for A and another for C genome, and DAPI staining has been used to characterise the introgression of chromosomal fragments among different genomes of rice species in somatic hybrids between *O. sativa* (AA) and *O. punctata* (BBCC). Chromosome painting is a powerful method for the detection of specific chromosome regions or entire chromosomes based on chromosome specific probes. It is a powerful tool to study genome duplication, chromosomal rearrangement and the evolution of species (Lysak et al. 2001). Uozu et al. (1997) utilised chromosomal painting to determine the distribution pattern of genome specific repetitive DNA sequences in twelve diploid species of rice with A, B, C, E and F genomes to show that the amplification of the repetitive DNA sequences causes the variation in the chromosome morphology and genome size of rice.

3.8 Genetics of Qualitative and Quantitative Traits

A basic understanding of the extent of genetic variability for traits as well as the nature of inheritance is very important for its use in crop improvement. Elucidating the mode of inheritance of traits is of utmost importance as it will help in planning an appropriate selection strategy for the improvement of the trait in rice breeding. Rice genetics is rich with valuable information on the genetics of a large number of traits as well as the linkage relationships, which have been documented systematically in the rice genetics newsletter since 1989. The sequencing of the rice genome through the international consortium initiative of the International Rice Genome Sequencing Project in 2002 has enabled map-based cloning as well as the identification of a large number of QTLs governing complex traits. A large number of genes and QTLs governing various agro-morphological traits, resistance to various stresses, and physiological traits have been identified and cloned in rice, which have been documented in QTL Annotation Rice Online (Q-TARO) database (http://qtaro.abr.affrc.go.jp). A snapshot of the functionally characterised genes and QTLs mapped is presented in Table 3.8.

3.9 Rice Research in India

The first experimental research for rice was initiated at Dacca (now in Bangladesh) in the year 1911. The first botanist in India to work on rice was Dr. Hector. However, Mr. Parnell was the first crop specialist (later designated as paddy specialist) fully devoted to rice research appointed at the Paddy breeding station (PBS), Coimbatore in Tamil Nadu, established in 1912. Dr. Ramiah succeeded him as paddy specialist at PBS (now a part of Tamil Nadu Agricultural University, Coimbatore). The establishment of the Central Rice Research Institute (presently ICAR-National Rice Research Institute), Cuttack, in 1946 is a landmark event in rice research in India. A large number of research stations/institutes were established across the country to cater to the needs of rice farmers from different regions and ecologies (Table 3.9). The All India Coordinated Rice Improvement Project (AICRIP) was established in 1965, which was upgraded as Directorate of Rice Research (presently ICAR-Indian Rice Research Institute), Hyderabad, with the mandate of development of an integrated national network of cooperative experimentation on various aspects of rice production with rice varietal improvement as a core activity.

3.10 Breeding Objectives

3.10.1 Yield

Rice yield is a complex trait that is a function of several traits including the number of panicles per plant, number of grains per panicle and thousand grain weight (Xing and Zhang 2010). Traditional rice varieties were in general tall, photosensitive, less

Table 3.8 Summary of functionally characterised genes and QTLs mapped in rice

Traits	Genes identified	Important genes	QTLs mapped	Important QTLs
Morphological traits				
Culm leaf	204	*nal7*, *lax*, *Osgl1–2*, *ygl7*	66	*qFL1–1*, *qPN1*
Plant height	214	*sd1*, *gid2*, *bu1*	47	*Eui*, *eui2*, *qPHT2*
Panicle flower	126	*sh4*, *Gn1a*, *WFP*, *IPA1*, *DEP1*	111	*qSH1*, *yld1.1*
Root	106	*SOR1*, *SLL1*, *OsRAA1*	60	*qRTH2*, *qSTA2*
Seed	80	*GS3*, *GW2*, *GW5*, *TGW6*	93	*qGL3a*, *qDTH6*
Seedling	51	*ysa*, *ylc1*	31	*qCER4–1*, *qRA6–1*
Others	5	*TDR*, *OsEXPA3*	–	–
Resistance or tolerance to stresses				
Bacterial blight resistance	81	*xa5*, *Xa7*, *xa13*, *Xa21*, *Xa38*	1	*qSB3*
Blast resistance	108	*Pi1*, *Pi2*, *Pi5*, *Pib*, *Pi9*, *Pi40*, *Pi54*, *Pita*	45	*qNBL5*, *qLN1–1*
Sheath blight resistance	4	*OsGLP8*, *Osoxo4*	6	*qSB9*, *qSBR3*
Other diseases	6	*rim1*	7	*qstv1*
Insect resistance	11	*Bph14*, *Bph 19*, *Bph20*	28	*qbph6*, *qbph10*, *qOVA4*
Drought tolerance	109	*Dro1*, *DST*	111	*qDTY1.1*, *qDTY3.1*, *qDTY12.1*
Cold tolerance	48	*qLTG3.1*	25	*qCT1*, *qLTG7*
Salinity tolerance	102	*SKC1*, *DST*	5	*Saltol*, *qSNC7*
Submergence tolerance	8	*Sub1A*, *SK1*, *SK2*	19	*qTIL12*, *qNEI12*
Lodging resistance	19	*SCM2*	2	
Soil stress tolerance	80	*PSTOl1*, *NRAT1*	72	*qDSR8*, *qDLTR11*
Other stresses	79	*ALS*, *OsBADH1*	9	*qTRA7*, *qUVR10*
Physiological traits				
Culm leaf	11	*OsGUN4*, *EPSPS*	–	–
Eating quality	65	*wx*, *BADH2*, *lpa1*, *flo2*, *LOX3*, *gpa2*	83	qER2, qGT6, qPGWC8
Flowering	76	*RFT1*, *Ehd2*, *Hd3a*, *Ghd7*	49	*qDTH8*, *QHd7*, *qEHd1*
Germination, Dormancy	36	*ARAG1*, *Osdsg1*, *Sdr4*	17	*qGRV8*, *qSD12*
Panicle flower	19	*cl7(t)*, *rip3*, *OsUDT1*	–	–
Root	14	*OSPT9*, *OsAMT3:1*	–	–
Seed and Seedling	21	*MET1a*, *OsWR2*, *OsJAR1*	–	–
Source activity	96	*FLO6*, *SS1*, *ADH1*	64	*qLCC7*, *qFRP4–1*

(continued)

Table 3.8 (continued)

Traits	Genes identified	Important genes	QTLs mapped	Important QTLs
Sterility	115	*TMS9–1*, *tms5*, *PAIR2*, *Rf3*, *sRf4*	45	*qSS7a*, *qLTSPS2*
Others	74	*OsMet1*, *OsDDM1a*	42	*Gy3a*, *qHUS1–2*

responsive to increased fertiliser application, poor yielding as well as highly susceptible to lodging at maturity. As a result of which, the average productivity of rice in India during 1950 was only a meagre 771 kg/ha. Therefore, ever since rice breeding started, one of the prime objectives has been the improvement of yield, to feed the large majority of rice eating populations of Asia, where more than 90% of the population is dependent on this crop. Genetic improvement for rice yield has been the focal point of rice breeding, employing five different concepts, viz.

(a) *Indica/japonica* hybridisation (including Tongil rice development through *japonica/indica* hybridisation)
(b) Development of semi-dwarf varieties.
(c) Breeding for new plant type.
(d) Super rice breeding.
(e) Heterosis breeding.

However, in the past century of rice breeding efforts, only two of the above major genetic improvement strategies have helped in realising quantum jump in rice yields, namely (1) semi-dwarf varieties through the incorporation of *sd1*, which helped in improving the plant architecture as well as the harvest index, and (2) production of hybrids that exploit heterosis.

3.10.2 *Indica/Japonica* Hybridisation

The first attempt of *indica/japonica* hybridisation was made in 1928 in Burma (Kirk and Silow 1951), involving D17-88/Shinriki, but discontinued due to sterility. Immediately after World War II, the shortage of food supplies and the immediate threat of population increase directed world attention towards finding ways to increase the production of rice. The International Rice Commission (IRC) was established on 4th January in 1949 within the framework of FAO with the objective of promoting national and international action in respect of production, conservation, distribution and consumption of rice. It was a milestone in the advance of cooperative rice research. Based on the recommendations of the first meeting of the working party of IRC held at Yangoon in 1950 (IRC, 1950), the Food and Agricultural Organisation (FAO) of the United Nations initiated one of the first major international efforts towards yield improvement for improving rice production. A collaborative project on *indica/japonica* hybridisation began in Southeast Asian

Table 3.9 Establishment of major rice research institutes/stations in India

Year	Institute	Location
1912	Paddy Breeding Station (Presently Department of Rice)	Coimbatore, Tamil Nadu
1913	Rice Research Station	Karimganj, Assam
1919	Regional Agricultural Research Station	Karjat, Maharashtra
1922	Rice Research Station (Presently Zonal Research Station)	Nagina, Uttar Pradesh
1922	Agricultural Research Station (Presently Tamil Nadu Rice Research Institute)	Aduthurai, Tamil Nadu
1923	Agricultural Research Station	Dharwad, Karnataka
1923	Rice Research Station	Titabar, Assam
1923	Rice Research Station	Ratnagiri, Maharashtra
1924	Rice Research Station	Kanpur, Uttar Pradesh
1927	Rice Research Station	Pattambi, Kerala
1928	Rice Section (Agricultural Research Institute)	Rajendranagar, Telangana
1935	Indian Agricultural Research Institute	New Delhi, Delhi
1936	Regional Research and Technology Transfer Sub Station	Jeypore, Odisha
1940	Rice Research Station	Kuttanadu, Kerala
1946	Central Rice Research Institute (Presently ICAR-National Rice Research Institute)	Cuttack, Odisha
1950	Mountain Research Centre for Field Crops	Khuwani, Jammu & Kashmir
1951	The Crop Research Farm	Masodha, Uttar Pradesh
1951	Agricultural Research Station	Ponnampet, Karnataka
1959	Rice gall fly (Regional Agricultural Rsearch Station)	Warangal, Telangana
1956	Agricultural Research Station	Gangavati, Karnataka
1960	Main Rice Research Station	Nawagam, Gujarat
1962	Regional Rice Research Station	Kapurthala, Punjab
1963	Regional Agricultural Research Station	Pattambi, Kerala
1963	Rice Research Station	Wangbal, Manipur
1965	Establishment of All India Coordinated Rice Improvement Project (AICRIP)	CRRI, Cuttack (Presently at IIRR, Hyderabad)
1965	Andhra Pradesh Rice Research Institute	Maruteru, Andhra Pradesh
1965	Rice Research Station, Directorate of Agriculture	Chinsurah, West Bengal
1966	Department of Genetics and Plant Breeding	Pantnagar, Uttarakhand
1968	Rice Research Station	Raipur, Chhattisgarh
1969	Agricultural Research Station, VC Farm	Mandya, Karnataka
1969	Regional Agricultural Research Station	Titabar, Assam
1970	Rice and Wheat research Centre (Kangra)	Malan, Himachal Pradesh`
1974	The Rice Research Station	Moncompu, Kerala
1975	Agricultural Rsearch Station	Kota, Rajasthan
1983	Directorate of Rice Research[a] (presently ICAR-Indian Institute of Rice Research)	Hyderabad, Telangana

[a]Instituted as AICRP and later given independent research status

Fig. 3.6 Pusa 2090—a high yielding genotype derived through *indica/japonica* hybridisation with typical short flag leaves and panicles at the top

countries in 1952 to combine the desirable attributes of *japonica* subspecies such as strong culm, dark-green erect leaves, high photosynthetic efficiency with high tillering and response to fertiliser with resistance to specific diseases and insects, and better grain quality of *indica* rice. A parallel scheme with similar objectives was adopted by the ICAR. These two projects used 192 improved *indica* varieties selected by the participating Asian countries and Indian states and produced a total of 710 *japonica/indica* hybrids (Parthasarathy 1972).

The F_1 seeds were distributed to the participating countries or states for growing the F_2 and subsequent generations and to breed varieties suited to local conditions. However, it could not result in a breakthrough owing to several reasons including (1) sterility induced due to cryptic structural hybridity (Oka 1957; Shastry 1964), (2) inability to screen the segregating populations under high fertility, (3) quantitative nature of the traits, (4) low population size in segregating generations leading to less selection efficiency, (5) inherent differences in the photo- and thermo-sensitive response of *indica* and *japonica* cultivars leading to the inefficient selection, (6) the incorrect belief at that time, that increased yield is associated with an increase in height and duration, (7) environmental influence on plant height and duration, (8) inherent differences in grain and cooking quality of the two subspecies, and (9) emphasis on yield improvement alone (Seetharaman 1981). Only a few rice varieties were developed and released including Malinja and Mahsuri in Malaysia, ADT 27 in India and Circna in Australia (Parthasarathy 1965). This could have been averted if tropical *japonica* genotypes adapted to Indian tropical environment such

as Taichung 65, Tainan 3, Herunchu, etc. were used in the hybridisation programme instead of the typical temperate *japonica* varieties such as Norin 6, Norin 8, Rikue 12, etc. (Rao and Nagaraju 1974) and possibly by attempting one backcross with the *indica* cultivars to reduce the spikelet sterility as well as improve the grain and cooking quality. With the discovery of wide compatibility (S_5) locus, the interest in *indica/japonica* hybridisation has been reinvigorated and efforts are underway to develop improved varieties (Fig. 3.6).

Parallelly, efforts were also made to introduce *japonicas* in India, but it was met with no success, except in the hills and some cool areas. *Japonicas* were both photoperiod and temperature sensitive, which flowered in 35–40 days leaving insufficient time for proper tillering and vegetative growth. Therefore, they were not as productive as *indicated* under Indian conditions. Another scheme was also launched by the Central Rice Research Institute (CRRI) in 1960 to breed high yielding fertiliser responsive hybrid varieties with *japonicas* in 11 states, which did not prove much successful either. At CRRI, Cuttack, a late-maturing tall and non-lodging rice variety, CR 1014, with medium slender grains and good cooking quality was developed from the *indica/bulu* (*javanica* or tropical *japonica*), cross between T 90 and Urang Urangan (Seetharaman 1981). However, the development and the introduction of semi-dwarf varieties into India brought an abrupt end to this scheme in 1966.

3.10.3 *Japonica/Indica* Hybridisation

On the other hand, the hybridisation involving *japonica* and *indica* varieties of *O. sativa* has been very successful in Korea in the development of the 'Tongil' cultivar, where the hybrid sterility in *indica-japonica* hybrids was overcome three-way crosses (Chung and Heu 1980). The *japonica* variety was crossed with semi-dwarf *indica* variety (possessing *sd1* gene, a breakthrough in rice improvement across the world, described in the following portion), which was further backcrossed or top crossed with semi-dwarf *indica* variety. This strategy of three-way crosses provided twofold advantage, namely (1) overcoming the grain sterility in *japonica-indica* hybrid populations and (2) improvement of the selection efficiency (Heu and Park 1973). The top cross, IR667 (IR8//Yukara/TN), showed good adaptability, and the line, Suweon 213, was released as 'Tongil' (literally meaning 'together one' in Korean) in 1971.

The development of Tongil rice resulted in a significant yield increase from 4 to 5 t/ha, corresponding to a 30% yield increase relative to the leading *japonica* varieties grown in Korea (Choi et al. 1974). Tongil rice is characterised by medium-long and erect leaves, thick leaf sheaths and strong culms, short plant height but relatively long panicles, open plant shape, lodging resistance and easily shattered grain (Chung and Heu 1991). However, the Tongil rice cultivars also possessed some undesirable characters, such as grain quality and susceptibility to low temperature. Through concerted efforts, these problems have been overcome and as many as 25 Tongil cultivars, including the popular Tongil varieties such as

Tongil, Milyang 23, Samgangbyeo, Jungwepnbyeo, Yongmoonbyeo, Dasnbyeo, Arumbyeo and Hanarumbyeo have been released till 2002 with yield improvement from 5.0 t/ha in Suweon 213 to 7.53 t/ha in Hanarumbyeo.

3.10.4 Development of Semi-Dwarf High Yielding Rice Varieties

A landmark event in rice yield improvement was the identification of a short statured genotype, Dee-geo-Woo-gen (meaning 'short legged, brown tipped') from a Taiwanese cultivar, 'Woo-gen' (Huang 1957). The first semi-dwarf rice variety, 'Taichung Native 1', was developed from the cross, Dee-geo-woo-gen/Tsai Yuan Chung made in 1949, aimed at combining the semi-dwarf statue and profuse tillering of Dee-geo-woo-gen with disease resistance of the tall cultivar, Tsai-Yuan Chung (Huang et al. 1972). It is semi-dwarf (83–85 cm), matures in 127–130 days possessing an average of 19 panicles/hill, with a panicle length of 20–22 cm and long (7.5 mm), medium grains. Taichung Native 1 along with two other semi-dwarf genotypes, Dee-geo-Woo-gen and I-Geo-tze were utilised in hybridisation at International Rice Research Institute, Manila, the Philippines, in 1960. IR 8-288-3, a semi-dwarf high yielding genotype derived from the cross, Peta/Dee-geo-Woo-gen, was released as IR 8 in 1965.

The release of IR 8 with a yield potential of upto 7.0 t/ha marked the beginning of an era where the semi-dwarf short statured varieties provided a breakthrough achievement in improving rice yields, which had long lasting impact across the world (Huang et al. 1972). Both IR 8 and Taichung Native 1 were introduced in India in 1966. These two varieties were utilised in the national programme leading to the development and release of 14 semi-dwarf high yielding rice varieties (including three introductions) in India between 1966 and 1970 (Freeman and Shastry 1972). Jaya and Padma developed from the cross, TN1/T 141 and the reciprocal cross, T 141/TN1, respectively were the first semi-dwarf rice varieties released in India in the year 1968 (Seetharaman 1981). The introduction of semi-dwarf varieties in the mid-1960s and their utilisation in the national programme for the development of new semi-dwarf high yielding rice varieties marked a new era of 'Green Revolution' (Coined by Dr. William Gaud of the US Department of Agriculture in 1968) helping to achieve self-sufficiency in food production in India.

Although mutants with short plant height, better tillering than the cultivar GEB24 and adapted to fertile soils were identified earlier in India, they were popular among the farmers because the critical tests of fertiliser response were not conducted then and the farmers preferred longer straw to feed the animals (Ramiah and Rao 1953). Therefore, the yield improvement was possible due to convergence of improved plant type along with appropriate management practices and production environments to enable them to realise their yield potential. During the decade, starting 1965, as many as 72 high yielding varieties suited for different ecosystems were released of which the majority of them were semi-dwarf rice cultivars. The adoption of these high yielding semi-dwarf rice varieties increased the average annual productivity from 0.67 t/ha in 1950–1951 to 1.12 t/ha in 1970–1971, thereby

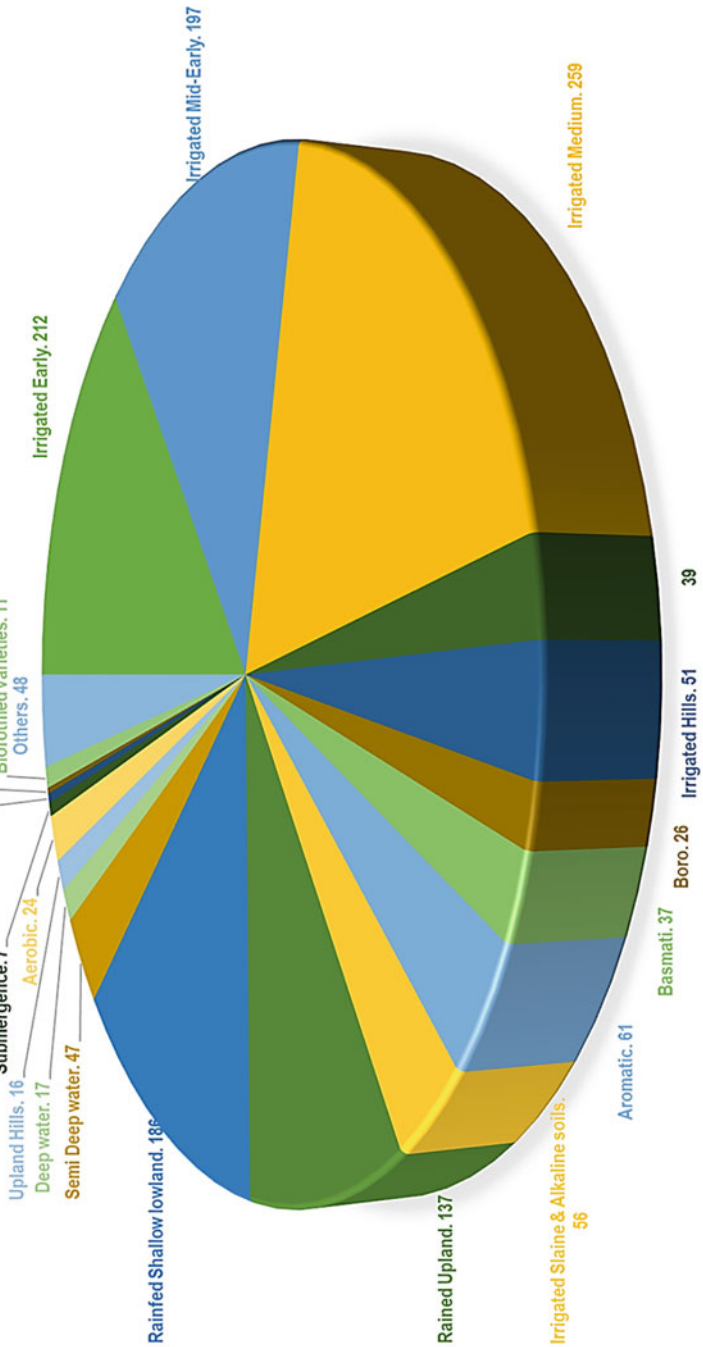

Fig. 3.7 A snapshot of the rice varieties released for diverse ecologies in India

doubling the rice production in India. In highly productive irrigated ecosystems of Punjab, the average yields improved to as high as 2.24 t/ha in 1970–1971. Till date, as many as 1439 rice varieties have been released for diverse ecologies across the country (Fig. 3.7).

3.10.5 New Plant Type Concept

Donald (1968) proposed the ideotype approach to plant breeding, wherein an 'ideotype' is an idealised plant type with a specific combination of characteristics for photosynthesis, growth and grain production based on the knowledge of the crop's physiology and morphology. In rice, it was observed that varieties with high yield potential and better fertiliser responsiveness had the short sturdy stem, and short, erect, narrow, thick and dark green leaves (Tsunoda 1962) based on which 'Plant type concept' was proposed (Yoshida 1972). Predictions using simulation modelling indicated that the yield potential can be increased by 25% through modification of certain physiological and morphological characters, such as (1) enhancing leaf growth with reduced tillering during early vegetative growth, (2) reducing leaf growth and increasing foliar N concentration during late vegetative and reproductive growth, (3) making the vertical N concentration gradient in the leaf canopy slope steeper with a greater proportion of total leaf N in the upper leaves, (4) increasing the carbohydrate storage capacity in stems and (5) creating better reproductive sink capacity as well as extending the grain-filling period (Dingkuhn et al. 1991). The key morphological traits of the basic ideotype was conceptualized by Janoria (1989). The characteristic features of ideotype includes tall stature, lodging resistance, upright growth habit, fewer but all effective tillers, fewer, well-spaced, thick, not-too-short, stiff leaves, heavy panicles, limited intraplant variation in panicle yield, and deep, extensively branched roots. These ideotypes were conceptualized for growing at closer spacing. He also developed the first prototype, Rewa 353 (PAU125-1-2/Lalco 14). It was 130 cm tall, with an average of six very upright, stiff tillers (all effective)/ plant. Highly synchronous panicles yield an average 4 g of long, slender grains with $< 10\%$ sterility and a harvest index of 0.50. It produced 4.3 t/ ha in 85 days over 19 locations, compared with 3.85 t/ ha produced by semidwarf check variety of same duration, Rasi.

The 'New Plant Type' (NPT) was conceptualised to improve yield potential by 15–20% in 1988 (Khush 2007). The proposed plant type included modifications in key traits such as plant height of 90–100 cm, low tillering (3–4 tillers under direct seeding), no/low unproductive tillers, 200–250 grains per panicle, thick sturdy stems, dark-green, thick and erect leaves, vigorous and deep root system, 100–130 days growth duration and increased harvest index (Peng et al. 1994). Breeding for the first-generation NPT lines began in 1989, with the identification of donors for these traits in 'bulu'/*javanica* (*tropical japonica*) germplasm from Indonesia and as many as 500 NPT lines were developed within 5 years (Khush 1995). Although the NPT genotypes were resistant to lodging with large panicles and few unproductive tillers, they were not released due to their poor yield. Low yield realisation was due

to low biomass production and poor grain filling, probably due to a lack of apical dominance within a panicle (Yamagishi et al. 1996), compact arrangement of spikelets on the panicle (Khush and Peng 1996), less number of large vascular bundles for assimilate transport and source limitation due to early leaf senescence (Ladha et al. 1998). Further they were also susceptible to diseases and insects and poor grain quality.

Consequently, in 1995, the development of second-generation NPT lines began by crossing first-generation tropical *japonica* NPT lines with new modern high-yielding *indica* varieties/elite lines. Genes from *indica* parents helped to reduce the panicle size, increase the tillering capacity, improving the grain quality, disease and insect resistance. From these efforts, an NPT line, IR77186–122–2-2-3, was released as NSIC Rc158 in the Philippines in 2007 (Peng et al. 2008). However, due to concomitant improvement in *indica* rice breeding, there was no significant difference in grain yield between the second-generation NPT lines and elite *indica* check varieties (Peng et al. 2004; Yang et al. 2007).

3.10.6 Super Rice Breeding

China established a nationwide mega project on the development of 'super' rice in 1996 (Cheng et al. 1998), with the objectives (i) to develop 'super' rice varieties with a yield of 9–10.5 t/ha by 2000, 12 t/ha by 2005 and 13.5 t/ha by 2015 from a large area of at least 6.7 ha. (ii) to develop 'super' rice varieties with yield potential of 12 t/ha by 2000, 13.5 t/ha by 2005 and 15 t/ha by 2015 from experimental and demonstration plots, and (iii) to raise the national average rice yield to 6.9 t/ha by 2010 and 7.5 t/ha by 2030. Additionally, the 'super' rice variety should outyield widely grown local check varieties by 10% with acceptable grain quality and pest resistance. Another goal of 'super' rice is to produce 100 kg grain/ha/day. Parallelly, a 'super' hybrid rice breeding programme was started in 1998 by Longping Yuan, by combining the ideotype approach with the use of intersubspecific heterosis (Yuan 2001).

The proposed 'super rice' ideotype aimed to combine several morphological traits, namely (1) moderate tillering capacity (270–300 panicles/m^2); (2) heavy (5 g per panicle) and drooping panicles at maturity; (3) plant height of at least 100 cm (from the soil surface to unbent plant tip) and panicle height of 60 cm (from the soil surface to the top of panicles with panicles in natural position) at maturity; (4) top three leaves featuring a flag-leaf length of 50 and 55 cm for the second and third leaves, which are positioned above the panicle height; (5) leaves remain erect until maturity with leaf angles around 58, 108 and 208 for the flag, second, and third leaves, respectively; (6) narrow and V-shape leaves (2 cm leaf width when flattened); (7) thick leaves (specific leaf weight of top three leaves = 55 g/m^2); (8) leaf area index (LAI) of top three leaves is about 6.0; and (9) harvest index of about 0.55 (Peng et al. 2008).

By 2001, seven 'super rice' varieties and 44 hybrids were released (Min et al. 2002). During 1998–2005, 34 'super' hybrid rice varieties were commercially

Table 3.10 Key morphological traits of the popular super rice hybrids, Xieyou9308 and Liangyoupeijiu

S. No.	Traits	Xieyou9308	Liangyoupeijiu
1.	Total duration (days)	150	135
2.	Plant height (cm)	120–135	115–125
3.	Flag leaf length (cm)	45	35–45
4.	Flag leaf angle	<10°	<10°
5.	Panicle length (cm)	26–28	24–26
6.	Panicles/m^2	250	200–250
7.	Spikelets per panicle	170–190	190–210
8.	Grain filling (%)	90	85
9.	1000-grain weight (g)	28.0	26.0–27.0

released, occupying an area of 13.5 mha and producing an additional 6.7 mt of rough rice in China (Cheng et al. 2007). Among these, two super rice hybrids, namely Xieyou9308 and Liangyoupeijiu, have gained popularity due to high yield and good grain quality. Xieyou9308 is a three-line intersubspecific super rice hybrid with Xieqingzao-A as female and Zhonghui9308 as the male parent, with a yield potential of 12.23 t/ha released in 1999 (Mao et al. 2003). Zhonghui9308 is an intermediate type with canopy morphology close to a *japonica* type and panicle morphology close to an *indica* type. Liangyoupeijiu is a two-line intersubspecific super rice hybrid with Pei'ai64S as the female and 9311 as the male parent, with a yield potential of 12.11 t/ha released in 1999 (Yuan 2001). The key morphological traits of the popular super rice hybrids, Xieyou9308 and Liangyoupeijiu are presented in Table 3.10.

The success of China's 'super hybrid rice' suggests that the ideotype approach in combination with intersubspecific heterosis is effective for breaking the yield ceiling of the irrigated rice. Unlike the NPT concept of IRRI, the emphasis was more on the top three leaves and panicle position within the canopy. Source–sink relationship was also well balanced in China's 'super' hybrid rice breeding project by improving photosynthesis, increasing the distance between panicle height and plant height (essentially by increasing plant height), and delaying leaf senescence of the top three leaves during the ripening phase.

3.11 Harnessing Heterosis and Hybrid Breeding

Jones (1926) reported heterosis in rice for culm number and yield. Even though heterosis to an extent of 20% or more was realised in rice through manual crosses, there was no interest in commercial exploitation in rice. The role of cytoplasm in causing male sterility in rice and its possible utility in breeding hybrid rice was first reported by Sampath and Mohanty (1954). Richharia (1962) also suggested clonal propagation as a practical means of exploiting hybrid vigour in rice. Even though the idea of commercial exploitation of heterosis in rice re-emerged in 1960, except in China, heterosis breeding in rice was not intensively pursued owing to the self-

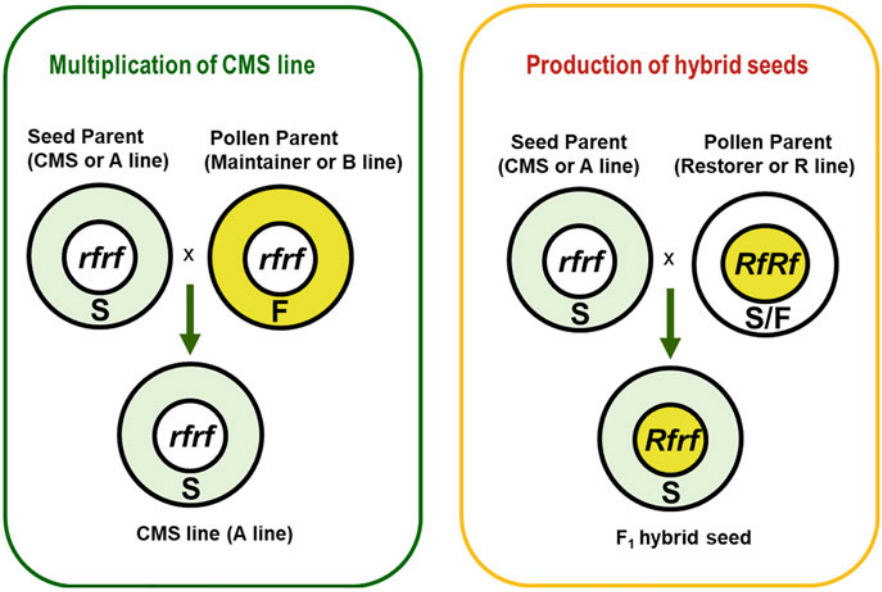

Fig. 3.8 Scheme for maintenance of cytoplasmic male sterility and production of hybrid seeds using three-line system

pollinated nature of the rice crop, there was difficulty in producing large quantities of hybrid seeds. Initially, hybrid rice seeds were produced using chemical hybridising agents (gametocides), such as ethrel, ethryl 4′ fluoro oxanilate, sodium methyl arsenate, zinc methyl arsenate, etc. in the 1970s; however, it was discontinued after the discovery of cytoplasmic male sterile systems. The basis of hybrid rice technology revolves around three basic genetic elements, necessary for commercial production of hybrid seed of crops, namely (1) a complete and stable cytoplasmic male sterility without any substantial negative effect on CMS lines and hybrids, (2) a genetic system that maintains the male sterility through a maintainer line or a particular environmental condition and (3) a genetic system that completely restores male fertility in hybrids. The male sterility in the CMS lines is due to aberrant mitochondrial genes encoding cytotoxic proteins.

The first cytoplasmic male sterile (CMS) line for producing commercial F_1 rice hybrids was developed from a naturally occurring male sterile plant in a population of wild rice (*Oryza sativa* f. *spontanea*) on Hainan island in 1970 in China (Yuan 1977). Designated as Wild Abortive or WA type, this was a landmark event in the history of hybrid rice breeding. This formed the basis of the first generation of hybrid rice technology involving a 'three-line system' consisting of a CMS line (A line), the maintainer line (B line) and a restorer line (R line). The CMS line (A line) is maintained by the isonuclear maintainer line (B line) while crossing the CMS line (A line) with the restorer line (R line) produces the hybrid seeds (Fig. 3.8). China released the first commercial rice hybrid, Nanyou 2 in 1973 (Yuan 1977). A large

number of WA-CMS-based hybrid varieties have been deployed for commercial rice production in China due to their yield advantage over the inbred varieties. Since the identification and commercial utilisation of the WA-CMS system, several CMS lines have been developed with various sources of CMS (Lin and Yuan 1980); however, only a few are in commercial production mainly due to the drawbacks, such as abnormal flowering behaviour of CMS lines/instability of male sterility expression over environments/non-availability of effective restorers (Table 3.11).

WA-CMS is the most widely used CMS system in hybrid rice production across the world. It encodes a mitochondrial protein, *WA352*, that inhibits the nuclear encoded mitochondrial transmembrane protein, OsOCX11, to induce pollen abortion through premature programmed cell death of tapetal cells at the uninucleate stage of microspore development (Luo et al. 2013). The pollen abortion in WA-CMS is determined by the genotype of the sporophytic tissues. The restorer lines possess specific fertility restorer (*Rf*) genes that repress the functioning of the CMS genes, either post-transcriptionally or post-translationally (Luo et al. 2013). A large number of *Rf* genes have been identified, mapped and cloned in rice (Table 3.12).

Hybrid rice research at the International Rice Research Institute (IRRI), Manila, the Philippines, was initiated in 1970; however, it did not receive much attention until 1979, when heterosis breeding gained significance as a means to improve productivity in rice after China's success with hybrid rice. But the Chinese source germplasm for hybrid rice development was neither adapted nor easily available to other tropical rice growing countries. Subsequently, several national rice research programmes in the tropical countries including India, Indonesia, South Korea, Malaysia, the Philippines, Thailand and Vietnam also joined the network with IRRI intending to harness heterosis in rice. A major collaborative effort between the Food and Agriculture Organization (FAO) with IRRI, China, Japan and selected national agricultural research centres from Indonesia, India, Brazil, Colombia, Vietnam, People's Republic of Korea and France promoted hybrid rice research and development, with emphasis on the rapid transfer of any major innovation in rice hybrid technologies.

Buoyed by the success story of hybrid rice in China, India launched a goal-oriented, systematic research on hybrid rice technology, which was initiated in December 1989 under the aegis of the Indian Council of Agricultural Research (ICAR), focusing on hybrids for irrigated cultivation (Janaiah 2002). This project was implemented through a well-organised national network system, comprising of 12 centres located across the country. Subsequently, various research programmes funded by the United Nations Industrial Development Organization (UNIDO) and Food and Agriculture Organization (FAO) during 1991–1996 and 1999–2001; the Mahyco Research Foundation (renamed the Barwale Foundation since 2005) during 1997–2000; the Asian Development Bank (ADB) and IRRI during 1999–2000; and the National Agricultural Technology Project and India's Ministry of Agriculture during 2003–2008 and 2015–2017 were taken in India to improve rice productivity through hybrid rice technology (Spielman et al. 2013). The first hybrid APHR 1 was developed and released for commercial cultivation in India in 1994, followed by MGR 1, KRH 1 and APHR 2 in the same year. Despite investments to the tune of

Table 3.11 Cytoplasmic male sterile (CMS) types, their origin and nature used in three line hybrid rice breeding

Type	Source of the cytoplasm		Popular CMS lines	Subspecies	Nature
	Species	Strain/variety			
WA- and WA-like CMS					
Wild Abortive (WA-CMS)	*O. sativa* f. *spontanea*	Male sterile wild rice	Zhenshan 97A, V20 A	*indica*	Sporophytic
Kalinga I (Kalinga I-CMS)	*O. sativa* L.	Kalinga I	CRMS32A	*indica*	Sporophytic
Dissi (D-CMS)	*O. sativa* L.	Dissi	D-Shan A, D62A	*indica*	Sporophytic
Dwarf Abortive (DA-CMS)	*O. rufipogon*	Dwarf wild rice	XieQingZao A	*indica*	Sporophytic
Gambiaca (GA-CMS)	*O. sativa* L.	Gambiaca	Chaoyang 1A, Gang46A	*indica*	Sporophytic
Indonesia (ID-CMS)	*O. sativa* L.	Indonesian paddy	II 32A, You 1A	*indica*	Sporophytic
Luihui (LX-CMS)	*O. sativa* L.	Luihui rice	Yue 4A	*indica*	Sporophytic
Maxie (Maxie-CMS)	*O. sativa* L.	Maweizhan	Maxie A	*indica*	Sporophytic
NX-CMS	*O. sativa* L.	Male sterile F_2 segregant from Wanhi88/ Neihui92–4	Neixiang 2A, Neixiang 5A	*indica*	Sporophytic
Yegong (Y-CMS)	*O. sativa* L.	Yegong	Y Huanong A	*indica*	Sporophytic
K-CMS	*O. sativa* L.	K52	K-17A	*japonica*	Sporophytic
Baotai (BT) and BT-like CMS					
Boro T (BT-CMS)	*O. sativa* L.	Chinsurah Boro II	Li-Ming A, Xu 9201A	*japonica*	gametophytic
Lead Rice (LD-CMS)	*O. sativa* L.	Lead rice	Fujisaka5A	*indica*	gametophytic
Dian1 (Dian1-CMS)	*O. sativa* L.	Yunnan high altitude rice	Yongjing 2A, Ning 67A	*indica*	gametophytic
HI	*O. sativa* f. *spontanea*	Common wild rice	QingSi-Ai A	*indica*	gametophytic
TI	*O. sativa* L.	E-Shan-Ta-Bai	Liu-Qian-Xin A	*japonica*	gametophytic
Others					
Honglian (HL-CMS)	*O. rufipogon*	Red awned wild rice	Yuetai A, Luohong 3A	*indica*	gametophytic

Table 3.12 Details of fertility restorer gene(s) identified for restoring fertility in different cytoplasmic male sterile (CMS) systems in rice

Rf genes	CMS restored	Restorers	Chromosome	Causative gene(s)	Encoded product	Gene based/gene linked marker	Reference
Rf1a, *Rf1b*	CMS-BT	IR24, BTR, MTC10R, C9083	10	PPR8–1, PPR791, *Rf1A*, *Rf1B*	PPR	InDel-Rf1a	Tao et al. (2013)
Rf2	CMS-LD	Kasalath, Minghui 63	2	LOC_Os02g0274000	Gly. Rich protein	CAPS42–1	Itabashi et al. (2011)
Rf3	CMS-WA	Swarna, PRR78	2	–	PPR	DRRM-Rf3–10	Katara et al. (2017)
Rf4	CMS-WA	IR 24, PRR 78	10	PPR782a	PPR	RM6100	Katara et al. (2017)
Rf5(t)	CMS-HL	Milyang 23	10	PPR791	PPR	RM3150	Liu et al. (2004)
Rf6	CMS-HL	–	10 & 8	–	–	RM5373	Liu et al. (2004)
Rf17	CMS-CW	CWR	4	PPR2	RNA interference	AT10.5–1, SNP 7–16	Fujii and Toriyama (2005)
Rf98	CMS-RT98A	RT98C	10	PPR762	PPR	UK	Igarashi et al. (2016)
Rf102	CMS-RT102A	RT102C, K102-*Oryza rufipogon. T*	12	UK	UK	UN	Okazaki et al. (2013)

over eight million US $, the hybrid rice development and delivery in India faces several challenges that have resulted in a delay of the Indian government's goal of spreading hybrid rice to about 25% of the cultivated rice area by 2015. Presently, the area under hybrid rice is estimated to be only 3.0 mha, which is only 7.0% of the total 44 million hectares under rice cultivation in India.

Presently, the national network on hybrid rice including a network of 20 voluntary centres represented by public, private and NGO sectors is coordinated by the ICAR-Indian Institute of Rice Research (IIRR), Hyderabad. Besides this, effective linkages have been established with various national and international agencies for the development and promotion of hybrid rice. As a result of concerted efforts for the past three decades, so far more than 127 rice hybrids have been released for commercial cultivation in India. The landmark hybrids developed and commercialised in India are presented in Table 3.13.

During the first decade of their release for commercial cultivation, the spread of rice hybrids was not as rapid as expected due to their poor grain quality, high seed cost, non-availability of quality seeds, low market price, susceptibility to pests and diseases, low head-rice recovery, and chaffy or sterile grains (Ou 1985; Spielman et al. 2013). In India, the adoption of hybrid rice has been slow; however, hybrid rice is gaining popularity among the rice farmers of northern Indian states, such as Uttar Pradesh, Bihar, Jharkhand, Punjab, Haryana and also in southern states like Maharashtra, Karnataka, Madhya Pradesh and Chhattisgarh, mainly due to it less fertiliser and water requirement as compared to the high yielding varieties (HYVs) (Hariprasad et al. 2011).

Three factors crucial for the commercial success of hybrid rice technology include high standard heterosis, stable male sterile source and efficient package for producing higher seed yields. However, CMS systems suffer from several intrinsic problems such as (1) a limited number of restorer lines in the germplasm (only 2–5% of rice germplasms possess *Rf* genes) and it is tedious to develop restorer lines as there is a need to assess combining ability of the restorers in each backcross leading to enormous workload. (2) It is difficult to breed new CMS lines, and (3) some of the CMS genes are unstable in different nuclear backgrounds, which makes it difficult to utilise in hybrid rice breeding.

In rice, a 'two-line system' has been successfully identified and utilised for hybrid seed production. The two-line system or the environment-sensitive genic male sterility (EGMS) is mainly dependent on environmental factors such as temperature and/or photoperiod, duration, or concentration at a sensitive stage such as panicle initiation during plant development (Nas et al. 2005). The EGMS makes use of photoperiod and/or temperature to control the sterility/fertility behaviour of the female parent. The EGMS was first identified from tomatoes (Rick 1948). Under permissive conditions (usually short photoperiod and/or low temperature), the EGMS line is male-fertile, enabling their multiplication by selfing without the need of a maintainer line as compared to the CGMS system. Whereas under restrictive conditions that inhibit male fertility, photoperiod- and thermo-sensitive genic male sterile (PTGMS) lines outcross with paternal lines to produce hybrid seeds (Fig. 3.9).

Table 3.13 Popular rice hybrids released in India

S. No.	Rice hybrids	Year of release	Duration (days)	Yield (t/ha)	Developed by	Recommended for (specialty)
1.	APHR 1	1994	130–135	7.14	APRRI, Maruteru	AP
2.	APHR 2	1994	120–125	7.52	APRRI, Maruteru	AP
3.	MGR 1	1994	110–115	6.08	TNAU, Coimbatore	TN
4.	KRH 1	1994	120–125	6.02	VC Farm, Mandya, UAS, Bangalore	KA
5.	CNRH 3	1995	125–130	7.49	RRS, Chinsurah	WB
6.	DRRH 1	1996	125–130	7.30	DRR, Hyderabad	AP
7.	KRH 2	1996	130–135	7.40	VC Farm, Mandya, UAS, Bangalore	BH, KA, TN, TR, MH, HR, UK, OR, WB, PY, RJ
8.	Pant Sankar Dhan 1	1997	115–120	6.80	GBPUAT&T, Pantnagar	UP
9.	PHB 71	1997	130–135	7.86	Pioneer Overseas Corporation, Hyderabad	HR, UP, TN, AP, KA
10.	PA 6201	2000	125–130	6.20	Bayer Bio-Science, Hyderabad	AP, KA, BH, OR, MP, UP, WB, TN, TR
11.	PA 6444	2001	135–140	6.11	Bayer Bio-Science, Hyderabad	UP, TR, OR, AP, KA, MH, UK (popular long duration hybrid)
12.	Pusa RH 10	2001	120–125	4.35	IARI, New Delhi	HR, DL, WUP, UK (long slender aromatic hybrid)
13.	Rajlakshmi (CRHR 5)	2005	130–135	5.84	CRRI, Cuttack	AS (Boro areas), OR (long duration hybrid)
14.	Ajay (CRHR 7)	2005	130–135	6.07	CRRI, Cuttack	OR (irrigated areas)
15.	JRH4	2007	110–115	7.50	JNKVV, Jabalpur	MP
16.	PA 6129	2007	115–120	6.58	Bayer Bio-Science, Hyderabad	PB, TN, PY
17.	Sahyadri 4	2008	115–120	6.80	RARS, Karjat (BSKKV)	HR, WB, UP, MH, PB

(continued)

Table 3.13 (continued)

S. No.	Rice hybrids	Year of release	Duration (days)	Yield (t/ha)	Developed by	Recommended for (specialty)
18.	DRRH 3 (IET19543)	2010	131	6.07	DRR, Hyderabad	AP, GJ, MP, OR, UP, CI (medium slender grain hybrid)
19.	US - 312	2010	125–130	5.76	Seed Works International, Hyderabad	AP, BH, KA, TN, UP, WB
20.	27P61 (IET21447)	2012	132	6.70	PHI Seeds Pvt. Ltd. Hyderabad	CH, GJ
Two line hybrid (TGMS system)						
21.	SAVA 127	2015	115–120	7.5	Savannah seed Pvt. Ltd.	UP (first two line hybrid released in India)

AS Assam, *AP* Andhra Pradesh, *KA* Karnataka, *TN* Tamil Nadu, *BH* Bihar, *TR* Tripura, *MH* Maharashtra, *HR* Haryana, *UK* Uttarakhand, *OR* Odisha, *PY* Pondicherry, *RJ* Rajasthan, *UP* Uttar Pradesh, *MP* Madhya Pradesh, *DL* Delhi, *WUP* Western UP, *PB* Punjab, *GJ* Gujarat, *CI* Central India, *CH* Chhattisgarh

Shi discovered the source material for the two-line system male sterile line, Nongken58S in rice in Hubei, China, in 1973 and studied in detail the influence of photoperiod and temperature on male sterility of this genotype (Shi 1981). EGMS is further classified as thermo-sensitive genic male-sterility (TGMS) and photosensitive genic male-sterility (PGMS). In the PGMS system, the plant behaves as sterile at longer photoperiod (>14 h) and fertile at shorter photoperiod (<13.75 h) as observed in Nongken58S (Shi and Deng 1986), a *japonica* type of PGMS source. In the case of the TGMS system, the plant behaves as sterile at a high temperature regime (>28°C) and fertile at a low temperature regime (<23°C) in AnnongIS (Tan et al. 1990), an *indica* type of TGMS source. The TGMS system is more preferable to take advantage of the wide temperature range through TGMS sources. Unlike the PGMS, wherein the phenomenon of fertility–sterility transformation is greatly influenced by temperature, TGMS is less affected by day length. Hence, the TGMS system is advantageous over the PGMS system (Huang et al. 2014).

The plant behaves male sterile in one set of temperature conditions (restrictive condition) while reverting to male fertility in another set of temperature conditions (permissive condition), which forms the basis for the use of the TGMS system as a two-line system for hybrid seed production. The plant behaves as male sterile at high temperature regime and fertile at low temperature regime in Annong1S (Tan et al. 1990), an *indica* type TGMS source. It is the first report of TGMS in rice due to a spontaneous mutant isolated from the Hunan Province, China. The mutant and its derivatives (5460S, R59TS, etc.) behave sterile at relatively higher temperatures (33–28 °C) and revert to male fertility under relatively low temperature conditions (27–22 °C) (Sun et al. 1989; Yang and Wang 1990). After the discovery of

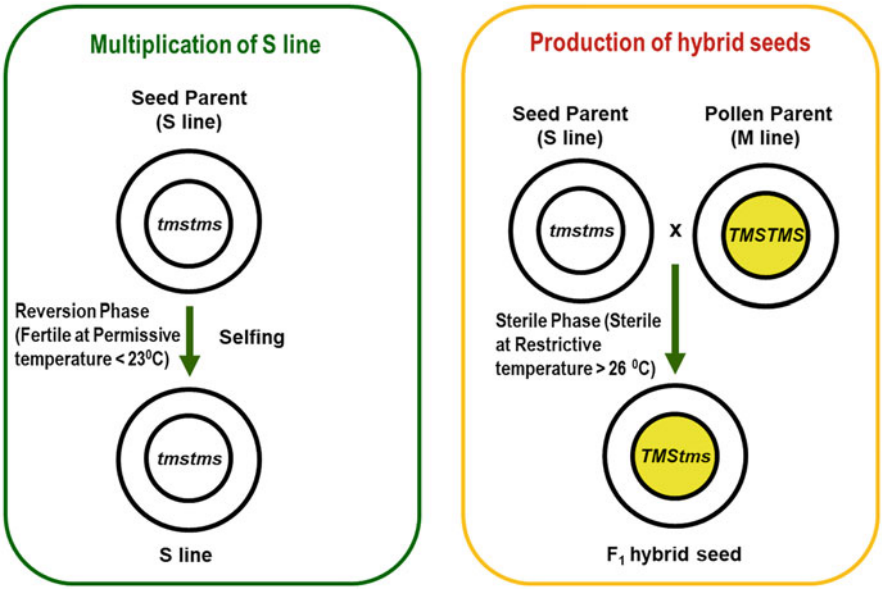

Fig. 3.9 Scheme for maintenance of TGMS line and production of hybrid seeds using two-line system

Annong1S, a gamma ray induced TGMS mutant 'NorinPL12' from Reimei, which is male sterile between 31 and 24 °C, partially fertile at 28–21 °C and completely fertile between 26 and 18 °C, was developed in Japan (Maruyama et al. 1990). To date, there have been more than 20 TGMS lines discovered/developed through spontaneous mutations, induced mutations as well as introgression breeding (Table 3.14).

Based on the critical temperatures for fertility transformation, the TGMS system can be classified into four types, namely (1) high CSP (critical sterility point)-high CFP (critical fertility point), (2) high CSP-low CFP, (3) low CSP-low CFP and (4) low CSP-high CFP based on the CSP, CFP and PRTA (photoperiod range for temperature activity). The line possessing a high sterility point, low fertility point and wide range of PRTA is considered as the best line for seed production and multiplication. A new source called JP38, which behaves sterile at low temperature and fertile at a high temperature known as reverse TGMS has also been reported in rice (Jiang 1988). However, it has not been used in commercial hybrid seed production. Precise information on the determination of critical temperature and stage of development sensitive to temperature and minimum duration of effective temperature treatment required for the reversion to fertile phase is not completely available in rice.

To date, around 14 genes governing thermo-sensitive genic male sterility have been mapped in rice (Table 3.15), which provides valuable tools for marker-assisted transfer of these genes for the development of new TGMS lines. Among these genes, *tms5* is the major gene involved in two-line breeding in China accounting for more

Table 3.14 TGMS sources in rice, their origin, and critical temperatures for sterility-fertility transformation

Source	Sub-species	Origin	CSP/CFP (°C)	Reference
5460 S	*indica*	Induced (R), China	28.0–26.0	Yang et al. (1990)
IR32364	*indica*	Induced (R), IRRI	32.0–24.0	Virmani and Voc (1991)
IR68945	*indica*	Introgression from Norin PL 12, Japan	30.0–24.0	Virmani (1992)
IR68949	*indica*	Introgression from Norin PL 12, Japan	30.0–24.0	Virmani (1992)
H 89–1	*japonica*	Induced (R), Japan	31.0–28.0	Maruyama et al. (1990)
Annong 1S	*indica*	Spontaneous mutation, China	30.2–27.0	Tan et al. (1990)
R 59TS	*indica*	Induced (R), China	–	Yang and Wang (1990)
Xianquang	*indica*	Breeding population, China	30.0–24.0	Cheng et al. (1995)
26 Zhi ZaoS	*indica*	Induced (R), China	23.0–25.0	Shen et al. (1993)
N5088 S	*indica*	Introgression from Nongken 58 S	30.0–22.0	Zhang et al. (1994)
SM 5	*indica*	Spontaneous, India	32.3–22.0	Ali et al. (1995)
SM 3	*indica*	Spontaneous, India	32.0–22.0	Ali et al. (1995)
JP 2	*indica*	Spontaneous, India	33.9–23.0	Ali et al. (1995)
SA 2	*indica*	Induced mutation (C) India	31.7–20.0	Ali et al. (1995)
F 61	*indica*	Induced mutation (C)	30.9–22.0	Ali et al. (1995)
JP 8-1A-12	*indica*	Breeding population, India	30.9–20.0	Ali et al. (1995)
JP 24A	*indica*	CMS, India	33.8–23.0	Ali (1993)
JP38	*indica*	Spontaneous mutation, India	24.0–30.5	Ali (1993)
Dianxin/A	*japonica*	CMS, China	23.0–20.0	Lu et al. (1994)
Hennong S	*indica*	Crossbreeding, China	30.0–29.0	Lu et al. (1994)
IV A	*indica*	Crossbreeding, China	24.0–28.0	Zhang et al. (1991)
J207S	*indica*	Spontaneous mutation, China	31.0 to >31.0	Jia et al. (2001)
B06S	*indica*	Spontaneous mutation, China	24.0 to >24.0	He et al. (2005)

CSP critical sterility point, *CFP* critical fertility point, *R* irradiation, *C* chemical mutagen
Source: Virmani et al. (2003)

than 71% of all two-line hybrid rice cultivars (~2.9 million hectares) grown two-line hybrid rice in China during 2011 (Zhou et al. 2014a). *tms5* is used in the development of a large number of TGMS lines, and they are used in as many as 71 commercial two-line hybrid rice cultivars in China. In India, development of TGMS lines and two-line hybrids is being actively pursued only at ICAR-IARI, New Delhi;

Table 3.15 Different sources of EGMS genes in rice, their chromosomal location and their linked markers

	Gene	Source	Chr.	LD (cM)	Markers	References
PGMS genes						
	pms1	32001S	7	3.5–15.0	RG477-RG511, RZ272	Zhang et al. (1994)
	^{pms}1(t)	Pei'ai64S	7	0.2, 0.2	RM21242, YF11	Zhou et al. (2011)
	pms2	32001S	3	10.6, 7.0	RG348, RG191	Zhang et al. (1994)
				0.1, 6.0	RG477/ R277, R1807	Liu et al. (2001)
	pms3	Nongken 58S	12	5.5, 9.0	RZ261/ C751, R2708	Mei et al. (1999)
		Nongken 58S	12	0.0	LJ47, LJ265	Lu et al. (2005) and Ding et al. (2012)
	pms4	Mian 9S	4	3.0, 3.5	RM6659, RM1305	Huang et al. (2008)
	p/ tms12–1	Pei'ai64S	12	–	PA301, PAIDL2	Zhou et al. (2012)
	rpms1	YiD1S	8	0.9, 1.8	RM22980, RM23017	Peng et al. (2008)
	rpms2	YiD1S	9	0.9, 0.9	RM23898, YDS926	Peng et al. (2008)
	rpms3 (t)	D52S	10	6.6, 4.6	RM5271, RM244	Joseph et al. (2011)
TGMS genes						
1.	*tms1*	5460S	8	6.7	TGMS 1.2	Wang et al. (1995)
2.	*tms2*	Norin PL12	7	5.0	R643A, R1440; RM11	Yamaguchi et al. (1997) and Lopez and Virmani (2000)
3.	*tms3*	IR32364	6	7.7	F18FM/ RM	Lang et al. (1999)
4.	*tms4*	TGMS-VN1	2	22.3	RM27	Dong et al. (2000)
5.	*tms5*	AnnongS-1	2	5.2	RM492	Wang et al. (2003)
6.	*tms5*	DQ200047–12	2	0.0	RM174	Jia et al. (2000)
7.	*tms6*	Sokcho-MS	5	5.8, 0.1	RM440, RM3351	Lee et al. (2005)
8.	*tms6(t)*	G20S	10	3.0, 1.1	RM3152, RM4455	Liu et al. (2010)
9.	tmsx	XianS	2	1.0, 2.0	RMAN81, RM7575	Peng et al. (2010)
10.	*rtms1*	J207S	10	3.6, 4.2	RM239, PRev1	Jia et al. (2001)
11.	*TGMS*	SA-2	9	6.2	RM257	Reddy et al. (2000)

(continued)

Table 3.15 (continued)

	Gene	Source	Chr.	LD (cM)	Markers	References
12.	ms-h	ms-h(t)	9		RG451	Koh et al. (1999)
13.	tms 9	Zhu 1S	2	30.2 kB	Indel 91, 101	Sheng et al. (2015)
14.	*p/ tms12–1*	PA64S	12	5.8 kB	PA301, PAIDL2	Zhou et al. (2012)

LD linkage distance, *Chr* chromosomal location

TNAU, Coimbatore; and GB Pant University for Agriculture and Technology, Pantnagar.

Two-line hybrids offer several advantages over three-line hybrids, which includes (1) the simplification in multiplication, as they do not need a maintainer line for propagation of the male sterile lines; (2) male sterility in PTGMS lines is mainly controlled by recessive nuclear genes, and when crossed with any plants carrying the wild type fertility gene, the fertility in hybrids is restored. Therefore, this system increases the frequency of heterotic hybrids, as there is no need for fertility restorer genes as in the case of the CGMS system and therefore, any fertile line can be used as a male parent; (3) it is free from cytoplasmic negative effects, if any, associated with the abnormal mitochondrial genes in the sterility-inducing cytoplasm are also eliminated (Yuan 1990; Siddiq and Ali 1999). Hence the EGMS system is the most promising and developing method of male sterility in many crops. Because of these advantages, it was quickly adopted for hybrid rice production, since the initial application in the 1990s, and hundreds of environment-sensitive genic male sterile lines and two-line hybrids have been released for commercial production.

Even though PTGMS systems are advantageous over CGMS systems, there are also some disadvantages associated with this system. Most of the commercial TGMS lines behave sterile under restrictive conditions of a long day and high temperature (critical sterility temperature >25 °C) and revert to fertile phase under permissive conditions of short day and low temperature (<24 °C) during the booting stage (Li et al. 2007). Stringent environmental conditions should be met for the multiplication of the PTGMS lines and production of hybrid seeds, as there is a very small window of the critical temperatures for fertility transformation (CTFT). Even a minor change in temperature at the booting stage can affect the production of PTGMS lines and hybrid seeds under unpredictable weather conditions (Liao et al. 2021). For example, during multiplication under locations with permissive weather conditions, a sudden increase in temperature can reduce fertility, thereby decreasing the yield of PTGMS seeds.

Alternatively, during hybrid seed production under locations with restrictive weather conditions, a sudden dip in temperature will enable the PTGMS line to revert to fertility, enabling self-pollination thereby causing genetic impurity of the hybrid seeds. Both these unforeseen weather conditions can cause failure in hybrid seeds production, which leads to huge losses. Further, the CTFT in PTGMS lines often shifts up after a few generations of propagation, and the increase in fertile

temperature brings a higher risk to hybrid seeds production. Therefore, there is a constant need for purifying PTGMS individuals of suitable CTFT repeatedly during production. Additionally, the CTFT trait is also influenced by minor QTLs, which imposes difficulty and uncertainty in breeding new PTGMS lines through marker-assisted transfer of these genes into different genetic backgrounds.

3.11.1 Fixation of Heterosis

In rice, the feasibility of asexual reproduction and maintenance of hybrids clonally through seed propagation has been demonstrated by combining genome-editing to substitute mitosis for meiosis (MiMe) with the expression of *BABY BOOM1* (*BBM1*) in the egg cell. The clonal progenies of rice retained genome-wide parental heterozygosity and the asexual propagation trait is heritable through multiple generations of clones (Khanday et al. 2019). Through simultaneous editing of four genes, namely *REC8*, *PAIR*, *OSD1* and *MTL* using CRISPR-Cas9 the rice seeds from F_1 hybrids, were able to proliferate clonally through seeds (Wang et al. 2019).

3.12 Genes Associated with Yield and Yield Component Traits

A large number of genes governing yield and yield component traits have been mapped and cloned in rice (Table 3.16). Most of them including *DEP1*, *Gn1A*, *GS3 and*, *GW5* negatively regulate yield. Spontaneous mutant alleles with desirable mutations have been selected by farmers and breeders to improve yield. Although these mutations have improved riceyields, there exists a possibility that more elite alleles of these cloned genes could be created through modern approaches like genome-editing and combined to further boost the yields in rice.

3.12.1 Rice Grain Quality

With major gains achieved in improving grain yield spurred by the green revolution, many rice eating countries across the world achieved self-sufficiency in food production. With food security and the subsequent improvement in affordability of the population, there has been an emphasis on the improvement of grain quality in rice. Rice grain quality is one of the major factors dictating the market value and plays a key role in the adoption and popularity of rice varieties. Grain quality in rice includes processing quality, physical appearance, biochemical, cooking and sensory quality as well as nutritional quality (Fitzgerald et al. 2009). Grain quality is a complex trait determined by numerous interrelated factors making rice quality improvement a challenge. Further, the perception of quality changes with the stakeholders. For producers, grain quality means the gross physical appearance which determines the price and profitability, whereas for rice millers priorities are on overall milling yield, head rice recovery and storage quality, while for the consumers, the processed

Table 3.16 List of yield-associated genes cloned and their mode of increasing grain yield in rice

Gene	Gene symbol	Locus ID	Trait	Mode of function	Reference
Tillering					
Tillering and dwarf 1	TAD1/TE	LOC_Os03g0123300	Shoot branching	G-A substitution, LoF	Xu et al. (2012)
Teosinte branched 1	TB1/FC1	LOC_Os03g0706500	Lateral branching	LoF	Takeda et al. (2003)
Dwarf high Tillering ability 34	DHTA34	LOC_Os03g0203200	High tillering, Dwarf	G-A substitution, LoF	Liang et al. (2019)
High Tillering and dwarf 1	HTD1	LOC_Os04g0550600	High tillering, Dwarf	C-T substitution, LoF AM growth	Zou et al. (2005)
Monoculm 1	MOC1	LOC_Os06g0610350	AM formation, Tillering	GoF	Li et al. (2003)
Tiller enhancer	TE	LOC_Os03g0123300	Tillering, Dwarf, Twisted leaf angle	Deletion and substitution, LoF	Lin et al. (2012)
Grain number/panicle size					
Grain number 1a	Gn1a	LOC_Os01g0197700	Grain number, cytokinin catabolism	Deletion, LoF	Ashikari et al. (2005)
Aberrant panicle organisation 1	APO1	LOC_Os06g0665400	Grain number	GoF	Ikeda et al. (2005)
Grain number, plant height and heading date 7	Ghd7	LOC_Os07g0261200	Flowering time under long day	GoF	Yan et al. (2013)
Wealthy farmer's panicle	IPA1/WFP (OsSPL14)	LOC_Os08g0509600	Panicle branching, Grain yield	GoF	Miura et al. (2010)
Grain number, plant height and heading date 8	Ghd8	LOC_Os08g0074500	Flowering under short day; delayed flowering under long day	GoF	Yan et al. (2011
Dense and erect panicles 1	DEP1	LOC_Os09g0441900	Dense and erect panicle, wider, shorter leaves, tiller number	Deletion, LoF	Huang et al. (2009)
Long panicle 1	LP1	LOC_Os09g0456100	Panicle length	GoF	Liu et al. (2016)

(continued)

Table 3.16 (continued)

Gene	Gene symbol	Locus ID	Trait	Mode of function	Reference
Grain size					
Grain size 3	GS3	LOC_Os03g0407400	Grain size, stigma exertion	Substitution, LoF	Fan et al. (2006)
Grain length 3.1	qGL3/GL3.1	LOC_Os03g0646900	Grain length	GoF	Qi et al. (2012)
Grain weight 2	GW2	LOC_Os02g0244100	Grain width and weight	Deletion, LoF	Song et al. (2007)
Grain size 5	GS5	LOC_Os05g0158500	Grain size	GoF	Li et al. (2011)
Grain weight 5	GW5	DQ991205	Grain size	Deletion, LoF	Weng et al. (2008)
Grain weight 6a	GW6a	LOC_Os06g0650300	Grain size	GoF	Song et al. (2015)
Oryza sativa squamosa promoter binding-like 13	OsSPL13	LOC_Os07g0505200	Grain size	GoF	Si et al. (2016)
Grain weight 8	GW8 (OsSPL16)	LOC_Os08g0531600	Grain size, grain shape	GoF	Wang et al. (2012)
Glutamine synthase 2	GS2/ OsGRF4	LOC_Os04g0659100	Salinity tolerance photorespiration	GoF	Hu et al. (2015)
Grain weight and filling					
Thousand grain weight 6	TGW6	LOC_Os06g0623700	Grain size, grain yield	Deletion, Lof	Ishimaru et al. (2013)
Oryza sativa squamosa promoter binding-like 18	OsSPL18	LOC_Os09g0507100	Grain weight and number	GoF	Yuan et al. (2019)
Grain incomplete filling 1	GIF1	LOC_Os04g0413500	Grain filling, grain size	GoF	Wang et al. (2008)

LoF loss of function, *GoF* gain of function, *AM* apical meristem

Table 3.17 QTLs/genes affecting milling quality parameters in rice

QTL/ Gene	Locus ID	Trait	Reference
qBRR10	LOC_Os10g0459800	Brown rice recovery rate; reduced glume thickness	Ren et al. (2016)
Chalk5	LOC_Os05g0156900	Increased chalkiness of grains	Li et al. (2014)
FLO5/ SSIIIa	LOC_Os08g0191433	White core floury endosperm	Ryoo et al. (2007)

rice appearance, cooking and eating qualities are important. More recently, the nutritional value of the rice grain includes the glycemic index, Zn, protein content and other nutraceutical properties.

The majority of the rice produced across the world is consumed after polishing as milled rice/white rice. Rice grains need to be processed by removal of husks and the bran portion by dehusking and milling in order to produce milled rice/white rice. Rice processing quality is a very important factor in determining the processing ability of a variety as it affects the milled rice yield. Even though there are differences in the consumer preference for grain quality, the processability of a rice variety into unbroken whole milled rice kernels is an important parameter for any rice variety. The grains harvested from the rice plant are known as paddy or rough rice. Rice hulls (lemma and palea) are easily removed from the grain using a deshusker. These dehusked rice grains are known as brown rice and the proportion of brown rice after dehusking is Hulling percentage (%). The brown rice is polished in a rice miller to remove the bran from the endosperm, which produces the milled rice and the proportion of milled rice after dehusking is milling percentage (%).

The process of removing husk as well as the bran from the rice grains to produce milled rice kernels is called milling. The proportion of unbroken white rice kernels (also known as head rice) from a given quantity of milled rice kernels is head rice recovery percentage (HRR %), which is a very important processing parameter in rice. The HRR (%) is an important factor in the trade as the majority of the consumers prefer unbroken milled rice. Poor milling quality can cause economic losses due to a reduction in the quantity of milled rice realised from the paddy harvested. As much as 30% loss in rice grain can happen due to breakage during the milling process (Hodges et al. 2011). All these parameters, namely hulling (%), milling (%) and HRR (%) are complex, showing quantitative inheritance, as a result of which show high environmental interaction. Advances in rice genomics, have enabled the mapping of QTLs governing these processing traits. As many as 26, 41 and 35 QTLs governing brown rice recovery rate (hulling %), Milling % and HRR, respectively have been mapped in rice (Zhou et al. 2019). Among them, the major genes affecting milling parameters in rice are listed in Table 3.17. *Chalk5* and *GS3* negatively affect the HRR in rice. Three QTLs for crack resistance, namely *qFIS1–1*, *qFIS1–2* and *qFIS1–8*, have been mapped by Pinson et al. (2013). Grain shape, uniformity and translucence are important parameters for consumers, millers, and traders, which are quantitative. More than 400 QTLs governing physical appearance

Table 3.18 Major genes governing physical appearance of grains that have been cloned in rice

Gene	Locus ID	Protein Encoded	Reference
Grain length			
GS3	LOC_Os03g0407400	Member protein with multiple domains	Fan et al. (2006)
qLGY3/ OsLG3b	LOC_Os03g0215400	MADS-domain transcription factor *OsMADS1*	Liu et al. (2018) and Yu et al. (2018)
GL3	LOC_Os03g0646900	Protein phosphatase with Kelch-like repeat domain (*OsPPKL1*)	Zhang et al. (2012) and Qi et al. (2012)
GW6a	LOC_Os06g0650300	A novel Histone acetyltransferase	Song et al. (2015)
TGW3/ qTGW3/ GL3.3	LOC_Os03g0841800	A GSK3/SHAGGY-like kinase	Ying et al. (2018)
GLW7/ OsSPL13	LOC_Os07g0505200	Plant-specific transcription factor *OsSPL13*	Si et al. (2016)
GL7/GW7	LOC_Os07g0603300	Protein homologous to *Arabidopsis thaliana* LONGIFOLIA proteins	Wang et al. (2015a, 2015b)
Grain width			
GW2	LOC_Os02g0244100	Ring-type E3 ubiquitin ligase	Song et al. (2007)
GS2/GL2	LOC_Os02g0701300	Plant-specific transcription factor OsGRF4	Che et al. (2016)
GW5/ qGW5	DQ991205	Arginine-rich protein of 144 amino acids	Weng et al. (2008) and Shomura et al. (2008)
GS5	LOC_Os05g0158500	Serine carboxypeptidase	Li et al. (2011)
GS6	LOC_Os06g0127800	GRAS-domain protein	Sun et al. (2013)
GW8/ SPL16	LOC_Os08g0531600	SQUAMOSA promoter-binding protein-like 16	Wang et al. (2012)
Grain chalkiness			
ms-h/ UGPase1	LOC_Os09g0553200	UDP-glucose pyrophosphorylase 1	Woo et al. (2008)
GIF1	LOC_Os04g0413500	Cell wall invertase	Wang et al. (2008)
Chalk5	LOC_Os05g0156900	Increased chalkiness of grains	
FLO5/ SSIIIa	LOC_Os08g0191433	Starch Synthase IIIa	Ryoo et al. (2007)

of the grains, including grain length, grain width and grain chalkiness, have been mapped and 17 of them have been cloned (Table 3.18), which includes as many as 10 genes affecting starch quality in the rice endosperm.

A large cache of biochemical traits influences the cooking, eating and nutritional quality of rice grain and several genes determine these quality traits that have been cloned in rice (Table 3.19). The differences in cooking and eating properties of rice are due to inherent differences in the composition and structure of starch granules in the endosperm. Amylose content is an important biochemical trait that determines the relative stickiness or fluffiness of the cooked rice kernels. The major gene

Table 3.19 Major genes governing grain, cooking, eating and nutritional quality in rice

Gene	Locus ID	Trait	Protein encoded	Reference
Wx/GBSS I	LOC_Os06g0133000	Amylose content	Granule bound starch synthase I	Hirano and Sano (1991)
ALK/SSIIa	LOC_Os06g0229800	Gelatinisation temperature	Soluble starch synthase IIa	Gao et al. (2003)
BADH2	LOC_Os08g0424500	Aroma (popcorn like)	Betaine aldehyde dehydrogenase 2	Bradbury et al. (2005)
BADH1	LOC_Os04g0464200	Aroma (popcorn like)	Betaine aldehyde dehydrogenase 1	Bradbury et al. (2008)
OsGAPDHB		Creamy popcorn-like aroma	Glyceraldehyde-3-phosphate dehydrogenase B	Lin et al. (2014)
qPC1/ OsAAP6	LOC_Os01g0878700	Grain protein content	A putative amino acid permease, *OsAAP6*	Peng et al. (2014)
OsZIP4	LOC_Os08g0207500	Zinc content	Zinc transporter	Ishimaru et al. 2005
OsZIP8	LOC_Os07g0232800	Zinc content	Plasma-membrane localised zinc transporter	Lee et al. (2010)
RPBF/ OsDof3	LOC_Os02g0252400	Lysine content	bZIP transcription factor, DOF transcription factor	Kawakatsu and Takaiwa (2010)
Kala4/OsB2	LOC_Os04g0557500	Anthocyanin content	bHLH transcription factor	Oikawa et al. (2015)
Kala3	–	Anthocyanin content	R2R3-Myb transcription factor	Maeda et al. (2014)
Kala1/Rd	Os01g0633500	Proanthocyanidin biosynthesis	Dihydroflavonol-4-reductase	Furukawa et al. (2006)
LPA1	LOC_Os03g0237250	Low phytic acid	Myo-inositol kinase	Kim et al. (2008)
LOX3	LOC_Os03g0699700	Grain storability (reduced lipid peroxidation)	Lipoxygenase	Suzuki et al. (1993)

governing amylose content in rice is *Waxy/GBSSI,* and as many as seven functional variants accounting for the range of 0–30% amylose content in diverse rice cultivars have been identified (Zhou et al. 2021). Gelatinisation temperature is another

important parameter affecting the resistance of milled rice to cooking. *ALK* regulates the gelatinisation temperature of milled rice kernels (Nakamura et al. 2005).

Aromatic rice varieties are premium quality rice fetching higher prices in the international markets. Although more than 300 volatile compounds have been identified from different aromatic rice cultivars, 2-acetyl-1-pyrrolidone (2-AP), is the key compound determining a popcorn-like fragrance in the cooked rice. Two genes, namely *BADH2* and *BADH1,* govern fragrance in rice (Bradbury et al. 2005, 2008). Anthocyanins are water-soluble pigments with high anti-oxidant activity. In black rice varieties, the pericarp is black due to the accumulation of anthocyanins.

Although rice grains possess diverse nutrients, they are generally low in proportion. Peng et al. (2014) cloned *OsAAP6*, regulating the content of storage proteins in rice. Homology based predictions have helped in identifying as many as 15 genes for glutelin (Kawakatsu et al. 2008), 34 prolamin genes (Xu and Messing 2009), 3 globulin genes (Sun et al. 1996) and 7 albumin genes (Alvarez et al. 1995) in rice. Kawakatsu and Takaiwa (2010) showed that free lysine content in rice grains can be increased by downregulation of the transcription factor, *RPBF/OSDof3*. Two micronutrients, Fe and Zn, play a very important role for human health. The major genes associated with iron content in rice grains are *OsFER1*, *OsNRAMP4*, *OsNRAMP5*, *OsNRAMP6*, *OsYSL6*, *OsYSL12*, *OsYSL4*, *OsZIP8* and *OsZIP10.* As many as eight ZIP proteins, namely *OsIRT1*, *OsIRT2*, *OsZIP1*, *OsZIP3*, *OsZIP4*, *OsZIP5*, *OsZIP7 and OsZIP8,* associated with zinc concentration in rice have been identified, among which *OsZIP4* and *OsZIP8* are particularly important for Zn transport to seeds. As many as nine rice varieties biofortified with zinc content ranging from 22.6 ppm to 27.4 ppm, one variety with 10.3% protein and two varieties biofortified with both protein and zinc have been developed through conventional breeding and released for commercial cultivation in India (Table 3.20).

Table 3.20 Biofortified rice varieties enriched with zinc and/or protein released in India

S. No.	Rice varieties	Content in milled rice
Zinc biofortified varieties		
1.	DRR Dhan 45	22.6 ppm
2.	DRR Dhan 48	24.0 ppm
3.	DRR Dhan 49	25.2 ppm
4.	Zinco Rice MS	27.4 ppm
5.	Surabhi	22.8 ppm
6.	CGZR 1	22.0 ppm
7.	CGZR 2	25.0 ppm
8.	HPR 2720	20.9 ppm
9.	CR Dhan 315	24.9 ppm
Protein biofortified varieties		
10.	CRR Dhan 310	10.3%
Both zinc and protein biofortified varieties		
11.	Protazin	20.9 ppm + 9.4%
12.	CRR Dhan 311	20.1 ppm + 10.3%

3.13 Basmati Rice Improvement

Basmati rice is a nature's gift to the Indian subcontinent, known for its exquisite quality with a harmonious combination of uniform kernels free from chalkiness, cooked kernels are sweet without bursting exhibiting kernel elongation ratio of nearly twice and neither too hard nor too soft eating quality (Ramaiah and Rao 1953; Singh 1994). The traditional Basmati rice varieties are low yielding, photosensitive, long duration and prone to lodging. Basmati 370 and Type 3 developed through pureline selection were the most popular Basmati rice cultivars with farmers till mid-1970s. Since 1965, systematic crop improvement with basic studies on grain and cooking quality traits at ICAR-Indian Agricultural Research Institute, New Delhi helped in understanding the basis of grain and cooking quality in Basmati rice (Sood and Siddiq 1978; Sood et al. 1979). Through a stepwise convergent breeding approach, the first high yielding semi-dwarf, weakly thermo and photosensitive Basmati rice variety, Pusa Basmati 1 was developed and released in 1989. Pusa Basmati 1121 is another popular Basmati rice variety with a milled rice kernel length of 8.40 mm and exceptional cooked rice length of 20.00 mm, which was developed and released for commercial cultivation in 2003 (Singh et al. 2018). Pusa Basmati 6 is a popular Basmati rice variety known for its unique cooking quality especially for its exceptional taste.

Two short duration Basmati rice varieties, namely Pusa Basmati 1509 (2013) and Pusa Basmati 1692 (2020) (Fig. 3.10), with excellent Basmati quality grains and cooking have also been developed and released by ICAR-IARI, New Delhi. As many as 37 Basmati rice varieties including 15 varieties through SVRC have been

Fig. 3.10 Pusa Basmati 1692—a short duration high yielding semi-dwarf Basmati rice variety

Table 3.21 Improvement in grain and cooking quality characteristics of the Basmati rice varieties compared to traditional Basmati rice variety Basmati 370

Variety	Year of release	Milling (%)	HRR (%)	KLBC (mm)	KBBC (mm)	KLAC (mm)	ER
Basmati 370	1976	72.50	53.00	6.89	1.85	13.40	1.97
Pusa Basmati 1	1989	68.90	58.50	7.38	1.80	14.30	1.93
Pusa Basmati 1121	2003	64.40	53.80	8.44	1.94	17.37	2.06
Pusa Basmati 1509	2013	68.10	49.50	8.19	1.86	18.20	2.22

released in India. The popularity of Pusa Basmati 1, Pusa Basmati 1121, Pusa Basmati 6 and Pusa Basmati 1509 have helped in significant improvement in the Basmati rice exports from India from a mere Rs. 317 crores during 1987–1988 to Rs. 29,840 crores during 2020–2021, revolutionising Basmati rice cultivation in India. The improvement in grain and cooking quality traits of Basmati rice varieties over the years are presented in Table 3.21.

3.14 Breeding for Resistance to Biotic Stresses

Rice is affected by more than 100 pathogens and pests, of which about 20 of them can cause yield losses to the tune of 30–40%. Bacterial, fungal and viral pathogens affect rice crop. Major disease of rice includes bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, blast by *Magnaporthe oryzae*, sheath blight by *Rhizoctonia solani*, false smut by *Ustilaginoidea virens*, brown spot by *Helminthosporium oryzae*, sheath rot by *Sarocladium oryzae*, stem rot by *Sclerotium oryzae* and rice tungro virus. However, the spectrum and intensity of diseases are changing continuously. A severe outbreak of brown spot disease in the winter rice crop of Bengal was responsible for the outbreak of the infamous Bengal famine in 1943. Till 1960, blast and brown spot was the major diseases reported in rice. With the introduction, development and adoption of high yielding fertiliser responsive semi-dwarf rice varieties during the green revolution, bacterial blight, followed by rice tungro, sheath blight attained the status of major diseases by 1990 in addition to blast and brown spot. By 2005, two more diseases, namely false smut and sheath rot, were causing major losses in rice. Of late, intensive rice cultivation with the adoption of high yielding rice varieties, and climate change have led to the emergence of several diseases, such as bakanae caused by *Fusarium fujikoroi*, glume discoloration caused by *Burkholderia glumae* and stem rot, have attained major proportions causing economic losses in yields in rice. The screening of a large number of rice germplasm in the disease nurseries over the years has helped in identifying rice

Table 3.22 Rice varieties with resistance to different diseases

Diseases	Resistant rice varieties/donors identified
Bacterial blight	Improved Pusa Basmati 1, Pusa Basmati 1718, Pusa Basmati 1728, Pusa Basmati 1847, Pusa Basmati 1885, Pusa Basmati 1886, Pusa 1927, Pusa 1592, Improved Samba Mahsuri, Ajaya, ADT 39, CR 837, HKR 95-128, HKR 95-131, IRBB 58, IRBB 59, IRBB 60, IR 64, OR 2329-22, PAU1061-19-22, PR 110, PR 111, PR 114, PR 118, PR 120, Pant Dhan 10, Pant Dhan 11, Saket 4, Sita, PR 4141, Bhudeb, Khitish, Sabita, ADT 39, ADT 36 and Co 43
Blast	Pusa Basmati 1637, Pusa Basmati 1847, Pusa Basmati 1885, Pusa Basmati 1886, Pusa 1884, Pusa Samba 1850, Rasi, IR 36, IR 64, Sasyasree, Srinivas, Tikkana, Simhapuri, Parijatha, Salivahana, Gauthami, Haryana Mahak 11, Tadukan, Tetep and Zenith
Sheath blight	Swarnadhan, Vikramarya, Radha, Pankaj, Manasarovar, IR 40, IR 64683—87-2-2-3-3, RP Bio Path 3, RPHR 25-104-1-2 and Tetep
Sheath rot	Bala, Cauvery, Kakatiya, Janaki, Tella Hamsa, Sabarmati. Swarnadhan, Vikas, Rajavadlu, Phalguna, Vikramarya
False smut	Bala, Cauvery, Sabarmati, Prakash, Pankaj, HKR 47, HKR 127, HKR 98-418, IR 48725-B-B-86-2-2, IR 65907-191-1-B
Brown spot	Rasi, Jagannath, IR 36
Stem rot	Jagannath, Sabarmati, Pankaj, Govind, Jalmagna, Cherno Fingo, CSR 30, IRBB 60, and RP Bio Path 3
Rice Tungro virus	Vikramarya, Manasarovar, Nidhi, Bharani, Nagarjuna, Srinivas, Janaki, Radha, Annapurna, Badami, Ghanteswari, Kshira, Lalat, Nilagiri, Parijat, Prachi, Rajeswari, Vanaprabha, Barathidasan, ADT 38, ADT 44, ASD 16, ASD17, Dinesh, Ambemohar 102, Kataribhog, Latisail and Pankhari 203

Table 3.23 Genes/QTLs governing resistance to different diseases mapped in rice

Diseases	No. of QTLs	No. of genes	Genes cloned	Effective genes/QTLs
Blast	~350	118	25	*Pi9*, *Pi2*, *Pi54*, *Pita*, *Pita*^{2}, *PigmR/S*, *Pi40*, *Pi21*
Bacterial blight	–	42	9	*xa5*, *xa13*, *Xa21*, *Xa38*, *Xa7*, *Xa4*, *Xa33*
Sheath blight	45	–	–	*qSBR11–1*
Bakanae	12	–	–	*qBK1.1*, *qBK1.2*
False smut	2	–	–	–
Brown spot	19	–	–	*qBS9*, *qBS11*
Bacterial grain rot	13	–	–	–
Rice tungro virus	2	–	–	–

varieties with resistance to different diseases (Table 3.22). Further, a number of genes and QTLs, governing resistance to different diseases, have been mapped in rice, which offers possibilities for molecular breeding for the development of cultivars (Table 3.23).

3.14.1 Pests

Insect pests affecting rice crop have been continuously evolving with the evolution and use of high yielding varieties. By early 1965, there were only three pests of major concern, namely gall midge, stem borer and green leafhopper. However, intensification of rice cultivation through the adoption of high yielding semi-dwarf rice varieties and cultivation practices have resulted in as many as 19 pests, including brown plant hopper (BPH), yellow stem borer, pink stem borer, gall midge, white backed plant hopper (WBPH), green leaf hopper, mites, case worm, gundhi bug, hispa, thrips, mealy bug, root weevil, black bug, blue beetle, army worm, leaf miner and chaffer beetle, attaining epidemic proportions and some of them have achieved major pest status in recent years. Several multiple pest resistant donors, such as Velluthacheera, Banglei, Aganni, Chennellu with resistance to gall midge, BPH and WBPH, have been identified in rice. The earliest reference to breeding for resistance to insect pests is from India. In Uttar Pradesh, the rice bug *(Leptocorisa varicornis*) was causing extensive damage to rice at ripening stages. A cleistogamous variety, 'Saathi', whose ripening grain was protected by a leaf sheath enclosing the panicle without emergence was identified as tolerant and used in breeding for resistance to rice bug (Sethi et al. 1937). Utilising the multiple pest resistant genotypes as donors, many multiple stress-resistant high yielding rice varieties like Suraksha, Vikramarya, Rasmi and Daya have been developed. The screening of a large number of rice germplasm in the pest screening nurseries over the years has helped in identifying rice varieties with resistance to different pests. A number of genes and QTLs governing resistance to different pests have been mapped in rice, which offers possibilities for molecular breeding for the development of cultivars (Table 3.24).

3.14.2 Weed Stress

Weeds cause significant yield losses especially in upland and direct seeded conditions. Although direct seeded rice helps in saving water and labour needed for puddling and transplanting, respectively. Weeds compete with the crop for water, nutrients, light and other resources leading to stress on the crops thereby affecting its yield significantly. If the rice variety is not competitive, it can result in significant crop loss. Moreover, majority of the notorious weeds in rice fields are C_4, and rice is a C_3 plant. Under elevated CO_2, the C_4 weeds produce higher biomass and broader leaves, stifling the rice crop (Korres et al. 2016). Weeds can be managed through weedicides, but it will increase the cost of production. Weed control may account for upto 30% of the cost of cultivation for rice in India (Rao and Chauhan 2015; Rao and Matsumoto 2017).

To stay competitive, a rice cultivar needs to germinate fast, develop roots faster and deeper, possess slightly broader leaves and be vigorous so as to enable early above ground cover, with rapid leaf area and establish the canopy faster. Majority of these traits are quantitative in nature. Early seedling germination and early seedling vigour are the key traits for the weed competitive ability of rice. As many 29 QTLs

Table 3.24 Genes/QTLs governing resistance to different pests mapped in rice and resistance donors

Pests	No. of QTLs	No. of genes	Genes cloned	Effective genes/QTLs	Resistant varieties
Brown plant hopper	111	38	8	*Bph31*, *Bph33*, *Bph34*, *Bph17*, *bph19*, *Bph20*, *Bph21*, *Bph26*, *Bph27*, *Bph3*, *Bph4*, *Bph14*, *bph29*	Mudgo, PTB33, Rathuheenati, ASD7, Babawee, ARC10550, Swarnalata, T12, Chin Saba, Balamawae, Sinna Sivappu, 1R9-60, IR747B2-6-3, and IR 1154-243, Chaitanya, Krishnaveni, Vajram, Pratibha, Makom, Pavizham, Manasarovar, Co42, Chandana, Nagarjuna, Sonasali, Rasmi, Jyothi, Bhadra, Neela Annanga, Daya, Aruna, Kanaka, Remya, Bharatidasan, Karthika, Vijeta, Cotton Dora Sannalu, ADT37
White backed plant hopper	88	14	–	*Wbph1*, *Wbph2*, *Wbph3*, *Wbph4*, *Wbph5*, *Wbph6*, *Wbph7*, *Wbph8*, *wbphM1*, *wbphM2*, *wbphAR*, *wbphN*, *wbphO*, *Ovc*	Laatha, Narendra 2002, Jitendra, Satyaranjan
Small brown plant hopper	34	–	–	–	
Green leafhopper	11	–	–	–	DM-27. DS-I, DK-I, and DNJ-27
Gall midge	11	7	–	*Gm4*, *Gm8*, *Gm1*, *Gm2*, *Gm5*, *Gm6*	ARC5984, ARC5833, W1263, Phalguna, RP2068-18-3-5, Abhaya, Jhitpiti, Sneha, Pothana, Kakatiya Erramallelu, Kavya, Rajendradhan 202, Karna, Ruchi, Samridhi, Usha, Asha, MDU 3, Bhuban, Samalei, Orugallu, Abhaya, Shakti, Suraksha, Daya, Pratap, Udaya, IR36, Shaktiman, Tara, Kshira, Sarasa, Neela, Lalat,

(continued)

Table 3.24 (continued)

Pests	No. of QTLs	No. of genes	Genes cloned	Effective genes/QTLs	Resistant varieties
					Phalguna, Mahaveer, Vibhava, Divya, Dhanya Lakshmi, Surekha, Vikram, Kunti, Triguna, Sita, Samleswari, Karma Mahsuri, Dhanarasi, Mahamaya, Jyothi
Stem borer	12	–	–	–	TKM6, PSBRc68, IR46, CO14
Root knot nematode	32	–	–	*qYR5.1*, *qYR11.1*, *qMGR4.1*, *qMGR7.1*, *qMGR9.1*	Phule Radha, IR78877–208-B-1-2, LD24, Khao Pahk Maw, NKSWR30, NKSWR259

associated with early seed germination traits and 15 QTLs with early seedling vigour including two QTL hotspots, one in chromosome 11 and another in chromosome 2 have been identified in rice (Dimaano et al. 2020). QTLs governing early uniform emergence (*qEMM11.1*) and early vigour (*qEVV9.1*) have been mapped in rice (Dixit et al. 2015; Sandhu et al. 2015).

Alternatively, weeds can be controlled by using herbicides. Selective herbicides such as Pendemthalin and Bispyribac Sodium have been used in rice crop for managing weeds. Broad spectrum non-selective herbicides offer better weed control at lower doses, flexibility in timing of application, are cost effective and eliminate the possibility of any injury to the crop. However, to use non-selective herbicides, phytotoxicity to the rice crop needs to be avoided through the use of herbicide tolerant mutants. Fortunately, in rice, herbicide tolerance genes with different modes of action have been identified (Table 3.25), which can be incorporated into popular rice varieties through marker-assisted backcross breeding to develop an herbicide tolerant rice variety (Grover et al. 2020). Imidazolinone group of herbicides (imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin, etc.) kills weeds by inhibiting acetohydroxyacid synthase (AHAS) enzyme, also called acetolactate synthase (ALS). It is a critical enzyme involved in the biosynthesis of branched-chain amino acids such as leucine, isoleucine and valine in plants. Imidazolinone group is most widely used for weed control in crops like soybean, groundnut, etc., which possess natural tolerance to these herbicides. However, rice is highly sensitive to imidazolinones. Allelic variants of *AHAS* conferring tolerance to imidazolinone herbicides have been created through mutagenesis and commercialised as Clearfield® rice. Imidazolinone herbicides offer broad spectrum control of grass and broadleaf weeds in imidazolinone-tolerant crops (Tan et al. 2005). Besides, AHAS/ALS, mutations in two more genes have been found to provide herbicide tolerance in rice (Table 3.25).

Table 3.25 Mutations in major genes governing herbicide tolerance in rice

Gene	Locus ID	Mutant/genotype	Mutation	Amino acid substitution	Target herbicide group	Reference
AHAS/ ALS	LOC_Os02g0510200	93-AS3510	G-A	G654E	Imidazolinones	Croughan (1998)
AHAS/ ALS	LOC_Os02g0510200	IMINTA1, IMINTA4	G-A	A122T	Imidazolinones	Tan et al. (2006)
AHAS/ ALS	LOC_Os02g0510200	Robin	G-A	S627N	Imidazolinines	Shoba et al. (2017)
AHAS/ ALS	LOC_Os02g0510200	JD164	G-A	S627N	Imidazolinines	Piao et al. (2018)
ACCase	LOC_Os05g0295300	Sabbore R line	G-T	W2027T	fops, dims, phenylpyrazolin (ACCase Inhibitors)	Andrade et al. (2018)
HIS1	LOC_Os02g0280700	Koshihikari	28-bp deletion	Nonsense mutation	β-triketones (4-HPPD inhibitor)	Maeda et al. (2019)

3.15 Breeding for Abiotic Stress Tolerance

Rice is affected by various abiotic stresses tolerance such as drought, submergence, salinity, extremes of temperature (cold/heat stress), low nutrients and high radiation. Drought is one of the most important abiotic stress limiting rice yields especially under upland rainfed ecologies (Vinod et al. 2019). Rice in general has a small fibrous root system, thin cuticle and swift stomata closure, which makes it highly susceptible to drought stress. Therefore, breeding for tolerance to drought is one of the major breeding objectives for rainfed ecologies. Rice is highly sensitive to salt stress both at seedling and reproductive stages, which necessitates the introgression of tolerance at both seedlings as well as reproductive stages (Vinod et al. 2013). Rice is semi-aquatic, but most of the genotypes are susceptible to complete submergence for even a short period. Flash floods cause more mechanical damage than inundation. However, waterlogging for a longer period can result in substantial yield loss. More than 16 million ha of rice in South and Southeast Asia is prone to floods (Dwiyanti and Yamada 2013).

In India, the eastern part of the country including eastern Uttar Pradesh, Bihar, West Bengal and Assam constitute the submergence prone ecologies under rice. Rice is highly sensitive to high temperature stress, particularly during the reproductive stage (Cao et al. 2008), resulting in poor panicle exsertion and spikelet sterility causing major yield loss. Reproductive stage heat stress not only results in yield loss but also there is a serious deterioration of grain quality, such as increased chalkiness, shrivelling of grains, as well as reduced storage proteins. Rice in general is adapted to the tropics and therefore is sensitive to low temperature. Low temperature tolerance is a key trait in hilly ecologies, where a sudden drop in temperature can result in productivity losses. Rice utilises significant quantities of nutrients from the soil for grain production (60% for N, 67% for P and 15% for K), and the balance is retained in the straw (Dobermann and Fairhurst 2000) followed by P, Ca, Mg and S. Genetic improvement of rice to improve nutrient use parameters under low fertility conditions by enhancing nutrient foraging, faster uptake, solubilising ability, microbial symbiosis, and terminal mobilisation of nutrients to grains thereby increasing yield conversion efficiency. Rice is endowed with a wealth of germplasm possessing tolerance to these major stresses (Table 3.26). Tolerance to a majority of these stresses show polygenic inheritance. Advances in genetics and genomics have enabled mapping a large number of genes/QTLs for tolerance to each of these stress factors in rice. Several drought tolerant rice varieties have been developed through conventional breeding and released for commercial cultivation in India (Table 3.27).

3.16 Breeding Approaches for Rice Improvement

Genetic variability is the foundation for any crop improvement. Rice is endowed with enormous variability. The selection and release of GEB24, a spontaneous mutant from a landrace ‘Konamani’ marked the beginning of rice breeding. Since

Table 3.26 Genes/QTLs mapped and tolerance sources for various abiotic stresses in rice

Abiotic stress	No. of QTLs	No. of genes	Genes cloned	Effective QTLs/ genes	Tolerant varieties/ genotypes
Drought	874	2	2	*qDTY1.1*, *qDTY12.1*, *qDTY2.1*, *qDTY3.1*, *qDTY2.2*, *Dro1*, *OsDREB1a*	Nagina 22, Vandana, IR94225-B-82-B, Annada, Varanideep, Azucena, PTB42, PTB43, PTB55
Salt	876	1	2	*Saltol*, *SKC1*, *SalT*	Pokkali, FL478, Nonabokra, CSR11, KR1–24, PTB44
Submergence	23	1	4	*Sub1A*, *OsTPP7*	FR13A, Dhalputtia, Nimbuiya, Beller, Amghaud, Malhi qud, Ramkajari
Heat	208	–	–	*TT1*, *qHTSF1.1*, *qHTSF4.1*	Nagina 22, NL44, NH 219, Dular, Nipponbare, WAB56–12
Cold	491	42	–	*qPSST3*, *qPSST7*, *qPSST9*, *qCTS4a*, *qCTS4b*	K-39, K-78, K348, Geumobyeo, W1943, M202, Leimaphou
Aluminium toxicity	5	1	1	*NRAT1*	Ca 902/B/21, Goria, Karkati 87, DM59, and P 737
Low Phosphorus	4	–	–	*PSTOL1, qPU5.2*	Kasalath, Rasi
Deep water	–	2	2	*Snorkel1*, *Snorkel2*	Jalmagna, TCA 269, TCA4, Jalnidhi, PTB47, PTB48
Oxidative stress	353	31	–	–	–
Nitrogen deficiency	113	–	–	*NRT1.1B*, *OSNR2*, *OsTCP19*, *NGR5*, *SMOS1*, *GRF4*, *qNU5.1*	9311

then, various strategies have been adopted by the rice breeders, to create variability as well as to recombine the available variability in the rice germplasm to develop rice varieties with improved productivity. Although yield improvement remains a major focus, the priorities in rice breeding have been changing from time to time. Rice improvement has also seen the adoption of various breeding approaches at different periods based on the priorities. The major breeding approaches in rice include (a) introduction, (b) pureline selection, (c) pedigree breeding, (d) mutation breeding, (e) marker-assisted breeding, (f) population improvement and (g) genomic selection, and more recently (g) genome-editing. The breeding approach for rice improvement has changed with the new scientific discoveries and molecular tools not only

Table 3.27 Major abiotic stress tolerant varieties released for commercial cultivation in India

Variety	Year	Genotype	Parentage	Ecosys
Drought				
Nagina 22	1978	–	A selection from Rajbhog	UL
BR34	1985	–	Pureline selection from Dolangi	RL
TKM10	1993	IET12270	CO31/C-22	UL
Birsa Dhan 106	1996	IET12052	Bala. Black Gora. OS-36/ CH1039	UL
MDU5	1997	–	*O. glaberrima*/Pollali	UL
Vandana	2002	RR 167-982	C-22/Kalakeri	UL
Anjali	2002	IET164030	PR-19-2/RR-149-1129	UL
Jaldi Dhan 13	2006	PNR 591-18	–	–
Sahbhagi Dhan	2010	IR74371-70-1-1	IR55419-04*2/Way Rarem	RL, UL
DRR Dhan 43	2014	IR83876-B-F3	IR03L03/IRRI148	RL
DRR Dhan 44	2014	IR93376-B-B-130	IR71700-247-1-1-2/IR03L120	UL
CR Dhan 201	2014	IR83380-B-B-124-1	IR72022-46-2-3-3-2/PSB RC 18	UL
CR Dhan 202	2014	IR84899-B-154	IR78877-208-B-1-1/IR55423-01	UL
CR Dhan 203	2014	IR84899-B-185	IR78877-208-B-1-1/IR55423-01	UL
CR Dhan 204	2014	IR83927-B-B-279	IR78888-208-B-1-2/CT6510-24-1-2	UL
CR Dhan 205	2014	IR86931-B-578	Nagina22/Swarna	UL
Tripura Hakuchuk 1	2014	IR83928-B-B-56-4	–	UL
Tripura Hakuchuk 2	2014	IR82589-B-B-138-2	IR55423-01/IRRI148	UL
Swarna Shreya	2016	IR84899-B-179-16-1-1-1-1	IR78877-208-B-1-1/IR55423-01	RL
Submergence (Flash flood)				
Tulashi	1989	IET 8548	CR-151-79/CR-1014	RL
Swarna *Sub1*	1999	CR AC 2539-1	Swarna/FR13A//Swarna*3	RL
Samba *Sub1*	2011		BPT5204/FR13A/? BPT5204*3--	RL
Ranjtih *Sub1*	2016		Ranjith/Swarna Sub1// Ranjtih*3	RL
Bahadur *Sub1*	2016		Bahadur/Swarna Sub1// Bahadur*3	RL
CR1009 *Sub1*	2015	IET 22187	CR1009/FR13A//CR1009*3	RL
CO43 *Sub1*	2018		Co43/FR13A//Co43*3	RL
Deep water rice with tolerance to prolonged flooding				
Jaladhi 2	1982	–	Pureline selection from Baku	DW
Jalnidhi	1994	–	Local selection from Goantis	DW
Jalpriya	1994	–	IET5060/Jalmagna	DW
Jalprabha	1997	IET11870	Selection from composite	DW

(continued)

Table 3.27 (continued)

Variety	Year	Genotype	Parentage	Ecosys
Salinity/Sodicity				
CSR13	1998	IET10348	CSR1/Basmati370??CSR5	SD
CSR23	2004	IET13769	IR64//IR4630-22-2-5-1-3/ IR964-45-2-2	SD
CSR27	1998	IET13765	Nona Bokra/IR565-33-2	SD
CSR30	2001	IET14720	BR4-10/Pak. Basmati	SD
CSR36	2005	IET17340	CSR13/Panvel//IR36	SD
Cold				
Leimaphou	1990	KD 2-6-3	Moirangphou/Lawagin	HE
Sanaphou	1999	KS 2-7-6-2	Moirangphou/Lawagin	HE

RL rainfed lowland, *UL* upland, *SD* sodic, *DW* deep water, *HE* hill ecology

enabling precise selection of genotypes but also the precise creation of superior alleles at various clones genes aided by the advances in rice functional genomics and molecular biology in rice. However, the utility of these breeding approaches is still relevant even today in order to meet the challenges imposed for improving as well as sustaining rice productivity.

3.16.1 Introduction

The introduction is one of the simplest methods in breeding, wherein the high yielding varieties released from other countries/states within India are directly introduced and released as varieties. It helps address two important objectives of breeding, namely improving productivity as well as enriching the variability of the elite germplasm available for further crop improvement. In the early 1950s, a large number of temperate *japonica* rice varieties were introduced from China and Japan for the hill ecologies of Himachal Pradesh, Kashmir and Uttarakhand, some of which became very popular. The noteworthy introductions from China include Shinei, China 45, China 988 and China 1039, and Japanese varieties include Koshihikari and Narahikari. During the green revolution era, several semi-dwarf high yielding rice varieties were introduced, which were used as the source germplasm for semi-dwarfing genes in India. Some of the landmark varieties were Taichung Native 1, Taichung 65, Tainan 3, Dee-Gee-woogen and IR8 from IRRI, Philippines. One the *indica*/*japonica* derived 'Mahsuri' was also introduced in India and has been utilised in breeding mega varieties, such as Swarna and BPT5204. IR20, IR36, IR50 and IR64 are some more varieties introduced and released as varieties in India, out of which IR36 and IR64 are still popular among the farmers. Many of the varieties such as Jaya, Rasi, Padma and Swarna from India were also introduced in several countries across the world (Table 3.28).

Although introductions were easy during earlier years, with the Intellectual Property Rights gaining importance in the agriculture sector, the exchange of

Table 3.28 Varieties from India introduced and adopted in countries across the world

Countries and varieties introduced	Year of release	Released as (variety name)
Afghanistan		
CR 44-11, Cauvery, Padma	1975	[a]
Cambodia		[a]
OR 142-99	–	[a]
PR China		
M114	1981	8085
Myanmar		
Mahsuri mutant	1977	Ma Naw Thu Kha
RP 1057039301	1995	Yezin
RP 1674-690-39-14	2005	Shwe Myanmar
Nepal		
CR 123-23	1978	Durga
Rasi	1981	Bindeswari
K-39-96-1-1-1-2	–	Khumal 3
Pakistan		
Khitish	1984	DR82
Vietnam		
Jaya	–	[a]
Iran		
Sona	1982	Amol 3

[a] Released in the same name

germplasm between countries across the world during recent years has come down drastically. The International Network for Genetic Enhancement of Rice (popularly known by the acronym INGER), an open access initiative by IRRI established in 1975 has been a valuable source of advanced pre-variety breeding lines as well as varieties developed by IRRI and other NARES partners involved in rice improvement. INGER has provided more than 70,000 breeding lines to 600 research stations in about 85 countries, which has facilitated more than 1300 varieties in different rice producing countries. The INGER nurseries are shared through a standard material transfer agreement under FAO International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA).

Introductions of varieties released by SVRC for one state to another state within India have also been highly successful including Swarna from Andhra Pradesh to Odisha, Madhya Pradesh, Uttar Pradesh, Bihar and West Bengal; BPT5204 released in Andhra Pradesh has been successfully adopted in Karnataka, Tamil Nadu, Uttar Pradesh, Bihar, Odisha and West Bengal. Both these varieties are very popular attaining the status of mega varieties in India. During recent years, the varieties released by SVRC, such as MTU1010, CO51 and PR114, have gone on to become popular in other states.

3.16.2 Pureline Selection

Pureline selection advocated by Johannsen (1903) was one of the most widely adopted and successful breeding approaches for rice improvement during the early quarter of the nineteenth century. This approach helped in bringing out the cream from the traditional rice varieties, with wide adaptation, high yielding under low inputs and resistant to local insect pests and diseases. However, they were all tall and lodged under heavy manuring. One of the first rice varieties to be released by pureline selection was CO1 by Paddy Breeding Station, Coimbatore, in 1923, following which as many as 14 rice varieties from this station were released till 1940 through pureline selection. A drought tolerant variety CO2 and a blast resistant rice variety CO4 were released through pureline selection (Parthasarathy 1972). Owing to the selection from the germplasm adopted to specific ecologies, most of the pureline selections were released for that particular state only. More than 50 varieties were developed and released through pureline selection from 1923 to 2002. Some of the popular rice varieties developed through the pureline selection approach are presented in Table 3.29. Most of the varieties developed through pureline selection before the green revolution were all tall and highly susceptible to lodging.

3.16.3 Pedigree Breeding

Pedigree breeding involves hybridisation and selection through controlled crossing of selected rice genotypes followed by selection in the segregating generations or fixed generations for traits based on the breeding objective. Hybridisation is formulated to ensure the recombination of desirable characteristics in the segregating generations providing scope for selecting superior genotypes with desirable characteristic features. The hybridisation can be effected through two-way crosses, which involve 2 purelines; three-way crosses, which involve crosses between purelines and an F_1 (also known as a top cross); double crosses, where two F_1s from two different crosses are intercrossed to generate segregating populations. Top crosses and double crosses are attempted to combine desirable traits from multiple parents. More recently multiparent crosses involving 8 parents, 16 parents called MAGIC (Multiparent Advanced Generation Intercrosses) have also been proposed to recombine superior alleles from as many parents as possible.

The F_1s generated by crossing selected parents are advanced by selfing, and phenotypic selection for highly heritable traits are carried out from the segregating generations till F_5 to F_7 generations, after which the selected lines are evaluated in preliminary yield trials (PYT), followed by station trials for 2 years (ST1, ST2), after which the genotypes are nominated for testing in the trials conducted by the All India Coordinated Rice Improvement Programme (AICRIP, described in detail later in this chapter). The various steps adopted in pedigree breeding are outlined in Fig. 3.11.

During the initial years of rice breeding, only one crop was raised per year especially under north Indian conditions during *Kharif* season only. If only one

Table 3.29 Some of the popular rice varieties developed through pureline selection approach

S. No.	Variety	Pureline selection from	Year of release	Eco-system	Salient features
1.	CO4	Anaikomban	1923	Irrigated transplanted	Resistant to blast disease
2.	Madhukar	Gonda	1969	Semi-deep water	Tolerant to stagnant flood
3.	T-23	Kala sukhdas	1975	Upland irrigated	Tall, long slender grains
4.	Giza-14	Giza-4	1978	Hill rice irrigated	Semi dwarf, short bold grains, good cooking quality
5.	Jalmagan	Barho	1978	Flood prone	Very tall (upto 4 m), short bold grains
6.	Nagina 22	Rajbhog	1978	Upland	Resistant to drought, tall, short bold grains
7.	Type 3	Dehraduni basmati	1978	Irrigated transplanted	Basmati rice, tall, long slender grains
8.	Type 100	Bhanslot	1978	Saline	Tall, short bold grains
9.	Vytilla 2	Cheruvirippu	1982	Saline	Tall (154 cm), medium bold grains, red pericarp
10.	Jaladhi 1	Kalakhersail	1982	Water logged	Very tall (upto 4 m), bold grains
11.	BR-34	Dolangi rice	1985	Rainfed	Tall, medium slender grains, moderately resistant to BB and SB, tolerant to drought
12.	SR26B	Kalambanka	1988	Rainfed shallow lowland	Salinity tolerant
13.	Improved white ponni	White ponni	1989	Irrigated transplanted	Medium slender grains, tall, resistant to RTV, moderately resistant to brown spot and blast
14.	Birsa Gora102	Gora rice	1993	Uplands	Tall, medium bold grains
15.	Taraori basmati	Karnal local	1996	Irrigated transplanted	Basmati rice, tall
16.	Ranbir basmati	Basmati370	1996	Irrigated transplanted	Early maturing basmati rice
17.	Sravani	IR 50	2000	Irrigated transplanted	Dwarf (80–85 cm), long slender grains, resistant to blast, brownspot, BB
18.	Maruteru sannalu	Oodasannalu	2000	Irrigated transplanted	Dwarf (90–95 cm), medium slender grains
19.	Mangala mahsuri	Mahsuri	2002	Irrigated transplanted	Tall (123 cm), Short bold grains, moderately resistant to SB and blue beetle

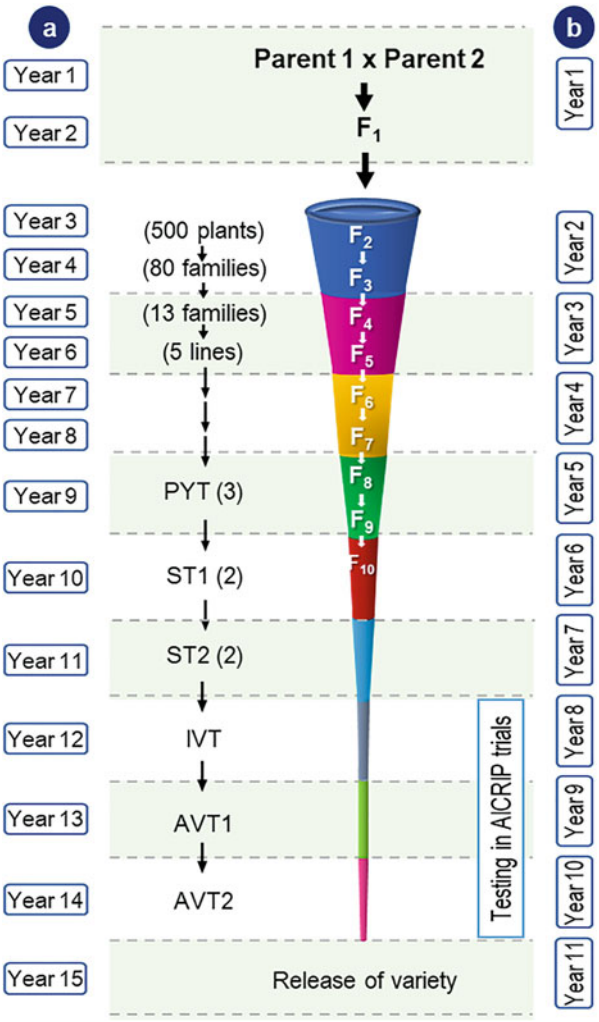

Fig. 3.11 A schematic overview of the pedigree breeding approach followed by testing for release of the variety. (**a**) indicates a situation, where only one crop is taken up per year and (**b**) indicates where two crops are raised in a single year

crop is raised per year, it takes 8 years from attempting crosses to develop elite breeding lines and including the testing in AICRIP trials, it takes 15 years to release a rice variety through pedigree breeding. However, extending the concept of shuttle breeding in rice, institutes like ICAR-IARI, New Delhi, has established an offseason nursery, Rice Breeding and Genetics Research Centre, IARI at Aduthurai, in the campus of Tamil Nadu Rice Research Institute, Aduthurai which has enabled taking up of two crops per season is being taken up since 1968. Shuttle breeding using the offseason nursery at RBGRC-IARI, Aduthurai has halved the number of years for the development of elite genotypes to 4 years and reduced the time taken for development and release of improved rice varieties significantly.

Further, shuttle breeding also offers the additional advantage of exposing the segregating populations to two different environments varying for soil, weather and disease pressure providing an opportunity for selection of better performing genotypes with better stability and adaptability. Several other methods have been utilised for rapid fixation of genotypes in rice, including doubled haploids (DH), single seed descent (SSD) (Goulden 1939) in combination with rapid generation advancement (RGA) and speed breeding. Among these DH enables rapid fixation of genotypes in a single step (Collard et al. 2017). However, it provides opportunity for only one cycle of recombination, whereas in other methods multiple cycles of recombination happens during the fixation process, thereby providing better chances of recombination of favourable alleles during the breeding process.

RGA under greenhouse conditions have been utilised in Japan and Korea (Heu et al. 1982; Ikehashi 1977). As many as 24 leading Japanese varieties including the popular *japonica* rice variety, Nipponbare (Used for generating reference genome of rice) were bred using RGA (Ikehashi and Fujimaki 1980). It could shorten breeding cycle thereby saving time while enabling breeding for cold tolerance as well (Heu et al. 1982). Recently, Field RGA enabling advancing multiple generations under field conditions have been utilised in IRRI and Bangladesh, thereby reducing the fixation time from 4 years to 2 years (Collard et al. 2017). RGA has several advantages, namely (1) technical simplicity, requirement of less resources including field and labour thereby saving time and money (Stoskopf et al. 1993); (2) no need for strict record keeping as in pedigree breeding; (3) only one step seed increase needed to produce sufficient seeds for evaluation. However, it has its own inherent disadvantages such as (1) retention of poor performing genotypes during generation advancement as there is no selection during line fixation; (2) a general perception that it does not capture adequate genetic variation; (3) lines through RGA are considered inferior compared to lines generated through early-generation visual selection (Jensen 1988).

Pedigree breeding is one of the most popular methods of breeding in rice improvement (Khush and Virk 2005). Large population size in the F_2 generation (around 2000 plants in 2-way crosses, 3000–5000 in 3-way crosses) must be raised to ensure desirable recombinants especially when the parents are contrasting for many traits of interest. However, in practice it is widely used due to several reasons, the availability of resources being one of the major constraints. During the initial years of the green revolution, consciously large populations of F_2 upto 13,200 plants were grown from the biparental crosses involving tall *indica* varieties, namely T90, GEB24, T141, SK20, SLO16, Basmati 370 and the semi-dwarf varieties, Taichung native 1, Dee-geo-woo-gen, IR8 (Freeman and Shastry 1972), so that a large number of semi-dwarf genotypes from the F_2 population could be selected for further selection in the following generations. However, the actual population size depends largely on the resources available to the breeders and the extent of segregation. In recent years, where the crosses between largely elite genotypes (elite/elite) fixed for favourable alleles at a given locus are attempted, reasonable population sizes of 500 are adequate to capture the desirable recombinants.

The crosses during the initial years of breeding at paddy breeding station, Coimbatore, were mainly aimed at the development of (1) blast resistant rice varieties and (2) non-lodging rice varieties suited for partly submersible ecosystems. These efforts resulted in the development and release of the rice varieties, namely CO14, CO15 and CO16 in 1940. CO14 from the cross, CO3/T275, was the first variety developed through pedigree breeding approach released for commercial cultivation in India in 1940. CO15 (Jada Molakolukulu) was the first blast resistant variety developed from the cross GEB24/ADT10 (also known as Korangu Samba) in 1940 (Parthasarathy 1972). ADT27 from the cross Norin 10/GEB24 is a landmark variety developed and released through pedigree breeding under the *indica/japonica* hybridisation programme funded by FAO. CR 1014, a late maturing tall and non-lodging rice variety with medium slender grains and good cooking quality was developed from the *indica/bulu* (*javanica* or tropical *japonica*) cross, T 90/Urang Urangan in the mid-1960s. Following the introduction of semi-dwarf rice varieties such as Taichung Native 1 (TN1), IR8 and Dee-Geo-Woo-Gen in 1965, a large number of crosses were attempted between the tall *indica* varieties and the three semi-dwarf rice varieties leading to the development of several popular semi-dwarf high yielding rice varieties (Table 3.30).

3.16.4 Mutation Breeding

Mutations either spontaneous or induced play a key role in creating variability which is vital for plant breeding. Mutation breeding has been utilised for creating useful mutants for rice improvement. Spontaneous mutations have also been utilised in rice varietal improvement. GEB24 was the first landmark variety identified and released in rice, which was a spontaneous mutant selected from a rice variety, 'Konamani' from Godavari delta with exceptional grain and cooking quality by Parnell and his associates at Paddy Breeding Station, Coimbatore in 1921. It is popularly known as 'Kitchili Samba' and has been widely used as a donor for grain quality in rice breeding programmes across the world. GEB24 is ranked fifth for use as a parent in the genealogy of released rice varieties till 1991. It is in the development of 554 rice varieties released across the world. It is also one of the parents of popular medium slender grain rice variety, 'BPT5204'. Another noteworthy variety developed through the selection of a spontaneous mutant from Basmati 370 is 'Ranbir Basmati', which is short duration Basmati rice variety released for Jammu regions of Jammu and Kashmir in 1996.

Ramaiah and Parthasarathy (1938) first used X-rays for producing mutations in rice. Thirty-six mutations affecting different characters, namely plant habit, height, panicle length, arrangement of spikelets in the panicle, spikelet fertility and 16 chlorophyll deficient types were generated. Induced mutagenesis gained importance in the late 1960s and 1970s with the funding and support from the International Atomic Energy Agency (IAEA) for generating basic knowledge and translating the potential of mutation in rice improvement.

Table 3.30 Some important rice varieties developed and released in India using the semi-dwarf rice varieties introduced from IRRI

Name of variety	Parentage	Year	Eco-system	Salient features
Jaya (IET723)	TN1/T141	1968	Irrigated	Dwarf, long bold grains, resistant to blast
Padma	T141/TN1	1968	Irrigated	Semi-dwarf, coarse grain, moderately resistant to BS
Bala	TN1/N22	1970	Rainfed upland	Semi-dwarf, tolerant to drought
Cauvery	TN1/ TKM6	1974	Rainfed upland or irrigated	Dwarf, short bold grains, resistant to BLS, moderately resistant to SB
Karishma	GEB24/ TN1	1974	Rainfed (late showing)	MS grains, very high head recovery, resistant to blast, leaf spot and SB
Ratna	TKM6 × IR8	1975	Upland and direct seeded	Dwarf, LS grains, moderately resistant to blast, LH and tolerant to SB
Saket4	TKM6/ IR8	1975	Upland irrigated or rainfed	Dwarf, LS grains, lodging resistant, moderately resistant to BLB, GLH and SB
Tella Hamsa	HR-12/ TN1	1975	Irrigated areas	LS grains, cold tolerant, resistant to SB
ORS11	T141/IR-8-246	1975	Rainfed coastal saline	Dwarf, long bold grains, moderately resistant to BLB, blast and GLH
Karuna	IR8/ ADT27	1976	Irrigated early	Dwarf, short stature, short bold grains
Jyothi	PTB10/ IR8	1977	Wetland areas	Dwarf, long bold grains, red pericarp, resistant to blast
Rasi	TN1/ CO29	1978	Rain fed upland areas	Semi dwarf, MB grains, resistant to blast, moderately resistant to RTV
Bharthi	PTB10/ IR8	1978	Irrigated early	Semi dwarf, long bold grains, red pericarp
Bhavani	Peta/BPI-76	1978	Irrigated medium	Semi tall, long bold grains, moderately resistant to blast, BLB
Gautami	IR8/SLO 13	1978	Coastal areas	Dwarf, short bold grains, resistant to blast
Pankaj	Peta/ Tongkai Rotan	1978	Low-land areas	Semi dwarf, long bold grains, moderately resistant to blast and RTV
Phalguna	IR8/Siam-29	1978	Coastal areas	Dwarf, LS grains, resistant to GM
Vasista	IR8/ SLO13	1978	Rainfed shallow	Grains: short bold, resistant to blast, moderately susceptible to BLB and SB.
PR 103	IR8/IR27	1982	Irrigated medium	Semi dwarf, LS grains, resistant to blast and *Helminthosporium*
Sarjoo 52	TN1/ Kashi	1982	Irrigated	Semi dwarf, erect, long bold grains

(continued)

Table 3.30 (continued)

Name of variety	Parentage	Year	Eco-system	Salient features
TKM9	TKM7/ IR8	1982	Irrigated early	Semi dwarf, short bold grains, red pericarp
ADT36	Triveni/ IR20	1982	Irrigated early	Semi dwarf, MS grains
Himalaya 2	Sabarmati/ Ratna	1983	Low and mid hills	Semi dwarf, long bold grains, aromatic, resistant to blast, SB and RH
KMP1 (Mandyavan)	CR-1014/ IR8	1984	Irrigated medium	Semi dwarf, super fine grains, tolerant to blast and BPH
Parijat	TKM6/ TN1	1985	High and medium lands	Dwarf, Medium slender grains
Dhan Narendra-1	Bella Patna/IR8	1983	Irrigated or rainfed	Dwarf, short bold grains, multiple stress resistant variety
Panvel 1	IR8/Bhura Rata 4–10	1985	Coastal saline area	Semi dwarf, short bold grains, resistant to neck blast
SKL6	Nagpur27/ IR8	1985	Irrigated areas	Semi dwarf, LS grains, resistant to blast and blight

Two most important traits for improvement through induced mutagenesis have been semi-dwarfism and earliness. Additionally, mutants for other traits such as high tillering, blast resistance, improved grain quality and photoperiod insensitiveness have also been derived through mutagenesis. Recently, Ethyl Methane Sulphonate induced mutant of Nagina 22, 'Robin' has been developed for tolerance to Imazethapyr, an herbicide (Shoba et al. 2017). Robin is currently being used in developing improved Basmati rice varieties suited for dry direct seeded rice cultivation. A semi-dwarf mutant, Reimei, derived from the variety, Fujiminori was the first mutant variety released in Japan in 1966. As many as 434 rice varieties have been developed through mutation breeding across the world. Many rice varieties have also been released through mutation breeding in India (Table 3.31), among which 'Jagannath' is very popular having been developed from T141 by X-ray mutagenesis. Jagannath is a semi-dwarf fertiliser responsive high yielding rice variety with medium slender grains.

3.16.5 Marker-Assisted Breeding

Rice is endowed with rich genetic and genomic resources, wherein a large number of genes governing economically important traits such as resistance to diseases, pests, grain quality, tolerance to drought, salinity, low phosphorus and herbicide have been mapped and many of them have been cloned. Availability of genes, a gene linked or gene-based markers and donor germplasm enable the use of marker-assisted breeding in rice. Marker-assisted breeding provides an excellent opportunity to the

Table 3.31 Mutant rice varieties released for commercial cultivation in India

Variety	Parentage	Year	Ecosystem	Salient features
Spontaneous mutation				
GEB24	Mutant of Konamani	1921	IR	Medium slender grain, exceptional grain and cooking quality
ADT41	Mutant of Basmati 370	1994	IR	Semi dwarf, extra LS grains, mild aroma
Ranbir Basmati	Mutant of Basmati370	1996	IR	Short duration Basmati, long slender grains and strong aroma
Induced mutation				
Jagannath	Mutant of T141	1975	CS	Semi Dwarf, MS grains, resistant to lodging, blast
CNM25	A mutant of IR8	1978	LA	Dwarf, long bold grains, moderately resistant to blast
CNM31	A mutant of IR8	1978	MLL	Semi dwarf, LS golden grains, resistant to BS
Lakshmi (CNM6)	A mutant of IR8	1982	UL	Dwarf, long bold grains
Biraj (CNM539)	Mutant	1984	RL	Tall, long bold grains
Prabhavati (PBN1)	Mutant of Ambemohar	1985	IU	Dwarf, coarse grains, aromatic, tolerant to Iron chlorosis
Padmini	Mutant of CR1014	1989	RSL	Superfine grains, highly tolerant to BLB
Lunisree	Mutant of Nonasai	1992	CSS, WL	Tall, Long Slender grains
Gautam	Mutant from Rasi	1996	IME	Dwarf, long bold grains
Malviya Dhan-36	Mutant of Mahsuri	1997	MLL	Medium Slender grains, resistant to major diseases
Radhi (CRM-40)	Swarnaprabha mutant	1997	IE	Long bold grains, tolerance to blast and BPH
Revathy (MO17)	Mutant of MO1	1998	IR	Resistance to BPH
Remanica (MO-15)	Mutant of MO1	1999	IM	Short bold grains, resistance to BPH and GM
Early Samba (RNRM7)	Mutant of BPT5204	2000	IM	Dwarf, Medium Slender grains
Kaum-20-19-4	Mutant of MO 1	2002	IR	Early, Dwarf, medium bold grains, resistant to BPH, ShB and ShR
CR Boro Dhan 2	Mutant of China-45	2008	IR Boro	Medium slender grains, cold tolerant (Seedling and tillering stages)
Anashwara (PTB 58)	Mutant of PTB 20	2006	IR	Semi tall, nonlodging, medium bold grains, and excellent cooking
	Mutant of MPR 7-2	2009	IR	Photoinsensitive, high yielding, long grains and aroma

(continued)

Table 3.31 (continued)

Variety	Parentage	Year	Ecosystem	Salient features
Malaviya Sugandh 105 (HUR 105)				
Malaviya Sugandh 4-3 (HUR 4-3)	Mutant of Lanjhi	2009	IR	Fine grain and mild aroma

IR irrigated, *CS* coastal saline, *UL* upland, *RL* rainfed lowland, *IU* irrigated upland, *RSL* rainfed shallow lowland, *WL* water logged, *IME* irrigated mid-early, *MLL* medium low land, *IE* irrigated early, *LA* lateritic and alluvial soils

present-day researchers for breeding new crop varieties by designing through the precise transfer of desirable gene(s) into popular rice varieties. Among the various breeding approaches, marker-assisted backcross breeding (MABB) helps in incorporating genes/QTLs governing resistance to disease/pest/improvement in quality, etc. into an already popular variety within a short span of 3 years.

Molecular markers are deployed in MABB for the foreground, background and recombinant selection (Singh and Krishnan 2016), where foreground selection is done for the target gene/QTL using a gene based/gene linked marker, background selection is exercised for recovery of recurrent parent genome in the plants selected with desirable alleles of target gene(s)/QTL(s) and recombinant selection is done to eliminate linkage drag, if any. Additionally, stringent phenotypic selection can also help in hastening the recovery of desirable phenotype in the improved near isogenic lines (NILs) derived through marker-assisted backcross breeding (Fig. 3.12).

Molecular marker-assisted breeding through the transfer of gene(s) for resistance to major diseases such as bacterial blight, blast, tolerance to drought, submergence, low phosphorus and herbicide has been very successful (Singh et al. 2011). As many as 39 improved rice cultivars developed through marker-assisted breeding have been released for commercial cultivation in India. Out of these, 17 varieties have been improved for bacterial blight resistance with x*a5*, *xa13* and *Xa21*, while five are improved with blast resistance, with *Pi1*, *Pi2*, *Pi9*, *Pi54* and *Pita* (Singh et al. 2019) and three Basmati varieties possessing resistance to both BB and blast diseases (Table 3.32).

Significant progress has also been made in breeding for resistance to bakanae and sheath blight, as well as against pests like brown planthopper. Markers have also been successfully utilised in transferring gene(s)/QTL(s) for tolerance to drought, submergence, salinity, low phosphorus and herbicide into popular rice varieties resulting in the release of as many as 14 improved rice varieties with enhanced tolerance to the above stresses.

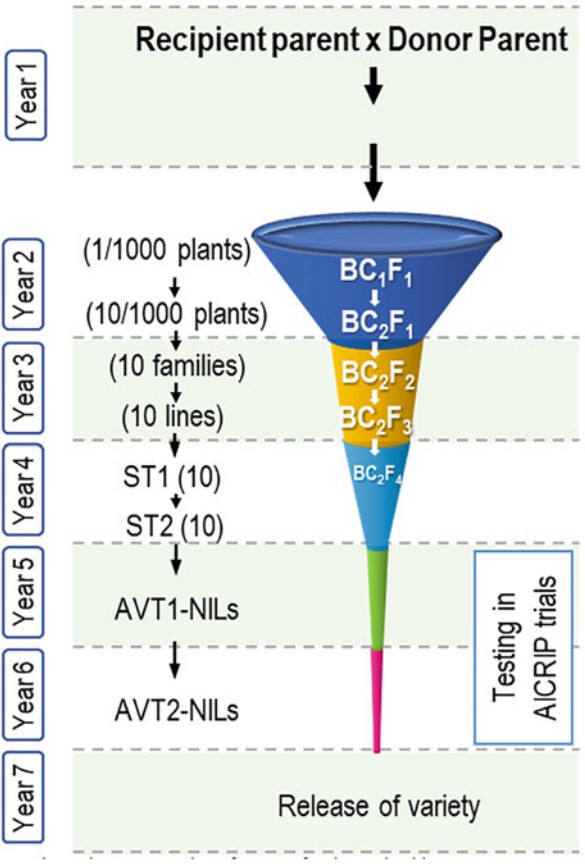

Fig. 3.12 Schematic representation of MABB for rice varietal improvement

3.17 Population Improvement

Breeding methods such as pureline selection, pedigree breeding, mutation breeding and backcross breeding have helped in making significant improvement in rice but also for other traits such as resistance to various biotic and abiotic stresses, grain and nutritional quality. However, these methods suffer from three inherent disadvantages, namely (1) limited size of the gene pool utilisation, as improvement is mainly through biparental crosses, (2) low genetic variability and limited recombination due to rapid fixation through inbreeding and (3) lack of crossing among hybrid progenies (Jensen 1970). This limits any further genetic gain and response to selection difficult for quantitative traits such as yield (Müller et al. 2017). Diallele selective mating system involving multiple parents, which helps in creating persistent gene pools, breaking undesirable linkages and accumulating favourable alleles with simultaneous infusion of variability into breeding population through use of

Table 3.32 MAS derived rice varieties incorporated with gene(s) governing resistance to biotic stresses released in India

S. No.	Improved rice variety	Genes incorporated	Year of release	Developer
Bacterial blight resistant rice varieties (17)				
1	Improved Pusa Basmati 1	*xa13*, *Xa21*	2007	ICAR-IARI, New Delhi
2	Improved Samba Mahsuri	*xa5*, *xa13*, *Xa21*	2008	ICAR-IIRR, Hyderabad
3	Improved Lalat	*Xa4*, *xa5*, *xa13*, *Xa21*	2012	ICAR-NRRI, Cuttack
4	Improved Tapaswini	*Xa4*, *xa5*, *xa13*, *Xa21*	2012	ICAR-NRRI, Cuttack
5	PR122	*Xa4*, *xa13*, *Xa21*	2013	PAU, Ludhiana
6	PR121	*Xa4*, *xa13*, *Xa21*	2013	PAU, Ludhiana
7	PR123	*Xa4*, *xa13*, *Xa21*	2014	PAU, Ludhiana
8	Pusa 1592	*xa13*, *Xa21*	2015	ICAR-IARI, New Delhi
9	PR124	*Xa4*, *xa13*	2015	PAU, Ludhiana
10	Pusa Basmati 1728	*xa13*, *Xa21*	2016	ICAR-IARI, New Delhi
11	Punjab Basmati 3	*xa13*, *Xa21*	2016	PAU, Ludhiana
12	CR Dhan 800	*xa5*, *xa13*, *Xa21*	2016	ICAR-NRRI, Cuttack
13	Pusa Basmati 1718	*xa13*, *Xa21*	2017	ICAR-IARI, New Delhi
14	Punjab Basmati 4	*xa13*, *Xa21*	2017	PAU, Ludhiana
15	Punjab Basmati 5	*xa13*, *Xa21*	2017	PAU, Ludhiana
16	PR127	*Xa45*(t)	2018	PAU, Ludhiana
17	DRR Dhan 59	*Xa33*	2021	ICAR-IIRR, Hyderabad
Blast resistant rice varieties (5)				
18	Pusa 6 (Pusa 1612)	*Pi2*, *Pi54*	2013	ICAR-IARI, New Delhi
19	Pusa Basmati 1609	*Pi2*, *Pi54*	2015	ICAR-IARI, New Delhi
20	Pusa Basmati 1637	*Pi9*	2016	ICAR-IARI, New Delhi
21	DRR Dhan 51	*Pi2*	2018	ICAR-IIRR, Hyderabad
22	Pusa Samba 1850	*Pi1*, *Pi54*, *Pita*	2018	ICAR-IARI, New Delhi
Both Bacterial blight and blast resistant rice varieties (3)				
23	Pusa Basmati 1847	*xa13*, *Xa21*, *Pi2*, *Pi54*	2021	ICAR-IARI, New Delhi
24	Pusa Basmati 1885	*xa13*, *Xa21*, *Pi2*, *Pi54*	2021	ICAR-IARI, New Delhi
25	Pusa Basmati 1886	*xa13*, *Xa21*, *Pi2*, *Pi54*	2021	ICAR-IARI, New Delhi

new genotypes (Jensen 1970). Generating synthetic populations and improving them through recurrent selection (RS) is an efficient alternative breeding strategy. Recurrent selection involves cyclical improvement through systematic selection of desirable plants from a breeding population followed by intercrossing them to enable further genetic recombination to form a new population better than the one in preceding cycle (Fehr 1987). It was originally proposed and extensively utilised in cross-pollinated crops such as maize (Hull 1945). Although this method has been utilised in self-pollinated crop like oat (Khadr and Frey 1965), effecting recurrent crosses between selected progenies to enable further recombination have been a major bottleneck. Gilmore (1964) suggested the use of male sterility to implement reciprocal recurrent selection in naturally self-pollinated species for both intra-population and inter-population improvement such as sorghum.

In rice, recurrent selection facilitated by the use of nuclear recessive male sterility gene was proposed by Fujimaki (1979). Singh and Ikehashi (1981) developed genic male sterile mutants in a popular rice variety, 'IR36', governed by a single recessive gene (*ms*). The use the male sterile mutants can facilitate genetic recombination naturally, and the naturally outcrossed seeds harvested from male-sterile plants can be used for recurrent cycles of improvement. Although these strategies were suggested for implementation of recurrent selection, it was not extensively used in rice improvement. The first recurrent selection programme mainly for intra-population improvement of rice was initiated in Brazil and Côte d'Ivoire in 1989, with the creation of first broad-based CMS *indica* rice population, CAN-IRAT4 (Taillebois and Neves 1989). This initiative resulted in the release of the world's first irrigated rice cultivar, SCSBRS 113 Tio Taka, selected from the genetically broad-based population CNA-IRAT 4, improved through recurrent selection (Rangel et al. 2007).

In 1996, rice population improvement through recurrent selection was undertaken as an alternative means of exploiting rice genetic resources, particularly in Latin America, with the creation of populations and training of scientists by CIAT, CIRAD and Embrapa Arroz e Feijão. Three populations were created, namely (1) PQUI-1 in Chile, combining European (France and Italy) with North American and Chilean germplasm (Hernaiz et al. 2000); (2) PCT-11, in Colombia, combining African, European and Brazilian lines of the *indica* and *japonica* subspecies (Ospina et al. 2000) and (3) GPCT-9, by CIAT and CIRAD combining commercial varieties and lines from several Latin American and Asian countries (Martínez et al. 1997). In order to maintain the gains, three suggestions have been made which includes, namely (1) maintenance and assessment of large number of families (250–300 families) of the population; (2) using appropriate evaluation strategy to improve accuracy of family assessments; and (3) using selection intensity to allow short term gains without losing genetic variability (Hideo et al. 2002). Even though, genetic gains for grain yield are reported through recurrent selection, till now only one cultivar has been released through recurrent selection. A novel dominant gene based male sterile line 'Jiabuyu' was utilised for population improvement of two populations, subjected to two cycles of recurrent selection through natural outcrossing. Even though, 45 lines with higher performance as compared to base

population was identified, the improved lines were inferior to the best yield check, NSIC Rc222 (Pang et al. 2017) (Table 3.33).

In order to improve the population improvement through recurrent selection, three modifications have been suggested based on the learnings from 15 years of recurrent selection (Vales 2010). First, adopting a modified method of Back recurrent selection (BCRS), a blend of backcross and recurrent selection which involves the progressive increase of recipient parent plants in the progressive cycles of population improvement in the ratio of two-third in the first cycle, which is increased to three-fourth in the next cycle leading to improvement of polygenic traits in the background of recipient cultivar. Second, is Simple and Efficient Breeding (SERB) based on the Narrow-Based Population (NBP) consisting of four or five progenitors with each possessing the very best for each targeted trait. It is simple, inexpensive, rapidly successful, easy to adjust and feasible. These populations can be readily adapted suited to breeders' normal practices, which allows breeders to closely monitor changes in breeding objectives and complementary tools. The first narrow-based populations were created in 2000 (Vales et al. 2000). In 2003, the Fundacion para la Investigacion Agricola (DANAC) of Venezuela extracted from the narrow-based population PCT-16, which led to the development of three cultivars, namely ACD 25–28, ACD 25–26 and ACD 25–40, released in Colombia, Venezuela, Panama, Costa Rica and Equator by Cultivos y Semillas el Aceituno (Aceituno) of Colombia (Gamboa et al. 2005). Third, is Plant-parasite reciprocal recurrent selection (2P2RS), which promotes the adaptation of the parasites (pathogen such as *Magnaporthe oryzae*) as a tool for selection of resistant plants during the breeding to produce genotypes with durable resistance. Alternately, SERB could be combined with the parallel and interlaced recurrent selection (PAIRS). In PAIRs, selection is exercised for earliness and grain size in S0 generation, for complete resistance to leaf blast in S1 plants, partial resistance to leaf and neck blast, earliness, yield components in S2 lines; gelatinisation temperature and alkali spreading value in S3 grains (Vales et al. 2009). Morais Junior et al. (2017) estimated a mean genetic gain of 1.98% for grain yield in CNA12S, a broad-based population of irrigated rice. Alternately, the efficiency of the recurrent selection method can be improved by reducing cycle time, increasing selection intensity or through recurrent genomic selection (Meuwissen et al. 2001).

3.18 Genomic Selection

Even though marker-assisted breeding has helped in shortening the time taken for rice varietal development, most of the success has been in transferring major genes/robust QTLs into popular rice varieties. It has found limited use in improving quantitative traits which are influenced by a large number of genes with small effects. Genomic Selection (GS) aims to address this limitation with the potential to improve genetic gain and breeding efficiency in crops. Genome-wide markers and phenotypic data of the target traits from a training population are used to predict breeding values (genome estimated breeding values; GEBVs) of the untested

Table 3.33 MAS derived rice varieties incorporated with gene(s)/QTL(s) governing with tolerance to biotic and abiotic stresses released in India

S. No.	Improved rice varieties	QTLs incorporated	Year of release	Developer
Drought tolerant rice varieties (1)				
1	IR64 Drt1 (DRR Dhan 42)	*qDTY2.2*, *qDTY4.1*	2014	ICAR-IIRR, Hyderabad
Submergence tolerant rice varieties (6)				
2	Swarna Sub1	*Sub1*	2009	ICAR-NRRI and IRRI
3	Samba Sub1	*Sub1*	2011	ICAR-NRRI and IRRI
4	CR1009 Sub1	*Sub1*	2013	ICAR-NRRI and IRRI
5	Ranjit Sub1	*Sub1*	2016	AAU, Jorhat
6	Bahadur Sub1	*Sub1*	2016	AAU, Jorhat
7	CO43 Sub1	*Sub1*	2018	TNAU, Coimbatore
Both Drought and submergence tolerant rice varieties (3)				
8	DRR Dhan 50	*qSub1*, *qDTY2.1*, *qDTY3.1*	2018	ICAR-IIRR, Hyderabad
9	CR Dhan 801	*qSub1*, *qDTY1.1*, *qDTY2.1*, *qDTY3.1*	2018	ICAR-NRRI, Cuttack
10	CR Dhan 802 (Subhash)	*qSub1*, *qDTY1.1*, *qDTY2.1*	2018	ICAR-NRRI, Cuttack
Both Bacterial Blight resistant and salinity tolerant rice varieties (1)				
11	DRR Dhan 58	*xa5*, *xa13*, *Xa21*, *Saltol*	2021	ICAR-IIRR, Hyderabad
Both Bacterial Blight resistant and low phosphorus tolerant rice varieties (1)				
12	DRR Dhan 60	*xa5*, *xa13*, *Xa21*, *Pup1*	2021	ICAR-IIRR, Hyderabad
Herbicide tolerance				
13	Pusa Basmati 1979	*Mutant AHAS allele*	2021	ICAR-IARI, New Delhi
14	Pusa Basmati 1985	*Mutant AHAS allele*	2021	ICAR-IARI, New Delhi

populations, which are yet to be phenotyped based on their genotypic data (Crossa et al. 2017). Unlike MAS, GS does not involve the detection of significant QTLs for different traits but enables breeding efficiency by enabling selecting elite genotypes based on GEBVs without the need for phenotyping large populations. GS involves rapid fixation of the genotypes by employing RGA/DH/Speed breeding from crosses between ~20 elite lines. A set of around 10,000 fixed lines are generated from 20 crosses, out of which a training population consisting of a subset of ~2000 lines are phenotyped, while all the fixed lines are genotyped. The phenotypic and genotypic data of the training populations are used for developing prediction models

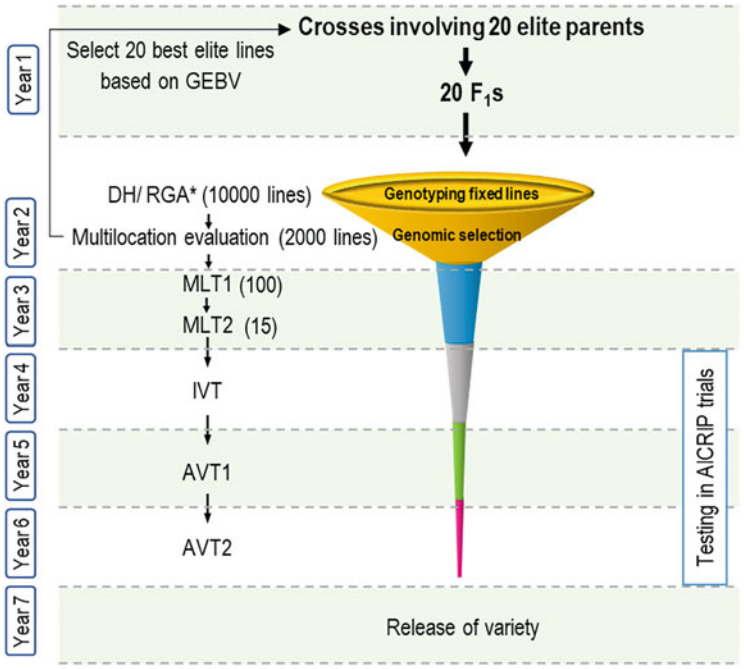

Fig. 3.13 Schematic representation of genomic selection for rice varietal improvement

based on which the GEBVs of the remaining 8000 lines are predicted. The top 20 lines are taken as parents for the next cycle of improvement, while a set of top performing lines are taken up for further multi-location evaluation and varietal improvement. The overall scheme for GS is schematically summarised in Fig. 3.13.

The prediction ability increases with higher marker density and a larger sample size. Effective GS models can be built using a training population from 2 to 13% of the total population (Guo et al. 2019). The prediction accuracy improves with the addition of related genotypes in the training population without compromising on the genetic variation than increasing the population size. Parametric models such as genomic best linear unbiased prediction (GBLUP), ridge regressions best linear unbiased prediction (RRBLUP), partial least square (PLS), least absolute shrinkage selection operator (LASSO), elastic net, and Bayesian methods including BayesA, BayesB, BayesC, BayesR, etc. and non-parametric models such as random forest (RF), support vector machine (SVM), reproducing kernel Hilbert space (RKHS), deep learning, etc. are used for GS. Comparison of the predictive ability of these methods has shown varying results. Therefore, it has been suggested to treat the GS method as a parameter and evaluate the predictive abilities of all methods using cross validation and select the best method with maximum accuracy for a particular breeding programme (Xu et al. 2017). In rice, GS has shown moderate to high predictive ability, with as high as 0.8 for culm length (Onogi et al. 2015) to as low as 0.3 for grain yield (Spindel et al. 2015).

GBLUP has been used for predicting a set of top 100 crosses with yield grains of upto 16% from a set of 21,667 untested hybrids, using a set of 278 hybrids derived from 210 RILs as training population (Xu et al. 2014). GBLUP has been effectively used for predicting the performance of 100 hybrids derived from half diallel cross, involving 21 parents that are different from the parents of hybrids used for developing models in the training set for six agronomic traits (Cui et al. 2020), which has demonstrated the use of GS in hybrid rice breeding as well. Genotyping consumes substantial costs of breeding in GS. SNP arrays of different densities including 44 K, 50 K, Rice 6 K, RiceSNP50, C7AIR and 1 k-RiCA. Optimised experimental designs, precise high throughput phenotyping platforms, low cost genotyping technologies and improved models can help in improving rice varieties using GS (Xu et al. 2021a).

3.19 Genome-Editing

Spontaneous and induced mutagenesis has been used for generating random mutations of limited density in the genome, out of which only a few of them are economically useful (Jacob et al. 2018; Shoba et al. 2017). Spontaneous mutations in the crop genome have been creating a large number of nucleotide substitutions, which are detected as single nucleotide polymorphisms (SNPs) through genome sequencing. Among them, a large number of loss of function (LoF) alleles have been identified from whole genome resequencing data. In rice, 198,609 premature stop codon inducing single nucleotide variants were found from the 3010 rice genotypes sequenced (Monroe et al. 2020). LoF alleles from spontaneous mutations have been greatly helpful in the evolution of modern-day rice crop through loss of shattering habit, prostrate growth habit, increase in grain size and grain number, better grain quality (Table 3.34). However, the selection process during domestication and improvement has created a genetic bottleneck that has reduced the variation available in the crop for utilisation in further improvement (Shan et al. 2014).

Targeted genome-editing using clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (CRISPR/Cas) such as *Cas9* enables the creation of precise and high-density mutations in the gene of interest (Jinek et al. 2012), which hold enormous potential for the creation of hitherto unknown superior alleles in economically important genes, so as to enable the development of desired plants with higher yield, improved grain quality, and resistance to herbicides, diseases and insect pests (Huang et al. 2018). Gene editing also helps in increasing the allelic diversity in functional genes of interest, which can help in easy and rapid improvement of crop like rice, where functional characterisation of a large number of genes have been accomplished (Hu et al. 2016).

The CRISPR/Cas gene editing creates double strand breaks and two repair pathways, namely nonhomologous end joining (NHEJ) and Homology directed repair (HDR) are utilised in creating mutations in the target loci. NHEJ generates random insertions and deletions at target sites with extreme precision and is a popular pathway for generating mutants. In contrast, HDR offers generation of

Table 3.34 Some examples of naturally occurring beneficial loss of function (LoF) alleles caused by spontaneous mutations that have helped in the evolution and improvement of rice

Gene	Mutation type	Mutation effect	Reference
LoF alleles created by single nucleotide substitutions			
Bh4	SNP—premature stop	White hull	Zhu et al. (2011)
PROG1	SNP—premature stop	Erect growth	Wu et al. (2018)
OsWaxy	SNP—splicing defect	Glutinous grains	Zhou et al. (2016)
SLR1	SNP—premature stop	Dwarf plant	Wu et al. (2018)
GS3	SNP—premature stop	Long grain	Lu et al. (2013)
LoF alleles created by Insertions and Deletions (InDels)			
qSH1	InDel—frameshift	Non-shattering	Konishi et al. (2006)
OsGn1A	InDels—frameshift	Increase grain number	Ashikari et al. (2005)
DEP1	InDels—frameshift	Reduce length of the inflorescence internode, more grains per panicle	Huang et al. (2009)
bHLH	Indel—frameshift	White pericarp	Sweeney et al. (2006)
OsLG3b	Indel—frameshift	Increased grain size	Yu et al. (2018)
Pi21	InDels—frameshift	Blast resistance	Fukouka et al. (2009)
Bad2	Indel—frameshift	Fragrant grains	Chen et al. (2008)

Domestication, diversification and improvement

desired mutations precisely at the target site. Additionally, base editors can also engineer efficient and precise nucleotide substitutions (especially CG to TA, AT to GC), while prime editing can introduce all types of point mutations and InDels at target gene without the need for double stranded breaks or donor DNA templates in rice (Hua et al. 2018; Hua and Tao 2019; Hua et al. 2020a, b; Lin et al. 2020). Genome-editing approaches involving the CRISPR/Cas9 system and its modifications including base editing and prime editing have been utilised in rice for the creation of novel alleles for various agro-morphological traits such as plant architecture, panicle architecture and grain characters, resistance to biotic stresses such as blast, bacterial blight and RTSV, tolerance to abiotic stresses such as drought, salinity and cold stress, improvement of grain, cooking and nutritional quality and herbicide tolerance (Table 3.35).

Table 3.35 Application of genome-editing for improving agro-morphological and stress tolerance traits in rice

S. No.	Target trait	Target gene(s)	Trait improvement in mutants	References
(1) Genome-editing using CRISPR/Cas9 system				
(a) Agro-morphological traits				
1.	Plant Architecture	*SD1*, *SCM1, SCM2*, *SCM3*, *OsTB1*	Semi-dwarf plant height sturdy stem, No. of tillers	Hu et al. (2016) and Cui et al. (2020)
2.	Panicle architecture	*DEP1*, *IPA1*, *Gn1a PYL1*, *PYL4*, *PYL6*	Panicle branching, grain number per panicle,	Miao et al. (2018)
3.	Grain characters	*GS3*, *GW2*, *GW5*, *GW6*, *GW8*, *OsBADH2*	Grain size, grain weight, aroma	Zeng et al. (2020), Xu et al. (2016) and Usman et al. (2021)
(b) Resistance to biotic stresses				
4.	Blast disease	*OsERF922*, *OsPi21*, *OSALB1*, *OsRSY1*	Resistance to blast disease	Wang et al. (2016), Foster et al. (2018) and Li et al. (2019)
5.	Bacterial blight disease	*OsSWEET11*, *OsSWEET13*, *OsSWEET14*	Resistance to bacterial blight disease	Kim et al. (2019), Olivia et al. (2019), Xu et al. (2019) and Li et al. (2020)
6.	Rice Tungro Spherical Virus	*eIF4G*	Resistance to Rice Tungro Spherical Virus	Macovei et al. (2018)
(c) Tolerance to abiotic stresses				
7.	Drought	*OsPYL9*, *DST*. *OsSRL1*, *OsSRL2*	leaf cuticle wax, stomatal conductance, transpiration rate, reduced stomatal density, enhanced leaf water retention, reduced number of stomata	Usman et al. (2020), Liao et al. (2019) and Kumar et al. (2020)
8.	Salinity	*OsRR22*, *DST*, *OsmiR535*	Salinity tolerance, shoot length, shoot fresh and dry weight osmotic tolerance osmotic tolerance	Kumar et al. (2020), Yue et al. (2020) and Zhang et al. (2019)
9.	Cold tolerance	*OsAnn3*, *OsMYB30*	Tolerance to cold	Shen et al. (2017) and Zeng et al. (2020)
(d) Improving rice grain quality				
10.	Physical parameters	*GS3*, *GW2*, *GW5*, *TGW6*, *OsFWL*	Increasing grain length and width	Gao et al. (2020), Xu et al. (2016) and Zeng et al. (2020)
11.	Eating and cooking quality	*OsWaxy*, *OsBEI*, *OsBEIIb*,	Decrease in amylose, high amylose, increase in total	Zhang et al. (2018), Huang et al. (2020), Wang et al. (2020),

(continued)

Table 3.35 (continued)

S. No.	Target trait	Target gene(s)	Trait improvement in mutants	References
		ISA1, *OsAP6*, *OsAAP6*, *BADH2*	soluble sugar, enhanced aroma	Sun et al. (2017) and Ashokkumar et al. (2020)
12.	Nutritional quality	*OsOr*, *OsPLDα1*, *OsFAD2.1*, *OsNramp5*	Increased β-carotene content, High linoleic acid; Low Cadmium accumulation	Endo et al. (2019), Khan et al. (2019), Abe et al. (2018), Tang et al. (2017) and Chang et al. (2020)
(2) Genome-editing using base editing approach				
13.	Grain yield and its components	*SPL14*, *SPL17*, *SPL16*, *SPL18*, *SPL17*, *SLR1*	Grain weight, size, shape, number and quality	Hua and TaoX 2019 and Hua et al. 2020a, 2020b)
14.	Nitrogen use efficiency	*NRT1.1B*	High NUE	Lu and Zhu (2017)
15.	Grain quality	*SBEIIb*, *Waxy*	High amylose	Li et al. (2017) and Xu et al. 2021a, 2021b
16.	Herbicide tolerance	*OsALS1*, *OsACC*, *OsTubA2*	Tolerance to imidazolinones, Aryloxyphenoxypropionate and dinitroaniline	Kuang et al. (2020), Liu et al. (2020) and Liu et al. (2021)
(3) Genome-editing using prime editing approach				
17.	Panicle architecture	*OsALS*, *OsKO2*, *OsDEP1*, *PDS*	Dense panicle architecture	Tang et al. (2020)
18.	Herbicide tolerance and Amylose	*OsPDS1*, *OsACC1*, *OsWaxy1*	Tolerance to imidazolinones Aryloxyphenoxypropionate and amylose content	Xu et al. (2020a, 2020b)
19.	Herbicide tolerance and Nitrogen Use efficiency	*OsALS*, *OsACC*, *OsDEP1*	Tolerance to imidazolinones, Aryloxyphenoxypropionate, high NUE	Xu et al. (2020a, 2020b)
20.	Herbicide tolerance and panicle organisation	*OsALS*, *APO1*	Tolerance to imidazolinones and high grain number	Hua et al. 2020a, 2020b

3.20 High Throughput Phenotyping in Rice

Accuracy of phenotyping has been a challenge in rice breeding research from the beginning. High throughput phenotyping is a contemporary development in crop research, which involves development of an accurate, non-destructive method for grain-trait analysis and capability of handling large populations is the key for improving different traits in rice. With the rapid development of novel sensors, imaging technology, and analytical methods, a number of platforms have been developed for phenotyping (Song et al. 2021). Modern integrated sensors (visible light, near-infrared, far-infrared, fluorescence, multispectral, laser, hyperspectral, etc.) help in acquisition of plant dynamic growth and development phenotypic datasets, which contain valuable information about plant genetics and mutations. These sensors are widely used in the analysis of plant height, chlorophyll content, LAI, disease susceptibility, drought stress sensitivity, nitrogen content, and yield of crops. Hyperspectral (such as near-infrared) photos can be combined with multi-spectral analysis to non-destructively estimate the nutrient components in rice such as amylose and other carbohydrates. The main application of phenotyping techniques is integrating a variety of phenotypic technologies to evaluate abiotic and biotic stress, as well as measure traits associated with yield potential to promote rice genetic improvement and breeding. Panicle segmentation under field conditions in rice is challenging due to the complexity of the field environment, involving illumination differentials and panicle shape deformations. Panicle-SEG-CNN model based on rice plot images have been developed for robust and accurate results for rice panicle segmentation in the field (Xiong et al. 2017). A labour-free instrument to thresh rice panicles, evaluate rice yield traits has been developed, which could calculate number of filled spikelets, kernel length and width, and 1000-kernel weight based on an RGB camera and weight sensor, with a throughput of 1440 plants per day (Duan et al. 2011). Applications have been developed for high throughput evaluation of tolerance to drought, salinity, disease detection and yield estimation in rice (Table 3.36).

Deep learning (DL), a subset of Machine Learning, is a versatile tool for providing reliable predictions of complex and uncertain phenomena by assimilating large amounts of heterogeneous data. DL have been applied for analysis of phenotyping data in a wide range of areas from organ counting to crop disease and pest recognition in rice (Table 3.37). At ICAR-IARI, Nanaji Deshmukh National Plant Phenomics Facility has been established with different sensors for high throughput imaging of plants, enabling continuous phenotyping of large number of plants throughout the developmental stages.

3.21 All India Coordinated Rice Improvement Project System

The All India Coordinated Rice Improvement Project (AICRIP) was established in 1965 at Hyderabad, with the responsibility to organise multi-disciplinary, multi-location testing for developing suitable varietal and production technologies was one

Table 3.36 Application of high throughput phenotyping techniques in rice

Application	Traits	Reference
Drought tolerance	Shoot biomass related traits, leaf stay-green-related traits, morphological traits, histogram texture traits, and texture traits derived from a gray-level co-occurrence matrix	Guo et al. (2018)
Salinity tolerance	Transpiration rate, plant growth rate, total shoot area, senescent shoot area	Hairmansis et al. (2014) and Al-Tamimi et al. (2016)
Diseases detection	Colour and morphology, transpiration rate, photosynthetic rate, spectral vegetation indices	Zhou et al. 2014a, 2014b
Direct yield estimation	Panicle traits, spike number, ear density, grain number and size, 3D spike morphological phenotypes	Xiong et al. (2017) and Duan et al. (2011)
Indirect yield estimation	Canopy height and structure; leaf area index	Zhou et al. (2017)

Table 3.37 Applications of deep learning (DL) in high throughput phenotyping in rice

Broad areas	Key issues	Preferred DL model	References
Organ identification and counting	Rice detection and counting in the field	An improved R-FCN model	Zhou et al. (2019)
	Flowering panicle detection and heading date estimation	CNN (ResNet-50)	Desai et al. (2019)
	Leaf and panicle segmentation, and leaf to panicle ratio (LPR) calculation	FPN-mask	Yang et al. (2020)
Crop seed variety classification	Identification and differentiation of ten major varieties of basmati rice based on seed images	iRSVPred	Sharma et al. (2020)
Crop yield prediction	To quantify rice kernels per panicle	Faster RCNN (ResNet101)	Wu et al. (2019)
Weed and crop recognition and segmentation	Rice seedling and weed image segmentation at the seedling stage in paddy fields	FCN (SegNet)	Ma et al. (2019)
Crop diseases and pest recognition	To construct video detection system for plant diseases and pests	faster-RCNN	Li et al. (2020)

CNN convolutional neural networks, *FCN* fully convolutional network, *FPN* feature pyramid networks, *iRSVPred* a web server for artificial intelligence based prediction of major basmati paddy seed varieties, *RCNN* region-based convolutional neural network, *ResNet* residual network, *R-FCN* region-based fully convolutional network

of the milestones in the history of rice research in India. The coordinated testing programme under AICRIP was envisaged with seven key features (Freeman and Shastry 1972), which includes:

3.21.1 System of Coordinated Testing

The system involves planning by the group conducting the trials and those interested in testing the performance of their entries in the trials, to organise the trials during annual workshop sessions, centralised pooling of seed for trials and dispatch by the coordinating centre, well-defined trial plans from layout to data collection forms, assembly of data from various centres for compilation and calculation of national and zonal yields and presentation of data in timely progress reports issued annually.

3.21.2 Intent of State and Central Institutes Towards Achieving the Common Objective

The development of a working memorandum of understanding between the state centres and the coordinating centre. Basic to the effectiveness of a written memorandum is the 'intent' of the parties involved and the spirit of cooperation that exists among them. The cooperation had been achieved to a great extent before a memorandum of understanding was evolved, since both state and centre agencies usually have one common objective of rice improvement.

3.21.3 Designing Research for Solving Problems in Rice Production

The research programmes at the coordinating centre and other national and state centres are designed to solve problems of rice production that will provide early pay-off in farmers' yields and national production.

3.21.4 Emphasise the Flexibility of Action

The programme emphasises flexibility of action, with more flexibility than normally exists in governmental agencies is required for a coordinated programme to function effectively.

3.21.5 Team Spirit Among Rice Workers

The team spirit among rice workers has developed from the exchange of seed materials, sharing of ideas in workshops, and information in progress reports.

3.21.6 Multi-disciplinary Approach with Inter- and Intradisciplinary Exchange of Ideas

A multi-disciplinary approach to the study of the rice crop is used. Some rice problems such as insects and diseases need to be tackled from several angles, so efforts of the workers in different disciplines at different locations focus on the same problem are coordinated. Without the cooperation of breeders on the one hand, and of pathologists and entomologists on the other, a considerable range of host-plant resistance could not be developed. Furthermore, it aids intradisciplinary coordination in studies of the disease organism or of the insect concerned. While more coordination is required to realise benefits more quickly in both phases, the progress made in these two aspects of coordination is exemplary. Coordination was pursued more vigorously to attain rapid progress in solving national problems in rice.

3.21.7 Sharing of Resources for Identifying Productive and Stable Genotypes

The merit of the multi-location approach helps in testing a large number of entries across several locations. Limitations in resources of local experiment stations did not provide a means to quickly evaluate materials or to create populations large enough to permit the identification of the best strain for a locality. Under the coordinated system, the crossing done at many locations creates enough progeny for effective selection, and evaluation at several locations allows potential varieties to be identified regardless of their origin. More the kinds of environment under which a variety has been proven, the more stable its performance is likely to be when grown by farmers in many different environments.

AICRIP was started with 22 centres (19 main and 3 testing centres) with 7 zonal centres and 12 regional centres. Currently, there are 45 funded centres and 99 voluntary centres. AICRIP capitalised upon the available research infrastructure in different states of India for multi-location testing and successfully introduced a national perspective in technology development and testing. AICRIP was later elevated to the status of Directorate of Rice Research (DRR) from April 1975, with the added mandate of pursuing research on irrigated rice. The Directorate was upgraded to ICAR-Indian Institute of Rice Research (ICAR-IIRR) in December 2014.

Realising the complexities in the rice production ecosystem, the prevalence of insect pests and diseases as well as grain quality requirements of rice consumed, AICRIP evolved need-based programmes/trials over the years to identify improved genotypes with better yield potential along with appropriate crop management practices for release as varieties. Initially, emphasis was on improving yields through improved plant type and management practices largely for irrigated ecosystems, which led to the development and release of a large number of short statured high yielding varieties (HYVs), which heralded the ‘Green Revolution’ in India. Following the success in improving yields, priority was given for sustaining the yield and quality improvement. As a result improved rice varieties with inbuilt resistance to

major biotic stresses and desirable grain and cooking quality were developed, which includes the successful development and release of the first semi-dwarf high yielding Basmati rice variety (Gopalakrishnan et al. 2008).

The 1990s witnessed efforts in developing hybrid rice technology and multi-location testing to validate their superiority over varietal checks at least by 10%, and also non-Basmati grain quality to meet the export markets. At the beginning of the century, trials to address soil stress problems and aerobic trials were initiated for developing and testing genotypes for water limited environments and hill trials for testing genotypes with cold tolerance. With advances in varietal development through effective use of modern biotechnological tools, guidelines for rapid testing and release of near isogenic lines (NILs) in the background of popular rice varieties incorporated with genes governing resistance to biotic and abiotic stresses have been developed. Separate trials for testing of rice NILs developed through marker-assisted selection (MAS) have been initiated in 2008 and biofortification trials for testing genotypes biofortified with micronutrients such as zinc, iron along with protein started since 2012.

3.22 Varietal Testing in AICRIP Trials

AICRIP led by ICAR-IIRR, Hyderabad, enables testing of promising elite materials (varieties, hybrids, etc.), thereby helping identify the stable, high-yielding, or superior genotypes suited for different agro-climatic conditions. A total of 35 varietal trials and 4 hybrid trials are being conducted in different ecologies, namely Irrigated, Rainfed Upland (direct seeded), Rainfed Shallow lowland, Semi-deep water, Deepwater, Hills, Saline alkaline and Boro. The irrigated ecology trial is further classified into Early transplanted, Mid-early, Medium and Late based on the duration of the crop season. Additionally, trials are conducted for grain types including Basmati, Aromatic short grain and medium slender grain. Separate biofortification, low phosphorus, low nitrogen, new plant type (NPT) and aerobic trials are also conducted along with NILs trials for Drought, Submergence, Combined stresses, Bacterial blight and blast diseases. These trials are being conducted at 123 locations across seven zones in 28 states and 2 union territories across India (Table 3.38). Among all these trials, Basmati trials are restricted to the regions notified as Basmati Geographical Indication (GI) vide GI No. 145 of the Geographical Indication Registry, Government of India through certificate No. 238 dated 15.02.2016, spanning seven states, namely Delhi, Haryana, Punjab, Uttarakhand, Himachal Pradesh, Jammu and Kathua districts of Jammu and Kashmir, and 27 districts from western Uttar Pradesh.

Thus, the varieties are identified and released, not only for the zones/regions from where they have been bred but also for other regions if they are found to perform in different zones across the countries. This also helps in complementing the efforts of the centres in respective zones, where the breeding programme is either not strong or still in its infancy and caters to the needs of the specific region. In the process, the best material gets identified for release in different regions/states.

Table 3.38 Details of zonation and the areas covered under each zone in the AICRIP trials

Zone No.	Name of the zone	States
Zone I	Hilly zone	Hilly regions from the states of Himachal Pradesh, Manipur, Nagaland, Sikkim, Meghalaya, Hilly regions of Jammu and Kashmir, Uttarakhand, West Bengal and Karnataka
Zone II	Northern zone	Northern India including Delhi, Haryana, Punjab, Rajasthan, Plains of western Uttar Pradesh, plains of Uttarakhand and Jammu and Kashmir
Zone III	Eastern zone	Eastern India comprising of Odisha, Bihar, Jharkhand, West Bengal, parts of eastern Uttar Pradesh
Zone IV	North Eastern zone	Northeastern India including Assam, Manipur and Tripura
Zone V	Central zone	Central India including Madhya Pradesh, Chhattisgarh, parts of Maharashtra
Zone VI	Western zone	Western India including Gujarat, Goa and parts of western Maharashtra
Zone VII	Southern zone	Southern India including Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, Kerala, Puducherry, Andaman and Nicobar Islands

AICRIP adopts a unique model that facilitates joint planning and implementation of multi-location testing along with an exchange of breeding and germplasm material. The 'National Evaluation System' is a three-tier system involving 3 years of testing in the trials including Initial Varietal Trial (IVT) for 1 year followed by 2 years of advance varietal trials (AVT 1 and AVT 2). Every entry nominated the first year of trial, either IVT or AVT-1 NILs are assigned an initial evaluation trail (IET) number. Issuing IET numbers to cultures was first initiated in AICRIP, which is now adopted by coordinated testing projects in other crops as well. The entries in the respective trials, which produce consistently superior performance over the best checks are identified. Under the present system, a given entry needs to exhibit 5% yield superiority over the best check either on an overall or regional basis for varieties. For hybrids, there are four Initial Hybrid Rice Trials (IHRT), namely IHRT-E (Early duration <120 days), IHRT-ME (mid-early duration, 121–130 days), IHRT-M (Medium duration, 131–140 days) and IHRT-MS (Medium Slender Grains) are constituted based on duration and grain type, respectively. The IHRT comprises only the experimental hybrids and the corresponding checks, while the hybrids promoted are tested in AVT 1 and AVT 2 trials under irrigated transplanted conditions, and the promising hybrids are compared with the promising elite inbred lines also. The hybrid entries should exhibit 10% yield superiority over the best varietal check and 5% superiority over the hybrid check. Parallelly, they are also screened for resistance to major insect pests and diseases at hot spot locations, as well as controlled conditions with artificial inoculation for 2 years in NSN 1 (National Screening Nurseries 1) and NSN 2 to assess their reaction to evolving pests and diseases. The entries were also tested for their grain quality and agronomic performance (Babu et al. 2016).

The promising genotypes identified to perform superior in more than two states where the performance in the third or final year of testing either overall/regional/

states, based on the extensive evaluation for 3 years in AICRIP trials. The concerned breeder submits the proposal for identification of the said promising variety in the prescribed proforma along with all the supplementary data and relevant information for consideration by the Variety Identification Committee (VIC) chaired by either Deputy Director General (Crop Sciences), which meets annually during the Annual Rice Group Meeting. Alternately, the genotypes with superior performance in a specific state either through AICRIP or the State trials are submitted for release by the State Variety Release Committees (SVRC). The superior test entries identified in the crop workshop/group meeting or the SVRC are then submitted for release and notification in the prescribed proforma, along with their DNA fingerprinting data in comparison with a check variety, Indigenous Collection (IC) number issued by ICAR-National Bureau of Plant Genetic Resources, New Delhi, by submitting 100 grams of seeds of the candidate variety and good quality photographs for consideration by the central sub-committee on crop standards, notification and release of varieties (CSCS&NRV). The proposals are thoroughly discussed by the CSCS&NRV in the meeting, chaired by the Deputy Director General (Crop Sciences) and the committee recommends the release and notification of the varieties either as Central or as State releases, which is then notified in the Gazette of India under the section 5 of the Seeds Act, 1966 (54 of 1966) by the Ministry of Agriculture and Farmers Welfare (Department of Agriculture, Cooperation and Farmers Welfare). The process for varietal testing, identification and release is depicted schematically in Fig. 3.14.

AICRIP is a continuously evolving system by taking into consideration the technological developments from time to time and the need in devising testing protocols for new rice varieties in different rice ecologies through multi-location testing. Considering the fact that near isogenic trials (NILs) are developed in the background of popular rice varieties, which have already been tested in the AICRIP trials, NILs trials have been initiated for fast tracking the products developed through marker-assisted backcross breeding. The NILs are tested only for 2 years by testing in AVT 1 NILs and AVT 2 NILs, along with the recurrent and the donor parents for validating the trait, which has been incorporated through MAS.

3.23 Seed Supply Chain in Rice

Seed is the basic and the most critical inputs for sustainable rice production. Seed is an important input affecting rice production and it contributes 15–20% of the crop yield. Therefore the quality of seeds plays a very important role in bridging the gap between potential yield and realised yield. While breeding addresses the varietal replacement rates by producing improved varieties with better productivity, seed replacement rate addresses the adoption of these improved varieties with quality seeds. Seed system encompasses the framework of institutions (either public/private)/and farmers groups and their role in seed multiplication, processing, quality assurance and marketing of seeds. There exist formal, informal and hybrid (formal and informal) seed systems in rice. The formal seed system is a systematic,

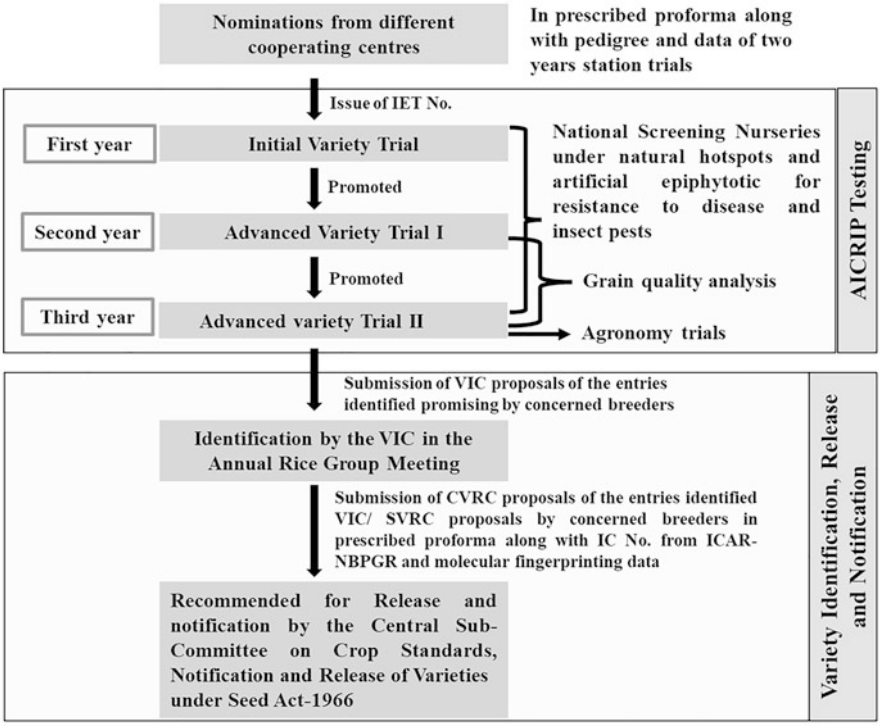

Fig. 3.14 Testing the nominations under the AICRIP system, identification, release and notification of a Central Release rice variety

well-organised system involving a series of activities leading to the production and supply of certified seeds of varieties notified under section 5 of the Seeds Act, 1966 (54 of 1966). It starts with the development and release of varieties/hybrids, and their systematic maintenance guided by the principles of maintaining varietal identity, genetic purity, optimal physical, physiological and sanitary quality (Reddy et al. 2007). At present, the formal seed delivery system in rice consists of 2 national corporations, namely National Seed Corporation and State Farm Corporation of India, 16 State Seed Corporations, 25 Seed Certification Agencies, 2 Central and 132 State Seed Testing Laboratories in addition to 187 private registered seed companies.

The Indian Council of Agricultural Research (ICAR) is mandated to produce nucleus and breeder seed as per the indent received from the Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare (DAC & FW), Government of India. The nucleus seed of the rice varieties is maintained as per standard procedures by the respective centres/institutes from where the rice variety/hybrids are released. The breeder seed is produced based on indent from different agencies. The breeder seed production is demand driven and the indents received from National Seed Corporation, State Seeds Corporations,

State Farm Corporation of India, other public as well as private sector organisations such as Seed Association of India, National Seed Association of India, etc. are consolidated by the seeds division of DAC, which is forwarded to ICAR. In the ICAR, the Crop Science Division coordinates the breeder seed production of rice in India, with the cooperation of various State Agricultural Universities (SAUs) and public sector crop-based institutes (NAAS 2018). The breeder seed indents of rice varieties received from ICAR are allocated for production to the respective institutes/SAUs and/or their centres after thorough discussion at the Annual Rice Group Meeting held every year in April/May. Based on the allocation of breeder seeds to be produced for different rice varieties, the Breeder Seed Proforma I (BSP-I) is prepared and sent to the respective centres by the Crop Coordinator of rice, ICAR-IIRR, Hyderabad, with the details of the quantity of breeder seed allotted for production for each variety/hybrid by the institute/SAU, which is also uploaded in the seed net portal (https://seednet.gov.in). Upon receiving the BSP-I proforma, the seed production is planned at the respective centres and the scientist responsible for the production of breeder seed plans and takes up the planting of the rice varieties based on the indent and prepares the BSP II proforma, indicating the name of the rice variety and the area planted, date of planting, under each variety for breeder seed production based on the indent.

When the rice crop is at flowering, monitoring of the crop is undertaken with a monitoring team comprising of originating breeder/sponsoring breeder along with one representative each from the National Seed Corporation and the respective State Seed Certification Agency for inspecting the breeder seed production plots. Based on the inspection by the monitoring team, the inspection report is prepared in the BSP-III proforma indicating the name of the varieties, area sown, location of the field, authority under the date of BSP-I, date of proforma BSP-III report of the monitoring team which is signed by every member of the monitoring team is distributed to ADG(Seeds), ICAR; Deputy Commissioner (Seeds), DAC; Director, ICAR-IIRR, Hyderabad; Director, ICAR-IISR, Mau; the competent authority of the institute/SAU, Nodal officer (Seeds), Chief Seed Certification Officer of the state and the area manager of National Seed Corporation. After the harvesting, threshing, processing (in case processing is not complete, estimated likely quantity of processed seed is indicated in BSP IV) and cleaning of the seeds of different rice varieties, the scientist responsible for the production of breeder seed production prepares the BSP IV indicating the quantity of breeder seed produced for each rice variety against the quantity allocated and distributes to all the concerned as in BSP III. Based on the allocation, the respective agencies lift the breeder seed produced for a given rice variety and finally, the BSP-V is filed indicating primarily lifting and non-lifting position of breeder seed by various agencies. The breeder seed is used to produce foundation seeds (either one or two stages as FS-I and FS-II), which are then utilised for the production of certified seeds, which are distributed to the farmers. Besides certified seeds, significant quantities of truthfully labelled seeds of different rice varieties are also produced under the supervision of the breeders/seed scientists which are distributed to the farmers. A schematic diagram presenting the

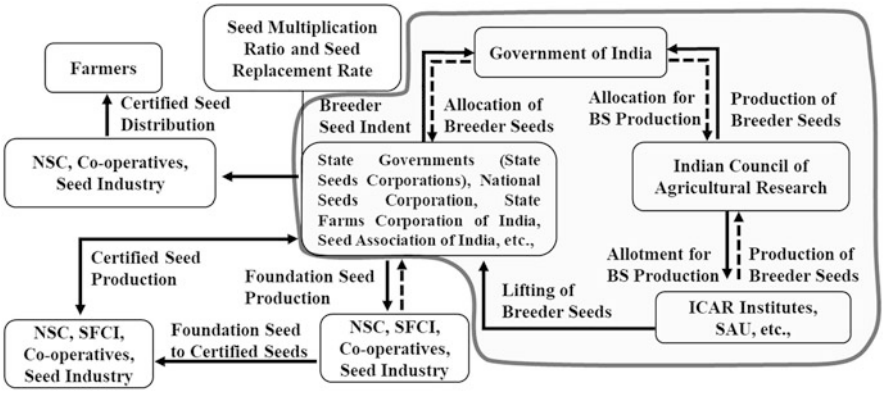

Fig. 3.15 The seed supply chain for rice varieties

interlinkage between different organisations involved in the production of quality seeds is presented in Fig. 3.15.

Of the 1436 rice varieties released in the country as many as 320 rice varieties are in the breeder seed production (BSP) chain. Among these, 63 varieties have maximum adoption with the farmers within one state or across several states in terms of their spread. Based on their popularity among the farmers, consumers, some of these varieties have achieved the status of mega-varieties. These include Jaya, IR36, IR64, MTU7029 (Swarna), BPT5204 (Samba Mahsuri), MTU1010 and Pusa Basmati 1121.

3.24 Emerging Challenges in Rice Breeding

Sustaining rice productivity in the face of climate change is one of the major challenges in rice production not only in India but also across the world. Broadly, the key issues to be addressed in rice research are the decline in arable land, increasing irrigation water scarcity, global climate change, shortage of agricultural labours, high labour wages for cultural operations and increasing consumer demand for high-quality rice (which often comes from low-yielding varieties). The major problems confronting rice production in India and other countries across the world is the narrow genetic background and yield plateau. The increased incidence and rapid changes in pest and disease populations as well as emergence of hitherto minor pests and diseases as major ones is another major challenge for rice improvement. Therefore, the rice improvement needs a paradigm shift from enhancing productivity to profitability which considers several factors including:

(a) Economising the cost of cultivation through development of rice varieties with inbuilt resistance to biotic stresses (major insect-pests, diseases and weeds) as well as tolerance to abiotic stresses.

(b) Improving the grain and nutritional quality and value addition.
(c) Development of rice hybrids for productive environment and enhancing area under-hybrid rice cultivation.
(d) Improving the water productivity and sustaining soil health by developing technologies suited for aerobic/limited water and nutrient environments including herbicide tolerant rice varieties.
(e) Developing varieties suited for managing the rice value chain, effectively.

Overall, the focus of rice breeding should be on the development of improved rice varieties with high yield potential with resistance to major diseases and insects, and to major abiotic stresses such as drought, submergence and heat, and better grain and nutritional quality. To do this effectively with limited resources, needs the adoption of modern tools and techniques enabling rapid and precision breeding for improvement of rice.

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Maize Breeding

4

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Abstract

Maize has emerged as an important crop for food, feed and various applications. Utilization of hybrid technology has resulted in a quantum jump in grain production worldwide. However, ever-increasing population pressure coupled with climate change warrant many fold increase in productivity in a shorter time frame. Emergence of newer diseases and insect-pests further pose a great challenge to even sustain the production. Malnutrition has become a major health issue, thereby causing severe socio-economic losses. However, discovery of new genes and quantitative trait loci (QTLs) for higher grain yield, plant architecture, resistance/tolerance to various biotic and abiotic stresses, nutritional quality and specialty traits, and also availability of suitable donors provide great opportunity to breed improved hybrids with higher productivity, better resilience to biotic and abiotic stresses, and higher nutritional quality. Genomics-assisted breeding, doubled haploid and gene editing technology provide great impetus to further accelerate the breeding cycle. Here, we discussed the present status, opportunities and challenges in maize breeding.

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_4

Keywords

Zea mays · Corn · Genomics · Markers · Gene · QTL · Specialty traits

4.1 Introduction

Maize assumes great significance as food, feed and raw material for diverse industrial applications, thereby serving as a source of livelihood to millions of people worldwide (Prasanna et al. 2020a; Hossain et al. 2021). Globally, maize is produced to a volume of 1148 million metric tons with cultivation of 197 million hectares area (FAOSTAT 2019). Maize is an important cereal crop in as many as 169 countries across North America, South America, Africa, Asia and Europe. Maize grains are consumed in various forms such as flat bread, porridge, boiled and roasted grains/cobs (Hossain et al. 2019a). Besides, maize possesses diverse usage as food in the form of sweet corn, baby corn, popcorn, waxy corn, high amylose maize and high oil maize (Hossain et al. 2019b; Pal et al. 2020; Chhabra et al. 2021). Maize, together with rice and wheat, provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Shiferaw et al. 2011). Considering the share of calories among all-staple crops, maize contributes 61% in Mesoamerica, 45% in Eastern and Southern Africa, 29% in the Andean region, 21% in West and Central Africa and 4% in South Asia (Shiferaw et al. 2011). The contribution to protein by maize is also in the similar tune (Mesoamerica: 62%, Eastern and Southern Africa: 43%, Andean region: 28%, West and Central Africa: 22% and South Asia: 4%). Maize grains are the principal component of animal feed, especially in the poultry and piggery industry (Gao 2002; Panda et al. 2013; Rajasekhar et al. 2020). Besides, it serves as a source of raw material to corn syrup, emulsifier, textile, paper and adhesive industries (Bao et al. 2012; Devi et al. 2017). To meet the requirement of ever-increasing population and growing animal feed industry, the demand for maize will be doubled by 2050 in the developing world (Rosengrant et al. 2009).

Gradual progression from open pollinated (OP) varieties to single cross hybrid technology helped to realize the highest potential for grain heterosis (Andorf et al. 2019). Furthermore, modification in plant architectural traits from wide leaf angle to shorter leaf angle enabled the maize hybrids to grow under high density, thereby further enhancing the grain yield. Maize production is increased by two-fold during the past 40 years due to enhanced grain yield resulting from improved crop cultivars coupled with the better responsiveness to fertilizer, water and pesticides (Evenson and Gollin 2003). However, climate change quite often increases frequent crop failures and decreases grain production. Lack of well-distributed rainfall pattern would further lead to water scarcity and eventually occurrence of drought stress which may lead to complete failure of the crop (Brown and Funk 2008; Funk and Brown 2009). Increase in heat has become a major issue to healthy crop production as each degree day spent above 30 °C reduces the final yield by 1% under optimal rain-fed conditions and by 1.7% under drought conditions (Lobell et al. 2011).

Incidence of biotic stress has also become a major limiting factor to maize production.

Of various biotic stresses, fall army worm (FAW) has recently caused havoc throughout the world, causing as much as complete loss of crop (Israni et al. 2020). Considerable loss has also been attributed to diseases like grey leaf spot (GLS) and maize lethal necrosis (MLN). Post-harvest loss up to 80% has been reported in the tropics due to infestation by grain weevils and grain borers (Hossain et al. 2007; Zunjare et al. 2014, 2015a, b, c, 2016). Malnutrition caused due to consumption of unbalanced diet has emerged as one of the major health concerns particularly in the developing and under-developed world (Bouis et al. 2019; Virk et al. 2021). Globally, around two billion people suffer from malnutrition (Global Nutrition Report 2020). Nearly 45% of deaths among children under age 5 are linked to malnutrition (Global Nutrition Report 2018). Globally, 149.2 million (22.0%) children (<5 years) are stunted, while 45.4 million (6.7%) children (below 5 years) possess child wasting (UNICEF-WHO-WB 2021). Malnutrition contributes to loss in 11% gross domestic product (GDP) in Asia and Africa, while malnutrition in all its forms could cost society up to US$3.5 trillion per year (Global Nutrition Report 2016, 2018). Among various avenues, breeding for improved maize hybrid would remain as the most sustainable and cost-effective avenues to combat the combined challenges of increasing demand, frequent occurrence of biotic and abiotic stresses and nutritional disorders.

4.2 Origin of Maize

Maize, as a crop is documented to be grown for 10,000 years (Schnable et al. 2009). Adult plant morphology of maize is very unique among all cereals, having single culm, separate male and female inflorescence and human-dependent seed dispersal, which are thought to have been evolved through few major mutations (Doebley 1992). Morphological resemblances and cytological studies have confirmed its close relatedness to teosinte (Doebley and Stec 1991). Role of humans in developing and establishing maize as a crop species has also been documented because of presence of great variability in maize and continuous selection by ancient people (Yu and Buckler 2006). Establishing the scientific theory of origin and domestication of maize was a daunting task because of absence of very close and morphologically similar plant species (Wang et al. 2017a). Several theories regarding origin of maize have been proposed and described below.

4.2.1 Tripartite Hypothesis (Mangelsdorf and Reeves 1938)

This hypothesis states that maize originated from an unknown plant which was having similar ear morphology. Such unknown plant is now extinct. Accordingly, teosinte is the offspring of maize and *Tripsacum*.

4.2.2 Catastrophic Sexual Transmutation Hypothesis (Iltis 1983)

According to this hypothesis, sudden sexual transmutation in teosinte was responsible for development of maize plant. The ear of maize is result of morphological change in central spike of teosinte resulting from a phenomenon called "genetic assimilation".

4.2.3 *Tripsacum–Zea Diploperennis* Hypothesis (Eubanks 1995)

It is the modified version of tripartite hypothesis. According to this hypothesis, maize plant evolved from the cross between *Zea diploperennis* and *Tripsacum dactyloides*.

4.2.4 Teosinte Hypothesis (Beadle 1939)

According to this hypothesis, ancient people cultivated teosinte and selected the morphologically useful plant forms. Maize plants arose due to major mutations in few loci of teosinte.

Of these, teosinte hypothesis is the most accepted and validated, using modern genomics associated studies (Doebley 1990; Doebley and Stec 1991; Dorweiler et al. 1993; Matsuoka et al. 2002; Wang et al. 2005; Dong et al. 2019). Beadle (1939) had predicted teosinte as a sole progenitor of maize and carried out a planned experiment using a very large recombinant population (F_2) derived from crossing teosinte with a primitive maize landrace. He reported that at least five major mutations were responsible for converting teosinte into maize. Later on, during the 1990s, a research group led by Prof. J.F. Doebley at University of Wisconsin, Madison, United States, had redeveloped and studied the recombinant mapping population using the same parent as done by Prof. Beadle in the 1930s. They also confirmed the role of at least five major mutations in developing maize from teosinte in a series of selection events practiced by ancient cultivators. Several studies have now been carried out to understand the molecular basis of domestication of maize (Dong et al. 2019).

The mutation present in *teosinte glume architecture1* (*tga1*) gene found in chromosome 4S was responsible for development of naked kernels (Dorweiler et al. 1993). This gene encodes a SBP (SQUAMOSA promoter binding protein-like) family transcription factor. A single nucleotide polymorphism (SNP) at position 18 of the ORF in this protein caused a gain-of-function mutation acting as transcriptional repressor responsible for naked kernel in maize (Wang et al. 2015). Similarly, single culm developed in maize with respect to tillering habits in teosinte was found to be governed by *teosinte branched1* (*tb1*) gene (Doebley et al. 1997). The gene *tb1* is a TCP-domain containing transcription factor whose higher expression in maize reduces the branching and tillering habits. The higher expression is due to the presence of "Hopscotch" and "Tourist" transposon at ~58–69 kb upstream of coding region (Studer et al. 2011). Similarly, other genes such as *grassy tiller1* (*gt1*),

enhancer of tb1.2 (*etb1.2*), *barren stalk1* (*ba1*), *barren stalk2* (*ba2*) have been found to be associated with branching habits in teosinte and maize intercross populations (Prakash et al. 2020).

4.3 Species Distribution

Genus *Zea* consist of five species, that is, *Zea diploperennis* (diploperennial teosinte, native of Western Mexico), *Zea luxurians* (Florida teosinte or Guatemalan teosinte, native of Mexico, Guatemala and Honduras), *Zea mays* (Southern Mexico, include cultivated maize, grown across the world), *Zea nicaraguensis* (Nicaraguan teosinte), and *Zea perennis* (perennial teosinte, native of western Mexico). Among these, *Zea mays* include four sub-species *Zea mays* ssp. *mays* (cultivated corn), *Zea mays* ssp. *parviglumis* (*Balsas* teosinte), *Zea mays* ssp. *mexicana* (Mexican teosinte), *Zea mays* ssp. *huehuetenangensis* (found on western highlands of Guatemala) (Hossain et al. 2016; Prakash et al. 2020). Among all the species documented under the genus *Zea*, maize (*Zea mays* ssp. *mays*) is the only one which is being cultivated. The other sub-species under *Zea mays* along with other species under *Zea* is collectively considered as 'teosinte'. It is now well established that maize is originated from *Zea mays* ssp. *parviglumis* (also known as balsas teosinte). All the teosinte species are commonly found in Mexico and northern part of South America.

However, maize has lost many variations present in these teosintes and acquired new variations arising out of mutations. Doebley (1990) has estimated 25% loss of gene diversity in maize from balsas teosinte. Later on, through various mutation and selection by indigenous people, several plant forms of maize were developed, such as waxy corn, popcorn, sweetcorn, pod corn (Smykal et al. 2018). Cultivated maize has spread to North America, Europe, Asia, Australia and Africa by European travelers of the colonial era. After introduction of maize into a different continent, the variations present in them had led to adaptation to local conditions, and temperate and tropical varieties began to evolve (Prasanna et al. 2010).

4.4 Types of Grains

Maize is of the most produced grain crops on this planet, and this has only been possible due to the presence of enormous diversity and mutant forms, making it suitable to be cultivated across environments and ecological conditions (Prasanna et al. 2012). The various maize forms used in cultivation are given below.

4.4.1 Flint Corn (*Zea mays* ssp. *mays* var. *indurata*)

Complete kernel including outer portion is made up of hard starch.

4.4.2 Dent Corn (*Zea mays* ssp. *mays* var. *indentata*)

Only kernel is made up of hard starch. It has dent-like structure on top of the grain.

4.4.3 Sweet Corn (*Zea mays* ssp. *mays* var. *saccharata*)

Sugar content is high at milking stage (up to 20% as compared to other maize types).

4.4.4 Flour Corn (*Zea mays* ssp. *mays* var. *amylacea*)

Kernel is made up of soft starch, and it is easy to grind and used in making chappatis.

4.4.5 Popcorn (*Zea mays* ssp. *mays* var. *everta*)

Small size kernel which burst and turn inside out after heating. The bursting during heating is because of formation of steam in the grain.

4.4.6 Waxy Corn (*Zea mays* ssp. *mays* var. *ceratina*)

These are waxy grains which are sticky after cooking. The waxiness of kernel is due to the presence of low amylose and high amylopectin content (almost 100%).

4.4.7 Pod Corn (*Zea mays* ssp. *mays* var. *tunicata*)

These are podded grains covered inside glumes.

The various forms of maize with specialized usage are a type of mutations selection occurring in the natural population. Most commonly used corn is of flint type. Sweet corn has evolved through mutation in genes regulating sugar metabolism, such as *sugary1* (*su1*) and *shrunken2* (*sh2*). Waxy corn is developed by mutation at *waxy1* (*wx1*) locus in maize. Similarly, pod corn is having mutation at *tunicate* locus (Yadav et al. 2015).

4.5 Floral Biology

Maize is a monoecious type of plant with separate female and male flowers borne on single plant. The main shoot of plant terminates into male inflorescence referred to as 'tassel', while the female inflorescence is called 'silk' that develop into the 'ear'. The modified leaf sheaths of several layers that surrounds around ear are known as 'husks'. Being protandrous in nature, male flowers mature before female flowers

with a gap of 2–3 days. The genetic mechanisms of monoecism and protandry are the key factors for anemophily (pollination due to wind) in maize (Kumar et al. 2011; Tripathi et al. 2011). There are two functional florets in each of the male flower spikelet, and each floret is composed of pair of thin layers (lemma and palea), three anthers, one rudimentary pistil and two lodicules. Kiesselbach (1949) has reported about 2000 to 7500 pollen grains per anther per floret.

Nearly 14×10^{-6} pollen grains can be produced by each plant tassel considering mean of 7000 anthers/tassel and 2000 pollen grains/anther (Kiesselbach 1949). The pollen grains are very small, lighter in weight and easily carried away by wind. The female flower formed in the row with development progresses toward the tip of the ear. The elongated style into thread-like structure known as 'silks' covered with numerous small hairs on which pollen grains fall leading to the base of the silk where fertilization occurs. The pollen grains germinate and enter the embryo sac within 12–28 h. Self- and cross-pollination are two important practices in maize breeding. In the breeding nursery, hand pollination is usually followed for accomplishing self- and cross-pollination, thus it is a labour and cost-intensive process for maize breeders. Self-pollination is required for development and maintenance of inbreds varieties, while cross-pollination is essential to generate variability and hybrid seed production.

To accomplish self-pollination, ear of plant is covered with 'silk bag' before the silk emerges out. The fresh pollen grains (yellowish in colour) from tassel of same plant are collected in the morning through bagging tassel with a 'tassel bag' wrapped the previous day evening. The collected fresh pollen grains are placed on silk hairs of the same plant with no outside exposure to ensure proper self-pollination. For cross-pollination, pollen grains collected from desired male parent is transferred to well-covered silk of desired female parent. The proper record of parents involved in crossing, date of crossing, and breeding scheme is to be clearly written with water-proof pencil on the tag fixed on the female parent. The plant-to-plant crossing is generally carried out for generation of genetic variability and introgression of genes. However, the large-scale hybrid seed production, fixed ratio of female rows to male rows sown in the field. Tassel of female plant is removed before initiation of pollen shedding, so that the pollen of male parent is used in the seed production plot. Removal of tassel as soon as it emerges out of flag leaf is called 'detasseling' and is necessary to avoid selfing in female rows.

4.6 Varietal Development

Most of the U.S. maize production traces to the U.S. Corn Belt dent corn, which was the product of hybridization between two corn races, Northern flints and Southern dents (Anderson and Brown 1952). Decades before the Mendel's laws of inheritance, farmer-breeders in the United States cherished the benefits of out-crossing, and by 1813, new cultivars were being developed through controlled pollinations. Lorain (1814) was the first to describe the effect of crossing dent and flint corns, and also elaborated the potential of crossed seeds for farmers. Later, corn producers and

breeders crossed the Northern flints corn and Southern dents corn to combine the favourable traits of both, and several new varieties of corn were developed for a quite long time. The new varieties were somewhat high yielding, early in flowering, more tolerant to drought and were more adapted to local environments (Anderson and Brown 1952). Sturtevant (1899) documented the popular use of 323 OP varieties of dent corn and 69 varieties of flint corn. Robert Reid crossed 'Gordon Hopkins', a semi-gourd dent corn and 'Little Yellow', a native Indian flint corn, and developed a new cultivar known as Reid's Yellow Dent (Troyer 1999). The OP varieties developed by farmers and corn breeders dominated the corn production in the U.S. Corn Belt for over 50 years. The OP varieties were the source material for the development of inbreds initially by successive generations of self-pollination/inbreeding.

4.7 Hybrid Development

In the early twentieth century, farmer breeders, U.S. public sector breeders and private seed producers bred OP varieties but with little gain in maize yield (Hallauer 2008). W.J. Beal was the first to use detasseling in crossing plots to make hybrids in 1881 (Beal 1881). Shull (1908) proposed that the maize yield can be increased by selfing of parental lines over several cycles to develop inbreds, and then crossing between inbreds to generate hybrids, the phenomenon termed as 'hybrid vigour', or heterosis (Crabb 1947). At the same time (1909–1912), Edward M. East also discovered the hybrid vigour at Harvard University's Bussey Institution in association with Connecticut Agricultural Experiment Station. The discovery of hybrid vigour proved to be a turning point for U.S. maize production and economy (Crabb 1947). Though the potential of hybrid breeding was known since eighteenth century, no commercial hybrid was developed until 1921. The reasons behind non-popularity of single cross hybrids were the parental inbreds with poor vigour, very low yield, low weed competitive ability and susceptibility to corn pests (Hallauer 2008). Furthermore, low yield of parental inbreds increased the cost of hybrid seed production.

The major breakthrough came in hybrid maize breeding when Donald F. Jones created double-cross hybrid 'Burr-Leaming' in 1918, which was first produced commercially in 1921 (Jones 1927). The use of double-cross hybrids greatly reduced the cost of hybrid seed production, making the hybrid technology feasible for farmers. A double cross hybrid is essentially the progeny of two single cross hybrids. The first-generation hybrids overcame the weaknesses of inbreds as they gave significantly higher seed yield with fewer diseases, insect-pest infestation and weed issues. At the same time that double cross hybrids were produced and commercialized, a number of technological advances were adopted in corn production viz., (1) use of chemical fertilizers such as inorganic nitrogen fertilizer since 1945 (Gardner 2009), (2) mechanization which facilitated uniform harvesting and other cultivation practices, thus saved time and labour inputs, and (3) improved agronomic practices which significantly improved maize productivity in the United States (Crow 1998; Troyer 2003).

Adoption of these technologies resulted in rapid shift of farmers toward double cross hybrids. In 1940, about 50% area of maize production in the United States was under double-cross hybrids, which reached to about 90% by 1950 (Griliches 1957). During the double-cross hybrid era, the U.S. national average production increased by ~1 bu./acre every year (Crow 1998). There were other contributing reasons, such as uniformity in flowering and plant stature, that were aesthetically pleasing and suitable for machine harvesting, and their better adaptation to different habitats (Crow 1998) and tolerance to drought than open pollinated varieties (Crabb 1947). Eventually, breeders could generate inbred varieties with higher productivity and adaptability which was enough to use them as seed producers to achieve commercial viability, and single cross hybrids (based on two parental lines) become popular and grown widely throughout the world (Crow 1998).

With the availability of more vigorous and high yielding inbred lines along with technological advances in maize production, the production of single hybrids became more feasible. This again shifted the trend from double cross hybrids to single cross hybrids in the United States. In the 1950s, many small- and medium-sized private seed companies were established, but initially dependent on the public sector hybrids to build their business (Fitzgerald 1990). The adoption of single cross hybrids resulted in significant increase in U.S. national annual yield by an average of 1.71 bu./acre (Crow 1998). It has now been established that the hybrids developed from genetically diverse parents are more often highly heterotic than generated from similar inbreds (Hallauer and Miranda 1988). Following this observation, genetically similar maize lines were grouped together to generate distinct diverse groups called 'heterotic groups'. A heterotic group denotes a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups (Melchinger and Gumber 1998; Ricci et al. 2007). The inter-group hybrids typically display more heterosis than intra-heterotic group hybrids. Several heterotic groups have been described in the United States, viz., Reid Yellow Dent, Lancaster Sure Crop, European flints and Minnesota 13 (Dubreuil and Charcosset 1999; Troyer 2006).

4.8 Genetic Resources

Maize is bestowed with enormous genetic diversity, and several landraces are reported across the globe that offer opportunity for genetic enhancement to meet the growing challenges. Wide variation for yield, stress tolerance and nutritional quality are present in a diverse array of landraces or populations worldwide. Landraces are heterogeneous in nature and are selectively grown by farmers for specific characteristics like adaptation, yield, use in a specific diet form, nutritive value and stress tolerance (Louette and Smale 2000). Some of the notable global land races of maize that have been used in maize improvement programmes include Tuxpeno, Bolita, Jala, Chalqueno, Nal-Tel, Palomero, Suwan-1, La Posta Sequia, Conica, Conica Nortena, Bolita, Oloton, etc., (Louette et al. 1997; Prasanna 2012).

Wide variation for landraces in maize has also been reported in India, particularly in Sikkim and northeastern Region of the country (Prasanna 2010). Some notable examples are Murli makai, Kaali makai, Rathi makai, Paheli makai, Seti makai, Putali makai, Chaptey makai, Gadbade makai, Bancharey makai, Kukharey makai, Kuchungdari, Kuchungtakmar, etc. (Prasanna and Sharma 2005). These landraces from India have been well-characterised both at phenotypic and molecular level for its effective use in the breeding programme (Sharma et al. 2010). One of the most promising landraces that has been well characterised is Murli makai (Sikkim Primitive) that possess prolificacy (more ears per plant) and stay green in character (Dhawan 1964; Prasanna 2012; Prakash et al. 2019; Prakash et al. 2021).

The most important consideration is use of these unique land races in the breeding programme for development of inbreds with specific target traits (Prakash et al. 2019). Broadening of germplasm offers novel trait combinations to the breeders, and it must be a continuous process. One such example is GEM' (Germplasm Enhancement of Maize) project, a cooperative effort of United States Department of Agriculture (USDA) with many institutions and industries that aim to utilise diverse maize genetic resources from around the world to widen the germplasm base of the commercial hybrid corn in the United States. Such efforts are required globally in the breeding programmes across the continents for germplasm enhancement and utilisation in maize.

Different methods have been used to improve germplasm and develop potential lines. The most important procedure for maize breeding is the recurrent selection scheme; wherein cyclical improvement of the lines can be achieved. Among recurrent selection schemes, the reciprocal recurrent selection (RRS) is the most useful in inter population improvement. The RRS applied may be half-sib RRS (HS-RRS; Comstock et al. 1949) or full-sib RRS (FS-RRS; Hallauer and Eberhart 1970) for developing lines with high general combining ability (GCA) and specific combining ability (SCA) and to develop heterotic pools. The homozygous lines developed by this are crossed to opposite heterotic pools, and highly heterotic hybrids can be commercialized. This traditional recurrent selection schemes are upgraded by marker technologies and using genomic selection (RRGS) which have shown lot of promise.

Once the improved lines are available, these lines can be utilized to produce an array of maize varieties and hybrids that include composites, synthetics, conventional hybrids (single cross, double cross, three-way cross, modified single and three way cross, and multiple cross) and non-conventional hybrids (inter-varietal hybrids, inter-family hybrids, top cross, double top cross, and poly cross). The procedure of development of composites and synthetics are similar, but they differ in the component lines used. Composites are the OP populations developed by inter-mating of outstanding lines (germplasm inbreds, OPVs, hybrids, advance generation lines) and subsequently maintained by mass selection from isolated plantings, while synthetic varieties are OP populations derived from the inter-crossing of selfed plants (homozygous lines) or lines and subsequently maintained by routine mass selection procedures from isolated plantings (Lonnquist 1961) and are proposed by Hayes and Garber (1919).

4.9 Key Loci for Economically Important Traits

4.9.1 Grain Yield and Component Traits

The ear and kernel related traits are the important contributing traits for maize yield. Two major quantitative trait loci (QTLs) for kernel row number (*KRN4* and *KRN1*) (Chuck et al. 2014; Wang et al. 2019) and one major quantitative trait locus (QTL) for kernel size and weight (*qHKW1*) (Raihan et al. 2016) have been cloned using map-based approaches. The ear and kernel-related genomic regions have undergone selection during maize domestication and improvement (Liu et al. 2015a; Wang et al. 2019). In addition, many secondary traits like nutrient uptake, photosynthesis, translocation, sink size, transpiration and respiration also influence the yield levels in maize. Grain yield is also affected by maturity duration, standability, and resistance to biotic and abiotic stresses (Gong et al. 2015). Besides, well-developed root system, strong stem, short plant height, low ear placement, ability to stay green at maturity, etc., contribute to good standability, which in turn contributes enormously toward higher yield.

High density planting is a practical approach to increase maize productivity per unit area. It is predicted that a gain of 20% in maize productivity is possible if planting density is increased by 15,000 plants/ha. Hence, breeding varieties suitable to high density planting by targeting leaf architectural traits is an important task. Among the multiple leaf traits important for high density planting, leaf angle is an important target trait (Li et al. 2015). Breeding for narrow leaf angle leads to more upright leaves, which helps in increasing the leaf area index and improve photosynthetic efficiency by reducing shade syndrome (Sakamoto et al. 2006). During the last century, maize breeders placed higher emphasis on shoot phenotypes (York et al. 2015). The recent understanding on root architecture makes it possible to modify root traits to make maize more water- and nutrition-efficient, and also suitable for high density planting.

The narrower xylem vessel reduces the root hydraulic conductance, and thereby saves soil moisture for later use (Meister et al. 2014). By controlling the expression of root-water channel-related genes, such as aquaporin, the water channels in the roots can be modulated (Hachez et al. 2012). Besides, QTL mapping has identified the maize genomic regions that govern root architecture in water uptake (Zurek et al. 2015). In dry and nitrogen- (N) deficient soils, a deeper root, reduced maize lateral root branching, a smaller number of crown roots would optimize N and water use (Lynch 2013; Zhan and Lynch 2015; Saengwilai et al. 2014). The shallow axial root growth angles many shorter laterals, and long root hairs will enhance phosphorus (P) utilization by maize (Lynch 2013).

4.9.2 Plant Architecture

The genetic variation for morphological traits is of great importance for plant breeders. The majority of the genes responsible for differences in morphology and

growth rate between maize and teosinte are either transcription factors or molecules regulating transcription factors (Yang and Xu 2013). Several candidate genes with regulatory function have been identified. The *liguleless1* (*lg1*) and *liguleless2* (*lg2*) genes are associated with leaf ligule and auricle development in maize (Tian et al. 2011). *ZmCLA4* is the functional gene for leaf angle QTL *qLA4–1* in maize (Zhang et al. 2014). *qLA4–1* is the major QTL that negatively controls the leaf angle by ~15° with semi-dominant effect. Two QTLs, *Upright Plant Architecture1* (*UPA1*) and *Upright Plant Architecture1* (*UPA2*), control the upright plant architecture in maize (Tian et al. 2019). The *brd1* (*brassino steroid C-6 oxidase1*) and *ZmRAVL1* are the underlying genes for these two QTLs.

The *UPA2* functions by altering the protein binding affinity of another leaf angle gene, *drooping leaf1* (Strable et al. 2017). *ZmGA3ox2* is the functional gene for plant height QTLs, *qPH3.1* and *dwarf1* in maize (Teng et al. 2013). *qPH3.1* is the major QTL for plant height with a 10.0 cm additive effect and 3.7 cm dominant effect. *qPH1* is another major QTL for plant height explaining 17.7 cm additive and 7.8 cm dominant effects (Xing et al. 2015). The presence of transposable element (TE) in *tb1* enhances its expression in maize which represses the axillary growth and leads to formation of female inflorescence, besides promoting apical dominance (Doebley et al. 2006). Another most important gene in maize domestication is *tga1*, which codes for a SBP transcription factor, and is responsible for naked grain phenotype in maize instead of kernels encased in a hardened fruitcase in teosinte (Wang et al. 2005). A single amino acid substitution (lysine to asparagine) at sixth amino acid of *tga1* changes its specificity to target site in maize leading to naked grain phenotype.

4.9.3 Flowering Time

Flowering time is a complex trait associated with adaptation of maize in different climatic regions. A large number of QTLs for flowering time in maize has been identified, of them three QTLs, vegetative to generative transition 1 (*Vgt1*), *ZmCCT10* (CCT transcription factor) and *ZmCCT9,* were mapped and cloned (Salvi et al. 2007; Hung et al. 2012; Yang et al. 2013; Huang et al. 2018). Association analysis identified significant association of three polymorphisms (*G/A/indel324*, *Mite* and *ATindel434*) in *Vgt1* with flowering time in maize (Salvi et al. 2007). The insertion of TE upstream of *ZmCCT10* changes the promotor methylation levels that would alter the expression pattern of *ZmCCT10* (Yang et al. 2013). *ZmCCT9* is the functional gene for days to anthesis QTL, *qDTA9* which was confirmed by knocking out of *ZmCCT9* function resulted in earlier flowering under long day conditions (Huang et al. 2018).

4.9.4 Nutritional Quality

Maize is an important source of carbohydrate, protein, lipids, minerals and certain vitamins (Prasanna et al. 2001). Several genes involved in starch biosynthesis in

maize endosperm have been mapped and cloned using well-known starch mutants (James et al. 2003). Among them, *su1* and *sh2* has been widely used in sweet corn breeding (Lertrat and Pulam 2007). The *sh2* gene encodes large subunit of ADP-glucose pyrophosphorylase and accumulates six times more sugars compared to ordinary maize (Bhave et al. 1990; Mehta et al. 2017). The *su1* gene codes for isoamylose-type starch debranching enzyme (DBE) and retains two to three times higher sugar and ten times higher water-soluble phytoglycogens than ordinary maize (James et al. 1995). Several maize mutants with reduced zeins and enhanced non-zeins have been well-characterized (Gupta et al. 2015).

The mutant *opaque2* (*o2*) located on chromosome-7 encodes less active leucine zipper transcriptional factor leading to enhanced lysine and tryptophan in maize endosperm (Schmidt et al. 1992). *opaque16* (*o16*), another recessive mutant of Robertson's Mutator (Mu) stock, located on chromosome-8 was found to be associated with enhanced lysine and tryptophan in maize endosperm (Yang et al. 2005; Hossain et al. 2008a, b, 2017; Sarika et al. 2017). These two genes have been shown to enhance lysine and tryptophan content in the introgressed versions of high yielding maize hybrids adapted to different climatic conditions (Sarika et al. 2018a, b; Prasanna et al. 2020a). Among the genes governing carotenoids biosynthesis, the favourable alleles of *lcyE* (*lycopene epsilon cyclase*) and *crtRB1* (*β-carotene hydroxylase*) have been found to be responsible for enhancement of provitamin-A (proA) carotenoids in maize kernels (Harjes et al. 2008; Yan et al. 2010).

The favourable allele of *lcyE* reduces lycopene flux by 30% in α-branch, and thereby diverts lycopene flux toward β-branch of the carotenoids biosynthesis pathway, resulting in three-fold enhancements in proA (Vignesh et al. 2013; Zunjare et al. 2017, 2018a, b). The favourable allele of *crtRB1* enhances proA concentration in maize kernels by limiting the enzymatic activity involved in β-carotene conversion in β-branch (Muthusamy et al. 2016; Zunjare et al. 2018c). The *ZmVTE4* and *ZmPORB2* encoding γ-tocopherol methyltransferase and protochlorophyllide oxidoreductase, respectively, enhance kernel provitamin-E (proE) content in maize (Li et al. 2012; Zhan et al. 2019). The favourable allele of diglyceride acyltransferase (*DGAT1–2*) which encodes type I acyl-coenzyme A: diacylglycerol acyltransferase leads to higher oil content in maize endosperm and embryo (Zheng et al. 2008). The insertion of an extra amino acid (phenylalanine) at position 469 of *DGAT1–2* causes enhancement of its enzymatic activity and results in increased oil and oleic acid. *Zmfatb* gene which codes for acyl-ACP thioesterase enhances palmitic acid content in maize (Li et al. 2011). An 11 bp *InDel* in the last exon of *Zmfatb* affects the enzyme activity by introducing a premature stop codon.

4.10 Specialty Traits

4.10.1 Sweet Corn

Two genes affecting the starch metabolism, viz., *su1* (chromosome 4) and *sh2* (chromosome 3), have been extensively used for development of sweet corn cultivars, where the *sh2* is located upstream of the pathway, while enzyme coded by *su1* affects step downstream of the pathway (Hossain et al. 2015; Chhabra et al. 2019a, b, 2020, 2021). Sugary varieties (*su1su1*) at the milky ripening stage contain nearly three times more reducing sugar and sucrose, ten times more water-soluble phytoglycan (WSP) and one-third of starch content of normal maize (Fisher and Boyer 1983; James et al. 1995; Feng et al. 2008). Besides, sugary kernels have creamy texture with good flavour and appear wrinkled and glossy upon maturity (Creech 1965). However, the sugar level after the harvest declines much faster in the *su1* types (Garwood et al. 1976). On the other hand, the *sh2*-based sweet corn types popularly called 'super sweet' or 'extra sweet corn' accumulate sugar in place of starch.

At milky ripening stage, the content of reducing sugars and sucrose in the kernel is about six-fold higher than the ordinary maize (Feng et al. 2008; Khanduri et al. 2011; Solomon et al. 2012). However, the content of WSP is similar to normal maize, and starch content is about one-third of the ordinary maize. By virtue of higher amount of sugars in *sh2sh2* mutant, kernels contain decreased amount of total carbohydrates at the mature seed stage, and the kernels get collapsed and look shrunken with degree of opaqueness (Creech 1965). The depletion of sugar level is much slower in *sh2* type even without refrigeration; thus, varieties have extended shelf life and are better suited for prolonged storage. Sweet corn cultivars with combination of *su1* and *sh2* have often been used in commercial sweet corn development (Lertrat and Pulam 2007). Furthermore, *sugary enhancer1* (*se1*) (chromosome 2), a modifier of *su1*, has been used in combination with *su1* in sweet corn development. Now, *brittle2* (*bt2*) (chromosome 4) based sweet corn cultivars have also been developed and commercialized worldwide. Furthermore, sweet corn has now been biofortified with high lysine, tryptophan, proA and proE (Feng et al. 2015; Mehta et al. 2020a, b, 2021; Baveja et al. 2021).

4.10.2 Popcorn

Popping percentage and popping volume are two most important characters for popcorn (Pal et al. 2020). Several mapping studies have reported many major genomic regions for popping traits with >10% phenotypic variance (Lu et al. 2003; Babu et al. 2006; Li et al. 2007a, b; Liu et al. 2007a; Yongbin et al. 2012). Recently, Meta-QTL analyses revealed three QTLs located on chromosome 1 (metaQTL1_1, metaQTL1_5 and metaQTL1_7) and one QTL on chromosome 6 (metaQTL6_2) as significant QTL responsible for popping traits (Kaur et al. 2021).

4.10.3 Baby Corn

Among various genes, *teosinte branched1* (*tb1*) has been identified as the key gene (on chromosome 1) determining prolificacy (Doebley et al. 1995, 1997; Doebley 2004). Wills et al. (2013) identified a '*prol1.1*' major QTL located on chromosome 1 for prolificacy. Prakash et al. (2021) identified a novel QTL '*qProl-SP-8.05*' from 'Sikkim Primitive' on chromosome 8. It is a prolific maize landrace with five to nine ears per plant. QTL mapping identified a major QTL (bin: 8.05) explaining 31.7% and 29.2% of phenotypic variance in two mapping populations.

4.10.4 Waxy Corn

Waxy maize is originated from the cultivated flint maize through mutation in *Wx1* locus (Fan et al. 2008; Zheng et al. 2013). *Wx1* is mapped on the short arm of chromosome 9 (Klosgen et al. 1986; Mason-Gamer et al. 1998). *Wx1* codes granule-bound starch synthase (GBSS-I) gene which catalyses amylose synthesis from ADP glucose in amyloplasts of maize endosperm. Different types of mutation, such as insertion of transposon, retroposon and fragments of few nucleotides and deletion of nucleotides, result in mutant allele (*wx1*) (Devi et al. 2017; Hossain et al. 2019b). These mutations create the synthesis of altered transcript with premature stop codon or change in amino acids in key domain or splicing or translational errors that in turn stops the activity of wild-type *Wx1* allele or inhibits the activity of GBSS-I, which results in lower amylose and higher amylopectin in grain (Bao et al. 2012; Zhang et al. 2013). Generally, GBSS-I is highly active in non-waxy maize and its product (amylose) cannot be fully changed into amylopectin by starch branching enzyme. GBSS-I coded by recessive gene *wx1* possesses reduced activity (Liu et al. 2007b). Most of the amylose synthesized by low activity of GBSS-I are transformed into amylopectin by starch branching enzyme. Amylopectin is only accumulated in endosperm, and the phenotype appears as waxy (Wessler et al. 1986).

4.10.5 High Amylose Maize

The recessive *amylose extender1* (*ae1*) mutation (present on chromosome 5) that codes for starch branching enzyme (*sbe2b*) enhances amylose to a level of 50–60% from 25 to 30% found in traditional maize (Li et al. 2008). The *ae1.1* is a null allele, also called *ae1-ref,* which does not produce a *SBE2b* protein product (Vineyard and Bear 1952). A second variant *ae1.2*, known as *ae1-Elmore*, produces a catalytically inactive and truncated protein (Liu et al. 2012). Increase in amylose content by *ae1* was only 50%. Maize starch with amylose content of >60% is the result of high amylose modifier genes in the maize *ae1* background. A major modifier gene of *ae1* has been reported to be *SbeIa*, which can increase amylose up to 70–80% in the presence of *ae1* (Garwood et al. 1976; Hedman and Boyer 1982). Second modifier called *modifier of amylose extender1* (*mae1*) has also been reported (Krzywdzinski 2016).

4.11 Biotic Stress Tolerance

Diseases are the most important factors for yield loss in maize. Many QTLs for disease resistance in maize has been identified; however, only few genes have been validated (Yang et al. 2017; Liu et al. 2020a). The maize *Hm1* gene governing resistance against maize leaf blight was the first gene to be cloned and elucidated at molecular level in plants (Johal and Briggs 1992). It is a dominant gene which is located on chromosome 1 and codes for NADPH-dependent HC-toxin reductase which inactivates HC toxin. The *Rp1-D* is another resistance gene conferring resistance toward common rust in maize (Collins et al. 1999). Six resistance genes, viz., *ZmWAK*, *ZmHtn1*, *ZmTrx*, *Rcg1*, *ZmCCT10* and *ZmAuxRp1,* with relatively large effects have also been cloned in maize (Zuo et al. 2015; Hurni et al. 2015; Wang et al. 2017b; Ye et al. 2019). The *ZmWAK* gene codes for a wall-associated kinase-specific 730 residue receptor-like protein which represses the fungal growth (*Sporisorium reilianum*) in above ground tissues (Zuo et al. 2015). The *ZmHtn1* gene mapped on chromosome 8 also encodes wall-associated receptor-like kinases to confer resistance toward northern leaf blight (Hurni et al. 2015).

The *ZmTrxh* mapped on chromosome 6 lacks two canonical cysteines in its thioredoxin active-site motif required to reduce disulfide bridges and provide resistance to sugarcane mosaic virus (Liu et al. 2017a). *Rcg1* is a major QTL located on chromosome 4 which encodes a NB-LRR domain and provides resistance to anthracnose stalk rot (Frey et al. 2011). *ZmCCT10*, a gene that controls the flowering time in maize is also associated with resistance to *Gibberella* stalk rot (Yang et al. 2013). The *CACTA*-like transposable element in *ZmCCT10* is the causal variant, which alters its DNA methylation and histone modification, and results in greater disease resistance (Wang et al. 2017b). *ZmAuxRP1* encodes DUF966 protein and also confers resistance to *Gibberella* stalk rot by affecting the biosynthesis of both IAA and benzoxazinoids (Ye et al. 2019). Among insect-pest, FAW severely limits maize production by causing severe damage to the growing young leaves. Womack et al. (2020) identified two major QTLs (bin: 4.06 and 9.03) that explained 35.7% of the phenotypic variance over all environments.

Resistance sources to foliar diseases of maize including maydis leaf blight (MLB, southern corn leaf blight, or SCLB) (Bhat et al. 2012), turcicum leaf blight (TLB,) northern corn leaf blight, or NCLB) (Ayiga-Aluba et al. 2015; Bhat et al. 2017; Kurosawa et al. 2017), gray leaf spot (GLS) (Dhami et al. 2015), polysora and common rust, downy mildew (DM), some viral diseases, *Aspergillus* contamination (Hooda et al. 2012; Badu-Apraku and Fakorede 2017) have been identified and are incorporated successfully through conventional breeding. In addition, multiple disease resistance (MDR) in maize has been reported (Martins et al. 2019). International Maize and Wheat Improvement Center (CIMMYT) has developed a multiple borer resistance (MBR) source population utilizing diverse germplasm obtained from different organizations.

MBR population was developed by adopting recombination and recurrent selection under artificial insect epidemics against Southwestern corn borer (SWCB), sugarcane borer (SCB), European corn borer (ECB) and fall armyworm (FAW)

Table 4.1 Secondary and tertiary gene pool resources of maize for biotic stress tolerance

S. No.	Biotic stress	Germplasm	Reference (s)
Secondary gene pool			
1.	Maize chlorotic dwarf virus	*Z. diploperennis*	Findley et al. (1982)
2.	*H. turcicum*, *H. maydis*	*Z. diploperennis*	Wei et al. (2003)
3.	*Corn smut disease* downy mildew	*Z. mays* spp. *mexicana*	Mammadov et al. (2018)
4.	*H. turcicum, H. maydis*	*Z. diploperennis*	Mammadov et al. (2018)
5.	*Fusarium* spp.	*Z.* spp. *mexicana*	Pasztor and Borsos (1990)
6.	Striga-parasitic weed	*Z. diploperennis*	Yallou et al. (2009)
7.	Northern leaf blight	*Teosinte*	Ott (2008)
8.	*Ustilago maydis*	*Teosinte*	Chavan and Smith (2014)
9.	Corn borer	*Z. mays* spp. *mexicana*	Pasztor and Borsos (1990)
10.	Asiatic corn borer	*Z. mexicana, Z. diploperennis. Z. perennis*	Ramirez (1997)
11.	Corn rootworm	*T. dactyloides*	Prischmann et al. (2009)
12.	*S. frugiferda*	*Z. diploperennis*	Farias-Rivera et al. (2003)
Tertiary gene pool			
13.	*C. graminicola, H. turcicum, H. maydis, E stewartii, P. sorghi*	*T. dactyloides*	Bergquist (1979)
14.	*Rust disesase*	*T. dactyloides*	Mammadov et al. (2018)
15.	*H. turcicum* (Ht gene)	*T. floridanum*	Hooker and Perkins (1980)
16.	*P. sorghi* (RpTd gene)	*T. dactyloides*	Bergquist (1981)

(Mihm 1985). As teosintes are more resistant to insects and pathogen than the improved maize cultivars, understanding the underlying defence mechanisms of teosintes would present novel strategies to breed for biotic stress resistance in modern maize. Exploring the genetic variation for resistance and other agronomic traits among wild and landrace of maize has long been advocated (Flint-Garcia 2013). Over the years, many research efforts have been directed towards the identification/development of resistant sources against various biotic stresses. Besides primary gene pool, resistant sources from secondary and tertiary gene pools have also been identified (Table 4.1).

4.12 Abiotic Stress Tolerance

Initial attempt of QTL mapping for drought tolerance in maize was executed using Polj17 (drought-resistant) and F-2 (drought-sensitive) to generate F_2 population (Lebreton et al. 1995). In this study, QTLs for abscisic acid (ABA) content and stomata conductance were mapped. Since then, numbers of studies have been recorded on QTL mapping in maize for important morpho-physiological traits drought stress condition. In different studies of QTL mapping, many QTLs were identified for morphological traits like male flowering, female flowering, anthesis and silking interval (ASI), yield and cob number (Ribaut et al. 1996; Ribaut et al. 1997; Agrama and Moussa 1996; Sari-Gorla et al. 1999). Later, considering the importance of root and its related traits imparting the tolerance to drought, different QTLs for root architecture and root-associated traits along with yield traits have been identified, viz., one QTL for root trait (Landi et al. 2010), 22 QTLs for root-associated traits, such as root density, root dry weight, sugar concentration and leaf ABA content through composite interval mapping in $F_{2:3}$ population (Rahman et al. 2011). Similarly, Trachsel et al. (2016) identified a total of 17 QTLs for stomatal conductance, leaf water content, ASI, and grain yield.

Besides, association mapping is another important approach for better resolution of QTLs as it utilizes the historical recombination events in natural populations known as association panel. Setter et al. (2011) conducted association mapping to identify single nucleotide polymorphisms (SNPs) related to genes involved in carbohydrate and ABA metabolite accumulation during drought stress. Aldehyde oxidase gene is found to regulate silk ABA concentration under drought stress. Later, SNP-based genome-wide association mapping was conducted using 5000 inbred lines as association panel (Li et al. 2016). The study revealed significant association of SNPs with drought tolerance associated candidate genes. However, most of the identified QTLs identified till date are of minor effect except few major QTLs. The mQTL study conducted using three populations and several environments revealed seven genomic regions for grain yield and one genomic region for ASI. Among these, six mQTL on grain yield mapped on chromosomes 1, 4, 5 and 10 under moisture stress and optimal environments and hence classify as stable QTLs.

Though hundreds of QTLs for drought tolerance have been mapped, only few of them have been cloned (Liu and Qin 2021). Two large effects on genes *SDG140* and *Hp322* were identified for drought tolerance in maize (Lu et al. 2010). *SDG140* gene which encodes a SET-domain protein is the underlying gene for *HP71* haplotype of drought tolerance. *Hp322* includes two closely linked SNPs from a gene encoding aldo-keto reductase and provides enhanced drought tolerance. Four genes, *ZmNAC111 ZmVPP1*, *ZmTIP1* and *Zmabh2,* have been identified to be responsible for seedling drought tolerance in maize (Mao et al. 2015; Wang et al. 2016; Zhang et al. 2019; Liu et al. 2020b). Two genes, *ZmPYL8* and *ZmPYL12,* which encodes the abscisic acid receptors, facilitate drought tolerance in maize (He et al. 2018). Maize *ABP2* (*ABRE binding protein 2*) gene codes for bZIP transcription factor which

enhances the expression of stress-responsive, and carbon metabolism-related genes result in enhanced tolerance to both drought and salt (Na et al. 2018).

Several QTLs for heat stress tolerance in maize has been mapped using biparental and genome-wide association mapping approaches, and few candidate genes have also been identified (Frey et al. 2016; Gao et al. 2019; Inghelandt et al. 2019; Longmei et al. 2021). Frey et al. (2016) identified two QTL hotspots separately on chromosome 2 and 3 for heat tolerance with respect to grain yield, which explained 7–13% of the variance. The low variance of these QTLs explained the multigenic inheritance of tolerance to heat in maize. Frey et al. (2016) also identified three heat tolerance genes (GRMZM2G115658; GRMZM2G537291 on chromosome 2; and GRMZM2G324886 on chromosome 3) in the above identified QTL hotspot regions. The gene GRMZM2G324886 codes for calcicylin binding protein involved in calcium signalling in response to stress condition. QTL for heat susceptibility index (leaf scorching trait) has been found on chromosome 9 and QTL for heat susceptibility index (grain yield) were reported on chromosome 2 and 3, which suggested the involvement of different genomic regions in regulation of genetic mechanisms for leaf scorching and grain yield (Frey et al. 2016).

Inghelandt et al. (2019) identified six QTLs for heat susceptibility index of the five traits at the seedling stage which exhibited 7–9% of the phenotypic variance. Eleven QTLs have been mapped for pollen germination and pollen tube (Frova and Sari-Gorla 1994). The pollen germination ability is correlated with the cellular membrane stability since QTLs for both the traits map to common region on the short arm of chromosome 8 (Ottaviano et al. 1991). Qin et al. (2007) reported that higher expression of ZmDREB2A gene in maize may induce heat responsive genes which may further provide adaptability under high temperature stress. QTLs controlling leaf temperature, ASI and grain yield have been mapped in maize on chromosome 7 (Sanguineti et al. 1999).

Transcriptome study in maize in response to heat stress identified 1029 upregulated and 828 downregulated DEGs, and the analysis indicated the central role of protein processing in endoplasmic reticulum in response to heat stress (Qian et al. 2019). They also identified 167 putative transcription factors associated with heat stress response of maize belonging to the TF family of MYB, AP2-EREBP, b-ZIP, bHLH, NAC and WRKY. Heat stress triggers the endoplasmic reticulum stress which causes heat-induced upregulation of *ZmbZIP60,* and study by Li et al. (2018) concluded that upstream region of *ZmbZIP60* is vital in its upregulation under heat stress.

Waterlogging tolerance in maize is governed by complex genetic mechanism with involvement of multiple genomic regions (Mano and Omori 2007). Mano et al. (2005) reported two QTLs on chromosomes 4 and 8 governing adventitious root formation under waterlogging condition. Mano et al. (2009) also identified three QTLs for adventitious root formation under waterlogging condition on chromosomes 3, 7 and 8 from the backcross population derived from the cross Mi29 × teosinte (*Z. nicaraguensis*). Composite interval mapping of F_2 population of cross between F1649 and H84 reported that a single QTL of chromosome 1 is responsible for the severity of leaf injury due to waterlogging (Mano et al. 2015).

Qiu et al. (2007) reported chromosomes 4 and 9 as the hot spots for several QTLs governing root dry weight, shoot dry weight, total dry weight and plant height. Zaidi et al. (2015) identified 22 candidate genes from several identified QTLs located on chromosome1, 3, 4, 5, 7, 8 and 10 for grain yield and other secondary traits associated with waterlogging tolerance.

A total of 7 out of 55 uniformly distributed QTLs among all the 10 chromosomes were identified as the candidate genes for waterlogging tolerance. These candidate genes were lying on the previously identified QTLs located on chromosomes 1, 4, 6, 7 and 9 (Osman et al. 2013). Transcriptome analysis has identified the rapid induction in TFs families bZIP, AP2/ERF, bHLH, NAC and MYB under waterlogging condition (Yu et al. 2020). A genome-wide analysis in the inbred line HZ32 revealed 38 out of 184 AP2/ERF genes identified in maize which responds to waterlogging stress (Du et al. 2014). In addition to all these genes and transcription factors, non-coding RNAs also play key role in making adaptive changes in morphology and metabolism of plants in response to waterlogging stress (Yu et al. 2020). The gene ZmEREB180 of the ERF-VII family in maize have been found to play key role in regulating the adventitious root growth, and overexpression of ZmEREB180 also enhances the tolerance level against waterlogging stress (Yu et al. 2019).

4.13 Genomics-Assisted Breeding

Conventional breeding achieved success in developing maize varieties with enhanced yield, quality and stress tolerance through crossing and selection over years (Liu and Qin 2021). However, this is tedious, time-consuming and inefficient for complex traits. Genomics-assisted breeding approaches, such as marker-assisted backcross breeding (MABB), forward breeding (FB) and genomic selection (GS), are considered to be efficient tools for accelerating genetic gain by increasing selection intensity and reducing selection cycles by two to three years (Gilliham et al. 2017; Prasanna et al. 2021). Bouchez et al. (2002) introgressed favourable alleles of three QTLs for earliness and grain yield through three cycles of marker-assisted backcrossing. Marker-assisted introgression of *o2*, *crtRB1* and *vte4* has been used to improve protein quality (Gupta et al. 2013; Hossain et al. 2018, 2019a, c; Sarika et al. 2018a; Jompuk et al. 2020), proA content (Muthusamy et al. 2014; Liu et al. 2015b; Zunjare et al. 2018a) and proE content (Feng et al. 2015; Das et al. 2021), respectively, in maize. In recent years, multi-nutrient-rich inbred lines and hybrids, especially combinations of quality protein maize (QPM), proA, proE and low phytate, have been developed through MABB (Bhatt et al. 2018; Goswami et al. 2019; Mehta et al. 2020b; Baveja et al. 2021; Das et al. 2021; Singh et al. 2021).

Several proA-rich and three QPM + proA-rich hybrids developed by MABB have been released for commercial cultivation worldwide (Prasanna et al. 2020a). MABB have also been used for improving disease resistance and abiotic stress tolerance in maize. Zhao et al. (2012) introgressed *qHSR1* QTL into 10 inbred lines of maize which resulted in significant improvement for head smut resistance. Marker-assisted

backcrossing of H5 haplotype of *ZmCCT* showed to enhance resistance to stalk rot in maize inbreds and hybrids (Li et al. 2017). The marker-assisted pyramiding of sugarcane mosaic virus (*Scmv1*) and *Scmv2* QTLs into F_7 maize line resulted in complete resistance to sugarcane mosaic virus (Xing et al. 2006).

Yang et al. (2017) developed near isogenic lines with *qMdr9.02* locus containing multiple disease resistance genes via maker-assisted selection, which possessed resistance to southern corn leaf blight and GLS. Using MABB, resistance to maize lethal necrosis (MLN) has been introgressed into over 30 elite drought-tolerant maize lines (Prasanna et al. 2020b). Similarly, Awata et al. (2021) introgressed QTL for resistance to MLN into nine elite but MLN susceptible lines. Ribaut and Ragot (2007) showed drought tolerance in MABB-derived test cross maize hybrids over control hybrids. Wang et al. (2016) intogressed *ZmVPP1* QTL into drought susceptible inbred, Shen5003 through four cycles of marker-assisted backcrossing. The improved progenies with homozygous *ZmVPP1* showed enhanced drought tolerance than Shen5003.

FB is a simple form of population improvement using molecular markers tightly linked to genomic regions of high importance. FB is being used for improvement of maize populations with favourable alleles of large effect genes/QTLs for disease resistance, such as maize streak virus and MLN, and nutritional quality traits, such as proA (Prasanna et al. 2020c). Duo et al. (2021) applied marker-assisted pedigree selection (MAPS) for improvement of proA in sub-tropically adapted maize inbreds. The resultant inbreds and hybrids showed significantly higher proA than traditional hybrids indicating the feasibility of MAPS for improving nutritional traits. Marker-assisted recurrent selection (MARS) is useful to accumulate favourable alleles from several genomic regions within a single population. Abdulmalik et al. (2017) applied MARS in a bi-parental population to increase the frequency of favourable alleles for drought tolerance.

GS is a powerful tool to take account of all the major or minor QTLs spread throughout the genome and is especially useful for complex traits (Santantonio et al. 2020). GS is conducted in a training population by combining genotypic and phenotypic data to estimate the marker effects (breeding values) of the individuals that have been genotyped but not phenotyped in a population to be tested (Meuwissen et al. 2001). GS helps in population improvement by rapid cycling and higher genetic gain per cycle through the use of markers. GS has been applied in maize for improvement of grain yield (Beyene et al. 2015; Zhang et al. 2017), drought and water logging tolerance (Vivek et al. 2017; Das et al. 2020), disease resistance (Technow et al. 2013; Sitonik et al. 2019; Nyaga et al. 2019; Liu et al. 2020c; Kuki et al. 2020; Holland et al. 2020), and kernel oil (Hao et al. 2019) and zinc content (Guo et al. 2020; Mageto et al. 2020).

4.14 Doubled Haploid Technology

Maize breeding exploiting the doubled haploid (DH) technology based on in vivo haploid induction has gained wide significance and become an invaluable tool due to the fastest and most efficient route to produce completely homozygous lines for maize breeding programmes (Ren et al. 2017). DH lines in maize is produced in four steps, viz., haploid induction using the haploid inducer line, identification of haploids, chromosome doubling and selfing of the doubled haploid maintain DH line (Chaikam et al. 2019). The natural occurrence of haploid maize plants (~0.1%) laid the base for in vivo DH production (Chase 1969). Haploid inducers may be the paternal (used as female parent and haploid produced retain genome from the male parent) or maternal inducers (used as male parent and retain genome from the female parent). Paternal haploid induction has been reported through mutation in the *ig1* gene (*indeterminate gametophyte1*) (Kermicle 1969; Evans 2007). However, it is not being commonly used due to low haploid induction rate (HIR) of 1–2%, as well as presence of anomaly in the cytoplasmic constitution of the resulting haploid (Kermicle 1973, 1994).

Maternal haploid induction is quite successful and is being frequently used in maize DH production due to high HIR of around 8–10%, and the haploid produced retain the cytoplasmic and nuclear genome of the female parent without any differences. At present, all the haploid inducers being used have been deduced from the ancestral haploid inducer line Stock6 (HIR of ~3% of maternal haploids). The currently available haploid inducers have the HIR of around 10%. In maize, two major quantitative trait loci, *qhir1* in bin 1.04 and *qhir8* in bin 9.01, govern significant haploid induction (Prigge et al. 2012). Dong et al. (2013) identified a 243 kb region with significant effect on haploid induction by fine-mapping a 3.57 Mb region between markers *umc1917* and *bnlg1811*, targeting the QTL *qhir1*. Kelliher et al. (2017), Liu et al. (2017b) and Gilles et al. (2017) reported *mtl/pla1/nld* is the gene underlying the *qhir1*. 4 bp insertion near the terminal end of the *mtl* gene causes the haploid induction. Zhong et al. (2019) cloned the gene underlying *qhir8* and found a non-Stock6-originating gene, *dmp*. They exhibited that SNP substitution in *dmp* from thymine (T) to cytosine (C), at 131 bp from the initiation codon ATG leads to amino acid substitution from methionine to threonine.

Enhanced ability of HIR by five to six times of *dmp* was observed in the presence of *mtl/pla1/nld.* The first identified inducer 'Stock 6' had HIR of 1–3%. Efforts have been made to enhance the HIR and several inducers like PHI (Rotarenco et al. 2010), RWS (Rober et al. 2005), UH400 (Prigge et al. 2011) and MHI (Chalyk 1999) were developed with HIR of ~6–15%, but all of them were adapted to temperate environment. Later on, tropically adapted inducer lines with HIR of around 6–14% were also developed with the efforts of CIMMYT and the University of Hohenheim (Prigge et al. 2012; Prasanna et al. 2012; Chaikam et al. 2016; Chaikam et al. 2018). DH technology is now the order of choice to all the maize breeders as lengthy 6–7 generation of inbreeding is bypassed, and completely homozygous plants are achieved in two to three seasons.

4.15 Gene Editing Technology

Mutagenesis through physical and chemical agents and through Targeting Induced Local Lesions in Genomes (TILLING) has been used to create variability (Slade et al. 2005). Site-directed mutagenesis systems are now available for targeted genome editing. Present-day genome editing tools such as zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs) and clustered regularly interspaced palindromic repeats (CRISPR)-CRISPR-associated (Cas) systems (Georges and Ray 2017). ZFN technology has been used to reduce phytate content in maize seeds by targeting one of the inositol phosphate kinase (IPK) homologues (Shukla et al. 2009). TALENs approach has been used to generate heritable changes in maize *gl2* locus (Char et al. 2015). In maize, various traits such as grain composition, male sterility, lignin biosynthesis, herbicide tolerance, secondary metabolism and drought tolerance have been modified through CRISPR technology (Chilcoat et al. 2017).

The first use of CRISPR technology was reported for the maize IPK gene (Liang et al. 2014). In addition, Shi et al. (2017) used the native maize GOS2 promoter to both replace and supplement the native ARGOS8 promoter to achieve altered expression of *ARGOS8* and yield gains under drought high yield levels under well-watered conditions. CIMMYT has started the MLN gene editing project which uses CRISPR/Cas9 with the goal of deployment of resistant cultivars by 2025. Besides, transgene-free semi-dwarf maize plants were generated using CRISPR-Cas9 technology by editing *GA20ox3* gene (GA biosynthetic gene). Qi et al. (2020) edited waxy locus in genetic background of ZC01 genotypes using in vivo CRISPR/Cas9 tool and successfully obtained progenies rich in kernel amylopectin. Recently, Liu et al. (2021) obtained genetic gain in multiple maize grain-yield-related traits by engineering quantitative variation for yield-related traits. This was done by engineering weak promoter alleles of *CLE* genes (a family of genes that act as a brake to stop stem cell growth) and a null allele of a newly identified partially redundant compensating *CLE* gene, using CRISPR-Cas9 genome editing. Very recently, a new genome editing technology that does not require double-stranded break in the DNA has been developed and is termed as prime editing. This can achieve different types of editing such as any transition and transversion mutations, as well as small indels. As this technology has wide flexibility to obtain different types of edits in the genome, it holds a great promise for developing superior maize varieties with high yield, resistance to various abiotic and biotic stresses, and quality of plant products (Marzec et al. 2020).

4.16 Future Thrust

Significant progress in understanding the genetics and efforts toward adopting newer breeding methodologies and effective integration of *omics* tools have led to a substantial genetic improvement of maize both in terms of productivity and total production. Continued yield enhancement in maize with newer varieties year-after-

year has led to an imperative growth in area under cultivation of the maize crop. The primary challenge in the future is going to be further enhancement of yield with increased adaptation to the newer challenges. Despite the persistent yield growth, breeding for stress tolerance particularly biotic stress is continuing to be an area that need immediate attention. Although efforts have been made to understand the genetics and to identify the gene(s)/loci governing disease resistance, their actual use in breeding for resistant cultivars is still to reach its full potential.

With the changing climatic conditions and evolution of new races of pathogen, breeding for resistance to disease of greater concern in the region is highly important. In the most recent times, outbreak and rapid spread of FAW have created havoc in maize cultivation as delay in control measures has led to a greater reduction in yield and sometimes complete failure of the crop. This has also necessitated excessive use of chemicals which has increased cost of production besides posing serious environmental pollution. Besides, tolerance to abiotic stresses, particularly drought, heat and water logging are very important as maize crop is sensitive to both moisture stress and excessive moisture. Thus, widening of germplasm for multiple stress resistance/tolerance and their use in crop improvement programme to breed high yielding maize hybrids coupled with stress tolerance is very important.

Recently, considerable success has been achieved in development of high yielding hybrids with enhanced nutritional quality (with one or more grain quality traits). This offers hope for simultaneous improvement of multiple traits, and these germplasms also need to be improved for stress tolerance for its wider adaptation and adoption. Weeds continue to remain as one of the major yield-reducing factors in maize production. Development of herbicide-tolerant maize genotypes will greatly facilitate use of herbicides for weed management anytime during the crop growth. This requires large-scale screening of available germplasm for herbicide tolerance or use of mutation as a tool to create variation for such economically important traits. Until recent times, breeders have been largely practising inbreeding for development of more and more inbreds from the source population. By the virtue of process and associated genetic effects, complete homozygosity could never be achieved in the inbreds. Use of DH technology in the maize-breeding programmes needs to be strengthened for rapid development of completely homozygous productive inbreds. This will help in greatly reducing the time taken for inbred development besides maximum realisation of heterosis. With the availability of gene-editing technology, accelerated development of genotypes with target traits is very much possible, and this will greatly facilitate in plant breeding. Furthermore, 'speed breeding' needs to be explored in maize to accelerate the breeding cycle and development of hybrids in a much shorter time frame.

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Barley Breeding

5

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Abstract

The importance of barley stemmed mainly from the diversified use of its grain and the plant pertaining to food, feed and forage. In many countries around the world, this crop is often considered the only possible rain-fed cereal crop under low input and stressful environments, such as drought, heat and cold. Therefore, this old crop is likely to have new future in current situations of climate change and ever-increasing population pressure on food supply. During the early 1920s, the barley improvement program was started in India using pure line selection method. Most of the developed barley varieties are of six-row types and are primarily used for feed purpose, while two-row malt-purpose barley varieties are of recent origin. Globally, barley improvement programmes now see a great potential as industrial crop, and barley breeding activities were directed to develop malt-type barley varieties. Besides conventional breeding, the development in the fields of barley genomics is rapid, and researchers are having more choice to identify, characterize, clone, annotate and edit the genes of interests for the development of better varieties.

Keywords

Biotic and abiotic stress tolerance · Breeding objectives · Coordinated system of testing · Genetic resources · *Hordeum vulgare*

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_5

5.1 Introduction

Since the beginning of sedentary civilization, cereal grains have been the integral part of human diet providing a major proportion of dietary energy (50% of the total) and nutrients including carbohydrates and protein (Laskowski et al. 2019). During the course of metamorphosis of early humans from hunter gatherers to agrarian sedentary civilizations, an array of cereal species had been domesticated and brought under organized cultivation mainly through persistent selection in the form of pick and choose. Apart from maize, rice and wheat, barley (*Hordeum vulgare* L. emend Bowden.) is one such cereal grain that was domesticated very early and eventually assumed unprecedented importance in the survival and well-being of the early settlers. Therefore, it is not only one of the first domesticated crops but also antique among cereals. The use of barley in making of beer was discovered early on, which is the third most popular beverage around the world after water and tea, even today. In the Sumerian and Babylonian civilizations, barley grains were even used as a currency.

Evolutionary progression of barley has rendered this crop to be accustomed to diverse eco-geographical environmental conditions as compared to other crop species because of its resilience to extreme environmental variations. The adaptability of barley to extreme environments and marginal growing conditions has led to extensive cultivation of this cereal all over the world (von Bothmer et al. 1995). The cultivation of barley extends from the tropics to high latitudes (>60°N) in Iceland and Scandinavia, as well as in high altitudes up to >4450 m above sea level (masl) in the Himalayas (Ceccarelli et al. 2008; von Bothmer et al. 2003).

Barley is a nutritious cereal grain that contains many important nutrients and vitamins. It is characterized by its rich nutty flavour and chewy consistency. This versatility of barley use makes it a vital grain commodity in the international markets.

Although the main uses of barley are in animal feed and brewing purposes, but owing to its nutritional value, specially being rich in dietary fibre, it is also consumed as a staple food in the North and Sub-Saharan Africa (SSA), hilly areas of Central Asia, South-West Asia and Northern Africa. The projected increase in global population, food consumption and dietary changes (Godfray et al. 2010; Tilman et al. 2011) are expected to broaden the gap between supply and demand of the food grains only to be aggravated by the shrinking acreage available for agriculture amidst the rampant global climate change (Ray et al. 2015). The world agricultural production needs to be doubled by the year 2050 to keep pace with the demographic expansion that is unfolding on the world (Gerland et al. 2014).

The IPCC (Porter et al. 2014) reported that for the major crops (wheat, rice and maize) in tropical and temperate regions, without adaptation, climate change will result into serious consequences for production when local temperature would increase by 2 °C or more. Therefore, the climate change may gradually upsurge the inter-annual variability of crop yields in some regions, resulting in an increased risk of more severe impacts on food security (Porter et al. 2014; Ray et al. 2015). The global climate change will cause few areas to experience high precipitation, while

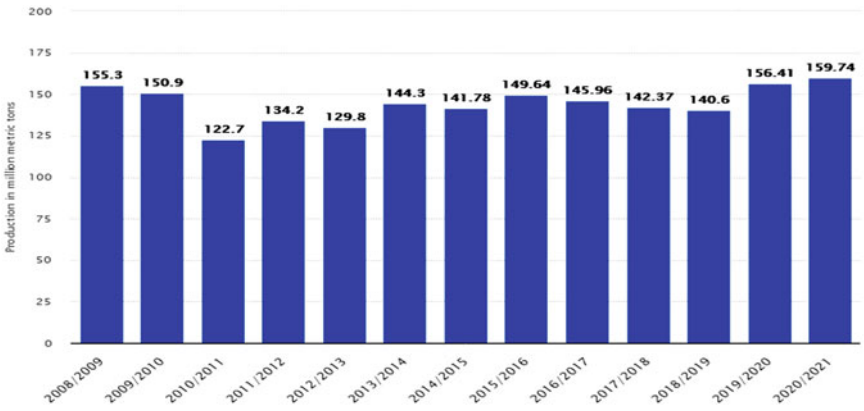

Fig. 5.1 Year-wise global production statistics of barley

few others facing prolonged droughts. In such a scenario, barley is one of the hardiest cereal crops that can adapt well to the hostile climates through its genetic plasticity (Garstang et al. 2011; Ingvordsen et al. 2015).

In fact, this crop can thrive well in the regions such as arctic and subarctic zone to subtropical regions where the other cereals such as maize, wheat and rice can hardly be grown. It is considered as the only available alternative in the harsh deserts owing to its ability to thrive in environments of limited moisture availability, heat and cold. Even more, barley when displaced by wheat particularly in the irrigated areas has found a new niche role in nutraceuticals, distillery and ethanol fuel production making it a potential candidate as an industrial crop around the world. Owing to its nutritional composition, the barley grains are up for being the next nutraceutical in a scenario of people being more health-conscious day-by-day. Lowering blood cholesterol, blood pressure and glycaemic index have been considered to be the major health benefits of barley because of presence of high amount of soluble fibre and beta-glucan (Baik and Ullrich 2008).

The largest proportion of the global barley production is utilized for animal feed, followed by the use in malting industries and various food products (Blake et al. 2011). Currently, barley is the fourth most important food crop of the world after maize, wheat and rice (FAO 2020; Shabrangy et al. 2021). During the year 2020–2021, 159.74 million metric tons of barley was produced globally as compared to 156.41 million metric tons in the year 2019–2020. The year-wise global production statistics are presented in Fig. 5.1. In 2020–2021, the top five barley producing countries were the Russian Federation (20.63), Australia (13), Canada (10.74), Turkey (8.1), and Ukraine (7.95) (https://www.statista.com/statistics/271973/world-barley-production-since-2008/) with the production in million metric tons. Figure 5.2 represents country-wise production of barley in the year 2020–2021. The largest consumer of barley is the European Union followed by Russia and the consumption pertains mainly to malting purposes.

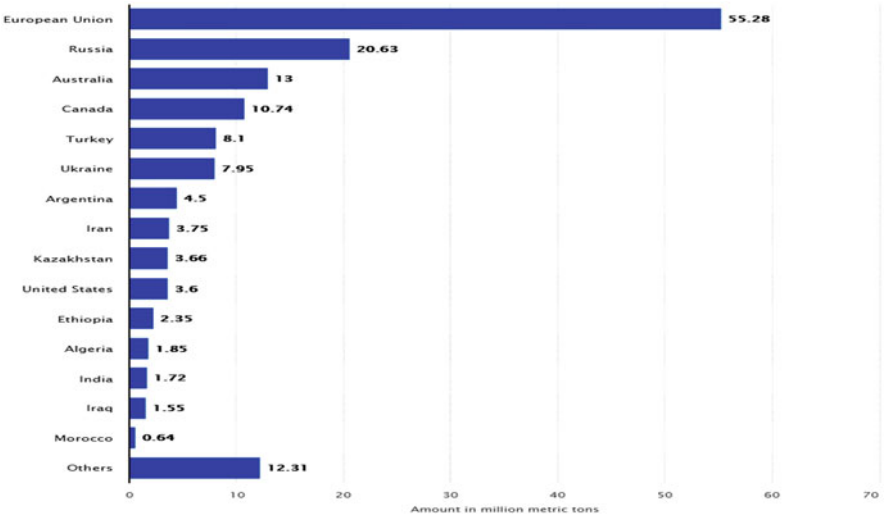

Fig. 5.2 Country-wise production statistics of barley during 2020–2021

The world barley trade in the form of exports and imports across the years has been presented in Table 5.1 (export) and Table 5.2 (import). The barley export trends are more or less similar across last 5 years where the European Union, Russia and Ukraine have been the largest exporters barring the year 2020–2021 where Australia held rank one among the barley exporting countries if European Union were to be considered not as a single country. The total global barley exports were stable from 2017–2018 to 2019–2020 and then increased in 2020–2021 and have shown a stable performance during the current period of 2021–2022.

Table 5.1 World barley export October/September year (thousand metric tons)

Country	2017/18	2018/19	2019/20	2020/21	2021/22
Argentina	2537	3001	2598	2900	3500
Australia	6088	3666	3231	6700	5000
Canada	1868	2269	2520	3800	4500
European Union	5894	5809	7579	7600	7300
Kazakhstan	1411	1762	1292	1000	1300
Russia	5661	4320	5141	5400	5200
Syria	0	0	300	250	150
Ukraine	3188	4407	4990	4300	5100
United Kingdom	896	1538	1397	1425	1000
Uruguay	12	62	41	200	150
Others	343	246	144	407	192
World Total	28,000	27,181	29,388	34,257	33,667

Source: https://apps.fas.usda.gov/psdonline/circulars/grain.pdf

Table 5.2 World barley import October/September year (thousand metric tons)

Country	2017/18	2018/19	2019/20	2020/21	2021/22
Algeria	439	467	503	800	700
Brazil	584	608	647	700	700
China	8144	5181	5969	10,000	10,600
Colombia	324	341	308	320	350
European Union	979	1762	1089	1400	1300
Iran	2700	3200	2300	2500	2400
Israel	234	236	358	300	350
Japan	1253	1158	1253	1150	1250
Jordan	788	928	564	700	800
Kuwait	521	474	522	350	500
Libya	438	888	891	900	700
Mexico	1	82	346	350	350
Morocco	363	299	1073	700	300
Qatar	229	288	349	400	450
Saudi Arabia	7700	5700	7300	7500	7500
Thailand	147	206	685	1150	600
Tunisia	674	487	751	800	700
Turkey	774	375	1007	650	1000
United Arab Emirates	263	476	443	470	500
Vietnam	177	133	198	750	400
Others	1551	1846	1309	1492	1344
World Total	28,000	27,181	29,388	34,257	33,667

Source: https://apps.fas.usda.gov/psdonline/circulars/grain.pdf

As already pointed out, the rich genetic diversity in barley enables this crop to adapt to diverse conditions. This genetic diversity of barley has always been an important subject of study for geneticists and breeders in order to identify new characters that can help improve the sustainability of the crop. This allowed early spring varieties suitable for environments with a prolonged cold weather and short spring-summer seasons and tardier winter varieties able to fully exploit the productive potential of temperate climates.

The good resistance to drought has also allowed the species to adapt to the environments such as those of North Africa and the Middle East. In fact, barley has a vast area of cultivation, from the humid regions of Europe to South America and the arid areas of Africa and Asia (Kling and Hayes 2004; Tricase et al. 2018). Nevertheless, the barley yield depends upon the favourable climatic conditions, soil characteristics and agronomic practices, and because of the deployment of high yielding varieties and other associated technological innovations, world barley productivity has increased from 1.33 t/ha in 1961 to 2.95 t/ha in 2018. Countries such as Belgium, Netherlands, New Zealand, Saudi Arabia, Ireland, Chile, Switzerland and France have recorded more than 6.0 t grain yields per hectare in 2018 (Fig. 5.3) (FAO 2020).

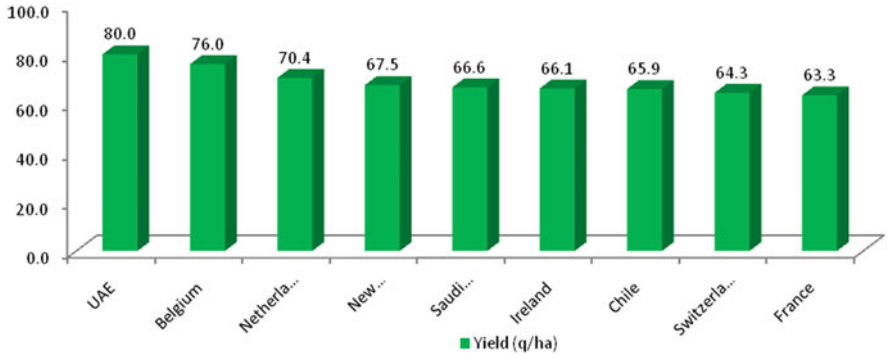

Fig. 5.3 Countries with high barley grain yields (q/ha) in 2018

5.2 Types of Barley and Versatility of Their Use

The genetic diversity in barley pertaining to morphological as well as agronomic attributes is immense, and different barley types are cultivated in different parts of the world depending on the climate buffering capacity and end use of the produce. Accordingly, barley can be classified depending on various factors major being the growth habit (spring or winter types), number of rows of grains in spike (two-row and six-row barley), spike compactness, hull adherence (hulled, presence of an outer husk attached to the grain or hulless/naked with removal of the outer husk during threshing), presence/absence or size of awns (awned, awnletted or awnless varieties), end-use (malting, feed or food) and aleurone colour (black, purple or white kernels) due to the different level of anthocyanin (Arendt and Zannini 2013; Ciccarelli et al. 2008; OECD 2004).

The two-row is the original ancestral variant which was domesticated and six-row is a mutant selected later on. In the two-row type, the lateral two florets are sterile while only the central floret is fertile. In the six-row type, all the three florets in a spikelet are fertile. The spike fertility is conditioned by a gene *Vrs1*. The two row barley grains are plump and the size is more uniform and lower in protein and high in starch, therefore considered suitable for malting purposes. The six-row barley is mainly used for feed and food with some good varieties also being utilised for malting because of its higher enzymatic activities. The hulless barley varieties in which the lemma and palea do not adhere to the pericarp strongly are preferred as food. The hulled condition is controlled by the *Nud* gene. In the malt barley varieties, hulled types are preferred as the hull which helps in faster moisture absorption in steeping, protection of growing plumule in germination stage of malting process, as well as it acts as a natural filter during mashing and brewing. Same is the case when the barley is meant for the feed purposes because of the yield advantage. The hulless barley constitutes a very small fraction of the total world barley production and is a

staple food crop in the Himalayan region, the Andes, north African countries and the Ethiopian highlands.

Barley can also be classified based on grain composition (normal, waxy or high amylose starch types, high lysine, and high beta-glucan or pro-anthocyanin free). Barleys of different classes often differ widely in both their physical and compositional characteristics, and as a result they have different processing properties and end uses. Globally, about 70% barley produce is used as animal feed and 6% as human food, while around 25% have industrial use in malting industry (Tricase et al. 2018). As a feed, barley is used in both ruminant and non-ruminant livestock, as well as in poultry and fish. For malt barley, mostly two-row types are used which normally yield less than the six-row varieties used for feed and food, however in recent years, several two-row malting barley varieties have been developed in India with comparable yield levels under optimum management.

Early uses of barley pertained to human food only to be followed by the malt purposes and its use as feed although came in the last but has become the most important one in the present day. The use as feed is global across developing and developed countries, while the use as food is in few developing countries of Africa and Asia. The utilization of barley for production of renewable biofuels has added tremendously to the versatility of the crop although the utilization data for the world or countries is not available. The wide adaptability of barley crop has contributed in its major part of production being animal feed apart from the quality considerations of the grain. Barley can be grazed, made into hay or silage while still green and can be used as straw after the grain is taken out. It grows up to 120 cm in height and due to its broader leaves, the leaf: stem ratio is quite high, that is, 0.88. The awnless/hooded barley varieties are considered to be safer and of higher nutritive quality as forage, as sometimes the pieces of the awns cause injury in mouth while animals are being fed the dry straw.

The grain protein generally ranges from 10 to 20% while that of the starch component is 50–70%. This nutrient composition is ideal for the livestock as well as for poultry and fish industries. The barley straw is also used as feed and contains 2–6% protein and 80–86% neutral detergent fibre (NDF). At flowering, the protein and NDF content has been reported to be 12% and 63%, respectively, which decrease to 9% and 56% at the time of dough formation stage, and also the forage is low in cell walls, ADF and lignin compared to other cereal forages (Ditsch and Bitzer 2005). In forage-deficient regions, which are usually the water-deficit areas, barley can be grown for obtaining both the forage and grain in the same crop, and this type of barley is called dual purpose barley. It is an alternative approach ensuring the availability of forage as well as acceptable amount of grain yield (Moustafa et al. 2021). The first cut of forage can be taken after 6–7 weeks of sowing, that is, the first node appearance stage, beyond that the yield of grain from regenerated crop is drastically decreased.

In India, scarcity of feed and fodder is one of the major constraints particularly in resource-poor, rural areas. Evidences indicate that feed-related problems accounted for about 36% (per annum in value terms) in dairy animals, and losses due to scarcity of dry and green fodder were estimated to be 11.6% and 12.3%, respectively (Birthal

and Jha 2005). The green fodder deficit of 65% and dry fodder deficit of 25% has been predicted in India by the year 2025 (Singh et al. 2013). Given the nutritional quality of barley, green fodder as well as straw and the ability of crop to withstand poor soils and drought, barley seems to be the trouble-shooter crop in the Indian context. These issues coupled with a rise in demand for dairy products due to urbanization and human population growth have warranted research on development of high yielding forage crop varieties with enhanced quality of feed and fodder. Under these circumstances, 70% of the total barley grain production of which is utilized as feed globally, as well as in India is an important feed and fodder crop particularly in winter when supply of green fodder is in shortage.

5.3 The Indian Context

In India, barley ranks fourth after wheat, rice and maize among the cereal crops. In 1961, a total of 2.82 million tons of barley grains were harvested from 3.2 million hectares with a yield level of 1.14 tons per hectare as compared to 1.78 m tons from 0.66 million hectares with yield of 2.7 tons per hectare in the year 2018 (FAO 2020). Though there was about 37% fall in the total grain production of this crop during this period, chiefly due to sharp decrease of 79% in its acreage brought about by increased irrigation facilities in the country leading to replacement of barley area by *rabi* crops mainly by wheat. It coincided with the green revolution, in which a spectacular increase of 206% in the yield levels propelled mainly through the development of high yielding barley varieties insulated with inbuilt resistance to important biotic and abiotic stresses was witnessed (Table 5.3). However, the area under barley consistently decreased since 1961 only to stabilize in the last two decades and drifts around 0.67 m ha (Table 5.3).

In India, barley is cultivated in the plains (Rajasthan, Uttar Pradesh, Madhya Pradesh, Punjab, Haryana and Bihar) and on the hills up to an elevation of around 4000 m (Himachal Pradesh, Uttarakhand and Jammu and Kashmir). In the Himalayas, the six-row husked barley types are commonly cultivated while the two-row types, both husked and huskless, are grown only to a limited extent. Cultivation of the six-row husk less forms are confined to the higher Himalayan ranges. In these areas, barley is generally a crop of marginal, low-input, drought-

Table 5.3 Decade wise % increase/decrease, for area, production and yield in India

Period	Area	Production	Yield
1961–1970	−13.7	−3.6	11.7
1971–1980	−30.7	−41.7	−15.9
1981–1990	−45.2	−35.2	18.2
1991–2000	−24.7	−11.4	17.7
2001–2010	−19.8	−5.3	18.0
2011–2018	−6.3	7.0	14.2
1961–2018	−79.4	−36.9	206.2

Source: FAO (2020)

Table 5.4 Recent high yielding varieties developed in India

Type	Production conditions	Varieties	Areas recommended for
Malt barley	IRTS	DWRUB52, RD2668, DWRB92, DWRB101, RD2849, DWRB123, DWRB160, DWRB182 (all 2-R)	NWPZ
	IRLS	DWRUB64, DWRB73(2-R), DWRB91 (2-R)	
Feed barley	IRTS	BH902, BH946, DWRB137, K1055, PL891[a]	NWPZ
	IRTS	DWRB137, HUB113	NEPZ
	IRTS	PL751, JB1, RD2715,[b] DWRB137, RD2786, RD2899, BH959	Central zone
	RFTS	RD2660	NWPZ
	RFTS	K603, JB58 (MP)	NEPZ
	IRTS (saline soils)	NDB1173, NDB1445, RD2794, RD2907	NWPZ and NEPZ
	RFTS (hills)	BHS380,[b] BHS400, UPB1008, VLB118, VLB130,[b] HBL713	NHZ

IRTS irrigated timely sown, *IRLS* irrigated late sown, *DP* dual purpose, *2-R* two-row type, *NWPZ* North Western Plain Zone (Punjab, Haryana, Delhi, Rajasthan, Western Uttar Pradesh), *NEPZ* Eastern UP, Bihar and Jharkhand, West Bengal (excluding hills), *Central Zone* Madhya Pradesh, Gujarat, Kota and Udaipur division of Rajasthan, Jhansi division of Uttar Pradesh, *NHZ* Uttarakhand, Himachal Pradesh and J & K

[a]Huskless

[b]Dual purpose

stressed environments. The landraces grown in these areas are favoured by farmers for their quality, both as grain and straw. However, the area under the husk-less barley landraces, which were very popular and widely grown in higher Himalayan ranges about four decades ago, has declined considerably (Kant et al. 2016).

The barley improvement program in India was initiated in the early 1920s, where pure line selections were followed. Thirty-one barley varieties were developed for cultivation in different states, before the inception of AICBIP. The organised breeding program was initiated with the inception of All India Coordinated Barley Improvement Programme (AICBIP) during 1966–1967 and since then more than 105 barley varieties have been released in India for commercial cultivation so far (Kumar et al. 2017). Majority of these varieties are six-row types, developed for feed purpose while two-row barley varieties meant for malt purpose are of recent origin. Most of the recently developed (last 15 years) varieties for different purposes, regions and production conditions (Table 5.4) are in seed chain and are being cultivated as per recommendations. However, a significant reduction in annual breeder seed indents of barley has been recorded (Table 5.5), which might prove a cause of concern for this crop considered to be a climate change crop (Fig. 5.4).

Indian barley improvement programme has been very successful in raising genetic potential of feed barley from 35 q/ha grain yield recorded in the variety ‘Vijaya’ to 67.44 q/ha in the variety ‘DWRB137’ released in the years 1972 and

Table 5.5 Barley breeder seed indents and production during last five cropping season in India

Year	DAC indent (*q*)	Varieties	Production (*q*)
2015–2016	1138.43	36	1123.57
2016–2017	1140.75	38	1521.86
2017–2018	1048.25	35	1452.20
2018–2019	827.85	29	1421.05
2019–2020	524.97	26	997.25
2020–2021	638.06	24	830.0

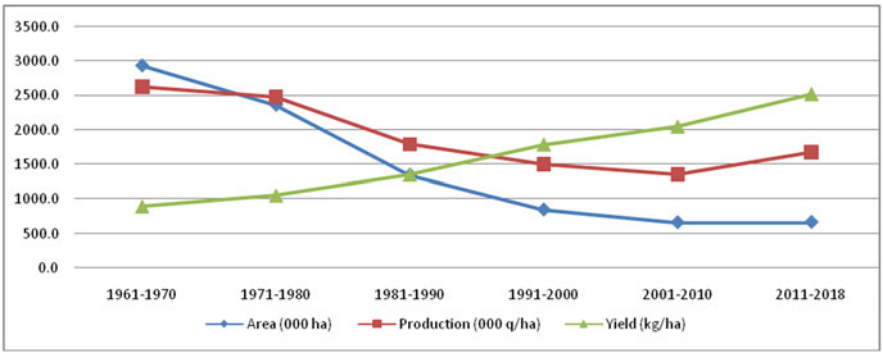

Fig. 5.4 Decadal average area, production and yield of barley in India (*Source*: FAO, 2020)

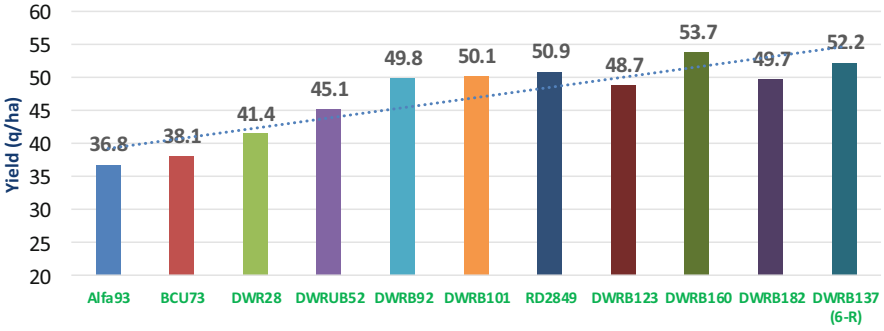

Fig. 5.5 Genetic gain in two-row malt barley cultivars released between 1994 and 2020 in India

2018, respectively. Similarly, in case of two-row malt barley improvement programme, the significant genetic gain has been made between 1994 (Alfa 93) and 2020 (DWRB182) (Fig. 5.5) initially utilizing the two-row × six-row hybridization program, which was initiated to improve the local adaptation of the introduced superior malting quality two-row barley cultivars in India. The yield gap between improved six-row feed barley and two-row barley has been almost bridged in northern plains under optimum management conditions as evident in Fig. 5.5,

where the average yield of latest six-row barley variety DWRB137 and two-row barley varieties is at par.

5.4 Origin and Evolution of Cultivated Barley

Belonging to the grass family Poaceae, barley is assumed to be domesticated circa 10,000 years ago, that is, the dawn of human civilization, making it one of the first domesticated crop plant species providing an anchor for development of early agriculture through the Neolithic period (Wang et al. 2015). Barley finds a mention in the oldest text available, that is, the Rig Veda supposed to be authored circa 5000 years ago and *Yavagu* a sour gruel somewhat similar to the modern-day beer is believed to be made from barley grains at that time as well (Nene 2012). The origin of cultivated barley is not conclusively, known and there are many claims about it having originated from the Fertile Crescent which is the Middle East and northern Africa (Jordan, Israel, Lebanon, Syria, Turkey, Iraq and Iran) in the present day (Zohary and Hopf 2000; Badr et al. 2000).

However, another hypothesis of origin of barley in the South-Eastern Asia (present-day China and Nepal) has been propounded by Clark (1967) and was later on supported by Bothmer and Komatsuda (2011). Azhaguvel and Komatsuda (2007) proposed diffuse (polyphyletic) origin of *H. vulgare, that is,* oriental and occidental barleys originating independent of each other. Moreover, the discovery of hulless six-row barley in Mehrgarh (Pakistan) dating 7000 to 6500 BC has made the subject matter of origin of barley highly debatable (Mehra 2007). The Central Asia and Tibetan plateau are also candidates of independent domestication events of cultivated barley narrowing down the debate that barley has multiple origins (Dai et al. 2014). Using sequences for NAM-1gene and grain protein content (GPC), Wang et al. (2015) concluded that Tibet is one of the origin and domestication centres for cultivated barley. The wide adaptability of barley to different climates and soils is one of the major factors why this crop was among the first ones selected for domestication by the early human settlers. Barley can naturally tolerate a variety of abiotic stresses including drought, soil salinity, high and low temperatures and can be termed as a minimum effort cereal crop. Therefore, where cultivation of wheat is not possible due to prevailing climatic stresses, barley can perform better.

For any crop to survive the onslaught of climate change and introduction of new crops, the genetic base needs to be large enough to incorporate new adaptive and agronomic alleles apart from the quality. This is true in case of the genus Hordeum which comprises of 31 species as opposed to *Triticum* (wheat) with only 10 species. The broad genetic base is at the core of very high adaptability of barley and its eventual spread as successful crop plant species to new geographies. Barley is cultivated from Scandinavia to Sub Saharan Africa to Central Asia to the Himalayas and to the Thar Desert. The progenitor barley species remains unknown, and the wild barley *H. spontaneum* is considered to be an intermediate stage frozen in evolution. Although in some studies, *H. spontaneum* has been considered to be the progenitor of cultivated barley (Wang et al. 2015). Ethiopia is considered to be the secondary

centre of diversity, while India is the regional centre of diversity for barley. The western Himalayas exhibit significant barley diversity for cold and drought tolerance and aleurone colours particularly variations of black–and–blue colours. The main domestication syndrome traits in barley are non-brittle rachis, hull-less (free threshing) grains and large seed size (Salamini et al. 2002).

5.5 Barley Wild Relatives

Crop and produce uniformity have become an essential requirement for modern agricultural production systems and this has led to a decline in the genetic diversity leading to increased susceptibility of the crop plants to abiotic and biotic stresses. Barley is no exception to this. To this effect, the barley crop wild relatives are potential donors of new genes for better adaptation (Rehman et al. 2019). Barley wild relatives possess good diversity for agronomic traits, resistance to diseases, physiological growth, salinity tolerance, water and nitrogen use efficiency. According to the gene pool concept (Harlan and de Wet 1971), the genus *Hordeum* has 33 listed species arranged in three gene pools. The primary gene pool (natural crossing occurs and hybrids are vigorous and fertile) in barley is composed of *Hordeum vulgare* (landraces and varieties) and the progenitor species *H. spontaneum* (Vincent et al. 2019; Rehman et al. 2019). Therefore, in barley breeding programmes, *H. spontaneum* or the wild barley is of very much importance as donor of desirable alleles to the cultivated barley and had been a regular feature of the barley breeding programmes aimed at both abiotic as well as biotic stresses. The *Rph16* gene conferring resistance to all the known races of leaf rust fungus *Puccinia hordei* and *Mla-6* and *Mla-14* against powdery mildew were sourced from *H. spontaneum* (Ivandic et al. 1998; Jørgensen 1992).

Currently, the species is having above 30,000 accessions in different gene banks around the world. The secondary gene pool (natural crossing generally does not occur and the hybrids are weak/non-fertile) in barley is composed of only one species, i.e., *H. bulbosum*. *H. bulbosum* has been historically utilized in developing of double haploids in barley, as well as wheat but no significant gene transfer incidence has been reported. Different studies have reported *H. bulbosum* as a valuable source of disease resistance genes. The remaining diploid, tetraploid and hexaploidy, 31 species of the genus *Hordeum* make its tertiary gene pool which still remains to be utilized in the breeding programmes. The fourth gene pool represents no living species but includes individual genes that may be transferred to barley by genetic transformation from any species including animals and microorganisms (Hernandez et al. 2020).

As far as the distribution of wild barley species is concerned, they are spread in the Americas, Europe, the Mediterranean, Central and Middle-Eastern Asia and Africa. These species possess very strong barrier to successful crossing including non-pairing of the chromosomes during meiosis and eventual hybrid non-viability or breakdown. Therefore, *H. bulbosum* in the secondary gene pool assumes very high importance as a donor of desired alleles to *H. vulgare*. The interspecific

incompatibility, chromosome elimination, endosperm degeneration, hybrid instability and chromosome pairing, hybrid infertility, reduced recombination and linkage drag are the major constraints of the *H. vulgare* × *H. bulbosum* crosses (Pickering and Johnston 2005). In spite of these constraints, many important genes have been identified from these interspecific crosses including biotic stresses such as leaf rust, powdery mildew, stem rust, *Septoria* speckled leaf blotch, barley yellow dwarf virus, barley mild mosaic virus, barley yellow mosaic virus and abiotic stresses such as drought tolerance (Rehman et al. 2019).

The primary gene pool, therefore, seems to be the most obvious choice for utilization in the breeding programmes and the landraces from this have been successfully utilized to breed powdery mildew-resistant varieties possessing *mlo11* gene (Jørgensen 1992). The *H. spontaneum* germplasm has also been reported to be a rich source of resistance genes against major diseases (leaf rust powdery mildew, scald), aphid (*Rhopalosiphum maides*) and abiotic stresses mainly the drought and salinity, as well as quality. Although the linkage drag is a persistent nuisance here also, still extensive use of *H. spontaneum* has been made as a donor of useful traits to the cultivated barley. *H. spontaneum* could be a good source of cold tolerance and novel lipoxygenase (LOX) alleles (Hirota et al. 2008). The International Center for Agricultural Research in the Dry Areas (ICARDA) has the global mandate for barley improvement and holds in-trust one of the largest collections of barley which includes unique and rich collections of landraces (18,935) and wild *Hordeum* (2392) species (Rehman et al. 2020).

The obstacles including cross incompatibility, infertility, reduced recombination and introgression of undesirable alien genome segments resulting in linkage drag have historically hampered the utilization of barley wild relatives in breeding programmes. A deeper and more practical understanding of these valuable wild relatives is necessary if these assets are to be used effectively in developing improved varieties. In general, current varieties and potential varieties have a narrow genetic base, making them prone to suffer the consequences of new and different abiotic and biotic stresses that can reduce crop yield and quality. Therefore, to achieve higher genetic gains in yield and quality in the changing climate scenario, the adaptation of the newly developed varieties must be very high in order to cope up with the prevalent abiotic and biotic stresses and the use of barley wild relatives in the breeding programmes might be highly useful to broaden the genetic base and to enhance the adaptation (Hernandez et al. 2020).

5.6 Barley Genetic Resources

Plant breeding requires sufficient genetic diversity for effective generation of novel, valuable and improved combinations of alleles. The genetic diversity in cultivated barley ranges from very low in regions of Europe (Garcia et al. 2003) to extremely high in other continents mainly Asia and Africa (Khodayari et al. 2012). Numerous factors such as mutations, selection (both natural and artificial), genetic drift, random mating, etc. have caused the origin of different forms in barley. For example,

mutation of ancestral wild-type two-row barley led to a recessive six-row type after domestication. Similarly, geographical separations and differential breeding for vivid end use led to different varieties of two- and six-row, and their further adaptation to different environments differentiated the spring and winter forms of barley. In barley, vast germplasm collections are accessible but their use for crop improvement has been historically limited and efficiently accessing genetic diversity is still a challenge.

The diverse barley genetic resources which include modern cultivars (currently in use), obsolete cultivars (often elite cultivars from the past that are frequently present in the pedigrees of modern cultivars), landraces, wild relatives, genetic and cytogenetic stocks, as well as breeding lines are essentially required to develop quality cultivars ensuring stable productivity. Therefore, it is imperative to collect, evaluate and maintain genetic resources available from different geographical areas throughout the world. The barley collection comprises accessions representing 20 species including wild relatives, of which 98.5% of the accessions represent common barley (*Hordeum vulgare* L.). Out of more than 400,000 accessions of *Hordeum* genus stored in 108 gene banks spread across 64 countries worldwide, include wild relatives (15%), landraces (44%), breeding materials (17%), genetic stocks (9%) and cultivars (15%).

The use of genetic resources for barley improvement programme started more than 70 years ago with the collection of local landraces. Landraces store a high level of genetic diversity and possess high potential for adaptation to environmental conditions, and thereby they are an important element of future breeding programmes. Landraces of inbreeding crops including barley are genetically heterogeneous populations comprising inbreeding lines and hybrid segregates generated by a low level of outcrossing (Nevo 1992). In barley, they represent the largest part of germplasm conserved in gene banks worldwide. Historically, landraces constitute important resource for introgression of different alleles into pure line varieties. In the initial collections of landraces held in gene banks, at least 50–60% of the total genetic variation captured resides within the landraces, the remainder being accounted for by differences between landraces (Brown and Munday 1982). Seven gene banks hold the largest landrace collections of barley with ICARDA (Morocco), having more than 15,500 landrace accessions. Other gene banks for barley landraces are Chinese Academy of Agricultural Sciences (CAAS), Institute of Biodiversity Conservation (IBC, Ethiopia), PGRC (Canada), USDA (United States), IPK (Germany), and Research Institute for Bioresources (RIB, Japan), each having more than 10,000 accessions. These landraces have been useful for varietal development for disease resistance, abiotic stress and root architecture features (Dziurdziak et al. 2020; Friedt et al. 2011).

Breeding materials are the second most frequent category of barley germplasm held in gene banks globally with 49,000 accessions. The largest collection of breeding material is conserved at CIMMYT in Mexico with 11,000 accessions, followed by PGRC (Canada), ICARDA (Morocco), USDA (United States), National Institute of Agrobiological Research (NIAR, Japan), and Institut National de la Recherche Agronomique (INRA, France).

As already mentioned, the crop wild relatives provide an important source of allelic diversity and enhanced levels of resistance to multiple stresses. Wild ancestors introduce new alleles from wild and old species to locally adapted germplasm and providing useful traits. For example, gene for fungal resistance has been introgressed from *H. spontaneum* in several barley cultivars released in Europe (Schmalenbach et al. 2008; von Korff et al. 2005). The majority of the wild relative collections are represented by the barley progenitor, *Hordeum spontaneum* (20,700 accessions), while out of 5900 accessions of wild barley species belonging to the secondary and tertiary gene pool, the largest collections are maintained in Canada and Sweden.

However, the alleles in barley wild relatives such as *H. spontaneum* and *H. gricrithon* have linkage drags, and their exploitation for cultivar improvement is limited by cross-incompatibility barriers and hence the potential of wild relatives for improving quantitatively inherited traits is largely unexplored (Fischbeck et al. 1976; Moseman et al. 1981). Many major genes from wild relatives have been transferred into the cultivated gene pools (Hajjar and Hodgkin 2007). The genetic stocks of barley include morphological mutants, genetic male sterile stocks, and various cytogenetic stocks, for example, trisomic, inversion and translocation stocks. Recently, the mapping populations are added to the list of the genetic stocks. The Nordic Genetic Resources Center or NordGen (formerly Nordic Gene Bank) in Sweden has the most extensive collection of barley genetic stocks which comprises about 10,000 accessions, and large collections of genetic stocks are also maintained at PGRC (Canada), USDA (United States) and NIAR (Japan).

The cultivars refer to the finished products (both advanced and obsolete) having desired traits and stability derived from plant breeding programmes. Numerous high-yielding barley cultivars have been evolved through extensive plant breeding efforts and strict selection approaches. Natural mutations in *H. vulgare* as well as mutations induced by radiation or chemical treatments have also been used for cultivar development. The largest cultivar collections of barley are held at N.I. Vavilov Research Institute of Plant Industry (VIR, Russia) with 9600 accessions (Tables 5.6 and 5.7).

Moseman and Smith Jr (1985) classified barley germplasm collections as either base or working/active collections. Base collections are maintained for long-term storage and are used as a benchmark for monitoring changes in genetic diversity over time due to genetic drift. The seed viability of accessions should remain at acceptable levels for at least 50 years. Active collections are available for distribution to scientists and are accessed frequently. Accessions in an active collection include cultivars, selections, breeding lines and landraces, which do not require special care for maintenance. The active collection is intended to preserve germplasm viability in medium-term storage conditions for at least 20 years.

Table 5.6 Share of *Hordeum* accessions based on different classification

Species collection	Species	Percent (%)
Genebank collections (Bockelman and Valkoun 2011)	*H. vulgare* ssp. *vulgare*	88
	H. vulgare ssp. *spontaneum*	10
	H. bulbosum	0.4
	other wild species	1.7
	Landraces	44
	Breeding lines	17
	Crop wild relatives	15
	Cultivars	15
	Genetic stocks	9
Natural v/s Created (Bockelman and Valkoun 2011)	Germplasm evolved in long-term interaction with local environments and farming practices	59
	Germplasm resulted from modern plant breeding and research	41

Table 5.7 Sample specifications for storage in base and active collection

	Minimum sample size for storage	
Type	Base collection	Active collection
Cultivated barley	Two packets of 25 g each.	1500 seeds (L flag)
Wild relatives	One packet of 25 g.	1500 seeds (L flag).

Source: https://cropgenebank.sgrp.cgiar.org/index.php/crops-mainmenu-367/barley-mainmenu-250/conservation-mainmenu-368/storage-mainmenu-448

Table 5.8 Composition of the barley core collection

S N	Category	Number of accessions
1	Cultivars	500–800
2	Landraces	500
3	*H. spontaneum* (Wild progenitor)	150
4	Other wild *Hordeum* spp.	50–80
5	Genetic stocks	150–200

5.7 Barley Core Collection

In order to facilitate the better access and utilization, development of Barley Core Collection (BCC) was initiated by van Hintum (1994). The USDA-ARS National Small Grains Collection (NSGC) is one of the largest collections of barley germplasm in the world (Munoz-Amatriain et al. 2014). The NSGC comprises of 33,176 barley accessions that have been acquired and maintained over the past 100 years. These include cultivars, breeding lines, landraces and genetic stocks from more than 100 countries (Bonman et al. 2011). The BCC has five (Table 5.8) proposed components of germplasm that represent the entire diversity of barley having 1700 accessions from different categories.

5.8 Floral Biology of Barley: Emasculation and Pollination Techniques

The barley inflorescence is called a spike and is composed of spikelets in triplets at each node of rachis. It is an annual, diploid ($2n = 2x = 14$) self-pollinating species with a genome of >5 Gbp in size. Barley is not only used as a model plant for genetic studies but the genome of Betzes cultivar of barley is being used as a reference genome for tribe *Triticeae* under family *Poaceae*. This is because of its large genome size (4873–5096 Mbp) unlike rice and maize and diploid ploidy level unlike wheat. The chromosome number of barley ($2n = 14$) is also lowest as compared to $2n$ chromosome numbers of wheat, rice and maize having 42, 24 and 20, respectively (Ullrich 2010). Barley has an incomplete flower because it lacks sepals and petals. The inflorescence of barley is known as spike, head, ear or panicle of spikelet. This flowering and fruiting unit emerges from the "boot," which is the sheath of the uppermost leaf on the culm (the flag leaf) (Fig. 5.6).

The flowers, group together in a central axis or rachis which is composed of nodes and internodes, which bears a group of three spikelets. Spikelets have only one flower. Each barley floret comprises of lemma, palea, lodicules, androecium and gynoecium in the model proposed by Forster et al. (2007). As described in the section "types of barley" above, the spikelet varies in six- and two-row barley types where all the spikelets develop grains in six-row while in two-row barleys, only the middle spikelet produces a grain, the remaining two outer ones being abortive. Each spikelet consists of a sterile boat-shaped bract called glume. At the end of each fertile spikelet, there is a long bristle called awn. A spikelet is partially enclosed by two

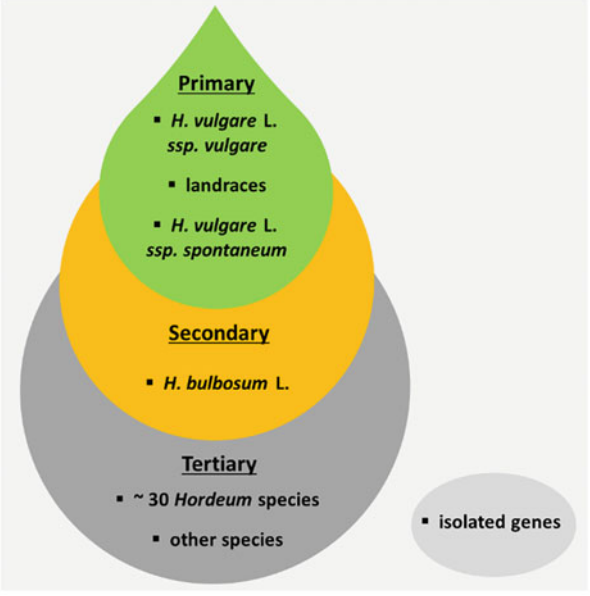

Fig. 5.6 The barley gene pools (*Source*: Hernandez et al. 2020)

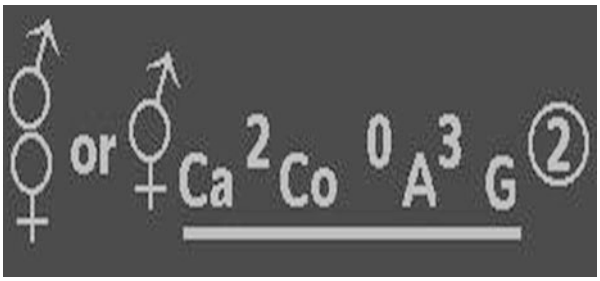

Fig. 5.7 Barley floral formula

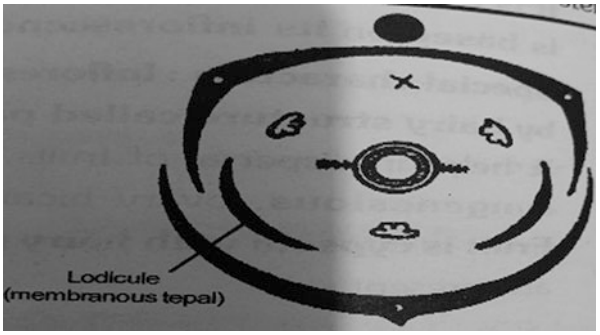

Fig. 5.8 Barley floral diagram

long narrow sheaths called "empty glumes," which do not get larger as development takes place. These empty glumes have two broad membranous sheaths inside which are enclosed all vital flower parts known as "flowering glumes" or "palea". Just within the base of the outer flowering glume are two small scale-like structures briming with long hairs, known as "lodicules". These lodicules have an important role in the opening and closing of the flower.

The barley floret is "perfect," meaning that it contains both male (stamen) and female (pistil) floral components (Figs. 5.7 and 5.8). The male and female flower parts are enclosed within the lemma and the palea. Barley is polyandrous and each androecium consists of three male organs or stamens having long slender stalk called "filament" and a two-lobed anther containing the pollen (Fig. 5.9).

The anther dehisces just after the emergence and shed pollen on feathery stigma. Pollen loses the viability very shortly after dehiscence. The ovary is superior, unilocular and has basal placentation, and the two stigmas are feathery and biforked. The stigma catches the pollen grains during flowering and serves as a medium for pollen germination. Stigma remains receptive at least for 2 days after anthesis. The hairiness of stigma varies among barley genotypes from completely covered to very few hairs (smooth awned barley). In the lateral spikelet of two-row barley, the ovary remains undeveloped. The ovary of barley encloses a single ovule. The seeds of barley are called caryopsis and are endospermic monocotyledonous. The upper flowers of the spike never develop and die off thereby, giving the barley ear its characteristic truncated appearance.

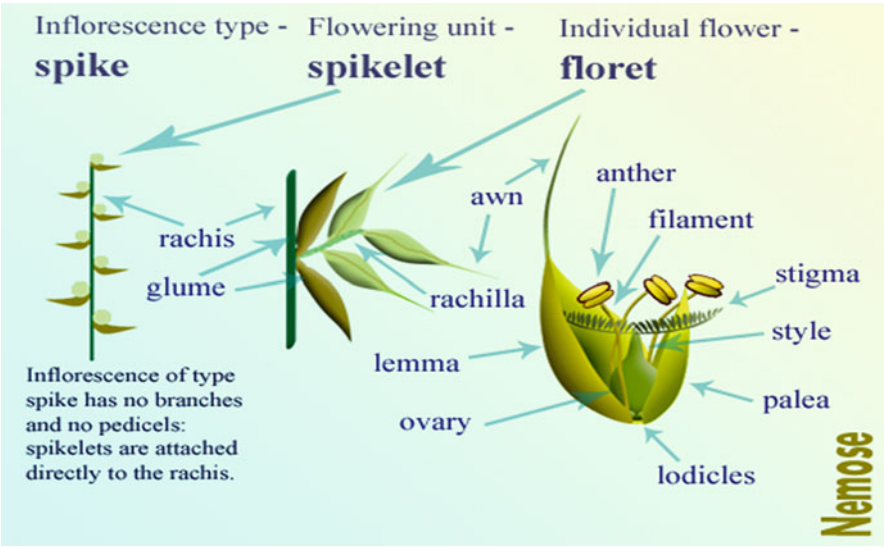

Fig. 5.9 Barley inflorescence (https://in.pinterest.com/pin/120400990013798413/visual-search/)

5.9 Mode of Pollination in Barley

Barley has hermaphrodite/bisexual flowers and is mostly autogamous (self-pollinated) crop, where the pollen from an anther shed within the floret on stigma of the same flower and fertilization takes place just before the opening of florets, and the phenomena is called chasmogamy. However, sometimes barley can be open pollinated, as the glumes open during flowering and the anthers are pushed outside the flower allowing the pollen to become wind-borne. The pollen can travel a few metres where it can land on an awaiting stigma of a neighbouring plant and cross-pollination then occurs. Cross-pollination may occur ranging from 1 to 5% in barley. In either case, once the pollen reaches the stigma, the fertilisation process begins and the fertilised cells begin their journey to become a grain. The cleistogamous state in barley is recessive and under the control of a single gene at the cleistogamy 1 (cly1) locus, which maps on the long arm of chromosome 2H (Turuspekov et al. 2004).

5.9.1 Selfing and Crossing Techniques

The generation and exploitation of new combinations of allelic variants at genetic loci is the fundamental step for getting superior recombinant progeny or in understanding the genetic control of key characters. As already pointed out, cultivated barley (*Hordeum vulgare* L.) is a diploid hermaphrodite where anthers tend to ripen and shed pollen inside the spikelet making barley a natural inbreeder with low rates

of outcrossing. The crop has cleistogamous behaviour where anthesis occurs before the anthers are exposed. In order to ensure complete selfing in barley, the inflorescence is covered with a butter paper as soon as it comes out of the boot and kept undisturbed till the flower opens completely. For crossing and development of hybrids, the following steps are generally adopted by the breeders:

5.9.1.1 Sowing

Plants for crossing programmes are grown in the field or in glasshouses. Crossing in field is advantageous in terms of cost reduction; however, one has to restrict to a limited crossing window and effect of weather. In contrast, crossing in the glasshouse is easier with flexibility of timing, no adverse weather effect and crossing success is often better as compared to that in fields. All entries are labelled containing information on the variety/nursery name and line number or code. Some of the winter barley lines require vernalization treatment therefore knowledge of the vernalization requirement has to be taken into consideration in a crossing schedule. Also staggered sowing should be practiced for matching of anthesis and pollination programme.

5.9.1.2 Tools Used for Crossing

To accomplish the selfing and controlled pollination, breeders need specific instruments such as sharp fine-pointed scissor, fine-pointed forceps, jewellers tags, glassine/cellophane/paper packets approximately 4 cm wide by 15 cm long and special magnifying glasses (to see spikelets and their anthers), a hollow tube approximately 2–3 cm internal width and 10–15 cm of length.

5.9.1.3 Emasculation

Removal of stamens/anthers or killing the pollen of a flower without affecting the female reproductive organ is known as emasculation. In bisexual flowers such as in barley, emasculation is essential to prevent self-pollination. Emasculation must start at 1–2 days before anthesis, when anthers are green. If the anthers near the centre of the spike appear pale yellow, the spike is too old to emasculate. Emasculation may be done normally before 10 AM in morning to avoid any chance of anther rupture in process. For emasculation, select the spike which is still enclosed in flag leaf sheath. The leaf sheath should be visibly swollen but the degree of swelling will be greater in a six-row parent than in a two-row one. Remove the flag leaf, lateral florets and very small florets at the base and at the tip of the spike. This is because the lateral spikelets under certain conditions can produce anthers that shed pollen and thus could potentially self-fertilize the emasculated spikelets.

With fine-pointed scissors, clip the top of the lemma and palea in an "egg-topping" approach. The idea is to cut at the top of the anthers (one can generally see the anthers through the lemma as a dark shadow against it). Remove the three anthers with a fine-pointed forcep from each spikelet without accruing any damage to the stigma. Bag the emasculated spike with glassine bag to prevent the contamination from the foreign pollen. Pickering (1982) observed significant improvement in seed quality and weight in barley when spikes were covered with brown paper

bags as compared to glassine and polyethylene. Attach a tag to the emasculated spike with information such as name of variety, date of emasculation and name of breeder. Emasculation of barley is labour-intensive and tedious, and usually requires skilled workers. Hot water emasculation is also another commonly used method for deactivating pollen. It is an easy method and does not necessarily require skilled labour (Tong and Yoshida 2008). The female emasculated spike will be ready to pollinate when the spikelets open showing a gap between the lemma and palea.

5.9.1.4 Pollination

Before pollination, check spike to determine if any of the spikelets has already set seed, these should be removed. Pollination is most successful if done with in 1–3 days following emasculation, when the stigma has maximum receptivity. Chose the male parent and find a spike that has not shed pollen where the anthers are pale yellow and are still at the base of each spikelet. Since anthers dehisce early in the morning, it is best to look for pollinating spikes as early in the day as possible. Clip the spikelets above the anthers, and after 5 min the anthers start to puff up. Thereafter, the filaments start elongating and force the anthers upward. One can check the shedding of pollen by gently tapping the spike and a cloud of pollen grains should fall from the anthers, these anthers are used for pollinating female parent. Barley pollination can be done by the twirl method or by anther transfer. In anther transfer method, the anthers are removed and placed above the open female spikelet and are broken. Since each anther contains numerous pollen grains, each anther can be used to pollinate 2, 3, or more female flowers. In twirl method, a 10–15 cm long tube that is 2–3 cm wide is placed over the emasculated female parent.

The spike with the extruded anthers is then held upside down over the top of the tube and is twirled and inserted into the tube simultaneously to shed pollen over the female spike. After the pollination, the glassine bag is replaced over the female spike and female parent and date of pollination is written. Sometimes there is no pollen available, so the emasculated spike may be kept in cold storage for up to 42 days. Similarly, non-emasculated detached spikes can produce viable pollen after up to 26 days in cold storage in barley (Pope 1939). If the cross is successful, the developing caryopses (kernels) will begin to be visible around 5 days after pollination and emerges from the cut spikelets by 7 days after pollination. The spike can then be left to complete drying and the F_1 hybrid seed picked off and packeted for subsequent sowing to produce F_1 plants. To reduce the time between crossing and sowing of F_1 hybrid seeds, the developing F_1 embryos can also be cultured.

Other crossing methods have also been tested by researchers such as male and female spikes are fastened together and enclosed in a bag. The bag is tapped or shaken periodically to disperse pollen (Hamilton 1953); or in the field, the approach method may be adopted for crosses between nonadjacent plants by cutting off the male culm and placing it in a bottle of water beside the female plant; or emasculated barley spikes can be cross-pollinated if male and female parents are planted in alternate rows and female spikes are emasculated (Omarov 1973).

5.9.2 Enhancing Crossing in Barley

- Crossing in barley can be accelerated by growing parent plants in control conditions.
- In winter barley, vernalization of seeds is needed for flowering to occur which can be accomplished by exposing germinating seeds to temperatures slightly above freezing.
- Tissue culture techniques such as embryo culture can be used for getting success in some wide crosses.

5.10 Barley Breeding Objectives

Framing breeding objectives to the targeted area and population for development of superior barley varieties is one of the most important planning steps in a breeding program. As in other crop breeding programs, barley breeding also involves the development and identification of short, intermediate and long-term objectives. The short-term objective aims at development of improved high-yielding cultivars in shortest possible duration, while intermediate and long-term objectives include development of germplasm for future use and involves programmes for incorporation of stable resistance genes to different biotic and abiotic factors, introduction of exotic germplasm and selection for adaptation. However, at times, most barley breeders are concerned primarily with short-term objectives (Wych and Rasmusson 1983). Also, breeding efforts on other aspects such as semi-dwarf plant development, lower protein content, higher malt extract are also equally important. Winter barley breeding programs involve improved hardiness, lodging resistance and disease resistance that are also suitable for harvesting as whole plant silage, thus having a place in double cropping systems.

Several breeding objectives depend on consumer preference and environment, and therefore is location-specific, for example, tolerance to acid soils and excess moisture at harvest time is essential in some areas, whereas drought resistance and suitability for irrigation are essential in other parts. Therefore, breeding objectives should not be rigid and should provide continuity of developing improved barley cultivars. In addition to high yield potential, barley breeding for yield stability, cold resistance in areas where non-hardy plants would be injured, resistance to drought in the dry areas, sturdy straw to prevent loss from lodging, resistance to soil stress in the presence of excess aluminium or toxic salts, or resistance to disease pathogens and insect pests that affect the plant's health are other very important barley breeding objectives. The major barley breeding objectives are described briefly in the sections which follow.

5.10.1 Increased Yield

Development of high-yielding cultivars of grain and forage is the primary objective of most barley breeding programmes because it directly affects the economic return to the growers. Success of breeding programme is measured by comparing modern cultivars with the older ones in terms of yield. In barley, substantial yield increase from 1.09 tonnes per ha in 1971 to 2.92 tonnes per ha in 2020, growing at an average annual rate of 2.55% have been demonstrated in India (https://knoema.com/atlas/India/topics/Agriculture/Crops-Production-Yield/Barley-yield). Similarly, in the Midwest, malting barley yields have increased at the rate of 2% per year over the past 40 years (Wych and Rasmusson 1983). This increase has been attributed to the yield contributing components including number of plants per unit area, number of heads per plant, number of kernels per head, and kernel weight (Woodworth 1931). Yield potential is generally expressed phenotypically through complex plant morphological, physiological functions and genetically complex quantitative character that interacts with the environment in which the plant genotype is grown.

Many studies in barley reported a positive correlation between grain yield and number of grains m^{-2} (Drikvand et al. 2011; Jabbari et al. 2010; Ruzdik et al. 2015). However, Purl et al. (1982) reported that the increase in grain yield was associated more with the weight than with the number of grains. Grafius (1965) described the plant yield increase in barley as the product of three components, viz., number of heads per unit area, number of kernels per head and kernel weight and proposed that performance of a cross could be predicted geometrically from parental yield components. Donald (1968) suggested uniculm ideotype for barley with a short, strong stem, high harvest index, few small erect leaves, erect spike and awns. Later, Casey-Common and Klinck (1981) suggested that the uniculm type is too restrictive, and as an alternative, proposed a limited-tillering ideotype.

The association of physiological traits such as increased vegetative biomass (Wych and Rasmusson 1983), harvest index (Martintello et al. 1987; Riggst et al. 1981) for improvement of the genetic potential of barley has also been highlighted. However, very fewer studies on yield gains were associated with increase in harvest index and grain number m^{-2} in barley. Nonetheless, these studies highlighted the genetic improvement contributed to the grain yield at the rate of 16 kg/ha/year in the United States (Boukerrou and Rasmusson 1990), from 18 to 20 kg/ha/year in Canada (Bulman et al. 1993; Jedel and Helm 1994) 19 kg/ha/year in the United Kingdom (Riggst et al. 1981) 41 kg/ha/year in Spain (Munoz et al. 1998), 74 kg/ha/year in Italy (Martintello et al. 1987), 41 kg/ha/year in Argentina (Abeledo et al. 2003), 21 kg/ha/year in Norway (Lillemo et al. 2010), 60 kg/ha/year in United Kingdom (Mackay et al. 2011) and in the Netherlands (Rijk et al. 2013).

However, Austin (1978) and Riggst et al. (1981) suggested that harvest index of barley may have reached a ceiling and that further increases in grain yield may have to come from increases in the biomass. A greater physiological efficiency by reductions in respiratory losses, increases in grain-filling duration, improved photosynthetic efficiency rate and greater pre-anthesis contribution to grain yield are the major suggested interventions in this regard. Breeding for high-yield potential in

barley is normally accomplished by crossing among genotypes with complementary genes to generate transgressive segregants with superior yield. Most selection for yield potential at early stage is based on the breeder's knowledge and experience, and the accuracy of his observations. In the early segregating generations, single plants are selected, which are then evaluated for yield potential by progeny tests. While constantly striving to improve the potential yielding ability in barley, it is also necessary to stabilize production by breeding for resistance to the adversities that may limit the final harvest.

Apart from genetic factors, environment may be responsible for positive phenotypic associations; therefore, the yield components should be evaluated in the environment in which selection is practiced. Most yield gains in barley have been achieved by selecting for yield itself, or for other factors which reduce yield-like resistance to lodging, shattering, disease resistance, etc.

5.10.2 Improved Malting Quality

Malting quality is a genetically complex trait, comprising of a series of different quality parameters and traits which follow a complex mode of inheritance (Schmidt et al. 2016). Malt quality is defined by the following specific criteria: Kolbach index, that is, enzyme activity (high), extract difference (low), extract content (high) and protein content (low). The trait, malting, is influenced not by genotype but by the environment and also the malting and brewing process. Hence, for a reliable description of this complex trait and its stability, multilocation trials are necessary, which need to be confirmed over several years of cultivation. Of different traits reported for malting improvement, the grain test weight (hectolitre weight) is important for achieving higher malt extract (Verma et al. 2008) followed by grain husk and protein content and can be used as a good selection criterion for large-scale germplasm evaluation and varietal improvement.

MacLeod (2000) reported negative correlation between malt extract yield and protein which is primarily due to *hordeins*. Diverse techniques based on multivariate analysis, principal component plots, etc. have been suggested for malting barley evaluation in breeding (Nielsen and Munck 2003), which give an easy-to-interpret picture about the correlation structure of the components for malting quality. The hulled barley is preferred by the malting and brewing industries, because the hulls are used as natural filters during the brewing process (Newman and Newman 2008). There has been a modest genetic gain for malting quality due to the development of few crosses and ultimately testing a limited number of advanced lines (Munoz-Amatriain et al. 2014). However, a new phase of breeding for high malting quality began with the introduction of the micro-malting method in the 1960s, which allowed a directed selection for processing quality based on small samples (Baumer et al. 2000). Because of the economic importance of malting barley, there has been a major effort to identify genes controlling malting traits and several references are available in the Barley Genetics Newsletters and in the Proceeding of the International Barley Genetics Symposia.

5.10.3 Resistance to Biotic Stresses

Resistance breeding is an important strategy for reducing crop losses caused by different biotic stresses. Genetic resistance is the most eco-friendly, durable and affordable method for low-income farmers, therefore, forms the major objective of most plant breeding programmes worldwide. Breeding for biotic stresses resistance involves different approaches based upon the economic importance of stress, the genetics of resistance, availability of resistance sources, expertise and the facilities available, etc. Also, effective selection depends on number of available genes and the screening environments. A list of major biotic stresses of barley is presented in Table 5.9. There are numerous successful examples wherein the conventional breeding approaches have done wonders; some of these include the resistant varieties against the rusts, smuts, powdery mildew, etc. Biotic stress in barley, specifically fungi, viruses, bacteria, nematodes, insects, arachnids and weeds, hinders the potential yield performance of the elite barley cultivars to a huge extent. These stresses cause varying degree of losses to the barley yield and quality. These agents can also cause minor reaction and loss below economic threshold level, however, sometimes devastating loses occurs that can cause epidemic by spreading to larger areas, even continent, forcing to famine-like situations. These biotic agents directly deprive their host of its nutrients leading to reduced plant vigour, and in extreme cases, death of the host plant. Therefore, breeding for biotic stress (Table 5.9) resistance is an important objective of barley improvement programmes.

5.10.3.1 Breeding for Disease Resistance

Under biotic stress, for most of the diseases, the genetics of resistance is relatively well known. For example, for barley yellow dwarf viruses, at least 40 major genes for resistance have been mapped till date. The QTL for resistance to all major diseases have also been discovered (Williams 2003). In barley breeding, for host resistance to different diseases each disease is treated as a separate breeding objective and based on severity of losses caused, the breeder establishes priorities on developing resistant cultivars. The breeding strategy depends on number of factors such as the resistance sources available, the number of loci involved, their mode of

Table 5.9 Major diseases and insect pests of barley

Type	Name of diseases/pests
Diseases	Net blotch (*Pyrenophora teres*), stripe rust (*Puccinia striiformishordei*), brown rust (*Puccinia hordei),* stem rust (*Puccinia graminis* f. sp. *hordei),* powdery mildew (*Blumeria graminis* f. sp. *hordei*), Scald (*Rhynchosporium commune*), head blight (*Fusarium heterosporium*), spot blotch (*Bipolaris sorokiniana),* covered smut (*Ustilago hordei*), loose smut (*Ustilago nuda hordei)*
Bacteria	Bacteria blight (*Xanthomonas campestris*, *Pseudomonas syringae*)
Virus	Barley yellow dwarf virus (BYDV), barley stripe mosaic virus (BSM)
Nematode	Cereal cyst nematode (*Heterodera avenae)*
Insects	Barley shoot fly (*Delia arambourgi* Seguy, *D. flavibasis* Stein.), Russian wheat aphid (*Diuraphisnoxia mordvilko*), and corn leaf aphid (*Rhopalosiphum maidis*)

inheritance and whether the objectives are for short-term or long-term protection. Generally, long-term resistance should be the ultimate objective of breeders, however, based on urgent need short-term breeding objectives may be taken on priority. Disease-resistant cultivars are developed by identifying resistant genes for the host species, or related wild species, and transferring it into adapted cultivars and breeding lines, normally by hybridization, tissue culture or chromosome engineering techniques. Quantitative resistance, which is durable (the chance of breaking the resistance genes due to changes in the pathogen over time is lower) is generally determined by multiple minor genes, should be chosen. Gene pyramiding approach, involving the introgression of different major and/or minor genes for resistance into one agronomically best cultivar is a useful strategy to assure long-term prevalence of the resistance.

In barley, the qualitative resistance conditioned by one major gene has also been durable, for example, the *Rpg*1 gene (also known as *T* gene), is still functional and has provided resistance to stem rust for more than 60 years (Zhang et al. 2006). Given the high significance of the disease, much of the work with disease resistance in barley is done in powdery mildew. Outstanding in this regard are the mutations induced at the *ml-o* locus. Multiline varieties and varietal mixtures offer another possibility that may be considered in extreme situations for decreasing vulnerability to attack from various pests of barley. For example, in the Netherlands, barley cultivar 'Grand Prix' was released as a mildew-resistant multiline based on 'Aramir' *Ml-ar* germplasm. The multiline is composed of three backcross derived lines with dominant mildew resistance genes from 'Monte Cristo' (C.I. 1017)- *MI-a*, 'Nepal' (C.I. 595)- *MI-n*, and 'Engeldow' (C.I. 9.3.1
7555)-*MI-a*.S.

Breeding objective for disease such as barley rust where the physiologic race specialization is present involves race-specific genes. These genes confer major resistance to a particular race and have simple inheritance. However, race non-specialization resistance in the host genotype is conferred by non-race-specific polygenes which are inherited quantitatively, each contributing a small increment toward resistance to the disease pathogen. Also, reduction in disease damage can be achieved by breeding for plant characteristics that enable the plant to escape or avoid disease infection.

5.10.3.2 Breeding for Resistance to Insect Pests

Host plant resistance is used to control insect pests that are difficult to control through cultural practices or use of pesticides. Breeding for host plant resistance is economical and environmentally safe method. The breeding methods employed for resistance breeding to an insect are generally similar to those used in breeding for resistance to disease pathogens. Resistance sources to the insect species are first identified and transferred to susceptible host genotypes by hybridization. During the selection process, the breeding lines are exposed to natural or artificial insect populations for efficient screening and to distinguish between resistant and susceptible genotypes. Breeding for genetic resistance to insects-pests has not been much emphasised because of availability of highly effective insecticides. However,

identification of resistance sources has been done since long. For example, feed barley with greenbug resistance has been grown widely since 1965 without breakdown of its effectiveness (Anglade 1978).

Aphid is a major insect problem in barley which causes heavy loss to the crop as well as reduces grain quality. Also, the cereal cyst nematodes cause heavy losses by reducing the tillering and ear head formation. Use of resistant varieties is generally encouraged to control the damage by nematodes. Lines resistance to aphids (Mornhinweg 2011); over 5000 cultivars resistance to Hessian fly Hill et al. (1952); 'Modjo' (CI 3212) to seed transmission of BSMV (Carroll et al. 1979) were reported early on. Improved resistance to different biotic stresses in barley through composite cross breeding has also been attempted, e.g., scald resistance (Zhang et al. 2019) and resistance against powdery mildew (Dreiseitl 2020) and blotch (Visioni et al. 2020). Resistance sources for different biotic stresses in barley are characterized and available (Lundqvist et al. 1997; Sharp 1985) and can be requested from gene banks. Also, the global barley improvement program of ICARDA provides diverse germplasm with resistance sources. Some of these resistant sources are in agronomically non-adapted backgrounds; however, to avoid the genetic gap, the barley breeders generally use the resistance genes already incorporated into adapted varieties.

5.10.3.3 Abiotic Stress Resistance, *vis-a-vis,* Climate Change

Climate change poses a major threat to global food security and also affects barley production. These climatic issues are very difficult to predict and their precise future effects on crop yields are unpredictable. The climate change parameters include: (1) physical parameters such as temperature, rainfall patterns and carbon dioxide; (2) changes in agricultural environmental systems such as loss of pollinators and increased occurrence of biotic stresses and (3) the adaptive responses of human systems (FAO 2016). Under changing climatic conditions, barley has emerged as a model plant mainly because of the broad and well-formed collections of landraces, wild genotypes and other *Hordeum* species which are important sources of new alleles (Dawson et al. 2015). Also, barley yield suffers less variation under climate change conditions than those of wheat and most other small grains, and therefore it is grown widely in semiarid regions (Cossani et al. 2009).

As far as drought stress is concerned, barley tends to mature earlier than other cereal crops and may escape drought during anthesis or early grain-fill. Also, plant might avoid drought stress by maintaining high internal moisture content. However, an optimum duration for barley maturity should be maintained because if a cultivar is too late in maturity, it may suffer from drought stress and if it is too early, it may fail to take advantage of available moisture. Therefore, breeder's job is to match the growth pattern of the crop to seasonal availability of moisture without disturbing the yield. Under field conditions, usually heat and drought stresses are correlated. Injury from heat stress is critical during the flowering period reducing pollen viability, stigma receptivity and seed formation.

Soil salinity also limits crop productivity in many arid and semiarid regions. Barley is, however, one of the most tolerant cereal crops and it is even used in

reclamation of saline soils and considerable variability for this trait has been reported. The resistance to soil salinity, generally referred to salt tolerance, is primarily due to avoidance mechanisms such as salt exclusion and salt dilution in barley (Levitt 1972). Initial breeding programme for salt tolerance involves a germination test in saline solution. Seedlings that survive this screening are then transplanted and grown to maturity with saline irrigation water. Breeding for resistance to deficient and toxic levels of aluminium and other minerals (boron, manganese or heavy metals) has received little attention in barley breeding programmes till now. Barley genotypes differ in their ability to take up and use nutrients (Perby and Jensen 1983).

Cultivars and breeding lines may be screened for aluminium tolerance in the laboratory by growing seedlings in a nutrient solution containing a high concentration of aluminium ions and selecting for plants with longest root and top growth. Minerals also affect other traits, aluminium, for example, inhibit root development and indirectly affect drought resistance, winter hardiness and nutrient uptake. Aluminium resistance (sometimes measured as resistance to low pH) is heritable in barley and may be screened for *in soil* or *in nutrient* solution cultures (McNeilly 1982).

Breeding for abiotic stress involves testing the germplasm at a specific or multiple growth stages on a particular climate. For example, at floral initiation, drought reduces the number of florets per spike and suppresses tillering, while after anthesis it reduces seed set (kernels per spike) and kernel weight. After identifying tolerant genotypes, a breeding programme starts by crossing the selected genotypes as donor parents. The trait (morphological or physiological or yield related) to improve drought tolerance must discriminate between drought-tolerant and drought-susceptible lines, should have high heritability estimates and positive significant correlation with final grain yield. The morphological and physiological traits such as plant height, kernel plumpness, harvest index, tillering capacity, root growth patterns, seedling vigor, stomatal size and density, stomatal control, diffusive resistance, transpiration rate, water potential, desiccation tolerance and proline accumulation may be associated with drought avoidance and tolerance.

There is interaction among these traits, and drought-resistant genotypes may obtain their resistance from a favourable combination of traits. Apart from tolerance to the abiotic stresses described above, the barley cultivars with inbuilt tolerance to lodging are also needed particularly in the areas prone to heavy rainfall, hail and windstorms. Lodging causes yield losses in barley, hence breeding to improve resistance to lodging is important breeding objective which involves changing the architecture of the plant. Breeding for lodging resistance involves developing plants with short culms, sturdy straw, a root system capable of anchoring the plant in the soil and acquiring resistance to diseases and insects as the later weakens the plant making it more susceptible to lodging.

Lodging resistance is a quantitative character with complex inheritance, although some of the plant characteristics associated with a reduction in lodging, such as dwarfing genes, or resistance to disease and insect pests, are often simply inherited. Improvement in lodging resistance of barley has been achieved through selection for

reduced plant height. For example, the Swedish cultivar 'Pallas' is an erectoides mutant and, in general, Japanese cultivars are semi dwarf ert-k.32 mutants which are resistant to lodging. Lodging-resistant semi-dwarfs are sometimes inferior to normal lines, and thereby incorporation of the short-straw trait into agronomically useful cultivars may require a long-term breeding effort.

Resistance to shattering (whole spikes or individual seeds) is imperative to prevent loss of yield before and during harvest in barley. Resistance to shattering is inherited as a complex quantitative character and depends upon environmental conditions such as wind velocity, timing and method of harvest. Klinner and Biggar (1972) observed that in certain environments, most shattering losses of barley result from breaking off of whole spikes. Harlan and Pope (1921) observed a positive association between rachis ash content and shattering. They found that awnless and hooded cultivars tended to shatter easily and that the rachis was more brittle and higher in ash than the rachis in awned cultivars. Therefore, they concluded that selection should be effective for this trait.

5.11 The Conventional Approaches of Barley Improvement

Barley is a self-pollinated crop and the breeding methods developed for such crops, for example, the pedigree and single seed decent apply and have been used for development of improved cultivars in barley as well. These conventional approaches of barley improvement have been the mainstay of barley breeders for almost whole of the breeding history. Both the techniques are based on the introgressive breeding plan which has been one of the most important methods of barley improvement since its domestication. The introgression has been defined as 'the transfer of one or several novel, favourable alleles from un-adapted germplasm to adapted germplasm' by (Hernandez et al. 2020).

As already mentioned, the domestication trait syndrome was used to determinate growth habit, high seed set percentage, more number of grains per spike, non-brittle rachis, bold seed and early germination (Harlan et al. 1973). These primary traits hold significant importance even in modern-day technologically advanced breeding programmes. The domestication lead to creation of locally adapted heterogeneous mixtures of nearly homozygous lines called landraces. These landraces evolve through artificial selection based on desired phenotype and 2% natural outcrossing in barley. Therefore, landraces are very important sources of genetic diversity that can be readily utilized to correct a specific defect of an otherwise good variety through introgressive breeding. For example, gene *Rpg1* providing resistance against the *Puccinia graminus* f.sp. *tritici* induced stem rust was sourced from "Chevron" (Steffenson 1992) and Russian Wheat Aphid (*Diuraphis noxia*) resistance was sourced from PI 366450 from Afghanistan (Bregitzer et al. 2005). Based on the source of starting genotypes, barley breeding can be elite × unadapted germplasm or elite × elite lines.

5.11.1 Pedigree Method

The pedigree method by far remains the most used method of breeding improved barley varieties. The pedigree method is hybridization-based and requires identification of better combining parents with complimentary traits. Therefore, there is a component of pre-breeding in utilization of pedigree method whose objective or outcome is a pureline having the desired traits from both the parents. In this method, phenotype-based plant selection is exercised from F_2 generation onwards until the segregating generations show no intraprogeny variations. In F_2 itself, the plants outperforming both the parents' transgressive segregants are selected. Ear-to-row is a basic pedigree method employed in case of barley improvement (Greveniotis et al. 2019).

The method is used in barley for improvement of a specific defect in a good genotype such as resistance to a particular abiotic or biotic stress, quality trait or any other trait contributing to the adaptation of the variety. From the F6 generations onwards, the progenies are tested in the preliminary yield trails/multilocation trials vis-a-vis the best local checks and the superior progenies having performance significantly higher than the check are identified. Unlike wheat, the shuttle breeding method (raising two generations in a year in geographically) is not successful in barley because of the vernalization requirement. Shuttle breeding is utilized to make the segregating lines homozygous in half of the period that will be required for developing a variety. In barley, the technique of double haploids is utilized for the same purpose which is described earlier.

5.11.2 Modified Bulk Pedigree Method (ICARDA/CIMMYT)

When the number of crosses to be handled in the segregating generations is in thousands in the international breeding programmes targeted for different mega environments then it becomes difficult to exercise individual plant selection in the early generations through pedigree method. Therefore, a modification of the pedigree method called modified bulk pedigree method was introduced and adopted at the CG centres, mainly the CIMMYT and the ICARDA for barley and wheat. It is in fact a local hybrid modification that combines the characteristics of both the bulk and pedigree methods, in that it involves individual plant/spike selection in F_2, F_6 and F_7 (pedigree) generations and bulking in F3, F4 and F5 (bulk) generations. The individual head to row plots from F_2 generation are sown to raise F_3 plots in which inter-plot selection is exercised for morphological, agronomic and disease resistance traits.

A total of 10–15 healthy spikes from the selected plots are harvested and bulked to raise F_3. In the similar fashion, the generation is advanced up to F_5. From F_6 onwards, the selection is made for individual plants and head rows are planted in the F_7. The main advantage of the method is that as the selection is omitted in the three generations, the logistic handling of the crosses becomes considerably easier and the resource inputs in the form of time, labour, expenditure on nursery preparation, land,

etc. are significantly reduced. Also, the early generation rejection probability of a potential line in this method is reduced because of bulking in the three consecutive generations. Therefore, it preserves considerable diversity up to F_5 generation from which selection can be made. From F_7 onwards, the lines are handled in pedigree method (Van Ginkel et al. 2002; Wang and Pfeiffer 2007).

5.11.3 Mutation Breeding in Barley

Improvement through mutagenesis (physical and chemical) has been an unparallel success story of barley breeding and some of the very popular varieties have been bred this way. The superior varieties have been successfully targeted to generate mutants which are more resistant to prevalent diseases, tolerant to various abiotic stresses, having bold grains with better malting quality, improved protein, starch and mineral composition and phenotypically having more number of effective tillers, number of grains per spike, dwarf type and an improved root structure leading to enhanced nutrient use efficiency. The extent of mutation research in barley has made it a model species for studying mutation genetics and breeding in plants. The IAEA-MVD Mutant Variety Database has listed a total of 316 barley varieties developed by mutagenesis (https://mvd.iaea.org/#!Search?Criteria[0][val]=barley%20 accessed 4 September 2021) including direct mutants and hybrids. The database also provides information on the pedigree of the varieties and their country of origin. The database includes direct mutants and their hybrids. The information includes the parent name, country and year of development among others.

Pallas the first mutant variety was developed using X-rays and registered in the United States in 1960 while the first chemically induced (diethyl sulfate) mutant barley variety, Luther was released in 1966 in the United States. Pallas has high lodging tolerance and higher yield to the parent variety Bonus. In the present times, Golden Promise (γ-irradiation of Maythorpe) released in the year 1960 and Diamant (Valticky irradiated by X-rays) released in the year 1965 remain two of the most popular barley varieties globally. The Golden Promise is characterized by short and stiff straw, salinity tolerance, high yield and good malting quality. On the other hand, the main characteristic of Diamant is dwarf type, high number of effective tillers and 12% yield advantage over the parent variety with good malting quality. These varieties have been part of different local breeding programmes, and thus form a part of pedigree of some of the latest barley varieties across the countries.

IZ Bori is a Bulgarian winter feed barley variety and was developed through chemical mutagenesis (sodium azide) and released in the year 2009. It is a variety with wider adaptation, disease resistance (powdery mildew and rusts), with a yield advantage ranging from 15 to 17%. It is frost resistance and nutritionally superior (high protein and lysine content). In India, the barley mutation research began in 1970s and the first variety RDB1 (mutant of RS17) was released in 1971 followed by PL56 (mutant of C164), HBL316 (mutant of HBL98). Presently, the mutation research in barley in India has taken a backseat and the tide has turned in the favour of utilization of available natural genetic diversity and the molecular tools.

5.11.4 Single Seed Descent (SSD)

In single seed decent method, one seed is taken from individual plant after F_1 generation and bulked together to raise the F_2 generation. This method was originally developed by Goulden (1939) for handling of the F_2 onwards generations of a cross made in self-pollinating crops. Basically, this method was devised to handle a greater number of crosses at any one given time but it suffers from lack of selection upto F_5 generation. In an interesting study, Lalic et al. (2003) compared pedigree method to the SSD in winter barley cross Timura* Osk.4.208/2–84. It was found that grain yield per plot was higher under two planting densities for pedigree method, however, upon comparison of top five lines from the crosses for both the methods, the lines developed by SSD had advantage. The lines, though, were poor in inheritance of traits with low heritability (yield per plant and effective tillers) which can be understood in terms of loss of variability and lack of selection in SSD. Conversely, the traits with high heritability such as grain weight and number of grains per spike were better preserved in case of SSD compared to the pedigree method.

5.11.5 Doubled Haploids (DH) in Barley

Genetic uniformity is the prerequisite of a line to be registered or released as a variety and in the pedigree and SSD methods, selfing and selection are the tools of achieving this uniformity. However, this is a very long and economically intensive process and usually takes no less than six generations to achieve this. On the contrary, the doubled haploid system is one of the quickest methods to achieve homozygosity in a line. In barley, the DH development was started by Clapham (1973) and since then it has come a long way contributing in not only the development of high yielding and genetically uniform barley cultivars as selection is easier among the lines but also making the barley plant a genetic model for the cereal crops because of the amenability of the DH for genetic studies, mutation and selection at the single cell level in cultures as well as in studying embryo development. 'Mingo' was the first cultivar developed and released in Canada in the year 1979 through DH technique based on *bulbosum* method which is described in the subsequent sub-section. The DH method is an alternative to the shuttle breeding method and makes all the loci homozygous in one single generation (Singh et al. 2021). Now the DH development has been taken up commercially by some institutes (e.g. Institut de Genech, France) or companies, following anther culture procedure of the F_1 plant.

5.11.6 Barley Haploid Development Techniques

5.11.6.1 Bulbosum Method

This method, discovered by Kasha and Kao (1970), is also called chromosome elimination method. This technique is based on the fact that in a *H. bulbosum* (Pollen donor) x *H. vulgare* (recipient) the chromosomes of the *H. bulbosum* are selectively

eliminated during the development of the embryo which eventually becomes haploid retaining only the chromosomes of *H. vulgare*. These embryos are then rescued some 2 weeks after pollination and cultured on artificial medium which is B5 minus 2,4-D and plus sucrose (20 g l^{-1}) and agar (7 g l^{-1}) (Gamborg et al. 1968) (Kasha and Kao 1970). This is followed by the colchicine treatment to make the DH fertile through doubling of the chromosomes. This method became an important tool of barley breeding soon after its discovery and was utilized to achieve homozygosity in the breeding pipelines and resulted in release of many cultivars across the world. At present, this method is utilized when DH from hybrids are to be developed particularly in the spring-type barley. It also has a utility in mapping, providing a random sample of gametes. *Bulbosum* method is an example of in vivo DH production and soon it was replaced by the in vitro methods including anther culture and isolated microspore culture. The major disadvantage of the method is that only a limited number of DH lines can be developed in a specific time.

5.11.6.2 Anther Culture

Guha and Maheshwari (1964, 1966) for the first time reported that anthers of *Datura metale* could be raised into haploid plants if cultured artificially. Soon, it was applied to barley by Clapham (1973). In anther culture, the anthers are taken from the spikelets which are already surface sterilized. The acetocarmine staining is used to identify the right stage of pollen development. In barley, the right stage is when the microspores within the anther are at uninucleate stage which coincides with plant developmental stage when the flag leaf and penultimate leaf is at a distance of 3–6 cm. This are then transferred to induction/nutrient media. The sporophytic stage is induced by both cold shock at 4 °C for 28 days or sugar starvation for 3–4 days in a 0.3–0.7 M solution of mannitol (Roberts-Oehlschlager and Dunwell 1990; Cistue et al. 1999), or alternatively with some macronutrients (Hoekstra et al. 1997). The culture plates are maintained at a 16:8 h of light:darkeness at 26 °C. After three to five weeks, the young embryos developing from the callus are transferred to the regeneration medium containing the basal medium plus sucrose and minus maltose. At the three to four leaf stage, the plants are transferred to glasshouse in pots. The frequencies of the spontaneous DH, polyploidy and haploids in barley is 60–80:8:remaining, respectively.

5.11.6.3 Pollen Culture (Isolated Microspore Culture)

The anther culture is a relatively easy technique to generate double haploids because of the high frequencies of spontaneous DH, but it suffers from the generation of polyploid plants from the anther tissue other than the microspores. This drawback is taken care by the pollen culture being high throughput (production of embryos in large numbers) and easier to undertake. The spontaneous chromosome doubling also reaches upto 80% making it a method of choice without the need of purposeful doubling through colchicine. However, the dependency of the IMC on genotype is major limiting factor restricting its routine use for development of DH in barley (Touraev et al. 2009).

5.12 Exploitation of Heterosis and Hybrid Development in Barley

Commercial barley hybrid production depends upon the degree of heterosis, frequencies of cross-pollination, availability of a practical system for inducing male sterility and (or) restoring fertility, and achieving acceptable quality for product end use. The initial release of hybrid barley was at the United States which was hindered by two major constraints; the balanced tertiary trisomic system allowed a few male-sterile plants in commercial fields, and ergot disease was a problem in the sterile heads. However, there are increased research efforts on hybrid barley because of a cytoplasmic male-sterile source found in *H. spontaneum* C. Koch (Ahokas 1980) and the availability of gametocides for inducing male sterility (Foster 1984). Numerous genes for genetic male sterility in barley have been documented (Hockett and Eslick 1968). Cytoplasmic male sterility in barley has been obtained from *H. jubatum* and *H. spontaneum* (Schooler 1967). Fertility restoration of the *H. spontaneum* source of cytoplasmic male sterility has been reported. The cytoplasmic male sterility and fertility restoration found in *H. jubatum* crosses were difficult to use because of extreme lateness (Schooler 1967). Inadiverse material from crosses of spring and winter barleys, over 100% heterosis was reported (Fejer and Fedak 1975). Yield advantage of 26% over five high-yielding varieties was reported by Foster and Fothergill (1981). Lehman (1981) found that two-row hybrids yielded 86 and 119% of the control cultivar during 1979 and 1980, respectively.

5.13 Application of Biotechnologies (Marker-assisted Selection, QTL Identification, Introgressive Breeding) in Barley Improvement

Most agronomic traits are inherited quantitatively and the loci controlling these are called quantitative trait loci (QTL). The identification of QTL for major traits and abiotic and biotic stress resistance is at the core of marker-assisted selection (MAS) and marker-assisted introgressive breeding in barley. The major techniques adopted when the QTL being explored are unlinked are the standard interval mapping (SIM) and multiple imputation (IMP) and when the QTL are linked on a chromosome then composite interval mapping (CIM) is used. The QTL discovery process is inherently statistical in nature and the calculated logarithms-of-odds (LOD values) are used to consider a region on genome to be a QTL controlling a specific trait.

The identified QTL are the main targets of MAS and introgressive breeding (Riaz et al. 2021). With the advent of the next-generation sequencing based on the SNPs, the process of QTL discovery has received a major, boost and its resolution has improved manifold compared to the RFLP and SSR markers. The major application of the MAS has been in the field of disease resistance in barley (Wang et al. 2019). In MAS, a particular phenotype is associated with presence/absence of a molecular marker at DNA level in the genome of barley, and once characterized, it circumvents

the necessity of field evaluation each single time, thus saving the time and resources apart from being highly accurate.

Powdery mildew was the first disease against which a QTL was reported (Heun 1992). It was followed by QTL for other diseases such as spot blotch by Steffenson et al. (1996). The identification of major genes is a straightforward approach for development of cultivars with inbuilt vertical resistance to major disease. The *ym4* gene conditioning yellow mosaic disease in barley from Franka cultivar was successfully introgressed into Igri by Ordon et al. (1995). Similarly, the *Rph7* gene conditioning leaf rust resistance in barley was also discovered by marker technology (Graner et al. 2000), and it was followed by genes conditioning other disease resistance such as powdery mildew, spot blotch and the rusts as well. A list of major QTL discovered for major barely diseases is presented in the Table 5.10.

The abiotic stresses such as drought and heat are becoming increasingly important for barley cultivation particularly in the tropical, sub-tropical and arid regions in the climate change scenario. Here also the marker technology has come into play for development of tolerant varieties suitable for a wide range of climatic regimes. QTL on different chromosomes have been identified for drought component traits such relative water content (RWC), Osmotic adjustment, Carbon isotope discrimination (CID) and proline accumulation (Visioni et al. 2020). The QTL identified for dry root weight under field conditions by Reinert et al. (2016) was later associated with the genes *HvCBF10A* and *HvCBF10B.* Other genes such as *HvNCED2, HVA1* and *cer* genes were also discovered and were found to regulate abscisic acid synthesis, leaf wilting and wax synthesis, respectively (Saade et al. 2018). Soil salinity impacts the barley productivity adversely in a significant area around the world and research efforts had been directed towards identification of markers associated with this complex trait in barley. The tolerance to soil salinity is conditioned by the loci regulating osmotic adjustment, generation of reactive oxygen species, ion transport and signal transduction transcriptional factors. The QTL controlling ionic action on root and shoot were identified by Nguyen et al. (2013), and in a later study, Saade et al. (2016) observed a 30% increase in salinity tolerance upon introgression of a wild 2H allele. Therefore, as far as tolerance to soil salinity is concerned, the role of barley wild relatives could not be underestimated. Similarly, the QTL conditioning the tolerance to frost have been reported on 5H chromosome. The tolerance to low temperatures (*Fr-H1*and *Fr-H2*) has been observed to be correlated with vernalization (*HvBM5A*) and flowering time. The genes *HvCBF2A* and *HvCBF4* in high copy numbers are responsible for imparting tolerance to frost in barley (Francia et al. 2016).

The improvement in quality traits constitutes a very important aspect of barley breeding. Malting quality is one of the most important barley quality traits and more than 200 QTL have been discovered for this. However, the utilization aspect of the discovered QTL is still lagging behind mainly because of the low phenotypic variation explained by the QTL and linkage drag. Cu et al. (2016) identified 63 QTL for 10 important quality traits in a barley DH population. The QTL on 1HS and 7HL chromosomes were found to be associated with α-amylase, soluble protein, Kolbach index, free amino acid nitrogen, wort β-glucan and viscosity.

Table 5.10 Disease resistance genes mapped in barley

Disease/pathogen	Gene	Chromosome location	Marker type/linked marker	Reference
Leaf rust *Puccinia hordei*	*Rph27*	4H	DArT-Seq	Rothwell et al. (2020)
	Rph26	1H	CM_1194	Yu et al. (2018)
	Rph24	6H	3,999,875, 3,265,068, 3,272,559, and 3,272,930	Ziems et al. (2017)
	Rph23	7H	bPb-8660 and bPb-9601; Ebmac0603	Singh et al. (2015)
	Rph22	2H	H35_26334 & H35_45139	Johnston et al. (2013)
	Rph21	4H	GBM1044 & GBM1220	Sandhu et al. (2012)
	Rph16	2H	GBR 1185	Perovic et al. (2004)
	Rph13	3H	*Hv*KASP_*Rph13*plus	Jost et al. (2020)
	Rph7	3H	TC2863–12.4 and ABG70	Mammadov et al. (2007)
	Rph5	3H		
	Rph6	3H	MWG2021 & BCD 907	Zhong et al. (2003)
	Rph3	7H	EBmac755	Park et al. (2003)
	$Rph_{MBR1012}$	1H	GMS021 & GBS546	König et al. (2012)
	RphC	5H	DART4872 and DART7508	Dracatos et al. (2014)
Stripe rust *Puccinia striiformis*	*Rdg2a*	7H	MWG2018	Arru et al. (2003) Tacconi et al. (2001)
Stem rust *Puccinia graminis*	*Rpg1*	7H	ABG704-MWG036B	Kilian et al. (1994)
	Rpg4	5H	ABG391	Kilian et al. (1997)
Erysiphe graminis f. sp. *hordei* (powdery mildew)		6H	DArT markers (4,793,171, 3,258,880 3,264,002 & 3,432,488)	Piechota et al. (2020)
	Mla	7H	GBM1126 & GBM1060 GBMS192 & GBM1060	Soldanova et al. (2013)
Leaf scald *Rhynchosporium commune*	*Rrs1*	3H	11_0010 and 11_0823	Hofmann et al. (2013)

(continued)

Table 5.10 (continued)

Disease/pathogen	Gene	Chromosome location	Marker type/linked marker	Reference
Yellow mosaic virus *Polymyxa graminis*	*Rym17*	3H	ABG070	Kai et al. (2012)
	Rym 18	4H	Bmag0490	Kai et al. (2012)
	Rym13	4H	HVM67 & GBM1015	Humbroich et al. (2010)
Loose smut Ustilago nuda	*Un8*		Un8 SNP4; 0498 L15 F8/R8	Zang et al. (2015)
Wheat stripe rust	*Rps6*	7H	FPC 320	Dawson et al. (2016)
Spot blotch *Cochliobolus sativus*	*Scs6*	1H	Bc183711 and Bc13291	Leng et al. (2018)
Spot blotch *Bipolaris sorokiniana*	*Rbs7*	6H	M13.06 and M13.37	Wang et al. (2019)

Similarly, for the trait hot water extract, QTL were located on the 1H, 2H, 4H, 5H, and 7H chromosomes. Two large effect QTLs explaining 48% phenotypic variation in the malt extract were identified by Wang et al. (2015) in a DH population. Before this, Zhou et al. (2012) identified major effect QTL explaining up to 53% phenotypic variation in malt extract. Other workers have reported QTL for KI, FAN, α-amylase, beta amylase and diastatic power on different barley chromosomes (Cu et al. 2016; Mohammadi et al. 2015; Panozzo et al. 2007; Wang et al. 2015; Zhou et al. 2016). The forage quality traits such as crude fiber (CF), acid detergent fiber (ADF), dry matter digestibility (DMD), crude protein (CP), dry ash (DA) and neutral detergent fiber (NDF) have also been subjected to marker analysis and QTL have been reported on different chromosomes explaining variable quantum of total phenotypic variance.

5.13.1 Genome-wide Association Studies in Barley

The genome-wide association study (GWAS) or linkage disequilibrium mapping takes the advantage of historical recombination events which occurred during the course of evolution. Generally, for gene mapping and marker trait association, the mapping populations such as RILs, NILs or DH have to be developed which are eventually subjected to the field evaluation for the trait in question. The development and maintenance of mapping populations is a time and resource-intensive endeavour, and given the limited recombination events that occur during the course of development, these studies suffer from low resolution as far as mapping of the QTL/gene is concerned. The GWAS takes care of these disadvantages in that any

random set of genotypes can be subjected to mapping and the resolution that is achieved is quite high. The invention of high-throughput genotyping (SNP, DArT) has enabled the researchers to scan any given set of barley genotypes for the trait of interest.

This accelerated growth in DNA-based research has been felt in both basic and applied studies for biotic/abiotic stresses resistance and quality in barley during last two decades. The GWAS has been applied to identify marker trait association for abiotic and biotic stresses and other agronomic and quality traits as well. A total of five QTL conditioning resistance for barley yellow dwarf virus were reported by Kraakman et al. (2006) in a set of 148 spring cultivars. Large and medium effect QTL for spot blotch resistance, leaf spot disease and net blotch have been reported by different workers using the GWAS (Adhikari et al. 2020; Gyawali et al. 2018; Tsai et al. 2020). In a recent study, Qian et al (2021), utilizing the latest version of barley genome discovered largest number (468) of NLR genes with chromosome 7 harbouring the most number (112) of genes present in multigene clusters. Such studies have immense implications as far as molecular breeding of barley is concerned. Among the abiotic stresses, significant marker trait associations have been reported for heat stress by Abou-Elwafa and Amein (2016), identification of nine haplotypes of HSP17.8 genes by Xia et al. (2013), heat and drought tolerance and stay green trait by Gous et al. (2016) drought tolerance at germination and seedling stage by Mwando et al. (2020) and drought tolerance under field conditions (Thabet et al. 2020) in diverse barley panels.

5.13.2 Genomics in Barley Improvement

As such the QTL mapping and GWAS are also the tools of genomics; however, here we consider the whole genome information as genomics for the sake of simplicity. The whole genome sequence of barley is published and molecular markers for different traits have been mapped in genetic and physical maps (Mascher et al. 2017). The genome sequence data is available in the public domain and is being used for deciphering gene function and identification of genes underlying the major traits by a myriad of researchers. The whole genome sequencing of barley has ushered the era of genomics-based breeding in barley. At present, there are several genomic databases such as EnsemblPlants, Nord-Gen, BARLEX, MorexGenes, GrainGenes, HvGDB, Bex-DB, BarleyDB and BarleyVarDB which are used to map genes/regions of interests in the whole genome. The major use of the reference genomes is to conduct a bulk segregant analysis (BSA) of different genotypes to analyse the extent of sequence variability resulting in identification of different alleles of the trait in question. Apart from the reference genome-based genomics, the bulk segregant ribonucleic acid (RNA) sequencing (BSR-seq) and specific-length amplified fragment sequencing (SLAF-seq) are also being standardized for barley. The transcriptome sequences are proving to be very useful for understanding of the gene function based on expression analysis of the candidate gene. The directional approach of the CRISPR/Cas9 technology is a futuristic tool for editing of the barley

genes to make them express-desired proteins. Collectively, the techniques of genome sequencing, genomic selection, GWAS and gene editing and cloning should cater to the challenge of molecular information not being efficiently translated into end products that is high yielding cultivars with desired quality traits and tolerant to the hostile environment and local diseases and pests mainly because of the complex regulatory framework of the traits and dispersal of genes across the seven barley chromosomes (Feng et al. 2019).

5.14 Coordinated System of Testing, Status of Varietal Development and Maintenance Breeding in India

The barley research was initiated way back during the nineteenth century in India and several improved varieties, such as NP13, NP21, BR22, BR32, C251, CN292 and CN294, were developed by pure line selection from indigenous landraces. Prior to inception of a national system of testing, the improved barley lines across the country in a coordinated pattern, about 31 barley varieties were already developed mostly through selection from the local germplasm. All India Coordinated Barley Improvement Project (AICBIP) was launched in 1966–1967 at IARI, New Delhi, which gave impetus to barley improvement programmes in different barley cultivation states. AICBIP was merged with wheat improvement programme in 1997 and a common coordinated programme for these two important cereals as All India Coordinated Wheat and Barley Improvement Project (AICW & BIP) came into existence.

Based on the agro-climatic conditions, barley cultivation regions have been divided into four zones, namely, North-western Plain Zone (NWPZ), North-eastern Plain Zone (NEPZ), Central Zone (CZ) and North Hill Zone (NHZ). The advanced lines found to be promising in the station trials conducted at the originating centre of the line are evaluated in the two-tier system of evaluation. All the entries are first evaluated in Initial Varietal Trial (IVT) across the zones except the NHZ where separate IVT is constituted. Based on the performance in a zone, the test entries are promoted to Advanced Varietal Trials first year (AVT I) to be conducted in that particular zone. Again, based on the performance in the AVT I, the entries are retained for second year of testing (AVT II). In each trial, the test entries are compared with the latest suitable zonal checks. Based on the superior performance across the yield evaluation trials (IVT and AVT), performance in the agronomic trials and their resistant reaction to diseases like yellow rust and leaf blights, the entries are identified for release by the Variety Identification Committee during the annual workshops of wheat and Barley. If all the requirements are met, the Central Sub-committee on Crop Standards, Notification and Release of Varieties of Agricultural Crops (CVRC) of Department of Agriculture Cooperation and Farmers' Welfare release this test entry as variety and notify it in its Gazzet for commercial cultivation. The originating centre of dropped test entries in the AICW & BIP testing, if are promising in their state, has option to release these as varieties for its

state through State Variety Release Committee (SVRC). After release by the SVRC, this is also notified in the central gazette of DAC & FW.

The centres namely IARI, New Delhi, Ludhiana, Kanpur and Sabour contributed significantly and developed several improved barley varieties, which paved the way for yield maximization, especially under rain-fed cultivation. Dolma a naked barley variety for food purpose was released in 1982. Barley improvement efforts have largely been concentrated on feed barley development, and a good number of feed barley varieties have been developed. The dual purpose varieties RD2715, BH380 and VLB130 have also been developed for feed and fodder purpose, while RD2035 and RD2552 released as normal feed barley were also found to be good dual purpose types. During the last decade of twentieth century, more efforts to improve naked barley for food purpose were made which led to the development of food barley varieties like Geetanjali, Sindhu, Norbo, NDB943, Karan16, HBL276, BH352 and PL891. Similarly, the barley improvement programme of the country envisaged huge potential of barley as industrial crop and barley breeding activities were steered to develop malt barley verities.

These efforts led to the release of malt barley varieties namely Clipper, Alfa 93, Rekha, DWR28, BH885, DWRUB52, RD2668, DWRB73, DWRUB64, DWRB91, DWRB92, DWRB101, RD2849, DWRB123, DWRB160 and DWRB182. Majority of the released barley variety are six row types. To utilize the grain-boldness of two-row barley for grain yield maximization, two-row barley varieties (Clipper, BH885, Alfa93, Rekha, DWR28, RD2668, DWRB73, UPB1008, DWRB91, DWRB123, DWRB160, DWRB182 and PL891) have also been released. As of now, 105 barley varieties have been released by CVRC (56) and SVRC (49) since 1966–1967, the year of launching AICW & BIP.

5.14.1 Maintenance Breeding

After release and notification, genetic purity of a variety is maintained by the originating institution. Seed from single spike of a variety is grown in a single row. Rows having doubtful plant characters deviating from the original variety characters are removed before flowering. Based on heading and maturity time also, the doubtful rows are rejected. At maturity also screening of rows is done for any deviation in the spike and awn characters. These rows are harvested separately and are evaluated for their grain characters. Only those rows which qualify for characters of the variety are taken as Nucleus Seed Stage I (NSS I). From the produce of a row, small seed plots are raised. Seed (NSS2) from only those plots is bulked which qualify for the variety characters. Genetic purity of all the varieties which are under cultivation is also maintained through grow-out tests. Seed samples of barley varieties under seed chain are sent from the originating institutions to ICAR-IIWBR, Karnal, where the seed unit arranges for the grow-out test of these varieties. At the end of the season, feedback about the genetic purity of a variety is sent to the concerned breeder who, in turn, takes need-based curative action.

5.15 Future Thrust and Conclusion

Barley is an ancient crop with inbuilt climate change buffering making it a food and feed crop of seemingly hostile future. The multifacet uses of barley in food, feed, forage, malt and other industrial uses make it a crop where the breeding efforts need to be invested for food and energy requirement of the demographically expanding world. The recent progress in barley genomics and particularly the whole genome assembly of the Golden Promise barley cultivar have been a morale booster of the researchers who now can more efficiently target and improve specific traits. These advances including the assembly of barley reference genome can well be utilized in understanding of the barley genetics and the trait which are being sought in the cultivar development programmes around the world. The understanding of genetics of important traits related to diseases, drought, heat and quality, etc. is not complete and functional characterization of the identified genes and their annotation in the reference genomes are still a challenge to the researchers. In this context, sequencing of additional genotypes including the barley wild relatives, landraces and segregating generations will bring to light more information available for uses by the barley workers. The conventional breeding approaches might be benefitted by these technologies in making the selection more rapid and accurate. They might also be very useful in identification of the parental material with the gene of interest. The CRISPR/Cas9 gene editing technology promises a plethora of theoretical advantages of which the practical and deliverable aspects remain to be seen in case of barley. With this technique, any gene of interest can be targeted for understanding of gene function in a non-transgenic fashion and thus the regulatory hurdles applied to the transgenic cultivars will not come into play in this case.

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Pearl Millet Breeding

6

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Abstract

Pearl millet is a rainfed crop grown and distributed worldwide over tropical zones covering nearly 30 countries. Globally, India is the leader both in terms of area under pearl millet cultivation and production. Due to unpredicted nature of weather, low seed cost, and more resilience for various abiotic and biotic stresses, open pollinated varieties are still very popular among the pearl millet farmers of India and arid regions of Africa. Pearl millet is often considered to be a 'super cereal' due to its rapid growth, high photosynthetic efficiency, balanced nutritional profile, and tolerance properties to extreme climatic conditions. Besides, pearl millet grains have immense medicinal value and have been encouraged by dieticians and nutritionists as a super food for the benefit of a large section of society. With the rise in some of the key global issues like malnutrition, climate change, global warming, pearl millet has received special attention amongst farmers, consumers, and policymakers during recent years as a crop of choice. This chapter mainly deals with the basics of pearl millet as a crop, its breeding strategies, variety developmental procedure, and also the key future researchable areas.

Keywords

Nutritional properties · Gene pool · Breeding techniques · Hybrid development · Biotic and abiotic stresses

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_6

6.1 Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br. syn. *Cenchrus americanus* (L.) Morrone] is the world's sixth most important cereal and the primary food source in the dryland farming systems of semi-arid and arid tropical environments. In India and Africa, it is primarily grown for food and forage, while in the American continents it is a main component of poultry and livestock sector (Serba et al. 2020). Pearl millet was domesticated over 4000 years ago in the West African Sahel, spreading later to East Africa and India (Sharma et al. 2020b). Now it is being cultivated over 30 million ha worldwide, with the majority of the crop grown in Africa (>18 million ha) and Asia (>10 million ha) (Raheem et al. 2021). Around 90 million people in the Sahelian region of Africa and northwestern India consume pearl millet grain as a staple food (Srivastava et al. 2020a). It is traditionally served as a thick porridge (toh), or to prepare unfermented breads and cakes (roti), steam-cooked dishes (couscous), fermented foods (kisra and gallettes), and snacks. In India, flour made out of it is used to make chapattis, porridge, boiled or roasted food, and even weaning mixtures (Pattanashetti et al. 2016).

In northern Nigeria, *Masa*, a popular fried cake is prepared from pearl millet (Ajeigbe et al. 2020) and its grains are also locally brewed to produce non-alcoholic or alcoholic beverages in Asia and Africa (Dwivedi et al. 2012). Alternative uses have increased (55%) mainly as animal feed for dairy both as a green fodder and dry stover for cattle. Green fodder is high in protein, calcium, phosphorus, and other minerals, with lower levels of oxalic acid. Stover has excellent forage quality with lower hydrocyanin content (Kumar et al. 2020a). Apart from its utility in human consumption and in livestock sector, it is used in industries for alcohol and fuel industry, starch industry, processed food industry, and has great export demand (Punia et al. 2021).

Pearl millet is often considered to be "super cereal" with respect to its rapid growth even with least input, high photosynthetic efficiency, inheritably good and balanced nutritional profile, tolerance to extreme climatic conditions and biotic stresses (Chandra et al. 2021). Pearl millet has C_4 photosynthetic pathway which is very efficient in energy production (50% higher photosynthesis efficiency than C_3 crops) in hot and dry climate (Wang et al. 2018). It is usually grown under the most adverse agro-climatic conditions where other staple cereal crop like rice and wheat fail to survive. The high dependency of people, mainly in arid and semiarid regions, on pearl millet is due to grain production capability even in the harshest conditions, including low soil fertility, high soil pH, high soil Al^{3+} saturation, low soil moisture, high temperature, high soil salinity, and scanty rainfall (Varshney et al. 2017). Pearl millet is more nutritious than commonly consumed staple crops such as wheat, rice, maize, and sorghum (Kumar et al. 2021a) and offers gluten-free grains with high and better protein in terms of quality and quantity. It is the only cereal whose flour is alkaline in nature with a low glycemic index (~55) making it a diabetic-friendly food. Also, it is filled with resistant starch, vitamins, antioxidants, essential micronutrients such as iron and zinc, and more balanced essential amino acid profile than maize or sorghum (Goswami et al. 2020; Punia et al. 2020). Because of its high

nutritional and nutraceutical value, it is renamed as 'Nutricereal' by Govt. of India through gazette No. 133 dated 13th April 2018 for production, consumption, and trade and included in Public Distribution System (PDS).

With the emergence of global issues such as malnutrition, climate change, global warming, a rise in lifestyle-related diseases, need for green fuel sources, and so on, millets have received more attention amongst farmers, consumers, and policymakers during recent years. According to Lesk et al. (2016), adverse climatic conditions such as a dramatic rise in temperature, drought, and other factors have reduced cereal crop production by 9–10% in recent years. Its effect can be seen not only in terms of quantity but also in terms of quality reduction. Many nutrient rich crops experienced to have 3–17% lower concentrations of protein, iron, and zinc in its grain. According to estimates, India will have a 2.2% increase in protein-deficient people (with 38.2 million new protein-deficient people) and a 2.9% increase in zinc-deficient people by 2050. About 106.1 million children and 396 million women would become iron-deficient (Beach et al. 2019; Moore et al. 2020). In all these context, millets can be saviour to mankind as it has lower carbon footprint than commonly consumed cereal like wheat, rice, and maize (Kritee et al. 2013; Saxena et al. 2018) and can provide food and nutritional security even in the hardest situation (Anuradha et al. 2017). Hence, millet is rightly termed as nutricereal. In addition, to include millets into the mainstream and exploit its nutritionally superior qualities and promote its cultivation, Govt. of India has declared Year 2018 as the 'Year of Millets' and FAO Committee on Agriculture (COAG) forum had declared Year 2023 as 'International Year of Millets'. With the importance of pearl millet in bringing food security and nutri-revolution to resource-constrained regions of Asia and Africa in mind, the following sections explain the various aspects and past and on-going pearl millet improvement programmes.

6.2 Origin, Domestication, Taxonomy, and Distribution

6.2.1 Origin and Domestication

Pennisetum violaceum (Lam.) Rich. [syn. *P. americanum* subsp. Monodii (Maire) Brunken] has been described as the wild progenitor of the cultivated pearl millet species, occurs in the Sahel zone in Africa from Senegal to northern Sudan (Brunken et al. 1977; Sharma et al. 2020b; Fuller et al. 2021). It was hypothesized that the origin of cultivated form of pearl millet occurred near Taoudeni Basin in western Sahara (6.61° E, 23.58° N). Burgarella et al. (2018) reported that in the Sahel zone, both cultivated and wild *Pennisetum* species coexists along with intermediate morphologies, and genotypes supported a Saharan cradle of pearl millet domestication. Wild pearl millet dating back to the middle Holocene (~5000 BC), was being exploited in northern Mali in the fifth millennium BC and that pre-domestication cultivation was probably established sometime in the fourth millennium BC. Domestication of pearl millet has been suggested to have occurred through single or multiple events between regions of Niger and Mauritania. By the middle of

the third millennium BC, morphological domestication traits had become well-established.

The domesticated pearl millet was introduced to adjacent regions, including the Hodh depression of Mauritania and the Lower Tilemsi Valley in Mali toward the end of the third millennium BC; and southern Mali, northern Ghana, and by the first half of the second millennium BC at the Lake Chad region. Domestication is said to have resulted in the first early maturing cultivars (Tostain and Marchais 1989). By 3000 BC, these early maturing forms of domesticated pearl millet were introduced to eastern Africa (Tostain and Marchais 1993; Tostain 1998) facilitated by their efficient adaptation to arid conditions (D'Andrea and Casey 2002) and then to India (D'Andrea et al. 2001; Khairwal et al. 2007).

India is regarded as the secondary diversity hotspot (Brunken et al. 1977). Around 2000 years BC, photoperiod-sensitive varieties were selected for further diffusion in the region near Lake Chad (on the Nigerian side) (Klee et al. 2004). This led to the development of a secondary center of diversity in this region. These late-maturing lines were transported further into the Sudanian zone of southwest Africa from northern Nigeria to southern Senegal (Tostain et al. 1987; Tostain and Marchais 1993). Further these lines were tailored to adapt under the humid conditions in the southern Sudanian zone (Tostain 1998; D'Andrea and Casey 2002). About 1000 years BC, pearl millet was extended towards the plateau of southern Africa via Uganda and to Namibia (Tostain and Marchais 1993; Tostain 1998). The United States and Brazil were the most recent countries to introduce this crop; records of its cultivation date back to the 1850s in the United States and the 1960s in Brazil (National Institute of Plant Health Management 2014).

Pearl millet shares many of the same morphological changes known for other cereal domestications. The domestication process of pearl millet is associated with frequent morphological changes such as suppression of spikelet shedding, size reduction of bristles and bracts, increase in seed size, increase in spikelet pedicel length, loss of dormancy, decrease in the number of basal tillers, and increase in spikelet length (Fuller et al. 2021).

6.2.2 Taxonomic Classification and the Gene Pool

The genus *Pennisetum* Rich. belongs to the family Poaceae, the subfamily Panicoideae, and the tribe Paniceae. This genus is closely related to the genus *Cenchrus* L. (Stapf and Hubbard 1934; Boer et al. 2007) and both are placed within the bristle clade in the tribe Paniceae along with ~23 other genera (*Ixophorus* Schltdl., *Paspalidium* Stapf, *Setaria* P. Beauv., and others) (Doust and Kellogg 2002; Bess et al. 2005). The characteristics such as degree of fusion of the bristles, the presence of pedicellate spikelets, and type of bristles (flat or stiff), are commonly used to separate *Pennisetum* from *Cenchrus* (Clayton and Renvoize 1982, 1986; Watson and Dallwitz 1992); however, none of them can be effectively used to segregate the two genera (Webster 1988). Kellogg et al. (2009) also placed the

genus *Odontelytrum*, harbouring only a single species, *O. abyssinicum*, in this clade along with two genera: *Pennisetum* and *Cenchrus*.

A study based on a combined nuclear, plastid, and morphological analysis proposed the unification of the three genera *Pennisetum, Cenchrus,* and *Odontelytrum* (Chemisquy et al. 2010). Similarly, based on the chromosomal and genomic characteristics along with phylogenetic relationships, Robert et al. (2011) favoured the inclusion of *Cenchrus* species in the genus *Pennisetum*. Therefore, it has been proposed to reconsider the taxonomic position of the *Cenchrus* species and to rename them into genus *Pennisetum* as previously known (Robert et al. 2011). The genus *Pennisetum* comprises ~80–140 species (Brunken 1977) with different basic chromosome numbers ($x = 5, 7, 8,$ or 9) (Jauhar 1981), ploidy levels (diploid to octoploid), reproductive behaviour (sexual or apomictic), and life cycle (annual, biennial, or perennial) (Martel et al. 1997). Phylogenetic analysis suggested that the chromosome complement in *Pennisetum* has evolved from a basic chromosome number of $x = 9$ with a short length (Martel et al. 2004). Species with basic chromosome numbers of $x = 5, 7,$ and 8 appear in the most recent divergent clades, suggesting that the genome structure in *Pennisetum* may have evolved towards a reduced chromosome number and an increased chromosome size (Martel et al. 2004), which is consistent with the chromosome evolutionary trend generally observed in grasses (Martel et al. 2004).

Based on morphological characteristics, genus *Pennisetum* is classified into five sections (Stapf and Hubbard 1934): *Brevivalvula* Döll (pan-tropical), *Eupennisetum* (tropical and subtropical Africa and Asia), *Gymnothrix* (P. Beauv.) Steud (pantropical), *Heterostachya* Schumach. (northeastern Africa), and *Penicillaria* (Willd.) Benth and Hook.f. nom. Superf. (tropical Africa and India), each having a variable number of species with variable basic chromosome number. The annual diploid cultivated pearl millet, *P. glaucum* (L.) R. Br. [former *P. americanum* (L.) Leeke; syn. *P. glaucum* ssp. *glaucum*] along with its diploid wild species, *P. glaucum* ssp. *monodii* (Maire) Br., the diploid weedy species, *P. glaucum* ssp. *stenostachyum* (Klotzsch ex Müll. Berol.) Brunken (all species with $2n = 2x = 14$) and the reproductively isolated perennial tetraploid *P. purpureum* Schumach ($2n = 4x = 28$) are placed in the section *Penicillaria* (Martel et al. 2004).

Based on the cross-compatibility relationship between cultivated pearl millet and crop wild relatives, these species can be put in three genepools: primary (GP1), secondary (GP2), and tertiary (GP3) as per Harlan and de-Wet (1971). Details of each member included in each genepool are provided in Table 6.1. Under sympatric conditions, members of GP1 could easily cross to each other resulting fertile F_1 showing normal chromosome pairing (Harlan and de-Wet 1971). Hence the members in GP1 have higher possibility for successful introgression of genes from crop wild relatives (CWR) into cultivated ones. Cultivated diploid species, *P. glaucum* ssp. *glaucum*, its wild progenitor, *P. glaucum* ssp. *monodii* with two ecotypes (1) *Pennisetum violaceum* (Lam.). L. Rich. (also known as *P. glaucum* ssp. *monodii* forma *violaceum*) and (2) *Pennisetum mollissimum* Hochst. (also known as *P. glaucum* ssp. *monodii* forma *mollissimum* Hochst.)] and a weedy form known as *shibras* [= *P. glaucum* ssp. *stenostachyum* Kloyzesh ex. Müll. Berol. Brunken]

Table 6.1 Details of species coming under different genepools in Genus Pennisetum

Species name	Common name	Section	Gene pool	Ploidy	Life cycle and reproduction
P. glaucum ssp. glaucum (L.) R. Br	Cultivated Pearl millet	Penicillaria	GP1	$2n = 2x = 14$	Annual, Sexual
P. monodii (Maire) Brunken, *Ecotype*: *P. violaceum*	Wild Pearl millet	Penicillaria	GP1	$2n = 2x = 14$	Annual, Sexual
P. monodii (Maire) Brunken, Ecotype: P. mollissimum Hochst.	Wild Pearl millet	Penicillaria	GP1	$2n = 2x = 14$	Annual, Sexual
P. glaucum ssp. Stenostachyum (Klotzsch ex Müll. Berol.) Brunken	Shibras	Penicillaria	GP1	$2n = 2x = 14$	Annual, Sexual
P. purpureum Schum.	Napier/ elephant grass	Penicillaria	GP2	$2n = 4x = 28$	Perennial, Vegetative/ Sexual
P. schweinfurthii Pilger.(= P. tetrastachyum)		Heterostachya	GP1	$2n = 2x = 14$	
P. squamulatum Fresen		Heterostachya	GP2	$2n = 6x = 54$ or $2n = 8x = 56$	Perennial, Apomixis
P. alopecuroides (L.) Spreng.	Swamp foxtail grass	Gymnothrix	GP3	$2n = 2x = 18$	Annual, Sexual
P. hordeoides Steud		Brevivalvula	GP3	$2n = 2x = 18$	Annual, Sexual/ Apomixis
P. pedicellatum Trin.	Deenanath grass	Brevivalvula	GP3	$2n = 4x = 36$	Annual, Sexual
P. polystachion L. Schult.	Mission grass	Brevivalvula	GP3	$2n = 6x = 54$	Annual/ Perennial, Sexual/ Apomixis
P. ramosum (Hochst.) Schweinf.		Gymnothrix	GP3	$2n = 2x = 10$	Biannual, Sexual/ Apomixis
P. cenchroides Rich.		Unknown	GP3	$2n = 4x = 36$	Perennial, Sexual
P. ciliare L. Mant. Syn: Cenchrus ciliaris L. Mant.	Buffel grass	Unknown	GP3	$2n = 4x = 36$	Perennial, Sexual/ Apomixis
P. clandestinum Hochst. Ex Chiov.	Kikuyu grass	Eu-Pennisetum	GP3	$2n = 4x = 36$	Perennial, Vegetative/ Sexual
P. divisum (Forssk.) Ex. Gmel.		Unknown	GP3	$2n = 4x = 36$	Perennial, Sexual

(continued)

Table 6.1 (continued)

Species name	Common name	Section	Gene pool	Ploidy	Life cycle and reproduction
P. flassidum Griseb.		Unknown	GP3	$2n = 4x = 36$	Perennial, Sexual/ Apomixis
P. hohenackeri Hochst. Ex Steud	Moya grass	Gymnothrix	GP3	$2n = 2x = 18$	Perennial, Sexual/ Apomixis
P. lanatum Leeke.		Unknown	GP3	$2n = 2x = 18$	Perennial, Apomixis
P. macrostachyum (Brongn.) Trin		Unknown	GP3	$2n = 7x = 63$	Perennial, Sexual
P. macrourum Trin.	Needle grass	Gymnothrix	GP3	$2n = 4x = 36$	Perennial, Vegetative
P. mezianum Leeke.		Gymnothrix	GP3	$2n = 4x = 32$	Perennial, Sexual/ Apomixis
P. orientale L.C. Rich.	White Fountain grass	Heterostachya	GP3	$2n = 4x = 36$	Perennial, Sexual/ Apomixis
P. setaceum (Forssk.) Chiov.	Fountain grass	Eu-Pennisetum	GP3	$2n = 3x = 27$ or $2n = 6x = 54$	Perennial, Sexual/ Apomixis
P. thunbergii Kunth.		Gymnothrix	GP3	$2n = 2x = 18$	Perennial, Sexual
P. villosum Fresen.	Feather top grass	Eu-Pennisetum	GP3	$2n = 5x = 45$	Perennial, Sexual/ Apomixis/ vegetative

forms GP1 members of the pearl millet. Napier grass (*Pennisetum purpureum*) and *P. squamulatum* are the members of GP2 in case of *Pennisetum. P. purpureum*, also known as Napier grass or elephant grass ($2n = 4x = 28$ with A′A′BB genome), and the apomictic and octaploid species *P. squamulatum* Fresen ($2n = 8x = 56$) (Kaushal et al. 2007). The GP2 includes an allotetraploid rhizomatous perennial species, which can be easily crossed with members of GP1 but their hybrids are highly sterile.

The GP3 includes the remaining species that are cross-incompatible with cultivated pearl millet. There are strong reproduction barriers between the members of GP3 and GP1 or GP2, and gene transfer is only possible by radical manipulations involving in vitro techniques or by using complex hybrid bridges. In GP3, *P. schweinfurthii* (= *P. tetrastachyum*) Pilg. is the only *Pennisetum* species to have $2n = 2x = 14$ large chromosomes with an annual growth habit (Martel et al. 2004) but its chromosomes are nonhomologous (Hanna and Dujardin 1986) with

different genomic localizations of rDNA probes (Martel et al. 1996). Tertiary gene pool species with a basic chromosome number of 9 ($x = 9$) are more likely to cross with pearl millet than those with $x = 5$ [*P. ramosum* (Hochst.) Schweinf] or $x = 8$ (*P. mezianum* Leeke).

6.2.3 Distribution

Pearl millet is a warm season millet grown as rainfed crop, and it prefers to grow in light-textured (sandy or light loam), well-drained soils. Pearl millet is distributed worldwide over tropical zones covering more than 30 countries and occupies an area of 27 million ha (Varshney et al. 2017) (Fig. 6.1). It serves as a significant crop in the arid and semiarid regions of South Asia particularly India and Africa. It occurs in Africa from north to south mainly concentrated in the west/central Africa (WCA) region (Nigeria, Niger, Chad, Mali, and Senegal) and east/southern Africa (ESA) which includes Sudan; rarely being found in the southern portions of the Arabian Peninsula, Spain, and the South-eastern United States (Brunken et al. 1977). The Sahel zone of West Africa exhibits the most significant share of genetic diversity, whereas the arid sections of India exhibit the highest hectarage. Western and Central Africa (WCA) is the largest pearl millet producing region in Africa and the world accounting for 95% of the total area in WCA (Jukanti et al. 2016). India occupies 32% of world pearl millet area followed by Niger (23%) and Nigeria (8%) of total cultivated area. The West and Central Africa (WCA) region has large areas under millets (15.7 million hectares), of which more than 90% is pearl millet. The crop is cultivated on more than two million hectares in the eastern and southern Africa region.

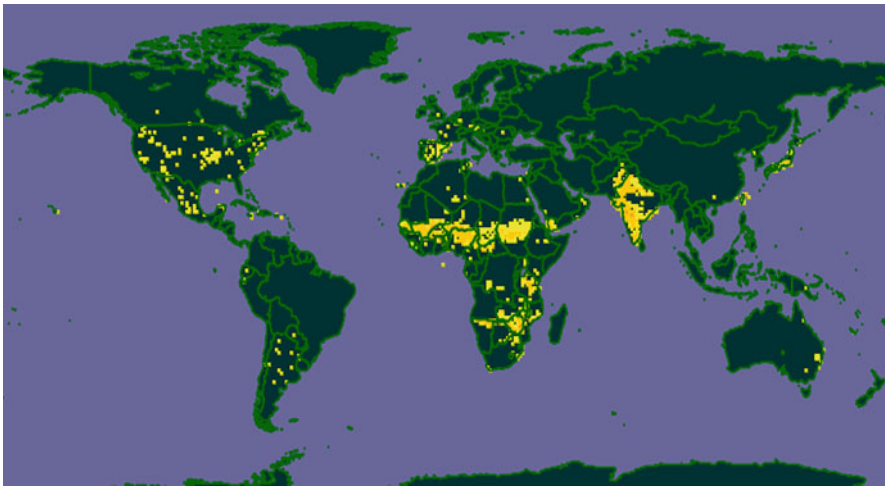

Fig. 6.1 Distribution of pearl millet in the world in which yellow regions indicate zone of pearl millet cultivation. (Source: Global Biodiversity Information Facility Network)

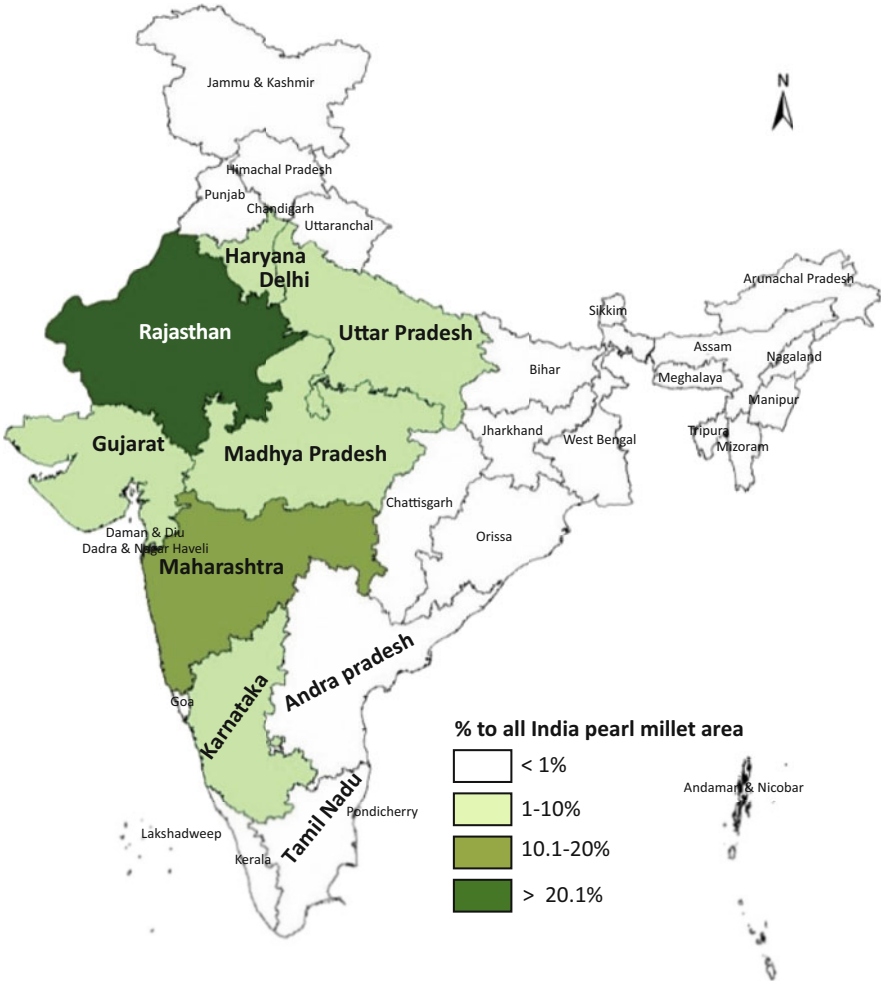

Fig. 6.2 Distribution of pearl millet in the India in which percentage area coverage indicated by strength of green shade. (Source: AICRP-PM 2021)

India has the distinction of being the largest producer of the crop, both in terms of area and production in the world. It is grown in 7.41 million hectares area with a production of 10.3 million tons during 2020–2021 (Directorate of Millets Development 2021). The major pearl millet growing states are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, and Haryana which account for more than 90% of pearl millet acreage in the country (Fig. 6.2). Amongst Indian states, Rajasthan ranks first in total pearl millet area of cultivation (57%) followed by Uttar Pradesh (13%) and Maharashtra (10%) with a production contribution of 45% from Rajasthan, 20% Uttar Pradesh, and 7% from Madhya Pradesh.

6.3 Nutritional and Nutraceutical Importance

Pearl millet, generally considered an orphan crop, is often regarded as 'powerhouse of nutrients'. It forms a good source of energy, carbohydrate, protein, fat, dietary fiber, vitamins, and minerals, especially iron and zinc in right quality and quantity required by human beings in order to fulfil their dietary requirement. Table 6.2

Table 6.2 Nutrient composition of major cereals (per 100 g) edible portion

Contents	Pearl millet	Sorghum	Maize	Rice	Wheat
A. Proximate composition					
Moisture (%)	12.40	11.90	14.90	13.00	12.80
Carbohydrates (g)	61.8	67.7	64.8	78.2	64.7
Energy (Kcal)	347	334	342	356	321
Protein (g)	10.9	9.9	8.8	7.9	10.6
Fat (g)	5.43	1.73	3.77	0.52	1.47
Dietary fibre (g)	11.5	10.2	12.24	2.8	11.2
B. Micronutrients					
Calcium (mg)	27.4	27.6	8.9	7.5	39.4
Phosphorus (mg)	289	274	279	96	315
Magnesium (mg)	124	133	145	19	125
Iron (mg)	6.4	3.9	2.5	0.6	3.9
Zinc (mg)	2.7	1.9	2.8	1.2	2.8
Copper (mg)	0.55	0.55	0.54	0.72	0.49
Sodium (mg)	10	7	4.4	3	18
Potassium (mg)	402	321	291	110	349
Thiamine (mg)	0.25	0.35	0.33	0.05	0.46
Riboflavin (mg)	0.20	0.14	0.09	0.05	0.15
Niacin (mg)	0.90	2.10	2.69	1.70	2.70
Folic Acid (μg)	36.1	39.4	25.81	9.32	30.1
β-Carotene(μg)	293	212	893	16.87	3.03
Vit E (mg)	0.24	0.06	0.36	0.06	0.77
C. Amino acids + (g/16 g N)					
Lysine	3.19	2.31	2.64	3.7	2.6
Tryptophan	1.33	1.03	0.7	1.27	1.4
Methionine	2.11	1.52	2.1	2.6	1.75
Threonine	3.55	2.96	3.23	4.36	3.01
Isoleucine	3.45	3.45	3.67	3.28	3.83
Leucine	8.52	12.03	12.24	8.09	6.81
Cysteine	1.23	1.06	1.55	1.84	2.35
Phenylalanine	4.82	5.1	5.14	5.36	4.75
Valine	4.79	4.51	5.41	6.06	5.11
Arginine	4.54	3.96	4.2	7.72	5.13
Histidine	2.15	2.07	2.7	2.45	2.65
Tyrosine	2.67	3.61	3.71	4.36	3.12

Source: NIN Hyderabad, 2017 and 2018

summarizes the nutritional composition of pearl millet grains compared to other staple cereals. Pearl millet is a rich source of energy (350 Kcal/100 g) comparable with sorghum, wheat, rice, and maize. The carbohydrate content of pearl millet is 61.8 g/100 g; with 60–70% starch comprising 28.8–31.9% amylose amongst which 14.6–17.2% form complex with native lipids. It encompasses a comparatively higher water absorption capacity and swelling index than the other cereal starches (Kajla et al. 2020). Free sugar, such as sucrose, glucose, fructose, and raffinose, forms 2.6–2.8%. Pearl millet contain adequate amount of dietary fibers (11.5 g/100 g) amongst which most of them belong to insoluble form, when compared with other grains (National Institute of Nutrition 2017). It helps to overcome acidity due to its alkaline nature. Pearl millet is having low glycaemic index score of ~55 and significantly rich in resistant starch. Hence, pearl millet can be excellent diet for diabetics, constipations, obesity, and celiac diseases (Nambiar et al. 2011).

The protein content of pearl millet is about 11 g/100 g, comparable to wheat but higher than rice. The amino acid profile of pearl millet protein includes most of the essential amino acids, which is comparatively higher than wheat and maize proteins. Similar to other cereals, lysine is one of the limiting amino acid. It is rich in methionine but poor in other sulphur containing amino acids. With low prolamin fraction, pearl millet is a gluten-free grain and is the only grain that retains its alkaline properties after being cooked which is ideal for people with gluten allergy. Pearl millet is rich in fat content (5–7 mg/100 g) with better fat digestibility as compared to other grains. Pearl millet grains have proportionally very large germs than other cereals where most of the lipids are located. It is rich in unsaturated fatty acids (75%) with higher content of nutritionally important omega-3 fatty acids such as oleic acids (25%), linoleic acid (45%), and linolenic acid (4%) than other cereal grains.

The ash content of whole pearl millet grain ranges between 1.6% and 3.6%. In terms of actual minerals, pearl millet grain, like other cereal grains, is an adequate source of dietary minerals, such as phosphorus, calcium, magnesium, iron, zinc, and copper (Serna-Saldivar 2016 and Pujar et al. 2020). Most of these minerals were located near to pericarp, and dehulling of the grain will lead to considerable loss of these mineral nutrients. Pearl millets are a rich source of phosphorus which is an important mineral for energy production. It is an essential component of ATP, the energy currency of the cell. It also forms a part of the nervous system and cell membranes. A well-cooked cup of millet gives 26.4% daily need for magnesium and 24% daily need for phosphorus.

Magnesium from pearl millets helps in relaxing blood vessels and maintains the blood pressure, enhances nutrient delivery by improving the blood flow, and thus further protects the cardiovascular system. Magnesium increases insulin sensitivity and lowers triglycerides. It also acts as a cofactor for more than 300 enzymes. Iron (Fe) and zinc (Zn) deficiency affects 50% of the world population resulting in anaemia, diarrhoea, impairment of physical growth, and suppressed immune function. Pearl millets are rich in these minerals can reach up to 5.5 mg/100 g and 3.2 mg/100 g, respectively (Minnis-Ndimba et al. 2015). However, like all cereals, pearl millet contains phytate, an anti-nutrient that chelates with minerals forming

complexes hence reducing their effective absorption and utilization by humans (Kent 1994). The typical ranges of phytate content in pearl millet are 443–1076 mg phytate/100 g (El-Hag et al. 2002).

Even though phenolic acids of pearl millet have detrimental effect on micronutrient bioavailability, they have considerable interest due to their impacts on human health through important biological and pharmacological properties, such as anti-hyperlipidemic against cardiovascular diseases and anti-carcinogenic activities against cancer. It is rich in brain cell promoting factors which can alleviate Parkinson's disease such as galic acid (15.3 μg/g), chlorogenic acid (16.0 μg/g), syringic acid (7.4 μg/g), *p*-coumaric acid (1350.88 μg/g), ferulic acid (199.56 μg/g), cinnamic acid (41.30 μg/g), ellagic acid (14.47 μg/g), querctin (5.9 μg/g), and apigenin (9.08 μg/g) sample of pearl millet flour. Furthermore, pearl millet has plenty of health-promoting attributes owing to its nutritional composition and hence has an immense potential toward medicinal uses. As for example, its high fibre content can make it a potential component in the diets of patients suffering from constipation, obesity, and gallstones (Ambati and Sucharitha 2019). Further due to its anti- or hypo-allergic properties, it can be safely incorporated in the diets of celiac patients, pregnant women, infants, lactating mothers, elderly, and convalescents. Thus, pearl millet grains have immense medicinal value and should be aggressively promoted by dieticians and nutritionists so that a large section of society could be benefited.

6.4 Botanic Description and Floral Biology of the Crop

Pearl millet is a diploid ($2n = 2x = 14$) annual monocot grass (Family-Poaceae) with a short life span of 75–100 days. It can achieve a height of 1.5–3 m tall but can grow up to 5 m. However, improved cultivars such as OPVs and hybrids are reportedly shorter. Culms can have a simple or branching habit with either slender or stout girth having a smooth or hairy surface. Leaf sheaths, collars, and blades may also be smooth or hairy. Its leaf blades are simple, minutely serrated, alternate, flat, green can be up to 1.5 m long and 8 cm wide. Generally, panicle emerges around ~35 to 70 days from the day of sowing depending on the maturity type in pearl millet (early/medium/late). Panicle emergence is marked by the rapid elongation of the peduncle and the inter-node below it and by the appearance of the flag leaf or boot leaf which requires nearly 6 days emerging from the leaf sheath (Fig. 6.3a). The inflorescence, usually regarded as panicle or spike are mostly cylindrical to conical in shape, stiff, and very dense and usually range from 10 to 45 cm long and up to 7–9 cm. Typical feature of *Pennisetum* spp. can be seen in case of involucres with bristles each of which enclosing 1–9 spikelets (Brunken et al. 1977).

Spikelets are short pediceled, come two in a fascicle, and are 3.5–4.5 mm long, ovate, and turgid. Spikelets on a panicle can vary between 500 and 3000 in number depending upon the genotype. Each spikelets have two kinds of flowers, staminate floret which is sessile in nature with single lemma and 3 stamens and is borne below

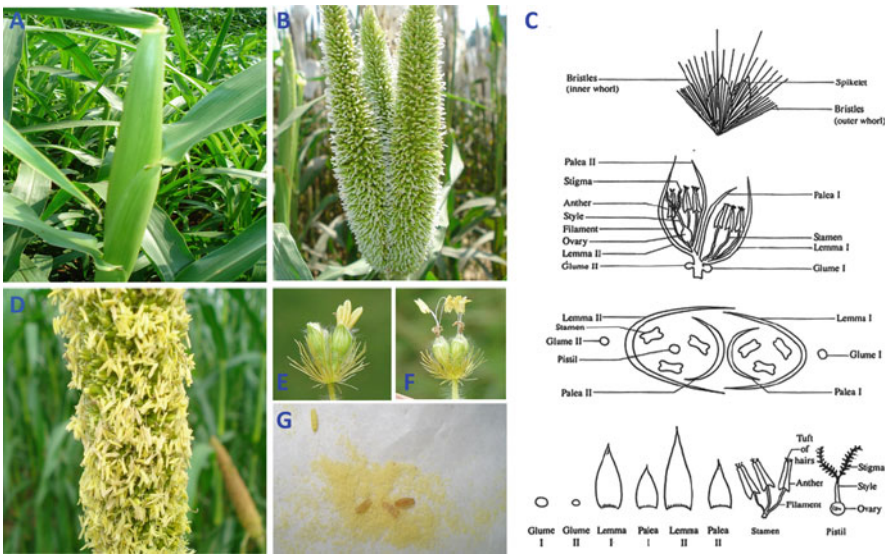

Fig. 6.3 Floral biology of pearl millet (**a**) Booting of panicle (**b**) Stigma emergence stage (**c**) details of floral organs in pearl millet (**d**) Anthesis (**e**) Staminate spikelet (**f**) Hermaphrodite Spikelet (**g**) Pollen from the anther

shortly pedicelled hermaphrodite (bisexual) ones (Fig. 6.3e). Hermaphrodite floret comprises 1 pistil, 2 feathery style, and 3 anthers enclosed between the lemma and palea (Fig. 6.3f). Pearl millet seeds are small wedge-shaped to spherical, can be of various colours ranging from white or yellow, brown or even grey, and size can vary between 0.5 and 3 mm long and could weigh between 3 and 25 g/1000 seed. After 50% flowering, seeds could reach physiological maturity in 27–30 days.

It is a highly allogamous (cross-pollinated) crop with outcrossing rate more than 85% mainly because of its protogynous nature. Hence, a natural population of pearl millet genetically represent heterozygous and heterogeneously. The main mode of pollination is due to anemophily (wind pollination). The panicle emerges around 10 weeks after sowing; the styles begin to protrude 2–3 days later first at the top of the inflorescence and proceeds downwards. They take 2–3 days to complete the entire spike (Fig. 6.3b). Exerted stigma remains receptive for 12–24 h. Anthesis commences from one-third of the apex of spike and proceeds upwards and downwards (Fig. 6.3c). Bisexual flowers appear 2–3 days earlier than staminate flowers. Hence, the first male phase in which the anthers from perfect floret commence followed by second male phase from the staminate florets emerge and anthesis is over within 2–3 days. This is followed by the first male phase in which the anthers from the perfect florets emerge out. Second male phase commences on the fifth day of anthesis. Usually, anthesis lasts throughout the day and night with the peak between 8.00 p.m. and 2.00 a.m.

6.4.1 Selfing and Crossing Techniques

To ensure selfing, spikes may be bagged before emergence of the stigmas at boot leaf stage. Care should be followed to cover the lowermost spikelet using selfing bag in an elongating spike. Another procedure is to enclose within a bag two full spikes from the same plant, 1 day (or) 2 days older than the other and ready to shed pollen as the stigmas are emerging form the younger spike. Since the spikelet in pearl millet is small, emasculation in pearl millet is very difficult and laborious and due to overlap of the female flower with the male phase at the tip of spike, about four-fifths of the upper portion of the spike is removed and the rest is bagged before the styles appear to prevent contamination. Flowers are pollinated by dusting them with fresh pollen obtained from the desired male plant or by shaking a spike which is shedding pollen over the exposed stigmas.

6.4.2 Controlled Cross-pollination

Pearl millet does not require emasculation for making crosses. The female line will be covered before stigma emergence with butter paper bag. Without removing the butter paper bag, one could observe the emergence of stigma. After most of the stigma has emerged, pollen from desired male parent is collected and dusted on to the female line. Pollination is usually made in the morning. The crossed heads are labelled. Another method is instead of removing the selfing bag of female and dusting, the top of the cover clipped of desired male parent inflorescence in the process of pollen bursting is inserted to burst the stigma. Then the clipped top of the bag is folded and stapled. The crossed heads can be collected after 30–35 days.

6.5 Cytogenetics

Rao (1929) from root tip studies, determined the chromosome number of pearl millet as $2n = 14$. Later Rangaswamy (1935) studied the chromosomes in dividing cells of the microsporangium and confirmed the chromosome number as $2n = 14$. Reader et al. (1996) was the first to use fluorescence in-situ hybridization (FISH) for detailed characterization of the somatic complement of pearl millet. Pantulu (1958) studied the chromosomes at pachytene and grouped them into four classes on the basis of relative length and position of centromere (1) two pairs (Chromosome 1 and 2) which had the greatest length and median centromere (2) two somewhat shorter pairs (Chromosome 3 and 4) with median to submedian centromeres (3) two pairs medium-sized (Chromosome 5–6 with submedian centromeres; and (4) the shortest pair (Chromosome 7) with the nucleolus organizer. Most lines of pearl millet have one pair of nucleolus-organizing chromosomes (Pantulu 1960). Karytopic measurements of an inbred I–55 of pearl millet revealed that the seven pairs of chromosomes were numbered 1–7 according to their descending total length, chromosome 1 being the longest (5.51 μ) and chromosome 7 being the shortest (3.24 μ)

as recorded by Tyagi (1975). The shortest chromosome was the SAT chromosome (Pantulu 1960). Some African origin varieties have one or two of the longer pairs that reveal secondary constrictions in their long arms. The shortest chromosome pair of the complement carries the nucleolar organizer in the short arm. B-chromosomes occur only in pearl millet populations of African origin. These B-chromosomes exhibited nucleolus-organizing activity (Burton and Powell 1968; Pantulu 1960; Powell et al. 1975).

Jauhar (1970) suggested that chromosome complement of *P. glaucum* has been derived from a basic set of $x = 5$ chromosome based on the meiotic paring behaviour in haploids, interspecific hybrids, and autotetraploids where two bivalents per cell (some with two chiasmata each) were observed suggesting duplication of two chromosomes in the species. Jauhar (1968) also considered inter and intra-genomic chromosome pairing in an interspecific hybrid and its bearing of the basic chromosome number in *Pennisetum*. Manga and Pantulu (1971) did not report any bivalent in haploid plants, whereas Gill et al. (1973) considered that the occurrence of bivalents was the result of segmental homologies amongst some chromosomes. Powell et al. (1975) also reported pairing of chromosomes in haploids and attributed the pairing to the duplicate loci.

6.6 Breeding Objectives

Pearl millet breeding strategies have evolved over several decades, considering to meet ever-changing human needs and knowledge gained with respect to production constrains, access to novel germplasm, and accumulated knowledge in the fields of genetics, physiology, pathology, and so on. Even though various farmers participatory pearl millet breeding surveys conducted across globe over different periods had pointed out similar set of traits to be focused for future improvements (Ipinge et al. 1996; Christinck 2002; Basavaraj et al. 2015; Angarawai et al. 2016; Thangapandian et al. 2017; Drabo et al. 2019). Major trait to be focused was maturity earliness and drought resilience under low rainfall conditions. Farmers preferred the plant type (e.g., HHB 67 and RHB 177) as they could mature within 70–75 days, before the available moisture got exhausted. Early maturity is a preferred trait for fitting in any cropping system and also helps from the menace of bird damage. Under irrigated conditions, farmers preferred high yielding cultivars with bold grains (>10 g/1000 grains), medium tillering nature, long, thick, and compact panicles. Most of the farmers, particularly the women, preferred to select those cultivars which have good grain quality traits such as light or white grain colour, shape, ease to mill, sweetness, good keeping quality, and least prone to staling or firming (maintenance of softness) in bread once prepared. Since pearl millet is a part of subsistence farming, they also preferred to grow stay green, non-lodging cultivars with good stover yield and disease resistance (e.g., foliar blast). In African countries, farmers pointed out the infection was due to striga as major biotic factor followed by head miner infestation, whereas in India, downy mildew and foliar blast was reported as major biotic constrains. Even though chemical control is available for above mentioned biotic

constrains, it will add to the cost of production which is undesirable for farmers with poor resources.

6.7 Centres Involved in Pearl Millet Breeding

6.7.1 Genesis of AICRP (Pearl Millet)

In the early 1940s, efforts were initiated by the Indian Council of Agricultural Research for pearl millet crop improvement in India. ICAR-All India Coordinated Millet Improvement Project (AICMIP) was established in the year 1965 with its headquarters at the Indian Agricultural Research Institute, New Delhi. Headquarter of the project was shifted to Pune in 1977. Later on, pearl millet was separated from the rest of the millet crops, and the All India Coordinated Pearl Millet Improvement Project (AICPMIP) was established in 1985 with its headquarters at Pune as an independent coordinated project. Later in July 1995, the headquarters of AICPMIP was shifted to Jodhpur in the state of Rajasthan, the state which occupies nearly half of pearl millet area of the country. AICPMIP has a network of 12 centers in Rajasthan, Maharashtra, Uttar Pradesh, Karnataka, Andhra Pradesh, Madhya Pradesh, Punjab, Haryana, Tamil Nadu, and Gujarat (Table 6.3). The All India Coordinated research project on Pearl Millet (AICRP) centers pursue mandated activities in pearl millet improvement, production, and protection. AICRP on pearl millet has played a pioneering role in developing a diverse range of improved breeding lines and parental lines of hybrids. These lines have been used extensively to develop and commercialize a large number of hybrids.

6.7.2 Role of ICRISAT in Pearl Millet Research

International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) started during 1972 had key role in pearl millet improvement programmes taken up in Indian and African countries. They conduct inter-disciplinary and partnership-based research for the genetic improvement of its mandate crops including pearl millet. Initial emphasis was given on: (1) developing a diverse range of trait-specific composites, based on the germplasm largely from the Western and Central Africa; (2) improving them by the process of recurrent selection, principally for grain yield and downy mildew (*Sclerospora graminicola* [Sacc.] Schroet) resistance; and (3) developing open-pollinated varieties (OPVs). The improved composites, OPVs, and breeding lines derived from them were disseminated for worldwide utilization. In 1990s, pearl millet improvement programme at ICRISAT adopted a regional strategy, by aligning its research and breeding product development priorities with the priorities of the regional players. In case of India, public sector breeding programmes in the National Agricultural Research System (NARS) were largely directed towards hybrid breeding as well as private seed companies, which

Table 6.3 List of AICPMIP centres involved in pearl millet research and extension in India

S. no	Name	Year of start	Research areas
1	AICRP coordinating unit Mandor, Jodhpur (Rajasthan)	1965	Breeding, Agronomy, Pathology, statistics
2	Agricultural Research Station, SK Rajasthan Agricultural University, Jaipur (Rajasthan)	1974	Breeding, Agronomy, Pathology, Physiology, Entomology
3	Agricultural Research Station, SK Rajasthan Agricultural University, Bikaner (Rajasthan)	1994	Breeding, Agronomy
4	College of Agriculture, Mahatma Phule Krishi Vidyapeeth, Dhule (Maharashtra)	1995	Breeding, Agronomy, Pathology
5	Regional Research station, Marathwada Agricultural University, Aurangabad (Maharashtra)	1974	Breeding, Agronomy, Pathology
6	Millet Research Station, Junagarh Agricultural University, Jamnagar (Gujarat)	1965	Breeding, Agronomy, Pathology, Physiology, Entomology
7	Department of Plant Breeding, CCS Haryana Agricultural University, Hisar (Haryana)	1965	Breeding, Agronomy, Pathology, Biochemistry
8	Regional Research Station, University of Agricultural Sciences, Vijayapura (Karnataka)	1978	Breeding, Agronomy
9	Downy Mildew Research Laboratory, University of Mysore, Mysore (Karnataka)	1978	Pathology
10	Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Anantapur (Andhra Pradesh)	1971	Breeding
11	Department of millets, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu)	1965	Breeding, Agronomy
12	College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (Madhya Pradesh)	1986	Breeding, Pathology
13	Department of Plant Breeding, Punjab Agricultural University, Ludhiana (Punjab)	1978	Breeding

were rapidly emerging as dominant players and were engaged only in the hybrid development.

Thus, pearl millet breeding at ICRISAT, Patancheru, made a strategic shift towards developing and disseminating a diverse range of high-yielding and downy mildew (DM) resistant, trait-based breeding lines, and hybrid parents (seed parents and restorer parents) while their utilization in hybrid development and commercialization was taken up by the NARS and private seed companies. Significant research progress made in achieving genetic gain in grain and fodder yield of hybrids through development of diverse parental lines with good agronomy and high combining ability, utilization of alternate male sterile cytoplasm and restorers, minimizing the severity of DM epidemics, biofortication of pearl millet with regard to grain

micronutrients, building genomic resource platform and development of basic, strategic and applied research methodology related to pearl millet improvement.

6.7.3 Other Centers

The details of other centers and their specific research objectives are listed in Table 6.4.

6.8 Breeding Achievements and Cultivar Development

Initially, its emphasis was on organizing research activities and conducting multilocation experimental trials to identify cultivars with high grain yield and broad adaptation OPVs by using simple plant selection and mass selection. Later, during the l 970s·and 1980s, the focus spread to the development of cultivars resistant to biotic and abiotic stresses along with yield enhancement. After discovery of cytoplasmic male sterility (CMS), the focus of NARS research shifted to development of hybrids to overcome downy mildew infestation and yield enhancement. The AICPMIP has played a significant role in developing a diverse range of improved breeding materials and parental lines of hybrids. These lines have been used extensively to develop and commercialize a large number of hybrids. List of hybrids and varieties released in India for commercial cultivation by farmers can be accessed through the link given http://www.aicpmip.res.in/pearl%20millet%20hybrids%20and%20varieties.pdf

6.8.1 Population Improvement and OPV Development

Due to unpredicted nature of weather, low cost of seeds, longer seed replacement period, and greater resilience towards abiotic and biotic stresses, open pollinated varieties (OPVs) in pearl millet are still popular amongst the farmers of India and arid regions of Africa despite of 25–30% less yield potential to corresponding hybrid developed for that area. In contrast to single cross hybrids of pearl millet, the intra-population variability in pearl millet OPVs contributes to greater resilience to a multitude of biotic and abiotic stresses. The genetic heterogeneity of OPVs offers genetic plasticity to adjust itself against the pressure by the limiting environments and disease epidemics (Freshley and Delgado-Serrano 2020). The genetic improvement of OPVs started in the 1930s and largely concentrated on improving the yield by mass selection and progeny testing and could not progress beyond a certain limit because of a narrow genetic base (Yadav et al. 2013, 2021).

ICRISAT during the 1970s, introduced African germplasms especially from Western Africa, and introgressed them with Indian germplasm to enhance the genetic diversity of this crop. Due to such focused efforts, composites and OPVs were developed largely based on these germplasm lines. These populations were

Table 6.4 List of some other major institutes relevant to pearl millet research

Sl. no.	Research Centre	Specific objectives related to pearl millet
International level		
1	Consultative Group for International Agricultural Research, France	Research to improve food security and nutrition, natural resources, and ecosystem
2	Institute of Biological, Environmental and Rural Sciences Aberystwyth University, Aberystwyth, Ceredigion SY23 3DA	Genome-wide association mapping of genes related to abiotic stress, grain quality
3	International Atomic Energy Agency, Vienna, Austria	Crop improvement by mutation breeding; Publication of protocol for mutation breeding in plants
4	International Center for Agricultural Research in the Dry Areas Beirut, Lebanon	Germplasm characterization and development of heat-tolerant lines
5	International Center for Tropical Agriculture, Cali, Colombia	Maintenance of crop biodiversity; co-ordination of HarvestPlus programme
6	International Food Policy Research Institute Washington, DC 20005–3915 USA	Research on impact on various economic policies on agricultural crops
7	International Institute of Tropical Agriculture, Oyo State, Nigeria	Maintenance of crop biodiversity; networking of food and nutritional programmes
8	International Centre for Genetic Engineering and Biotechnology, New Delhi, India	Research related to cloning and identification of plant genes related to abiotic stress
9	Kansas State University	Studies on abiotic stress tolerance in pearl millet
10	University of Georgia, Tifton, USA	Development of pearl millet cultivars, Basic work related to fertility restoration, apomixes, etc.
11	University of Florida and National Aeronautic Space Agency (NASA), USA	Exploration of plant materials for deep space acclimatization
12	Senegalese Institute of Agricultural Research (ISRA) (Institut sénégalais de Recherche Agricole), Dakar, Senegal	Development of pearl millet cultivar for drought prone areas of Senegal
13	Environmental Institute for Agricultural Research (INERA) Institut de l'Environnement et de Recherches Agricoles, Burkina Faso	Development of pearl millet cultivars for drought-prone areas of Burkina Faso
14	Institut d'Economie Rurale (IER), Bamako, Mali	Development of pearl millet cultivars for drought-prone areas of Mali
15	Centre d'étude régional pour l'amélioration de l'adaptation à la sécheresse (CERAAS), Senegal	Development of pearl millet cultivars for drought striga and downy mildew-prone areas of Senegal
16	Institut National de la Recherche Agronomique du Niger (INRAN), Niger	Development of pearl millet cultivars for drought striga and downy mildew-prone areas of Niger

(continued)

Table 6.4 (continued)

Sl. no.	Research Centre	Specific objectives related to pearl millet
17	Institut de Recherche pour le Développement (IRD), Montpellier, France.	Basic research work on domestication traits, earliness, root Phenotyping studies for drought tolerance in pearl millet.
18	Beijing Genomics Institute (BGI)-Shenzhen, Shenzhen, China	Generation of genomic resources in pearl millet
19	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany	Generation of genomic resources in pearl millet; wide hybridization studies in pearl millet
National level		
1	Central Arid Zone Research Institute, Jodhpur, Rajasthan, India	Research
2	Central Institute of Post-Harvest Engineering and Technology, Ludhiana, India	Development of processing-related protocols
3	Central Research Institute for Dry land Agriculture, India	Research on agricultural technology related to drylands
4	Indian Institute of Millets Research Rajendranagar, Hyderabad-Telangana, India	Developing improved production and crop protection technologies in millets
5	Indian Agricultural Research Institute, New Delhi, India	Research, training, extension on pearl millet
6	Indian Grassland and Fodder Research Institute, Jhansi, India	Research in fodder pearl millet and related species
7	National Bureau of Plant Genetic Resources, New Delhi, India	Exploration, evaluation, conservation, genetic fingerprinting of plant genetic resources
8	National Institute on Plant Biotechnology, New Delhi, India	Biotechnological aspects of pearl millet

also a source for the breeding lines, which were widely used over the years by both the public and private sectors (Andrews and Kumar 1996; Rai et al. 2014). The Iniadi germplasm, acquired from western Africa, has been extensively used in India, the United States, and other places worldwide. Soon after their release, OPVs like WC-C75, Raj 171, ICMV 155, ICMV 221, ICTP 8203, Pusa composites-383, 443, 612, 701, CZP 9802, and JBV 2 became very popular with farmers (Reddy et al. 2021). In Maharashtra, a substantial proportion is still occupied by a biofortified OPV, Dhanshakti for its grain iron content.

However, the OPV option allows only partial exploitation of heterosis. Sustainable improvement in grain and fodder yield through OPVs faces a major dilemma. On the one hand, crosses between morphologically similar genotypes or lines generate little genetic variability to sustain significant genetic gains, though the pace in development of the OPVs will be rapid with having acceptable phenotypic uniformity. On the other hand, utilization of genetically dissimilar lines in crossing will create more heterotic combination and huge variability in subsequent

generations, but the population will take more generations to achieve desired phenotypic uniformity.

6.8.2 Hybrid Development in Pearl Millet

Pearl millet displayed high degree of heterosis for grain and stover yields (Virk 1988) and attempts were made to exploit heterosis in the 1950s utilizing the protogynous nature of flowering of this crop. The usual method at that time for production of hybrid seeds was growing the parental lines in mixture and allowing them to cross-pollinate. The resultant seed contained approximately 40% hybrid seed when the two parental lines had synchronous flowering at about same time. These chance hybrids thus produced, out-yielded local varieties by 10–15%. However, they could not become popular due to their limited yield advantage over OPVs, narrow range of adaptation and lack of seed production programmes. Exploitation of heterosis is the most efficient means for enhancing the productivity. Work on hybrid development in India started in the early 1950s and chance hybrids were released in Maharashtra and Tamil Nadu in the 1950s. Due to limited hybrid seed production, hybrids could not be cultivated at commercial scale. This limitation was overcome by the discovery of cytoplasmic male sterility by Burton in 1958.

The first male sterile line, Tift 23 A, was developed using Tift 23 B as a recurrent parent and was released in 1965 (Burton 1965). The above-mentioned limitations in the exploitation of heterosis were circumvented with the discovery of cytoplasmic nuclear male sterility and release of male-sterile lines Tift 23A and Tift 18A in early 1960s at Tifton Georgia, USA. These lines were made available to Indian breeding programmes. The male-sterile line Tift 23A containing Tifton male sterile cytoplasm (A_1) was extensively utilized, both at the Punjab Agricultural University and the Indian Agricultural Research Institute, because of its short stature, profuse tillering, uniform flowering, and good combining ability. This laid the foundation of pearl millet hybrid breeding in India. The demonstration of the first commercial pearl millet grain hybrid HB-1, developed by Punjab Agricultural University (PAU), Ludhiana, and released in India in 1965, had twice much grain yield as the then improved OPVs (Athwal 1965) boosted the shift in research and adoption of the single cross hybrids over the OPVs.

6.8.3 Different Phases of Pearl Millet Hybrid Improvement in India

There have been four conspicuous phases in hybrid development in India in which rate of improvement in grain yield in kg/ha/year during each phase (Singh et al. 2014; Yadav et al. 2019) is given by Fig. 6.4. The details regarding the four phase of pearl millet improvement is given below.

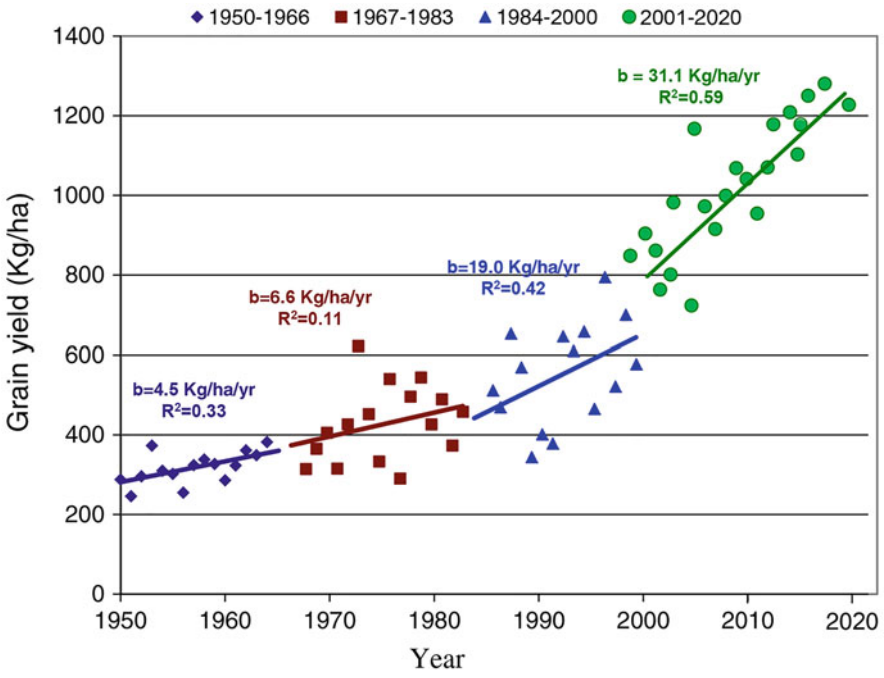

Fig. 6.4 Snapshot depicting increasing trends in pearl millet grain productivity during India's four phases of hybrid improvement, with numbers inside the figure indicating the rate of grain yield improvement in kg/ha per year for each phase

6.8.3.1 Pre-hybrid Phase (1950–1966)

The Indian Council of Agricultural Research (ICAR) was the first institution to take up the responsibility of pearl millet crop improvement in India. During the early 1940s and 1950s, research was sporadic and mainly aimed to improve productivity. Some periodic efforts were made in the 1940s towards varietal improvement through simple mass selection from locally adapted material aimed at improvement of yield. As a result, varieties like Vansari, Kopargaon Local, N 28–15–1, Co 1, K 1, Co 2, Co 3, AKP 1, AKP 2, RSJ, RSK and T 55 were developed and released. The same method was used with African populations, which resulted in the development of S 530 and Pusa Moti. In order to exploit heterosis, attempts were made to breed chance hybrids: The hybrids thus produced were released in India in the early 1950s (X1, X2, X3) and they outperformed local varieties by 10% in terms of yield. But these hybrids and improved varieties did not become popular because of their limited productivity, narrow range of adaptability, and lack of seed production programmes. Overall, there have been four phases in the development of pearl millet improved cultivars in India, covering a period of 60 years. During the pre-hybrid phase (1950–1966), improvement efforts largely concentrated on local traditional landrace materials and carried out simple mass selection. This resulted in the development and release of a total of 13 improved cultivars (3 hybrids and 10 OPVs). The average

improvement in pearl millet productivity during this period was 4.5 kg/ha per annum.

6.8.3.2 Second Phase (1967–1983)

During this phase, hybrid breeding received a major impetus when cytoplasmic male sterility (CMS) was discovered. Broadly, there have been three conspicuous phases of pearl millet hybrid development in India. ICAR established the AICMIP in 1965 to conduct intensive and systematic research on millets. The project played a pioneering role in developing a diverse range of improved breeding lines and parental lines of hybrids, conducting multilocation trials and commercializing a large number of hybrids. Later, the project also developed production and protection technologies specific to agro-ecological regions in different states. Male sterile lines Tift 23A and Tift 18A were released in the early 1960s. This laid the foundation for pearl millet hybrid breeding in India. At the same time, two additional male sterile lines, L 66A and L 67 A, were developed at Ludhiana. The male sterility line Tift 23A was extensively utilized because of its short stature, profuse tillering, uniform flowering, and good combining ability. The first hybrid HB 1 was released in 1965 followed by HB 2, HB 3, HB 4, and HB 5.

During this period, 16 hybrids and 13 varieties were developed, and India enjoyed the privilege of being the first country to release pearl millet hybrids. Most of the hybrid releases dominated the cropped acreage during that period. Amongst the hybrids of the HB series, HB 3 became the most popular because of its shorter maturity, bold grain, and adaptability to moisture stress conditions. Adoption of these hybrids led to a 75–100% increase in yield over local cultivars and boosted production from 3.5 million tons in 1965 to a record 8.0 million tons in 1970. In the initial years of pearl millet research after ICRISAT was established in 1972, the emphasis was more on population improvement and development of OPVs rather than hybrids and hybrid parents (Kumara et al. 2014). Overall, there was only a modest increase (6.6 kg/ha per year) in pearl millet productivity during this phase.

6.8.3.3 Third Phase (1984–2000)

In third phase of the development of pearl millet improved cultivars in India, the recurring problem of downy mildew epidemics that had been affecting hybrids till 1980 led to strengthening of research on genetic diversification of hybrid seed parents. As a result, large number of genetically diverse male sterile lines were developed and utilized in hybrid breeding programmes. With hybrids based on Tift 23A succumbing to downy mildew, another male sterile line 5071A, bred at IARI, Delhi, by mutational change from Tift 23A and showing less downy mildew incidence, was utilized. Three hybrids, NHB 3, NHB 4, and NHB 5, were developed from this line but they did not become popular because of their low yields and continued susceptibility to downy mildew. They were cultivated for not more than a year or two. Two more male sterile lines were developed and made available from Tift 23A. These lines, 5141A and 5054A, had good downy mildew resistance and were widely used. The hybrids BJ 104 and BK 560 (5141A) and CJ 104 (5054A)

were widely cultivated during 1977–1984. Hybrids based on 5141A were phased out in 1985 due to susceptibility to the downy mildew.

From the mid-1980s, the private sector started participating actively in pearl millet crop development and seed distribution. A major driver of this spurt in private sector participation was the strong public sector research support programme and supply of breeding material from national and international institutions. However, the partnership between ICRISAT, national institutes, and the private sector remained informal and passive. It was in the 1990s that ICRISAT changed its research focus from OPV development to hybrid parent development in alignment with the regional priorities of the NARS and the rapidly expanding private seed sector. A total of 38 hybrids and 16 OPVs were released during this period. Downy mildew was largely contained by using the genetic diversity of pearl millet. A critical analysis of the situation reveals that the absence of long-lasting resistance to downy mildew amongst hybrids was primarily due to the lack of diversity in their parental lines. This was due to the fact that most of the hybrids were based on Tift 23A and then on 5141A and 5071A.

Similarly, the same pollinators were also repeatedly used in combinations with different male sterile lines. For example, J 104 was used in four hybrids, K 560–230 in three, and K 559 in two hybrids. Thus, the outbreaks of downy mildew in hybrids were due to the use of a limited number of parental lines in hybrid breeding rather than the cytoplasm linked susceptibility (Yadav et al. 1993). Much greater efforts are now being made in developing genetically diverse male sterile lines. As a result, a large number of male sterile lines with A_1 source of cytoplasmic male sterility (CMS) have been used in hybrid breeding. In addition, CMS sources other than A_1 have also been used. The average productivity has been enhanced to about 19.0 kg/ha per annum during this period.

6.8.3.4 Fourth Phase (2001–2020)

During the fourth phase, greater emphasis was placed on genetic diversity by using a larger number of highly diverse seed and pollinator parents in hybrid development and targeting adaptation to specific niches in different zones. The highest number of cultivars (81 hybrids, 20 varieties) was released during this period. The hybrids released during the last decade were based on more than 12 generally diverse male sterile lines and a large number of diverse pollinators. Moreover, several hybrids developed by the private sector in its sound and well-established research and development programmes further helped in diversifying the genetic base of hybrids. Consequently, the problems of downy mildew epidemics were kept largely under control. As a result, improvement in grain productivity further increased to 31.1 kg/ha per year.

6.8.3.5 Hybrid Development at a Glance from 1950 to 2021

One-hundred-eighty hybrids were released in India from 1950 to 2021. Forty-two hybrids during 2015–2021; 35 hybrids during 2009–2014, 14 during 2004–2008, 17 during 1999–2003, 22 during 1994–1998, 11during 1989–1993, 14 during 1984–1988, 7 during 1979–1983, 10during 1974–1978, 5 during 1969–1973, and

3 hybrids during 1950–1957 were released by both public and private sector (Kumar et al. 2020b).

6.8.4 Search for New Sources of CMS and Male Fertility Restorers in Pearl Millet

Large-scale use of the single A_1 CMS source during the 1960s in all the hybrids had raised concern regarding its potential vulnerability to diseases and insect pests. As a result, continuing efforts were made to search for alternate CMS sources. This led to identification of A_2 and A_3 CMS sources from genetic stocks and their derivatives (Athwal 1961, 1966); Av and A_4 CMS sources from *P. glaucum* sub-species *monodii* (*violaceum*) accessions (Marchais and Pernes 1985; Hanna 1989); and Aegp and A_5 CMS sources from gene pools (Sujata et al. 1994; Rai 1995). Based on the differential male fertility restoration of hybrids using common restorers, it has been established that the A_1, A_2, A_3, A_v, A_4, and A_5 were distinctly different CMS system. Enormous differences in downy mildew incidence amongst male sterile lines based on Tift 23A, cytoplasm indicates that the cytoplasm is not associated with downy mildew susceptibility, and that it is nuclear gene resistance which is important. Experimental evidence confirms this assumption (Kumar et al. 1983). Thus, at present, there is no need to be alarmed about the vulnerability of Tift $23A_1$ cytoplasm to downy mildew. However, in the long run, the large scale and continuous use of a single cytoplasm source runs the risk of it becoming vulnerable to existing or unforeseen diseases. Hence, there is a need to diversify the cytoplasmic base of male sterile lines. Later discovery of A_4 and A_5 CMS system marked new landmark in heterosis breeding in pearl millet. These CMS systems were found most stable and imparting better fodder yield than corresponding counterparts. But their utilization is limited by shortage of their restorer lines. Later mapping of theses restorer genes were carried out (Pucher et al. 2018; Thribhuvan 2020) using molecular markers. Theses gene linked markers can serve as tool for diversification of pearl millet parental lines.

6.8.5 Breeding for Climate Change Resilience in Pearl Millet

Pearl millet meets the food and nutritional securities for resource-poor farmers of arid and semi-arid tropics. It is such a hardy crop that other major cereals fail to grow and survive. Usually such areas are affected by low soil moisture stress due to limited level and untimely rainfall, poor soil nutrient status, salinity, high temperatures, crust formation, etc. which badly affects the productivity of the crop and its grain quality. Looming climate changes makes environmental stresses even more prominent (Porfirio et al. 2018). Since, each environmental stress mentioned below have different genetic basis, proper breeding strategies need to encompass to address them.

6.8.5.1 Breeding for Drought Resistance

Drought is the most serious abiotic stress limiting the pearl millet productivity. Even though pearl millet has low water requirement of 350 mm when compared to other main cereal crops, rice (1250 mm) and wheat (450 mm) (Ullah et al. 2017), low soil moisture at seedling stage can result in poor crop establishment and at reproductive stage can results in spikelet sterility leading to reduction in grain number and grain size reduction due to reduction in grain filling period which ultimately reflects in reduced grain and fodder yield (Meena et al. 2021; Gupta et al. 2015). Studies on drought tolerance are very complicated because of uncertain nature of duration and intensity of dry spells and due to control under quantitative traits (Shivhare and Lata 2017). Hence, devising the screening methods for breeding for drought should be simple and reproducible for the target environmental conditions. Several drought screening methods such as screening germplasm using in vitro techniques with PEG treatment (Govindaraj et al. 2010), split plot technique/pot experiment in which a set of lines were given ample irrigation and withholding irrigation in another set containing same genotypes (Kholová et al. 2016; Choudhary et al. 2020), use of lysimeter (Vadez et al. 2013) and LeasyScan facility (Chaudhary 2020), employing rainout shelters (Ausiku et al. 2020) and multilocation screening of germplasms and breeding lines across drought-prone areas (Varshney et al. 2017). Various indicator traits are identified which are associated which explains the drought resilience in pearl millet such as high seedling growth and rapid canopy development (Vadez et al. 2015), early flowering (Bidinger et al. 1987), low threshing percentage and panicle harvest index (Bidinger 2002), low transpiration rate at high atmospheric evaporative demand (Kholova et al. 2010; Choudhary et al. 2020), involvement of specific aquaporins (Tharanya et al. 2018), high level of leaf abscisic acids and proline content (Vadez et al. 2015; Jaiswal et al. 2018), etc. Researchers could identify various genotypes and germplasms could serve as donor for imparting drought tolerance in pearl millet. The most studied and exploited one is PRLT 2/89–33 (Serraj et al. 2005) harbouring DT-QTL at LG-2 regulating the ionic uptake Na^+ by roots. Others were CZP 9802, 863B, ICMP 83720, ICMV 9413, and ICMV 94472 (Shivhare and Lata 2017) which serves as promising genotypes for breeding for drought tolerance in pearl millet.

6.8.5.2 Breeding for Seedling and Terminal Thermotolerance

Optimum temperature required by pearl millet for its germination, seedling emergence and for photosynthesis is ~35°C (Garcia-huidobro et al. 1982) which indicates its inherent capacity to survive in hot regions of Sahel and Thar deserts when compared to other staple food crops. But high soil temperatures higher than 45°C lead to heat girdling in pearl millet which apparently block the phloem prevent the channelling of carbohydrate to roots (Peacock et al. 1993). Further, the high temperature of seedbed has been established as a major cause of poor plant stand in pearl millet (Ong 1983). Various studies suggested existence of huge genetic variance for seedling thermotolerance in pearl millet (Sankar et al. 2014; Yadav et al. 2011; Sankar et al. 2021a). Field screening techniques for heat tolerance include the

seedling thermo-tolerance index (STI) (Peacock et al. 1993) and seed-to-seedling thermo-tolerance index (SSTI) (Yadav et al. 2011, 2013).

Howarth et al. (1997) developed laboratory-based screening methodology based on membrane stability index (MSI) for identification of thermo-tolerant genotypes amongst large number of germplasm and breeding lines. Accessions IP 3201 (Howarth et al. 1997), inbreds H77/833–2, 99HS-18, CVJ-2-5-3-1-3, 96AC-93, Togo-II, G73-107, and (77/371 × BSECT CP-1) (Yadav et al. 2014), MS 841B (James et al. 2015; Maibam et al. 2020), and WGI 126, TT-1 (Sankar et al. 2021a) are screened as heat tolerant at seedling stage. There is growing trend in summer cultivation of pearl millet in regions of Gujarat, Rajasthan, and Uttar Pradesh where high temperatures (>42°C) are of common occurrence during flowering. As similar to seedling thermotolerance, variation for supra-optimal temperature tolerance was observed while screening lines at reproductive stage. Genotypes such as ICMB 92777, ICMB 05666, ICMB 02333, IP 19877 showed less spikelet sterility (Gupta et al. 2015) and PI526279 and PI307704 showed higher pollen germination under high temperature stress given under growth chamber (Djanaguiraman et al. 2018). A few hybrids from some of the seed companies (e.g. 86M64 and Proagro 9444) were found to have good yield under summer cultivation in India.

6.8.5.3 Breeding for Salinity Tolerance

Soil salinity as well as irrigation with brackish water affects large areas of West Asia and North Africa (WANA) zones and in India, especially states of Rajasthan, Haryana, Punjab, and UP. It can cause around 33% reduction in grain yield. As compared to other cereal crops, only limited information is available on response to soil salinity in pearl millet. Reduced shoot N content and increased K^+ and Na^+ content is usually associated with salinity tolerance in pearl millet (Dwivedi et al. 2012). According to Krishnamurthy et al. (2007), shoot-biomass ratio associated with salt tolerance and shoot Na^+ concentration could be used as potential selection criteria for screening of pearl millet germplasm at vegetative stage.

Accessions such as 93,613, KAT/PM-2, Kitui, Kitui local, 93,612; 10,876, 10,878, 18,406, 18,570; IP 3757, 3732; Birjand pearl millet; IP 6112; IP 3616, 6104, 6112; ZZ ecotype found to be tolerant to salinity (Pattanashetti et al. 2016). Raipuria (2012) conducted pot experiments with 21 genotypes of pearl millet, in which 10 seeds of each genotype were planted in pots 12 cm high and 8 cm diameter (12 × 8 cm). The pots were irrigated at with varying NaCl solutions levels (50, 100, 150, and 200 mM) on alternate days. Observations were taken for shoot length, germination percentage, root length, root shoot length ratio, fresh and dry root and shoot weight, root shoot dry weight ratio, and seedling vigour index (Fig. 6.5a, b). Of the 21 genotypes studied, genotypes D 23 and DPR 18 exhibited salinity tolerance with good seedling vigour index and other seedling parameters tested (Fig. 6.5c, d). Pearl millet variety 'HASHAKI 1' is a good forage variety and has been released in 2012 for saline areas like Uzbekistan (Fig. 6.5e).

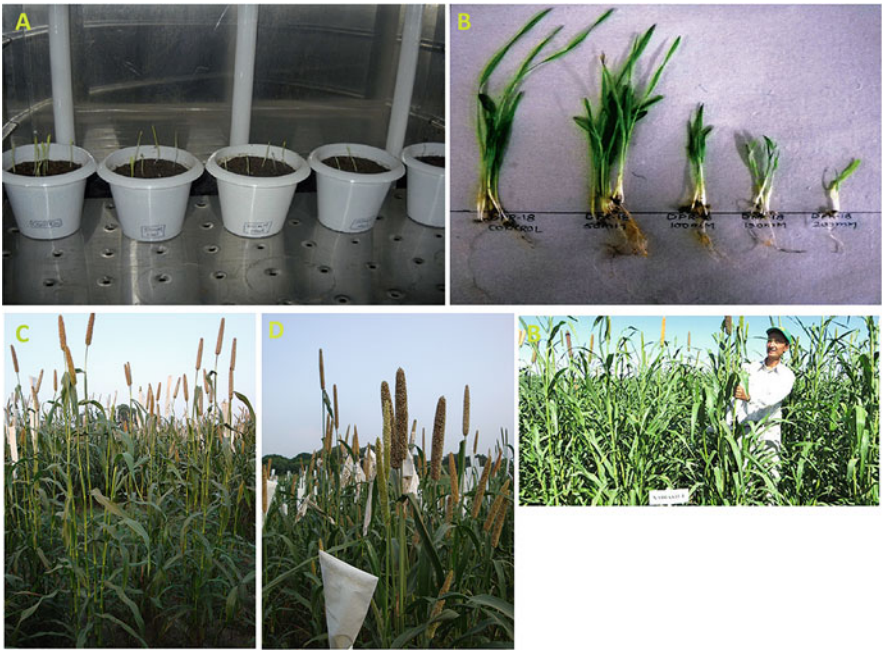

Fig. 6.5 Salinity stress tolerance studies in Pearl millet (**a**) Screening of pearl millet at different level of NaCl induced salinity under controlled conditions (**b**) Performance of salinity tolerant pearl millet genotype DPR-18 seedlings at different concentrations of NaCl. (**c**) Field view of DPR-18 (**d**) Field view of D-23 (**e**) Field view of "HASHAKI 1". (Photo source: Kristina Toderich)

6.8.5.4 Breeding Pearl Millet for Better Nutrient Use Efficiency

Semi-arid and arid regions are characterized by poor soil conditions with either limiting plant nutrients or present in unavailable form. Application of excess fertilizer to a nutrient irresponsive genotype makes economic loss to farmers as well as lead to various environmental concerns (Móring et al. 2021). Moreover, the sources of fertilizers especially phosphorus and potassium are under depletion (Manschadi et al. 2014). Hence, breeding effort needs to concentrate in research and development towards highly yielding and nutrient-efficient genotype. Nutrient use efficiency (NUE) is defined as the ability to produce economic yield per unit soil nutrients applied. NUE mainly depends on the results of two main processes, such as uptake efficiency and utilization efficiency. The understanding of grain yield response and its associated NUE traits performance is still largely lagging in the pearl millet diversity panel. However, few studies have explored genotypic variations for NUE at different levels of nutrients. In one such report, 20 diverse pearl millet genotypes and few high-yielding hybrids were screened under field conditions at two Nitrogen levels (Alagarswamy and Bidinger 1987). Recently, ICRISAT screened the germplasm panel to identify the genotypes and QTLs associated with higher NUE, could identify 25 Nitrogen-insensitive genotypes. Amongst which, Genotypes IP 10820,

IP 17720, ICMB 01222-P1, IP 10379, ICMB 89111-P_2, IP 8069, ICMB 90111-P_2, ICMV-IS 89305, and ICMV 221 proved to be the most efficient genotypes in terms of grain yield at low and high N_2 levels, and indeed shows their inherent genotypic plasticity toward N_2 application (Pujarula et al. 2021). Similarly, strategic research at ICRISAT in the Western and Central African region has been initiated to identify QTLs for enhanced phosphorous efficiency and to examine the stability of their expression across the genetic backgrounds and the environments (Gemenet et al. 2015, 2016).

6.8.6 Addressing Emerging Biotic Constraints

In comparison to other major cereals, pearl millet is a very hardy crop with few diseases and insect pests. The diseases that affect pearl millet, on the other hand, are capable of wreaking havoc. Amongst 111 diseases reported on pearl millet in India and Africa, downy mildew, foliar blast, rust, smut, ergot, and damage due to Striga are important. Downy mildew is important in India and to some extent in western Africa, and Striga is important in western Africa. Being a crop grown by resource-constrained farmers, diseases and pests can be best managed through host plant resistance (HPR).

6.8.6.1 Downy Mildew

Downy mildew or 'green ear' caused by *Sclerospora graminicola* (Sacc. Schroet.) is one of the most devastating disease of pearl millet causing maximum yield loss in India and Africa (Kumar et al. 2013). Downy mildew attacks plants results in general stunted and often undergo a transformation of flower organs into leaves (phyllody or witches' broom), and their effects may range from mild symptoms to catastrophes when large fields have been destroyed (Fig. 6.7a). The disease was first reported in India and was considered of minor importance till 1970 when HB3, a popular Indian pearl millet hybrid suffered severe yield loss from approximately 8.2 million metric tons in 1970–1971 to 4.6 million metric tons in 1971–1972 due to downy mildew epidemic (Dwivedi et al. 2012; Kumar et al. 2013). There is clear evidence that the A_1 cytoplasm is not associated with susceptibility or resistance (Kumar et al. 1983). There is ample evidence for nuclear genes controlling resistance to this disease. Except in one case, where resistance was reported to be recessive (Singh et al. 1978), reports generally confirm that resistance is inherited as a dominant trait and variation in segregating populations is continuous (Singh 1995). In a few cases where clear Mendelian segregations has been observed, the number of dominant genes governing resistance has been one or two (Deswal and Govila 1994). Quantitative inheritance studies have been more successful, and non-additive gene action is responsible for much of the heritable variability (Tyagi and Singh 1989; Deswal and Govila 1994; Kataria et al. 1994). Such non-additive gene action can contribute substantially to general combining ability (GCA), since parents having dominant resistance can be expected to have high GCA for this trait when compared to more susceptible parents.

Due to concentrated effort done by ICRISAT towards diversification of nuclear background with periodic monitoring of advanced breeding progenies with the help of glass house and field sick plot methods, finally derived B-lines and R-lines resistant to this disease (Rai et al. 2014). Several putative QTL have been identified that determine a significant proportion of DM resistance in pearl millet (Jones et al. 1995; Breese et al. 2002; Jones et al. 2002; Satyavathi et al. 2016; Chelpuri et al. 2019). Integrated conventional and marker-assisted back-crossing was taken up to introgress DM resistance QTL from sources IP 18293 and P 1449-2 into the genetic backgrounds of maintainer lines 81B, 843B, and PT 732B advanced by a generation following screens against highly virulent pathogen isolates from Patancheru and New Delhi. Resistance alleles at two DM QTL, one each on linkage groups 1 (LG1) and 4 (LG4) were added to the male parent (H77/833-2) of widely grown hybrid HHB 67 through marker-assisted back crossing from the resistance donor ICMP 451, and a DM resistant version was released as 'HHB 67 improved' (Yadav and Rai 2013).

6.8.6.2 Foliar Blast

Foliar blast caused due to *Magnaporthe grisea* took over as the major and top priority disease in case of pearl millet in India (Sankar et al. 2021b). It is widespread in different pearl millet-growing ecologies of India but became a very serious threat in both A_1 and A zones, where early- to medium-maturing cultivars are preferred (AICRP-PM 2020). The disease starts out as a small speck or lesion that grows larger and necrotic, causing widespread chlorosis and premature death of young leaves (Fig. 6.6). Lesions can appear as diamond-shaped white to gray or reddish-brown lesions near the leaf tips or margins, or both with reddish to brown borders that extend down and may enlarge, coalesce, and kill entire leaves (Nayaka 2021). Inheritance studies suggest the foliar blast resistance is governed by single dominant gene in *P glaucum* (Gupta et al. 2012; Mallik et al. 2021). But wild accession of *P. glaucum* subsp. *monodii* is reported to have three independent dominant resistance genes (Hanna and Wells 1989), and four landraces from Burkina Faso each had independent dominant resistance genes (Wilson et al. 1989). Sharma et al. (2020a) identified 17 accessions belonging to *P. violaceum* (Lam.) Rich found as highly resistant to foliar blast. Amongst two blast resistant accessions, namely IP 21544 and IP 21720, and four cultivated pearl millet genotypes [one germplasm line (IP 22269), one forage variety (ICMV 05555), and two hybrid parents (ICMB 94555 and ICMB 97111)], four advanced backcross populations were developed for multi-institutional evaluation of blast-resistant breeding lines (Sharma et al. 2020b).

6.8.6.3 Breeding for Striga Resistance

Striga spp., *S. hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze, known as witch weeds, are one of the most troublesome and damaging weeds affecting pearl millet in western and central Africa (Parker 2009). About 40% of the cereal producing area is severely infested with Striga and grain yield losses can go up to 100% in sub-Saharan Africa (Kountche et al. 2013). Similar to other biotic constraints, breeding for striga-tolerant genotypes is most economic and central

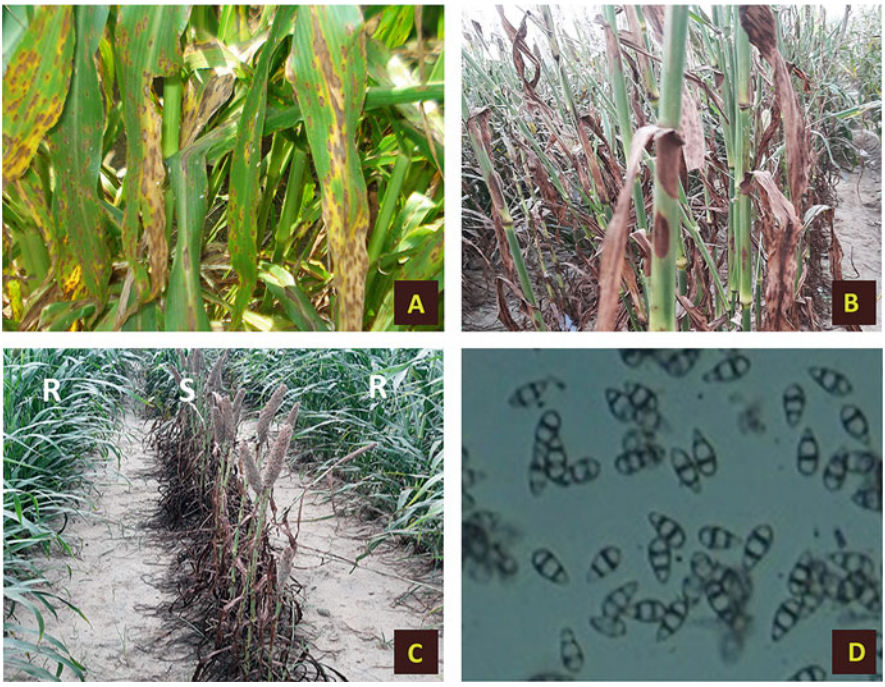

Fig. 6.6 Symptoms of foliar blast on infected pearl millet plants. (**a**) Infected leaves. (**b**) Sheath and stem infection. (**c**) Infected plants along with resistant lines. (**d**) Conidia of Magnaporthe grisea. (Photo source: Sankar et al. 2021b)

approaches in their management. Only vague information on inheritance of striga tolerance in pearl millet is existing and mostly suggests the partial dominance nature (Wilson et al. 2000, 2004). Since 2006, the ICRISAT in Niger, in partnership with the national agricultural research programme in Mali (Institut d'Economie Rurale, IER), has conducted recurrent selection in a diversified pearl millet genepool to increase the frequency of desirable alleles for Striga and downy mildew resistance and panicle yield (Kountche et al. 2013) thereby considerable improvement in the striga resistance in landraces such as M141, M239, M029, M197, M017, and KBH were made possible. Moumouni et al. (2015) constructed genetic linkage map of SNP markers together with SSR markers using a segregating population derived from a cross between a wild relative, resistant to Striga (Wilson et al. 2004), and a cultivated-susceptible pearl millet parent and the underlying QTLs were identified. This material will serve as excellent breeding source for the development of Striga-resistant genotypes through marker-assisted selection.

6.8.6.4 Other Minor Biotic Constraints

Other biotic constraints have received relatively low priority in breeding programmes targeted at the semi-arid tropical regions of Asia and Africa. These can be grouped into two categories. The first category includes ergot (*Claviceps*

fusiformis Lov) and smut (*Moesziomyces penicillariae* [Bref.] Vánky), diseases of pearl millet; for these, good resistance sources and effective screening techniques have been developed (Thakur et al. 2011). However, these have been shown not to have as large an impact on yield, on as large a geographical scale. Also, some of these can, to some extent, be managed by other means, for instance, by breeding for improved male fertility restoration in hybrids and by resorting to alternative cultivar options (e.g. OPVs, top-cross hybrids, and protogyny-based single-cross hybrids wherein seed is produced using protogynous flowering rather than by male-sterile seed parents) can be effective in reducing ergot and smut severity in pearl millet). Simply inherited recessive 'tr' allele, which conditions trichomelessness to most aboveground parts, including stigmas, confers a useful degree of smut resistance. Rust of pearl millet is of minor importance as it generally occurs after the grain-filling stage. Rust resistance has been demonstrated to be under the control of a single dominant gene (Andrews et al. 1985). One major QTL present on LG1 explaining 58% of total phenotypic variance has been identified for rust resistance but their deployment in the parental lines is yet to begin (Ambawat et al. 2016).

The second category includes stem borers of pearl millet (*Coniesta ignefusalis* Hampson); and head miner (*Heliocheilus albipunctella* de Joannis) for which confirmed sources of good resistance are not available and (or) the trait inheritance is too complex to permit its effective utilisation in breeding. This category has received little attention in breeding programmes in Asia and Africa. The details of biotic stress resistant pearl millet genotypes are given in Table 6.5.

6.8.7 Development of Genetic and Genomic Resources in Pearl Millet

Genetic and genomic resources and tools will be the raw materials for futuristic pearl millet improvement approaches towards mitigating the crisis of ever-increasing food and fodder yield, nutritional security, and sustainable crop production. Some of such genetic and genomic resources and tools are briefly discussed relating to its development and utilization in understanding pearl millet genetics and their use in pearl millet improvement.

6.8.7.1 Markers, Mapping Population, and Genetic Linkage Maps

Genomic resources such as DNA-based molecular markers, genetic linkage maps, and sequence information on gene are a prerequisite to conduct any genetic studies or marker-aided breeding programme in any crop. The first key milestone was laid with the use of RFLP as molecular tool for the development of a linkage map of pearl millet (Liu et al. 1994). In subsequent years, AFLP, RAPD, expressed sequence tag-based (EST) markers, sequence-tagged sites (STSs), simple sequence repeat (SSRs/microsatellites), DArTs, CISP, SSCP-SNP, and NGS-based SNP genotyping have been developed to distinguish genetic variability, linkage map analysis, and marker-assisted screening to expedite the breeding programmes (Devos et al. 1995; Allouis et al. 2001; Gale et al. 2001; vom Brocke et al. 2003; Qi et al. 2004; Bertin

Table 6.5 Biotic stress-resistant genotypes available in pearl millet

Biotic agents	Resistant genotypes	References
Downy mildew (*Sclerospora graminicola*)	ICML 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ICMPE 13-6-30, 134-6-9, 134-6-34, 13-6-27, 37, 71; ICMA 92666, ICMB 92666, ICMA 91333, 91444, 91555 (resistant to downy mildew, smut and ergot), WGI 52, WGI 148, ICMR 09999, ICMB 89111-P6 × ICMB 90111-P6	Thakur et al. (1982, 1992), Willingale et al. (1986), Thakur and King (1988a, b), Rai et al. (1998a), Khairwal and Yadav (2005), Satyavathi et al. (2016) and Chelpuri et al. (2019)
Blast (*Magnaporthe grisea*)	IP 7846, IP 11036, IP 21187, IP 4291, IP 15256, IP 22449, IP 5964, IP 11010, IP 13636, IP 20577, IP 5964, IP 11010, IP 13636, IP 21525, IP 21531, IP 21536, IP 21540, IP 21594, IP 21610, 21,640, IP 21706, IP 21711, IP 21716, IP 21719, IP 21720, IP 21721, IP 21724, IP 21987, IP 21988, IP 22160, IP 21544, IP 21720, IP 22269, IP 21544, IP 11353, IP 22423, IP 7941	Sharma et al. (2013, 2020a, b) and Sankar et al. (2021b)
Rust (*Puccinia* spp.)	IP 16438, IP16762; P310-17, P1449-3; IP18292, IP18293, IP700651; ICML 12 to 16, 22; ICMP 312, 423, 85410; 7042S; 841A; IP 9, 55, 104, 262, 253, 346, 336, 498, 545, 558; landraces like Desi Bajri-Chomu, Dhodsar local, Ardi-Beniya Ka Bas, 81B-P6, ICMP 451-P8, IP 21629, 21645, 21658, 21660, 21662, 21711, 21974, 21975, and 22038	Singh et al. (1997), Khairwal and Yadav (2005), Thakur et al. (2006), Sharma et al. (2007, 2020b) and Ambawat et al. (2016)
Smut (*Moesziomyces penicillariae*)	ICML 5 to 10; ICML 17 to 21; Tif leaf 3; Tift 3 (PI 547035) and Tift 4 (PI 547036); Tift 65 (resistant to leaf spot and rust)	Bourland (1987), Thakur and King (1988a), Wilson and Burton (1991), Burton and Wilson (1995) and Hanna et al. (1997)
Ergot (*Claviceps fusiformis*	ExB 46-1-2-S-2, ExB 112-1-S-1-1, ICMV 8282, 8283; ICMA 88006A and 88006B (resistant to downy mildew and smut); ICMA 91333 to 91555; ICML 5 to 10; SSC FS 252-S-4, ICMPS 100-5-1, 700-1-5-4, 900-1-4-1, 900-3-1, 900-9-3, 1300-2-1-2, 1400-1-6-2, 1500-7-3-2, 1600-2-4, 1800-3-1-2, 2000-5-2; ICI 7517-S-1, ExB 132-2-S-5-2-DM-1, P-489-S-3; SSC 46-2-2-1, SC 77-7-2-3-1, SSC 18-7-3-1	Thakur and King (1988c), Yadav and Duhan (1996), Rai et al. (1998b) and Khairwal and Yadav (2005)

et al. 2005; Senthilvel et al. 2008; Supriya et al. 2011; Sehgal et al. 2012; Rajaram et al. 2013; Srivastava et al. 2020a, b; Singhal et al. 2021). Currently, application of NGS-based sequencing platform such as GBS, RAD-seq, etc. are gaining popularity

for its rapid, high density genome coverage and low-cost marker development, and genotyping platform (Shivhare et al. 2020a, b). These genetic tools have also been used for diversity assessment (Liu et al. 1992; Bhattacharjee et al. 2002), studying recombination rates (Busso et al. 1995; Liu et al. 1996), analysing domestication syndrome (Poncet et al. 2000, 2002), and comparative genetics (Gale and Devos 1998).

Another key genomic resource is mapping population and the molecular linkage maps. A mapping population is a genetic tool used to develop genetic maps, which can be used later for gene/QTL mapping studies. Different types of mapping populations may be used for linkage map development and QTL mapping, each population type having advantages and disadvantages (Singh and Singh 2015). The first linkage map based on RFLP marker used an inter-varietal F_2 population (Liu et al. 1994). The first integrated map was constructed using four F_2 populations developed from LGD × ICMP 85410, 81B × ICMP 451, ICMB 841 × 863B, and PT 732B × P1449-2 crosses (Qi et al. 2004). The F_2 population of the ICMB 841-P3 × 863B-P_2 cross was also used to integrate a newly developed SSR marker in previous maps (Senthilvel et al. 2008).

Subsequently, RILs were used for genetic linkage mapping of pearl millet genome. RILs developed from Tift23DB x PI536400 cross (Pedraza-Garcia et al. 2010), H 77/833-2 × PRLT 2/89-33 (Supriya et al. 2011), H77/833-2 × PRLT 2/89-33 (Sehgal et al. 2012) crosses were used for genetic linkage mapping. Four RILs populations developed from ICMB 841-P3 × 863B-P2, H 77/833-2 × PRLT 2/89-33, 81B-P6 × ICMP 451-P8, and PT 732B-P2 × P1449-2-P1 were used to construct a consensus linkage map (Rajaram et al. 2013). An F_2 population of a cross between wild pearl millet (116_11-(PS202-14)-121) and a cultivated pearl millet (SOSAT-IBL-197) was also used to map high density map (Moumouni et al. 2015). Two downy mildew QTLs were mapped using genome-wide SSR and AFLP markers while phenotyping a RIL mapping population derived from the parents, WGI 52 and WGI 148, against *S. graminicola* Delhi isolate (*Sg561*). Similarly, the RIL population derived from the parents WGI 148 X ICMR 09999 exhibited four QTLs on LG1, 3 and 5 and two QTLs imparting resistance when screened against the Rajasthan isolate (*Sg384*) and the Gujarat Banaskanta isolate (*Sg445*), respectively (Fig. 6.7b–e; Satyavathi et al. 2016). Punnuri et al. (2016) developed 96-plex ApeKI GBS library from the 186 RILs and from their parents ('Tift $99D_2B_1$' and 'Tift 454') and F_1 population. DNA of these populations was sequenced and the results were used for the development of reference genetic map using 150 RILs that contained a total of 16,650 SNPs and 333,567 sequence tags spread across all seven pearl millet chromosomes.

The final map has a genetic distance of 716.7 cM with 23.23/cM overall average density of SNPs and 1.66 unique linkage bins per cM. This map was further used in mapping QTLs for flowering and resistance to *Pyricularia* leaf spot disease in pearl millet. Kumar et al. (2018b) used another population to construct a genetic linkage map with DArT and SSR markers using 317 RIL progenies derived from the (ICMS 8511-S1-17-2-1-1-B-P03 × AIMP 92901-S1-183-2-2-B-08) cross. Recently, same mapping population was utilized to agronomic traits by Kumar et al. (2021b) on

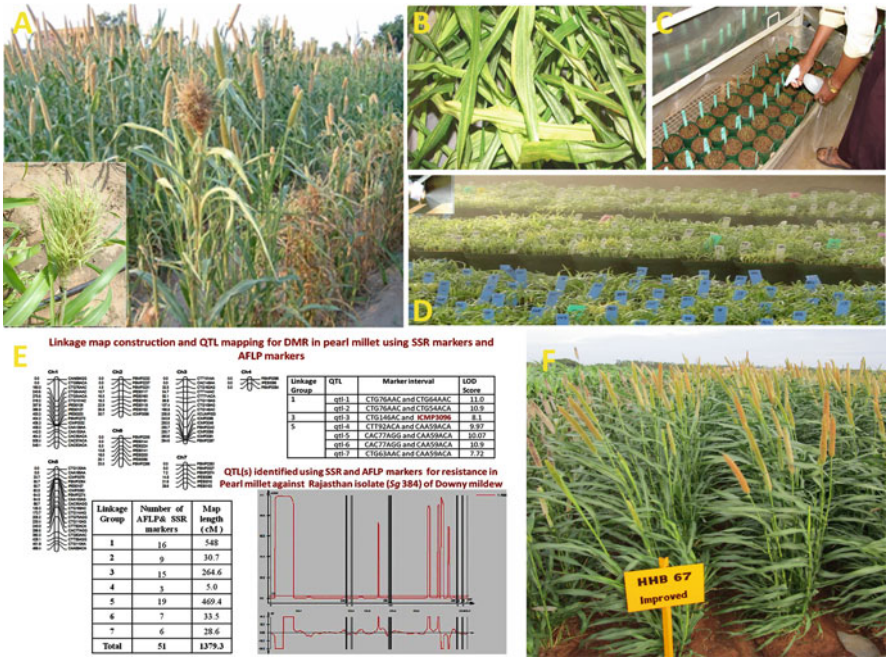

Fig. 6.7 QTL mapping for downy mildew resistance and MAS in pearl millet. (**a**) Symptoms of downy mildew on infected pearl millet plants. (**b**) Sporangia on infected leaves. (**c** and **d**) Screening biparental population for DMR through artificial inoculation. (**e**) Linkage map construction and QTL mapping (Satyavathi et al. 2016) (**f**) Field view of HHB 67 improved at ICRISAT

seven linkage groups. To circumvent the problems of segregation distortion and masking of minor-effect alleles/QTLs, a set of chromosome segment substitution lines (CSSLs) for all the seven LGs was developed by introgression of overlapping chromosome segments from 863B line into the genetic background of elite ICMB 841 line (Kumari et al. 2014). These are valuable genetic stocks for minor-effect QTL detection, fine mapping, and trait mechanism studies, especially for complex traits in pearl millet. The second-generation mapping populations such as nested-association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) populations are derived from multiple elite breeding lines with a combination of useful traits. These populations are useful for precise QTL mapping and for use in cultivar development. However, their development in pearl millet is not yet realized.

6.8.7.2 Mapping of Trait for Marker-Assisted Breeding

The molecular markers and linkage maps discussed above have been used to identify and map QTLs for terminal drought (Yadav et al. 2002), reduced salt uptake (Sharma et al. 2011, 2014), grain and stover yield (Yadav et al. 2002, 2003, 2004), and for downy mildew resistance (Jones et al. 1995, 2002; Breese et al. 2002; Gulia et al.

2007; Satyavathi et al. 2016), rust and blast resistance (Morgan et al. 1998, Punnuri et al. 2016), fertility restorer genes (Yadav 2005; Pucher et al. 2018;), grain micronutrient enrichment (Kumar et al. 2018b; Singhal et al. 2021). However, linkage analysis in most of these studies suggest presence of genes/QTLs at a distance of 10–40 cM from the closest markers making it difficult for MABB or functional validation of candidate genes (Dwivedi et al. 2012). Until now only a few reports showing that either fine mapping or validation of candidate genes associated with a particular trait have been identified in this crop-like drought tolerance QTL on LG2 (Sehgal et al. 2012), validation of grain Fe-Zn candidate genes, etc. (Mahendrakar et al. 2020; Singhal et al. 2021).

Molecular markers flanking identified QTLs and genes have successfully been deployed in MABC to further improve 'HHB 67 Improved' and GHB 538 hybrids from India. In fact, the first molecular marker-assisted cultivar released for commercial cultivation belongs to pearl millet hybrid 'HHB-67 Improved' (Hash et al. 2006). HHB 67 pearl millet hybrid was released in 1990 and soon became a farmer-preferred hybrid owing to its extra-early maturity that helped the plant escape end season drought. However, this hybrid became susceptible as the incidence of DM increased in western India, warranting improvement. The parental lines of HHB 67, namely ICMB 843 and H77/833-2, was improved for their DM resistance in which the former through conventional backcross breeding and latter through marker-assisted backcross breeding and the new reconstituted hybrid, with DM resistance, was released in 2005 in the name 'HHB 67 improved' (Fig. 6.7f).

Researchers at ICRISAT partnered with Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) and ICAR-All India Coordinated Research Project on Pearl Millet (AICRP-PM) for the second cycle improvement of HHB 67 Improved hybrid. Using genome-wide simple sequence repeat (SSR) DNA markers, three DM resistance QTLs were stacked along with drought resistance QTLs at LG-2. The latest improvement has been christened HHB 67 Improved 2–7 (meaning HHB 67 Improved second cycle improvement, seventh version). It completed testing in Essentially Derived Variety (EDV) multi-location, multiyear trials of AICRP-PM in A_1 and A (dry) zones of India. The new version hybrid shown to have around 58% improvement over downy mildew resistance and 11% over blast incidence, keeping the maturity earliness at par with earlier version HHB-67 improved (Srivastava, personal communication). Based on yield superiority of 15% in grain yield and 21% in fodder yield, with very low DM incidence (% disease incidence = 2.6%) in EDV trials, the HHB 67 Improved 2–7 (MH 2545) was identified for release for cultivation in Rajasthan. Haryana, and Gujarat under rainfed conditions in *Kharif* season (VIC proceedings on Pearl millet, AICRP-PM 2021).

6.8.7.3 Draft Pearl Millet Genome Sequence

With the effort of a global participation of various international institutes, a draft pearl millet reference genome sequence made available to public which serves as a valuable genomic resource for pearl millet improvement (Varshney et al. 2017). A global reference genotype, Tift $23D_2B_1$-P_1-P_5 has been chosen to develop its draft

genome sequence through whole genome shotgun and Bacterial artificial chromosome (BAC) sequence approaches. The genome size is roughly 1.79 Gbp and has around 38,579 genes. Along the draft reference genome, a set of 994 germplasm lines including its wild genotypes were re-sequenced and were also made available to public which harbours around three million SNPs. The genome sequence and resequencing data will facilitate the successful marker–trait association studies, defining heterotic pools, and predicting hybrid performance in future programmes. The data can be accessed through following weblink, http://cegsb.icrisat.org/ipmgsc/ and http://gigadb.org/dataset/100192.

While addressing any biotic constraints, the availability of genome sequence information of pathogen will complement the understanding on host–pathogen interaction. It provides a way and means for biotechnological manipulation of host (through gene editing via CRISPR-Cas) for their successful management. In this direction, whole genome sequencing was successfully conducted in two major pathogens in pearl millet, namely *Sclerospora graminicola* (causing downy mildew) and *Magnaporthe grisea* (causing foliar blast). The draft genome sequence of *S. graminicola* pathotype 1 had 299,901,251 bp length and was assembled having N of 17,909 bp with minimum of 1 kb scaffold size and overall coverage of 40× (Nayaka et al. 2017; Chakravartty et al. 2017). Draft genome sequence of *Sclerospora graminicola*, the pearl millet downy mildew pathogen. It had 47.2% GC content with 26,786 scaffolds and scaffold size of 238,843 bp was found to be longest amongst these. The genomic sequence and other related data of *S. graminicola* can be retrieved from NCBI through link given, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA325098/. Similarly, Prakash et al. (2019) sequenced genome of *Magnaporthe grisea* strain PMg_Dl and generated 13.1 Gb PE reads (number of reads, 43,962,401), 3.4 Gb mate-paired reads (number of reads, 17,160,010), and 1.1 Gb PacBio reads (number of reads, 148,768). *M. grisea* strain *PMg_Dl* genome sequence and relevant information can be found at NCBI through following link, https://www.ncbi.nlm.nih.gov/bioproject/PRJNA494483/. These genomic resources would potentially benefit in deciphering the pathogen's evolutionary pattern and effector evolution in order to develop successful durable resistance breeding programme in pearl millet.

6.8.7.4 Transcriptomics, Proteomic Approaches, and Genomic Database of Pearl Millet

Transcriptome database is available for large number of crops but no such database has been developed for orphan crop like pearl millet to be used as genomic resources in crop improvement research (Kumar et al. 2020b). Few researchers have attempted to unravel the molecular basis of drought, salinity, and heat tolerance in pearl millet using whole transcriptome approach (Jaiswal et al. 2018; Dudhate et al. 2018; Shivhare et al. 2020a, b; Shinde et al. 2018; Sun et al. 2020; Maibam et al. 2020). Mapping of sequenced reads against the foxtail millet genome, which has been relatively well-annotated, led to the identification of several differentially expressed genes under drought stress. Pathway and gene function analysis by KEGG online tool revealed that the drought response in pearl millet is mainly regulated by

pathways related to photosynthesis, plant hormone signal transduction, and mitogen-activated protein kinase signalling. They also identified 34,652 putative markers (4192 simple sequence repeats, 12,111 SNPs and 6249 InDels). Transcriptome has been used in the area of pearl millet for the development of first web-based genomic resource (http://webtom.cabgrid.res.in/pmdtdb/) which can be used for candidate genes-based SNP discovery programmes and trait-based association studies.

Recently, using transcriptomic studies, the key candidate genes, viz. *PglZIP*, *PglNRAMP, PglYSL*, and *PglFER* family genes were candidates for grain Fe content and grain Zn content were discovered for grain Fe and Zn metabolism in pearl millet (Mahendrakar et al. 2020). Ferritin-like gene (*PglFER-1*) was found to be the strongest most potent candidate gene for grain Fe Content. Expressed genes were correlated with major QTL co-localized on LG7 for grain Fe content and grain Zn content (Mahendrakar et al. 2020). Also, in order to dissect the molecular basis of flour rancidity, denovo transcriptome sequencing was carried out in contrasting genotypes of pearl millet to identify involvement of lipase towards hydrolysis of unsaturated fat present in pearl millet flour (Kumar et al. 2021a). The study provided a useful understanding of different Fe and Zn metabolism gene homologs and laid a foundation for functional dissection. Also, the role of small RNA towards abiotic stress was investigated by Shinde et al. 2020 and Kumar et al. 2018a.

Detailed studies of pearl millet with respect to whole genome proteomics is much limited than that of transcriptomics. A few literatures related to whole proteomics is available related to identification of key genes involved in drought tolerance (Anatala et al. 2015; Ghatak et al. 2021), downy mildew resistance (Anup et al. 2015), and grain quality (Bugs et al. 2004; Marcellino et al. 2002). Analysis of pearl millet lines using SDS-PAGE for the storage protein pattern profiling in popular pearl millet showed the appearance of most of the band in the range of 25–65 KDa. The alcohol soluble fraction of the storage protein was also separated on SDS-PAGE and observed very intact and prominent bands of prolamins in all the fractions of different genotypes of pearl millet.

Biophysical methods and structural modelling techniques have been used to characterize the prolamins from maize and pearl millet. The alcohol-soluble prolamin from pearl millet was extracted using a simple protocol and purified by gel filtration in a 70% ethanol solution. Two protein fractions were purified from seed extracts of pearl millet with molecular weights of 25.5 and 7 kDa, as estimated by SDS-PAGE. The high molecular weight protein corresponds to pennisetin, which has a high a-helical content both in solution and the solid state, as demonstrated by circular dichroism and Fourier transform infrared spectra. Fluorescence spectroscopy of both fractions indicated changes in the tryptophan microenvironments with increasing water content of the buffer. Low-resolution envelopes of both fractions were retrieved by ab initio procedures from small-angle X-ray scattering data, which yielded maximum molecular dimensions of about 14 nm and 1 nm for pennisetin and the low molecular weight protein, respectively, and similar values were observed by dynamic light scattering experiments (Kumar et al. 2020b).

6.8.8 Increasing Nutritional Value Through Biofortification

Biofortication of pearl millet with iron (Fe) and zinc (Zn) initially by ICRISAT supported by the HarvestPlus conducted in partnership with public and private sector research organizations, has become a role model to be adopted for the improvement of the nutritional profile in other staple food crops. Biofortification Priority Index (BPI) given by IFPRI indicates pearl millet is a major target crop for iron and zinc biofortification (Herrington et al. 2019). Screening the existing germplasms for Fe and Zn indicated a huge variability for Fe (31–125 ppm) and Zn (35–82 ppm) content whereas not a single popular cultivar is having grain Fe content more than HarvestPlus baseline of 77 ppm to be considered as High Fe Pearl millet cultivars. Hence, there was a good prospect of developing cultivars with higher levels of these micronutrients. In pearl millet biofortification programme, the initial strategy was to select and improve the existing OPVs to improve yield and grain micronutrient simultaneously through recurrent selections as the grain Fe and Zn content in pearl millet is under additive gene action. Most of the high Fe and Zn accessions were from Togo and Ghana that had Fe content of 95–121 ppm and Zn content of 59–87 ppm indicating *iniadi* germplasm as a valuable germplasm resource for genetic improvement of Fe and Zn contents in pearl millet.

First systematic breeding effort to develop a high Fe cultivar resulted in a world first high-Fe pearl millet variety 'Dhanashakti' was developed by utilizing the intra-population variability within ICTP 8203, an early-maturing, large-seeded, disease-resistant and high-yielding open-pollinated variety, was released in 2014 as Dhanashakti, which has been rapidly adopted by the farmers. The improved version of variety ICTP 8203, having an Fe content of 71 mg/kg without any change in Zn content. Likewise, variety ICMV 221Fe11-2, a better version of variety ICMV 221, has been developed with high Fe (81 mg/kg) and Zn (51 mg/kg) content. Simultaneously, ICRISAT had utilized the existing high-Fe hybrid parents in development of high-yielding and high-Fe hybrids which resulted in two hybrids, namely ICMH 1201 and ICMH 1301 with Fe content of 75 and 77 mg/kg, respectively. These hybrids had a yield advantage of 36% and 33% over variety ICTP 8203, respectively (ICRISAT, India). Later with the effort of AICRP partners, biofortified pearl millet hybrid HHB 299 was developed by CCSHAU, Hisar with an Fe content of 73 ppm and average grain yield of 39.5 q/ha, which was notified in 2018 (AICRP-PM 2020). Also, five biofortified hybrids, namely AHB 1200Fe, RHB 233, RHB 234, HHB 311, and AHB 1269, have been released during 2018–2020. These biofortified cultivars mark the first milestone in the mainstreaming of the grain mineral traits in the cultivar development process (Fig. 6.8; Table 6.6). With the great support of partners, especially MPKV-Dhule, AICRP-PM, Mandore and Nirmal seeds Pvt. Ltd., first high-iron variety Dhanashakti reached regular seed systems that annually send 200–300 kg of breeder seed to national partners; so far, they have reached >60,000 farmers in peninsular India. The truthfully labelled seed (TLS) production of Shakti-1201 is being undertaken by Shaktivardhak Seed Company for commercialization, and it has been adopted by 20,000 ha, mostly in Maharashtra and Rajasthan (Govindaraj et al. 2019). The significant progress

Fig. 6.8 Biofortified pearl millet cultivars released by ICAR for cultivation by Indian famers

made by AICPMIP regarding development and release of biofortified cultivars in India (Satyavathi et al. 2021) can be accessed through the link http://www.aicpmip.res.in/Micronutrient%20Pearl%20Millet.pdf

ICAR-AICRP on Pearl millet (AICPMIP) constructed a special module to test and release biofortified pearl millet cultivars in India (AICPMIP 2017). Furthermore, ICAR has endorsed a landmark decision on inclusion of the minimum levels of iron (42 ppm) and zinc (32 ppm) in varietal promotion criteria for future pearl millet

Table 6.6 List and salient features of biofortifed cultivars developed and cultivated in India

Sl. no	Hybrid/ variety	Breeding Station	Notification details	Area of adoption	Salient features	Days to flowering	Days to maturity	Grain yield (kg/ha)	Fodder yield (q/ha)	Grain Fe content (ppm)	Grain zinc content (ppm)
1	Dhanshakti	ICRISAT, India and MPKV, Dhule	S. O 1146 (E) 24.04.2014	Maharashtra, Karnataka, AP, Tamil Nadu, Rajasthan, Haryana, MP, Gujarat, UP and Punjab	Early maturing bold, globular, shining slate grey-coloured seed resistant to downy mildew disease	45	76	2199	53	81	43
2	HHB 299	CCS HAU, Hisar	S. O 1379 (E) 27.03.2018	Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu	Medium maturing, compact panicle greyish hexagonal-shaped grain and resistant to major diseases	50	81	3274	73	73	41
3	AHB 1200	NARP, Aurangabad	S. O 1379 (E) 27.03.2018	Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu, AP, and Telangana	Medium maturing, cylindrical panicle resistant to downy mildew and highly responsive to fertilizers	47	78	3170	70	77	39
4	AHB 1269	NARP, Aurangabad	S. O 1498 (E) 01.04.2019	Rajasthan, Haryana, Gujarat, Punjab, UP, Delhi, Maharashtra, Tamil Nadu, AP, Telangana, and Karnataka	Medium maturing, long cylindrical type panicle, bold grain and resistant to major diseases	50	81	3168	74	91	43

(continued)

Table 6.6 (continued)

Sl. no	Hybrid/ variety	Breeding Station	Notification details	Area of adoption	Salient features	Days to flowering	Days to maturity	Grain yield (kg/ha)	Fodder yield (q/ha)	Grain Fe content (ppm)	Grain zinc content (ppm)
5	HHB 311	CCS HAU, Hisar	S. O 3220 (E) 06.09.2019	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra, and Tamil Nadu	Medium maturing, compact panicle having grey-coloured hexagonal-shaped grain, Highly resistant to downy mildew and other diseases	50	81	3173	72	83	39
6	RHB 233	SKNAU, Jobner	S. O 3220 (E) 06.09.2019	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra, and Tamil Nadu	Medium maturing, grey globular-shaped grain; Highly resistant to blast and downy mildew diseases	49	80	3157	74	83	46
7	RHB 234	SKN AU, Jobner	S. O 3220 (E) 06.09.2019	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra and Tamil Nadu	Medium maturing, conical shaped compact panicle with greyish coloured hexagonal-shaped grain, Highly resistant to downy mildew	49	81	3169	71	84	41

varieties to be released in the country which is the first of its kind in the world. Thus, along with yield improvement, focus on the nutritional improvement was also taken care in pearl millet and in order to develop biofortified varieties/hybrids with enhanced Fe and Zn. With visionary breeding strategies along with proper policy intervention results in much greater progress in adopting high-Fe hybrids with a high-grain yield is seen in pearl millet.

6.8.9 Breeding Pearl millet for Better End-Use Consumer Acceptability

In spite of better nutrition profile the main constraints in utilization of pearl millet as whole or in multigrain food products has been the problem of rapid development of rancidity or bitterness in the flour after milling, poor flour rheological properties, presence of various anti-nutrients like phytate and polyphenol and typical grey colour of the pearl millet. The understanding regarding the genetics related to these traits are only preliminary. Extensive work related to reduction of flour rancidity in pearl millet was carried out by researchers of IARI, New Delhi, and CCS, HAU, Hisar. High-fat content coupled with highly active lipases causes hydrolysis of pearl millet fats to fatty acids. The typical fat content in pearl millet is about 5.1% on a dry weight basis. Pearl millet fat contains 74% unsaturated fatty acids, predominantly linoleic (C18:2) and oleic (C18:1). Lipase enzyme is concentrated in the pericarp, aleurone layer, and germ gets released upon grinding and accounts for the stepwise hydrolysis of these fatty acid fractions resulting in free fatty acids and acyl glycerols (Satyavathi et al. 2017). Rancidity results from the hydrolysis of these free short chain fatty acids and glycerols when exposed to air, light, moisture, or bacterial activity, leading to unpleasant odour and taste. Rancidity reflects the storage ability of pearl millet grain flour and its products affecting its nutrient composition. Sharma and Saharan (2006) reported that the rancidity development in pearl millet flour is caused by higher phenol contents and higher peroxidase activity. Enzymes such as polyphenol oxidases act on the phenolic compounds like C-glycosyl flavones resulting in browning of flour and the activities of peroxidases results in off odour in flour. Based on the degree of rancidity indicators such as comprehensive acid value (CAV), comprehensive peroxidase value (CPV), and activities of lipase and lipoxygenase (LOX), a rancidity matrix was generated using 93 pearl millet genotypes which can further help the pearl millet breeders in designing low rancid pearl millet cultivars (Goswami et al. 2020).

Even though pearl millet is considered as 'powerhouse of nutrients' their bioavailability is low, due to the presence of certain antinutritional factors. These antinutritional components are mainly found in the bran layer. There exists a negative correlation between the presence of antinutrients and in vitro protein digestibility. One of the antinutrients of pearl millet grains is phytate, with a range of 172–327 mg/100 g (Taylor 2004). Phytate binds multivalent metal ions such as calcium and iron, thereby interfering with their absorption in the gut (Lestienne et al. 2007). Several studies have investigated the phytate content of pearl millet grain. A

panel of 145 pearl millet inbred lines analysed for phytic acid phosphorus (PAP) revealed a large genetic diversity PAP content ranged between 0.198 ± 0.034 and 0.410 ± 0.036 g/100 g of seed flour with a relative standard deviation amongst the lines of 12.8% and an average PAP content of 0.281 g/100 g (Boncompagni et al. 2018). Two studies so far analysed the genetics of phytic acid content in pearl millet using diallel cross (Satija and Thukral 1985; Shanmuganathan et al. 2006). They showed highly significant differences amongst parents as well as the hybrids and demonstrated that both additive and non-additive gene effects were significant. It is advisable to follow a population improvement programme using recurrent selection to breed for low phytic acid content.

IARI identified a low phytic acid line, PPMI 1161 (TPR-14-12-4-5-3-B) having low PAP of 0.001 g/100 g of seed flour while screening 68 advance breeding lines and test single cross hybrids for grain Fe/Zn along with PAP (IARI-Annual Report 2020). Polyphenols are yet another factor that limits protein and starch utilization either by binding with proteins or by inhibiting digestive enzymes, especially trypsine and amylase. Polyphenols also form insoluble complexes with iron and cause inhibition of iron absorption (Brune et al. 1991; Cercamondi et al. 2014). Yadav et al. (2010) reported a lower concentration (50.87 mg/100 g) of polyphenol in cultivar HTP 94/54. The variability for grain minerals and its bioavailability traits in this crop opens the opportunity for breeding high bioavailable micronutrient-rich breeding lines and hybrid parents, and thereby high-iron cultivars for improved human nutrition in millet consuming regions.

Most of the hybrids and varieties available in commercial market are either grey or brown grain colour, due to the presence of a thick pericarp and the presence of pigments in the aleurone layer. Akingbala (1991) reported the presence of the phenolic compounds, C-glycosyl flavones concentrated in the outer layers of the grains, and they contribute to the grey grain colour (Taylor 2004). Grey colour in food products made out of pearl millet imparts undesirable consumer acceptance towards them. Pearly white grained genepool amongst pearl millet were first reported by Patel 1939. Later Mangath (1987) developed white grain inbred lines from segregating germplasm lines through selections. But these lines have heavy incidence of downy mildew which further hampered the development of white grain hybrids. Thinh (2011) reported that white grain lines were higher in reducing sugar level which may be the reason for its susceptibility to downy mildew disease. Satyavathi et al. (2015) developed *126* white grain pearl millet inbred lines with downy mildew resistance along good agronomic features that could be utilized for developing white grain hybrids. Lakkawar and Govila (1998) reported inheritance of white grain colour in pearl millet is under the control of single dominant gene. Now efforts were undergoing to map the gene responsible for the trait and to introgress them into elite parental lines.

6.9 Future Research Thrust Areas

6.9.1 Improvement of Grain Yield

The impressive use of hybrid technology in India is evidenced by continuing genetic gains of over 31.1 kg/ha/year in grain yield. Single-cross hybrids will continue to be the only commercial option, given the private sector's increasingly dominant role in hybrid development and seed production. As a result, parental line development with high-yield potential, DM and blast resistance, and regional adaptation should be prioritized. Hybrid development should prioritize better-endowed environments, while strategic research for marginal environments should be prioritized.

6.9.2 Diversification of Parental lines and Utilization of Alternate CMS Systems

Development of heterotic pool and diversification of different heterotic pool is crucial for developing the good heterotic hybrids. Various approaches (pedigree breeding, backcross breeding, mutation breeding, and population approaches) can be effectively employed for diversification of parental lines amongst pool. Also, A_4 and A_5 based CMS resources are found to have better stability and concern-linked markers were known and can be deployed through rapidly through marker-assisted backcrossing in diverse genetic background to exploit their commercial potential.

6.9.3 Exploitation of Pre-breeding Programmes

Past-breeding efforts utilized only the narrow genetic diversity existing in pearl millet found mostly in the cultivated types. Wild and wild relatives are the rich sources of novel traits for pearl millet improvements. Exploitation of wild types and wild relatives should be incorporated along with regular pearl millet breeding programmes to identify new gene sources of biotic and abiotic stresses. Novel breeding and biotechnological approaches like somatic hybridization, embryo rescue, speed breeding, gene editing techniques, etc. can put synergistic effect towards creation of new genetic material for further improvements in this crop.

6.9.4 Quality Trait Focus

Till date, breeding efforts was mainly concentrated on improving grain yield, earliness, and imparting biotic and abiotic resistance. Emphasis should be given for the development of biofortified pearl millet with high consumer acceptance. Success on iron-zinc biofortified hybrids in India can be kept as a role model for improvement of other grain quality traits such as protein content, pro-vitamin A, vitamin E, Niacin, etc. Recent research on improving the bioavailability of nutrients

and improvement of up-keeping quality of pearl millet flour is also impressive. Despite the fact that pearl millet has a high protein content of 11.6%, key amino acids such as lysine and tryptophan are deficient from these proteins. As a result, research into quality protein pearl millet (QPPM) should commence, following the advances made in quality protein maize (QPM). However, incorporation of consumer or end-use preferred traits into elite cultivars need to be emphasized.

6.9.5 Marker-assisted Breeding

Development of molecular markers tightly linked to economic traits is of prime importance in pearl millet. Identification of gene-based markers enable rapid introgression of target traits into parental lines belongs to different heterotic groups. Recent developments in sequencing technology and bigdata analytics provide a way to identify and utilize such trait-linked markers and pyramid them together against multi-disease complexes, abiotic stress, improvement of grain nutritional and up-keeping quality and ultimately the yield.

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Sorghum Breeding

7

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Abstract

Sorghum [Sorghum bicolor (L.) Moench] is the fifth most important cereal crop after wheat, rice, maize, and barley across the world. It is mostly cultivated in the arid and semi-arid tropics for its better adaptation to drought, heat, salinity, and flooding. It is the main staple food for the poorest and most food-insecure people of the world. In sorghum, commercial exploitation of heterosis has been possible owing to availability of a stable and heritable CMS mechanism enabling large-scale, economic hybrid seed production and sufficiently high magnitude of heterosis across a range of production environments for economic characters. The greater contribution of hybrids to yield, compared to improve and landrace varieties, has been demonstrated in almost every situation/condition. The hybrids besides being superior for grain yield and other traits of interest are stable across environments. In India, many improved high yielding hybrids and varieties of kharif, rabi, forage, and sweet sorghum, suitable to different zones/states, have been released for cultivation, which resulted in higher production and productivity. Trait-based approach for the genetic improvement of sorghum has been adopted by use of cutting-edge technologies of plant biotechnology and molecular biology to develop genotypes with improved performance under stress during crop growth and enhanced quality of the produce with extended shelf life of seed, grain, and novel sorghum products. Genomics has made rapid advances during the past decade. The sorghum genome has been sequenced, and important gene transcripts and regulatory mechanisms are being deciphered on a large scale worldwide.

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_7

The genetic diversity in sorghum provides an opportunity to search for new genes and alleles that are responsible for conferring desirable phenotypes. Genome profiling using molecular markers would provide a large number of DNA markers. Genomic selection programs would pave way for effective utilization of sorghum germplasm for crop improvement. The chapter on sorghum starts with introduction to the crop, history, its origin, evolution, and distribution of species and forms, wild relatives, and plant genetic resources. Information on floral biology, emasculation, and pollination techniques, insight into molecular cytogenetics and breeding, and genetic studies on qualitative and quantitative traits are exhaustively covered for the benefit of users. Breeding objectives including yield, quality characters, biotic and abiotic stresses, exploitation of heterosis, and development of hybrids and varieties through conventional and non-conventional breeding including genomics-assisted breeding are given exclusively for the students and sorghum researchers to serve as reference and use in crop improvement.

Keywords

Genetic resources · Germplasm · Hybridization · Purity maintenance · Varietal development · Genomics

7.1 Introduction

Sorghum [Sorghum bicolor (L.) Moench] is the fifth most important cereal crop after wheat, rice, maize, and barley across the world. It is mostly cultivated in the arid and semi-arid tropics for its better adaptation to drought, heat, salinity, and flooding. It is the main staple food for the poorest and most food-insecure people of the world. Sorghum is reported to be cultivated across 105 countries representing 41.1 million ha with an average production of 58.6 million, with productivity hovering around 1.60 tons/ha. With exceptions in some regions, it is mainly produced and consumed by poor farmers. India contributes about 16% of the world's sorghum production.

Sorghum is the fourth most important cereal crop in India. This crop was one of the major cereal staples during the 1950s and occupied an area of 17.36 million ha but has come down to 4.48 million ha in 2020. The decline has serious concern on the cropping systems and the food security of these dry land regions of the country. However, the productivity has increased from 387 kg/ha in 1955–1956 to 1018 kg/ha in 2019–2020, with threefold increase (Figs. 7.1, 7.2, 7.3, and 7.4). The total sorghum production in India has registered a constant growth rate of 0.10% per annum during the period from 1969–1970 to 2015–2016. Though, kharif sorghum yield growth rates were relatively higher, it could not offset the declining growth rates in production, as the growth rates in kharif sorghum area were negative and high. Just opposite is true in case of rabi sorghum where the area decline was not sufficient to undermine the yield growth, thus resulting in positive production growth rates. The overall increase in productivity of kharif is far more than rabi

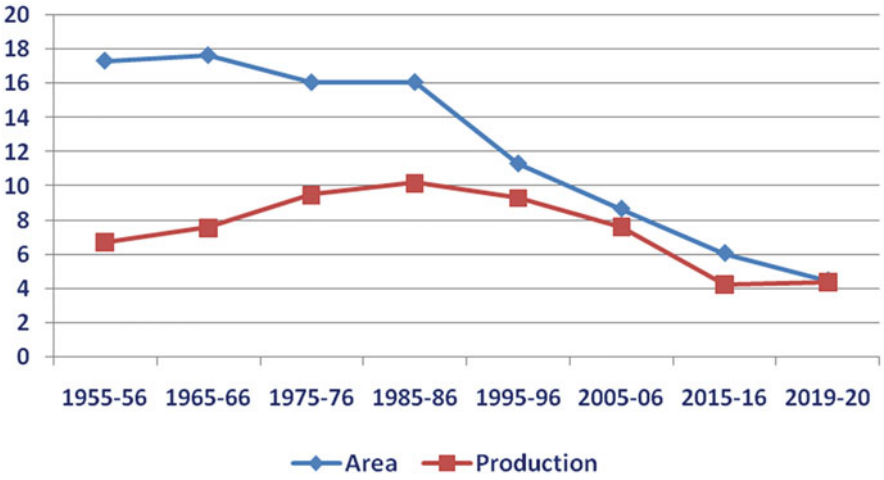

Fig. 7.1 Area and Production of Sorghum in India from 1955–1956 to 2019–2020 (*Area, million ha; production, million tons*)

Fig. 7.2 Yield of Sorghum in India from 1955–1956 to 2019–2020 (*Yield, kg/ha*)

sorghum. However, the loss in both area and production is greater in kharif sorghum than in rabi.

If we consider the recent 6 years, the area in kharif reduced from 2.3 million ha in 2014–2015 to 1.69 million ha in 2019–2020 and from 3.15 to 2.79 million ha in rabi in the same period, with percent change of −26.5% in kharif and −11.4% in rabi. However, there was improvement in productivity in both kharif (28.6%) and rabi (24%). The coverage with high yielding varieties (HYVs) of sorghum is nearly 80% in kharif, and potential under moderate input is also high (4–6 tons/ha). The area loss may be due to the expansion in irrigation which has made other crops such as rice,

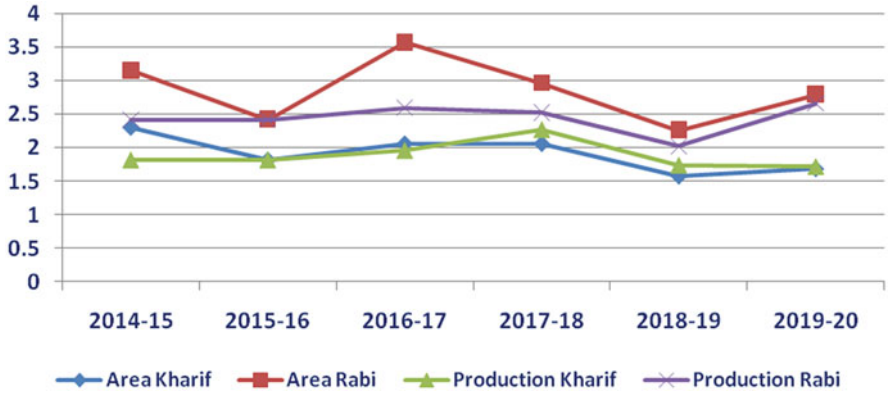

Fig. 7.3 Area and production of sorghum in kharif and rabi seasons in India from 2014–2015 to 2019–2020 (*Area, million ha; production, million tons*)

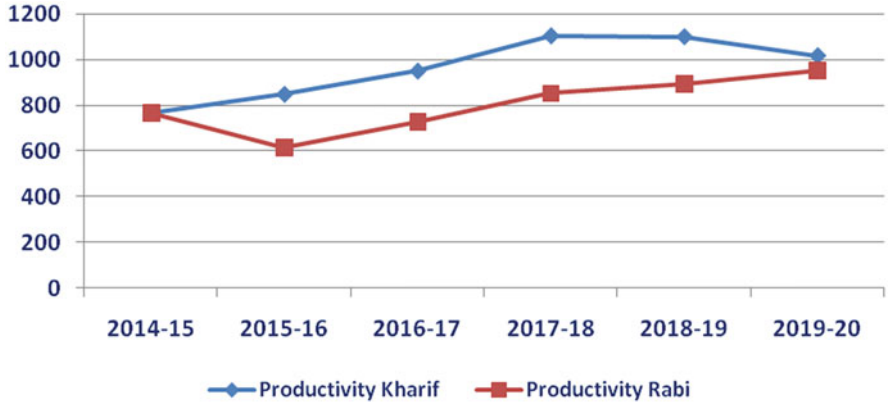

Fig. 7.4 Yield of sorghum in kharif and rabi seasons in India from 2014–2015 to 2019–2020 (*Yield, kg/ha*)

sugarcane, cotton, etc. more attractive and remunerative thus rendering sorghum to be less competitive. The decline in consumption demand of sorghum grain was also a major factor for the decline in area. The increased productivity of sorghum has not been able to compensate the loss in area turning the production to be negative.

7.2 Origin, Evolution, and Distribution of Species and Forms and Wild Relatives

Origin and early domestication of sorghum is hypothesized to have taken place around *5000–8000* years ago in northeastern Africa or at the Egyptian-Sudanese border. There is no argument against the African origin of sorghum, with the largest

diversity of cultivated and wild sorghum also found there (De Wet and Huckabay 1967; Doggett 1988; Kimber 2000). The secondary center of origin of sorghum is the Indian subcontinent, with evidence for early cereal cultivation discovered at an archaeological site in western parts of Rojdi (Saurashtra) dating back to about *4500* before 1950 AD (Kimber 2000).

Murdock (1959) has suggested that the Mande people around the headwaters of the Niger river might have domesticated sorghum. Doggett (1965) indicated that archaeological evidence suggests that the practice of cereal domestication was introduced from Ethiopia to Egypt about 3000 BC It is possible that domestication of sorghum began about that time. de Wet et al. (1970) studied archaeological reports but found only meagre information about sorghum. They suggested that sorghum had a diverse origin and probably arose from *Sorghum verticilliflorum*. *S. arundinaceum* is a grass of the tropical forests, and *S. aethiopicum* and *S. virgatum* are found in desert regions. These habitats are outside the major sorghum areas and probably contributed less to its domestication. *S. verticilliflorum* is usually found in areas where sorghum is cultivated. There is tremendous variation in *S. verticilliflorum*: and it, as well as the other wild species, readily crosses with cultivated sorghum. The races durra, guinea, and caffra are closely allied and may have arisen from *S. aethiopicum*, *S. arundinaceum*, and *S. verticilliflorum*, respectively. Murty and Govil (1967), using several different statistical procedures, classified the genus sorghum into nine groups: *S. roxburghii*, *S. conspicuum*, *S. arundinaceum*, *S. nervosum*, *S. durra*, *S. subglabrescens*, *S. sudanense*, *S. halepense*, and *S. virgatum* (OECD 2017).

7.2.1 Domestication

Sorghum, a grass of the steppes and savannas of Africa, was probably domesticated in the northeast quadrant of Africa, an area that extends from the Ethiopia-Sudan border westward to Chad (Doggett 1970; De Wet 1978). From there, it spread to India, China, the Middle East, and Europe soon after its domestication (Doggett 1965). The great diversity of *S. bicolor* has been created through disruptive selection (i.e., selection for extreme types) and by isolation and recombination in the extremely varied habitats of northeast Africa and the movement of people carrying the species throughout the continent (Doggett 1988). It has been diversified into a food source, sugar source, and construction material. Harvesting of entire panicles of sorghum by people altered the selection process (Kimber 2000). The basic morphological difference between a domesticated and a wild sorghum is the presence or absence of an abscission zone at the rachis, panicle, or spikelet nodes (Harlan 1972). The process of domestication involved a change in several characteristics of the plant. A tough primary axis (rachis) and persistence of sessile spikelet were introduced early in the process. It is likely that the transformation of a loose and open inflorescence into a compact type involved several changes: (1) an increase in the number of branches per node, (2) an increase in the number of branches per primary inflorescence branch, and (3) a decrease in the internode length on the

rachis. An increase in seed size was also probably a product of domestication, the seed becoming large enough to protrude from the glumes (House 1985).

7.2.2 Classification and Nomenclature

The word "sorghum" typically refers to cultivated sorghum (*Sorghum bicolor* [L.] Moench subsp. *bicolor*), a member of the grass family Poaceae, tribe Andropogoneae, and subtribe *Sorghinae*, which is grown for its grain (grain sorghum) and its sugary sap (sweet sorghum) or as a forage (forage sorghum). A variety of common names are used in different regions to refer to cultivated sorghum, including great millet, guinea corn, broomcorn, kaffir corn, durra, mtama, milo, jowar, or kaoliang (OECD 2017).

Cultivated sorghum is only one member of the genus *Sorghum*, made up of 25 species and separated into five taxonomic sections: Chaetosorghum, Heterosorghum, Parasorghum, Stiposorghumand, and Eusorghum. Eusorghum species are agronomically important, which include cultivated sorghum and its wild progenitor (*Sorghum bicolor* [L.] Moench). The nomenclature of cultivated sorghum and its wild and weedy relatives was thoroughly reviewed by Wiersema and Dahlberg (2007). Competing names and priorities were considered, and three subspecies were validated for *S. bicolor*: *S. bicolor* subsp. *bicolor*, *S. bicolor* subsp. *verticilliflorum*, and *S. bicolor* subsp. *drummondii*. *S. bicolor* subsp. *bicolor* comprises the cultivated sorghums; *S. bicolor* subsp. *verticilliflorum* comprises annual wild relatives of cultivated sorghum native to Africa, Madagascar, and the Mascarenes and introduced varieties to India, Australia, and the Americas; *S.* subsp. *drummondii* comprises annual weedy derivatives arising from hybridization of cultivated sorghum and *S. bicolor* subsp. *verticilliflorum*. A complete listing of the names of all known subspecies plus homotypic species names is provided by Wiersema and Dahlberg (2007).

Section Eusorghum also includes the rhizomatous taxa Johnson grass and *S. propinquum* (de Wet 1978). Although Johnson grass is native to southern Eurasia and India, its introduction to temperate regions and introgression with cultivated sorghums has caused it to become a troublesome weed (de Wet 1978). *S. propinquum* is generally restricted to Sri Lanka, southern India, and Burma east toward Southeast Asia (de Wet 1978; Doggett 1988). By natural crossing with cultivated sorghums in the Philippines, *S. propinquum* has also become a geographically isolated noxious weed (de Wet 1978).

Of the 25 recognized species of *Sorghum*, 17 are native to Australia and Southeast Asia, of which 14 are endemic to Australia (Lazarides 1991). Basic chromosome numbers vary from 10 to 40, and in some cases, such as within *S. timorense* (Kunth) Buse, there are multiple ploidy levels. These species are not within the Eusorghum section and previously were regarded as sufficiently distant to be sexually incompatible with cultivated sorghum. Recent studies have demonstrated that *S. bicolor* × *S. macrospermum* crosses are not only possible but that there is

significant genomic introgression of the wild germplasm into the cultivated species after backcrossing the hybrids (OECD 2017).

7.2.3 Description

Cultivated sorghum is a cane-like grass with diverse morphology. Plant height ranges from 0.5 meters (m) to 6 m. Culms (stalks) are erect and range from slender to stout. Tillers (adventitious stems originating from the plant base) can range in quantity from none to profuse. Leaf blades vary from linear to lanceolate and can be smooth or hairy, measuring up to 100 centimeters (cm) long and 10 cm wide with smooth to thinly pilose sheaths. The inflorescence consists of a single panicle with many racemes. Panicles may be either compact or open up to 50 cm long and 30 cm wide; panicle branches are stiffly ascending or spreading and pendulous, with the bottom branch being almost half as long as the panicle. At maturity, racemes have one to eight nodes and can be either fragile or tough. Spikelets may be glabrous or hirsute, elliptic to obovate, and up to 6 mm long (Fig. 7.5). Glumes (bracts) range from leathery to membranous, often with winged keels. Lower lemmas are approximately 6 mm long, while upper lemmas are slightly shorter and often awned. Both upper and lower lemmas of sessile spikelets are somewhat ciliate and translucent (Doggett 1988).

For many years, sorghum breeders have classified cultivated sorghum into races or working groups (Murty and Govil 1967) according to morphological characteristics. de Wet et al. (1970) described the various groups of cultivated sorghum and identified their historical geographic distribution. A system was then

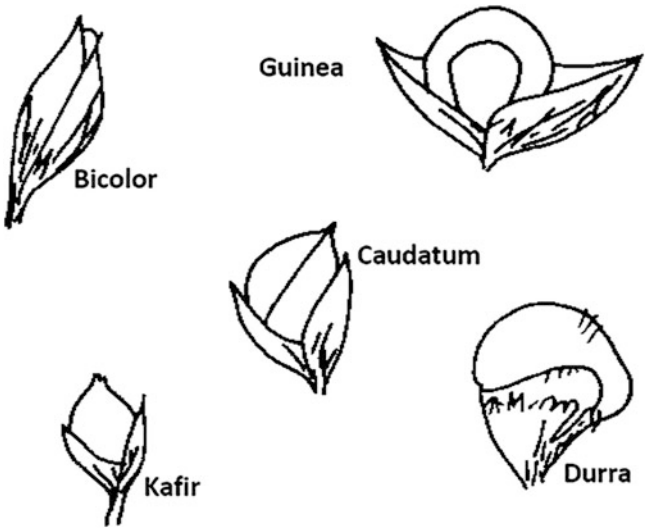

Fig. 7.5 Spikelet types of the five races of cultivated sorghum

developed dividing cultivated sorghum into five basic inter-fertile races (Bicolor, Kafir, Caudatum, Durra and Guinea) and ten intermediate races, based on floral morphology (Harlan and de Wet 1972). This classification system was widely adopted. An integrated classification of cultivated sorghum was proposed by Wiersema and Dahlberg (2007) following the morphological guidelines outlined above and simplifies their classification systems by presenting working groups numerically. A more detailed description of the characteristics of each of the five main races of cultivated sorghum can be found.

For cultivated sorghum, Harlan and de Wet (1972) have developed a simplified, informal classification useful to plant breeders for the cultivated sorghums and their closest wild relatives. The classification is based on five fundamental spikelet types.

7.2.3.1 Bicolor

Grain elongate, sometimes slightly obovate, nearly symmetrical dorso-ventrally; glumes clasping the grain, which may be completely covered or exposed as much as 1/4 of its length at the tip; spikelets persistent.

7.2.3.2 Guinea

Grain flattened dorso-ventrally, sub-lenticular in outline, twisting at maturity 90° between gaping involutes glumes that are nearly as long to longer than the grain.

7.2.3.3 Caudatum

Grain markedly asymmetrical, the side next to the lower glume flat or even somewhat concave, the opposite side rounded and bulging; the persistent style often at the tip of a beak pointing toward the lower glume; glumes 1/2 of the length of the grain or less.

7.2.3.4 Kafir

Grain approximately symmetrical, more or less spherical, not twisting, glumes clasping and variable in length.

7.2.3.5 Durra

Grain rounded, obovate, wedge-shaped at the base and broadest slightly above the middle; the glumes very wide, the tip of a different texture from the base and often with a trans-verse crease across the middle.

Harlan and de Wet (1972) developed a simplified classification of cultivated sorghum into *five* basic and *ten* hybrid races (Table 7.1) that proved to be of real practical utility for sorghum researchers. The *15* races of cultivated sorghum are identified by mature spikelets alone, although head type is sometimes helpful.

Sorghum is classified under the family Pinaceae, tribe Andropogoneae, subtribe Sorghinae, and genus *Sorghum* Moench. The genera is divided into five subgenera: Eu-sorghum, Chaetosorghum, Heterosorghum, Para-sorghum, and Stiposorghum. Chaetosorghum and Heterosorghum are found in single species primarily in Australia and the South Pacific. Para-sorghum includes seven species found in the eastern hemisphere and Central America, while Stiposorghum contains *ten* species

Table 7.1 Fifteen races of cultivated *Sorghum bicolor* subsp. *bicolor*

Basic races	Intermediate hybrid races
1. Race bicolor (B)	6. Race guinea-bicolor (GB)
2. Race guinea (G)	7. Race caudatum-bicolor (CB)
3. Race caudatum (C)	8. Race kafir-bicolor (KB)
4. Race kafir (K)	9. Race durra-bicolor (DB)
5. Race durra (D)	10. Race guinea-caudatum (GC)
	11. Race guinea-kafir (GK)
	12. Race guinea-durra (GO)
	13. Race kafir-caudatum (KG)
	14. Race durra-caudatum (DC)
	15. Race kafir-durra (KO)

Source: Harlan and de Wet (1972)

endemic to Australia. Lazarides (1991) present an excellent overview of the species in each of these four subgenera. The basic chromosome number of sorghum is 5, although striking differences in chromosome number, modes of origin, chromosome size, and geographic distribution of species are observed. Five is hypothesized as the lowest chromosome number in the Para-sorghum and Stiposorghum species, with polyploid proposed as autopolyploidy building by units of 10. *S. bicolor* spp. contain all the cultivated sorghums and are described as annual, with stout culms up to *5* m tall, often branched, and frequently tillering (Doggett 1988). The International Plant Genetic Resources Institute (IPGRI) Advisory Committee on Sorghum and Millets germplasm has accepted and recommended this classification to be used in describing sorghum germplasm.

Harlan (1972) reported that in Africa, the distribution of indigenous materials is rather consistent. Guinea is primarily West African with a secondary center in Malawi-Tanzania. Caudatum is most abundant from east Nigeria to eastern Sudan and southward into Uganda. Kafir is primarily a race of East Africa, south of the equator and southern Africa. Durra is dominant in Ethiopia and westward across the continent in the driest zones of sorghum culture near the Sahara. The hybrid races are found rather consistently in the expected places: e.g., guinea-caudatum occurs where guinea and caudatum overlap (Nigeria. Chad, Sudan), durra-caudatum occurs in northern Nigeria and parts of Sudan where durras and caudatums are also found, etc. The bicolor race occurs on a minor scale almost everywhere in Africa. The sweet types used for chewing are usually bicolors, and some are used for beer. On the other hand, the highland Ethiopian sorghums belong to the durra-bicolor race and are grown very extensively. Bicolor races are frequently reconstituted locally through introgression between grain sorghums and wild and weedy sorts that are very abundant in central and eastern Africa (OECD 2017).

Indian sorghums are mostly durra, guineas, and guinea-kafirs, with some bicolors grown on a minor scale. The American grain sorghums are now almost entirely kafir-caudatums. The Nigerian Kau-ras are durra-caudatums; the Zera zeras and Hegaris are caudatums. What is called Feterita in Sudan ranges from guinea-caudatum through caudatum to durra-caudatum. Broom corns, sorgos, and sudan grass fall

under race bicolor. Cultivated sorghums are more variable than the wild-weed complexes.

7.2.4 Wild Sorghum

The weedy and wild relatives of the grain sorghums, earlier classified primarily in series Spontanea Snowden, are now listed in subsp. drummondii and subsp. arundinaceum (de Wet 1978). The wild relatives included in the classification of Harlan and de Wet (1972) as races arundinaceum, aethiopicum, virgatum, and verticilliflorum are now included in subsp. arundinaceum; and propinquum has been recognized as a separate species of the genus *Sorghum*. The weedy taxa of subsp. drummondii are stable hybrids of the cultivated races and the wild taxa in *Sorghum bicolor*. These "species" have less tough racemes. The wild "species" have fragile racemes, and the plants usually inhabit natural grass vegetation but may invade cultivated fields.

Ng'uni et al. (2010) published a phylogenetic analysis showing the relationships between the taxonomic sections based on four regions of the chloroplast DNA (*trn*Y-*trn*D, *psb*Z-*trn*G, *trn*Y-*psb*M, and *trn*T-*trn*L) and the internal transcribed spacer region of the 18S-5 · 8S-26S nuclear ribosomal DNA from 21 sorghum species (Fig. 7.6). Germplasm accessions used in their study include wild sorghum species and several cultivated sorghums obtained from the Australian Tropical Crops Genetic Resource Centre, Biloela, Queensland, Australia, and the Zambian National Plant Genetic Resources Centre.

7.3 Plant Genetic Resources

Plant genetic resources are defined as the "genetic material of plants that is of value as a resource for the present and future generations of people." The importance of genetic resources was recognized at the intergovernmental platform under the umbrella of the Food and Agricultural Organization (FAO) of the United Nations as the "common heritage of mankind," which should be made available without restriction. Genetic resources have evolved as a product of domestication, intensification, diversification, and improvement through conscious and unconscious selection by countless generations of farmers. These landraces and improved cultivars provide the basic and strategic raw material for crop improvement the world over for present and future generations.

Sorghum has an immense range of genetic resources with much of the genetic variability available in the African regions where domestication first occurred and in the Asian region as an introduction. In Africa, the genetic variability occurs as cultivated species, wild crop progenitors, and wild species (de Wet and Harlan 1971). Landraces and wild relatives of cultivated sorghum from the centers of diversity have been rich sources of resistance to new pathogens, insect pests, and other stresses, such as high temperature and drought, as well as sources of traits to

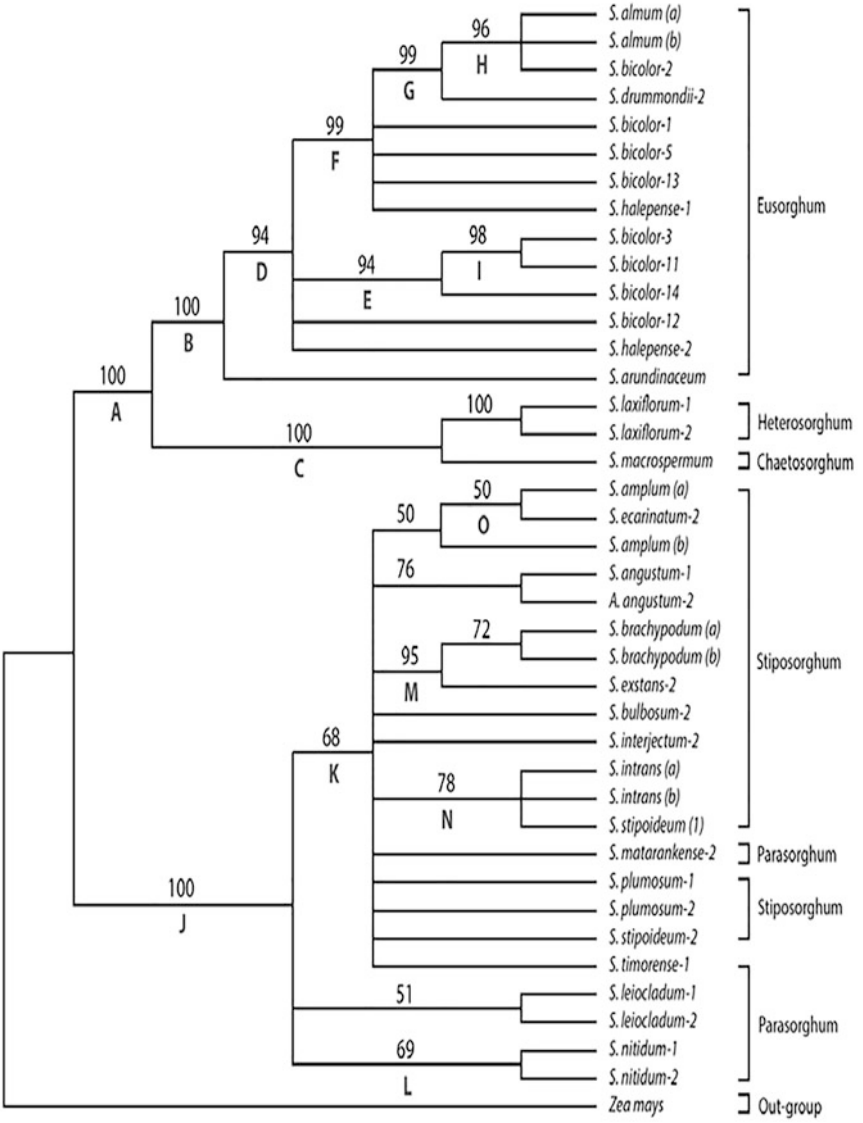

Fig. 7.6 Phylogenetic analysis of 21 sorghum species based on four regions chloroplast DNA and internal transcribed spacers of nuclear ribosomal DNA. *Clades are indicated by letters below the branches. Bootstrap values ≥50%, indicating the percentage likelihood that subgroups differ, are located above the branches. (Adapted from Ng'uni et al. 2010)

improve food and fodder quality, animal feed, and industrial products. However, this natural genetic diversity is by the adoption of improved varieties. To prevent the extinction of landraces and wild relatives of cultivated sorghum, the collection and

conservation of sorghum germplasm were accelerated about four decades ago. Since then, germplasm collection and conservation have become integral components of several crop improvement programs at both national and international levels.

7.3.1 Status of Genetic Resources

At the global level, sorghum germplasm collections consist of approximately *168,500* accessions; the largest collection (*21%* of global total) is held at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The total accessions consist of *18%* landraces, old cultivars, *21%* advanced cultivars breeding lines, and *60%* mixed categories of unknown material, while very few are wild relatives.

7.3.2 Genetic Resources at ICRISAT, Hyderabad

The gene bank at ICRISAT, India, that serves as a world repository for sorghum germplasm conserves 39,234 accessions from 93 countries, including 6249 from seven South Asian countries: Afghanistan (6), Bangladesh (9), India (6101), the Maldives (10), Nepal (8), Pakistan (90), and Sri Lanka (25). A total of 5340 geo-referenced accessions were used to identify gaps, and 5322 accessions that were characterized at ICRISAT were used to assess the diversity in the collection. Accessions of basic races varied widely than those of intermediate races for flowering in the post-rainy season, plant height in both rainy and post-rainy seasons, panicle exertion, panicle length and width, seed size, and 100 seed weight. Landraces from India were late flowering and tall and produced stout panicles and larger seeds. Landraces from Pakistan flowered early in both seasons and produced stout panicles, and those from Sri Lanka were late flowering and tall in both seasons, produced more basal tillers and stout panicles. A total of 110 districts in 20 provinces of India, 13 districts in three provinces of Pakistan, three districts in Bangladesh and five districts in four provinces of Sri Lanka were identified as geographical gaps. *Sorghum bicolor* subsp. *verticilliflorum*, *S. halepense*, and *S. propinquum* were identified as taxonomic gaps in the collection. Therefore, it is suggested to explore the districts identified as gaps to enrich the variability in the world collection of sorghum at ICRISAT (Reddy et al. 2008a, b; Upadhyaya et al. 2009).

7.3.3 Core and Mini Core Collections at ICRISAT

Core and mini core collections representing diversity in the entire collection of the germplasm of a given species preserved in the gene bank are an ideal resource for efficient conservation and utilization of plant genetic resources in crop improvement programs. Both core and mini core collections are available in sorghum. The core collection consisting of 2277 accessions constituted about 10% of the accessions of

the entire collection of a given species preserved in gene bank, while the mini core collection consisting of 242 accessions constituted 1% of the accessions of entire collection or 10% of the accessions of the core collection representing diversity of the core collection and entire collection of a given species preserved in a gene bank.

Core and mini core collections served as resource to discovering new sources of variations. Research to date suggests that core and mini core collections or genotype-based reference sets have been found useful in extracting germplasm with agronomically beneficial traits for use in crop improvement programs. The researchers at ICRISAT and elsewhere have extensively evaluated these subsets for resistance to abiotic and biotic stresses and for agronomic and nutritional traits and reported a number of germplasm accessions with agronomically beneficial traits. New sources of variations for resistance to abiotic and/or biotic stresses and of agronomic and nutritional (oil and protein, Ca, Fe and Zn, O/L ratio) traits have been reported for use in crop breeding. More importantly, a number of accessions with multiple resistance and nutrient dense types, some with specific adaptation (rainy and/or post rainy seasons), are available in ICRISAT gene bank, which can be accessed after signing with ICRISAT the Standard Material Transfer Agreement (www.icrisat.org/icrisat-ip-mta-htm). With the availability of abundant genomic resources on these crops, it is visualized that there will be increased use of genomics-based germplasm analysis to enhance use of germplasm and make impact in breeding programs in the near future.

7.3.4 Genetic Resources at ICAR-IIMR, Hyderabad

ICAR-Indian Institute of Millets Research (IIMR) is one of the National Active Germplasm Sites (NAGS) to act as a national repository for sorghum germplasm in India. The objectives of the Millets Gene bank are collection, augmentation, conservation, characterization, evaluation, documentation, distribution, and utilization of millets genetic resources (Elangovan 2020). A total of 43 explorations were undertaken and 1372 acc. of sorghum collected from 13 states, viz., Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Gujarat, Uttar Pradesh, Rajasthan, Uttarakhand, Madhya Pradesh, Odisha, Chhattisgarh, Bihar, and Jharkhand. For assembling, a total of 76,329 accessions of sorghum and other millets augmented from other national and international centers, in which 7708 acc. received from ICRISAT and 57,319 acc. from NBPGR. A total of 57,663 acc. of millets germplasm were characterized for different morpho-agronomic traits (25–27 traits). This includes *Sorghum* (39,974 acc.). Besides, as of 1 December 2020, the gene bank has 53,562 accessions of millets in bulk and 18,513 accessions in voucher sample.

7.3.4.1 Distribution

A total of 90,752 acc. were distributed to bonafide users during 2000–2020, which includes sorghum (70,645 acc.), wherein NRCS/DSR/IIMR received 49,776 acc. followed by AICRP on *Sorghum* (23,466 acc.), distributed to 16 State Agricultural Universities, 2724 acc. to ten ICAR institutes and two KVKs, etc. On utilization part,

the compilation from the published annual reports of NRCS/DSR from 2000–2001 to 2013–2014 revealed that 3585 accessions have been identified based on their uniqueness of trait. The compilation from the published AICRP on *Sorghum* reports from 2004–2005 to 2013–2014 revealed that 17,794 sorghum genotypes have been identified based on their uniqueness of trait in the AICSIP trials.

7.3.4.2 Database

Database on collection (passport), augmentation, characterization, evaluation, multiplication, conservation, distribution, utilization, and registration of all the millets germplasm accessions are maintained at MGB-IIMR-Hyderabad as primary database. Besides, it also maintains the passport and characterization database of millets germplasm maintained at ICAR-NBPGR-New Delhi and ICRISAT-Hyderabad as secondary database. Pedigree database of sorghum (1975–2017) are also available. The database of potential genetic stocks identified for utilization in sorghum under AICRP on sorghum (2004–2014), sorghum under NRCS/DSR (2000–2014), and millets under ICAR-IIMR (2014–2019) are also published. A total of 35 final products contributed using the ICAR-IIMR germplasm through selection/breeding by the AICRP on *Sorghum* trials during 2007–2020. There were a maximum of 18 rabi sorghum varieties followed by six sweet sorghum varieties, six kharif sorghum varieties, and three single-cut forage varieties. One each of sweet sorghum hybrid and dual-purpose varieties contributed to the trials.

7.3.4.3 Registration

Registration with ICAR-NBPGR-New Delhi: 16 genetic stocks were registered with ICAR-NBPGR in which 15 are sorghum for shoot fly resistance, good roti quality, multiple-disease resistance, high grain yield and biomass, MS line with bold seed, early MS line with compact ear head, yellow pericarp, and scented sorghum. The Institute facilitated ten Import Permit from NBPGR-New Delhi, 67 Standard Material Transfer Agreement (SMTA) from ICRISAT, and 931 Material Transfer Agreement (MTA) of NRCS/DSR/IIMR.

7.3.4.4 Mobile App Development for Germplasm Characterization

The Institute customized the free android mobile application "Field Book" developed by CIMMYT-Mexico and Kansa State University-USA for characterization of 70 agri-horticultural crops which include sorghum, pearl millet, finger millet, foxtail millet, barnyard millet, proso millet, little millet, and kodo millet germplasm. Also, the Institute developed spatial mapping for all the millet germplasm collection and merging of characterization data of sorghum germplasm and developed webpage with information on 5000 acc. of sorghum germplasm characterization for 25 traits along with panicle photos under CRP-Agro-biodiversity (Elangovan 2020).

7.4 Floral Biology, Emasculation, and Pollination Techniques

Sorghum is primarily a self-pollinated crop; however, outcrossing does occur. In varieties with compact or semi-compact panicles, selfing can be up to 90–95%, with 5–10% outcrossing (occurring more frequently at the tips of the panicles) (Doggett 1988). Varieties with loose or open panicles have higher rates of outcrossing: 30–60% (House 1985). In nature, the rate of outcrossing is affected by the wind as stigmas are most receptive during the first 3–5 days after their emergence, but can remain receptive up to a week or more after anthesis, depending upon temperature and humidity (Doggett 1988).

Hybridization, or crossing, of sorghum on a field scale is made feasible through genetic, cytoplasmic, and cytoplasmic-genetic male sterility systems (Ayyangar and Ponnaiya 1937; Stephens and Holland 1954). Limited scale crossing can be carried out through (1) emasculation with hot water and plastic bag technique or (2) hand emasculation and pollination techniques. Emasculation using hot water and plastic bags is cumbersome and requires a lot of preparation. It is safer to use hand emasculation, which can easily be done by unskilled staff with some training (House 1985).

Sorghum is normally self-pollinated, but some florets are *protogyny* resulting in cross-pollination averaging about 6%. So, it is classified as often cross-pollinated. The amount of natural cross-pollination varies from 0.6% to 50% in different varieties and places. The cross-pollination is more in loose panicles than in compact ones. Anthesis starts from tip to downward at the rate of 2–5 cm/day and completes within 7–10 days, with anthesis time 3–6 A.M. The pollen grains are viable only for short period, and stigma is receptive for 8–16 h.

7.4.1 Selfing

Head bagging becomes efficient for selfing the ear heads. Once the decision to bag heads has been made, all heads in a row should be covered. If a head has already begun to flower, the flowering portion should be cut off. During head bagging, boot leaf of the plant is usually removed prior to placing the bag.

7.4.2 Emasculation

7.4.2.1 Hand Emasculation and Pollination

Conventional crossing or hybridization of different sorghum varieties is carried out by simple hand emasculation of normal bisexual florets and then transferring the pollen from the chosen male parent (which usually is a pure line but not necessarily always) to the stigmas of the emasculated florets (Reddy 1997).

For this we need a pair of scissors, a secateur or a manicuring clipper, a blunt needle or a pencil, a pair of forceps, 7 cm × 3 cm × 15 cm butter paper bag, paper clips or a stapler, and a marking pen.

The steps are as follows:

- From the desired parental line, choose a panicle that has just started anthesis.
- Clip off the florets which have completed anthesis, with a secateur or scissors.
- Remove primary- or secondary- and tertiary-branch rachises in the lower portion of the panicle, leaving about 200–300 florets in the central portion of the panicle just below the clipped florets.
- Clip off all the pedicellate (sterile) florets from the central portion, leaving only the sessile (fertile) florets.
- Thin out the sessile florets by clipping off some of the tertiary rachises to make it easier to hold the sessile florets during emasculation.
- Grasp the sessile floret to be emasculated between the thumb and forefinger.
- Insert a blunt needle between the glumes below the middle portion of the floret, and move it slowly around the inner surface of the glumes so as to break the stamen filaments.
- Lift the needle out and upward, slowly pushing the detached anthers out of the floret.
- After emasculating the florets as described above, cover them with a butter paper bag, and clip or staple it as explained earlier in the selfing process. These bags should have the date of emasculation recorded on them.
- Inspect the emasculated panicles on the following day for any remaining anthers that might have emerged from the florets. Remove these florets along with the anthers, and once again cover the emasculated panicles with bags.
- On the third/fourth day after emasculation, take the pollen from the chosen parent into another butter paper bag. Slowly insert the emasculated panicle into the bag with the pollen, and with a hand over the bag clasping the peduncle at the base of the panicle, shake the panicle so that the pollen in the bag stick to the stigmas that would have emerged from the emasculated florets.
- Staple/clip as in selfing with the folded corners of the mouth of the bag clasping the peduncle. Make sure that the bag carries information on the date of emasculation, date of pollination, and the male parent used in crossing. It is useful to pollinate a second time on the following day to ensure pollination of all the florets.

When the seed is harvested from the pollinated panicle, make sure that the bag containing the seed is properly labeled and the male and female parents and the crosses are clearly indicated. The cross is usually denoted as follows:

Name of the parent female × male, where "×" denotes the cross. For example, IS 3541 ×IS 1052 means that IS 3541 as female has been crossed with IS 1052 as male. Care should be taken that the glume closest to the pedicellate spikelet is held facing away from the worker. Trimming should be done so that the individual sessile florets remain uniformly spread.

7.4.2.2 Emasculation with Hot Water and Plastic Bag Technique

This requires plastic sleeves, a pair of scissors, string, butter paper bags, paper clips or a stapler with staples, hot water in a thermos flask, and a thermometer.

The steps are as follows:

- Cut and trim the florets at the tip of the panicle. Take a bag made out of a plastic sleeve and, tie it closely around the peduncle to surround the panicle.
- Pour hot water (42 °C) into the closed plastic sleeve, and leave it for 10 min, soaking the panicle in hot water.
- The water is drained after 10 min, and the sleeve is tied over the panicle.
- The florets at the top of the panicle open after 2–3 days, and anthers emerge but do not dehisce and do not shed pollen; knock these anthers free from the panicle by tapping it.
- Remove the remaining unopened florets from the lower portion of the panicle.
- Get pollen from another panicle of the desired line in a butter paper bag, and put it over the emasculated panicle, tying it around the peduncle as in the selfing process.
- Before collecting the pollen, write the name of the pollen parent and the date of crossing/pollination on the bag.
- On the fourth day after pollination, check for selfed florets; these can be recognized by their distinctively superior size compared with the rest. Remove the selfed florets and rebag.

(Note: This is a cumbersome method and requires a lot of preparation. It leaves some selfs in the F_1 which need to be thoroughly checked and rogued out. It is always safer to follow the hand emasculation method which can be easily done by unskilled staff with some training.)

In this method, the panicle flowered tip and lower panicle branches are removed. About 50 florets (in clusters of two or three) are immersed in hot water at 48 °C for 10 min.

7.4.2.3 Plastic Bag/Mass Emasculation Technique

In this method, sorghum panicle is covered with plastic bag. This creates high humidity inside the bag. Under such humidity, the florets open, and the anthers emerge but shed no pollen. The anthers are knocked free of head by tapping. In this method, some selfing occurs. Therefore, marker genes are needed to identify the plants arising from selfed seed.

On a dry morning when pollen shedding is occurring between 6 and 7 A.M., the hand pollination may begin around 9.30 A.M. In rainy days, the operation may be started at 11.30–12.30 A.M. The pollen is collected in paper bags. Sorghum pollen kept in bags is viable for 10–20 min. For collection, appropriate heads may be selected and bagged in the previous night itself.

The selected male parent panicle will be covered with brown paper bag the previous day evening before dehiscence of anthers. Next day, the pollen will be collected by tapping the bag. The collected pollen will be dusted on to the

emasculated head and covered with butter paper bag labeled properly. Dusting of pollen is done for 2–3 days continuously.

7.4.3 Importance of Selfing and Crossing

Selfing and crossing are essential tools in the regulation of variability in plant breeding programs. The breeder must therefore know these techniques. When a flowering panicle is tapped with a finger, a cloud of yellow pollen grains can be seen. The wind carries the pollen grains to the stigmas, and pollination is achieved. Pollen is normally viable for 3–6 h in the anther and 10–20 min outside. In nature, occurrence of outcrossing varies from 1% to 10%; in wild types with loose/open panicles, it may be up to 30%. In normal compact or semi-compact panicles in improved cultivars, selfing can be up to 90–95%, with 5–10% outcrossing, occurring more frequently at the tips of the panicles.

Selfing and crossing or outcrossing are processes with opposite effects. Selfing promotes homozygosity and preserves the linked gene complexes, which helps maintain pure stocks of cultivars. Outcrossing or crossing promotes recombination and creation of new linkage gene complexes, leading to variability, which provides an opportunity to the breeder to select upon.

Outcrossing, referred to as crossing here, is a process of transferring pollen grains from a floret of one panicle to the stigma of a floret of another panicle. In nature, it is usually effected by the wind as stigmas remain receptive up to a week or more after blooming, depending on the temperature and humidity. Lower temperatures favor a longer period of receptivity of stigmas. However, stigmas are most receptive during the first 3–5 days after their emergence.

Hybridization of sorghum on a field scale is made feasible through the use of the genetic and cytoplasmic genic male sterile (CMS) system. There are a number of genes (ms_1, ms_2, ms_3, ms_4, ms_5, ms_6, ms_7, ms_8) which individually contribute in homozygous condition to male sterility. Also, another system, independent of the genetic system, called the CMS system, creates male sterility because of the interaction of genes in the cytoplasm with those in the genome.

7.4.4 Fertilization

Pollen grains germinate as soon as they come in contact with a receptive stigma; the pollen tubes grow through the stigmatic papillae down to the ovary through the stylar region. Only one pollen tube succeeds in reaching the micropyle. The sperm nucleus divides into two, one of which fertilizes the egg cell to give rise to the embryo ($2n = 20$ chromosomes in sorghum), while the other joins the two polar nuclei to form the endosperm ($3n = 30$ chromosomes, i.e., 20 from the female parent and 10 from the male parent). This process of embryo formation by the union of egg nucleus and sperm nucleus is called fertilization. The glumes close shortly after pollination. The ovule begins to develop as a light green, almost cream-colored

sphere, and about 10 days after pollination, it becomes bigger and turns dark green. The development of the embryo and endosperm continues for another 30 days when the seed reaches physiological maturity with the hilum region (a spot on the seed through which the seed receives nourishment of the plant) becoming black. During the development, the seed passes through milk, early-dough, and late-dough stages. The dried-up style may persist in some seeds up to physiological maturity (House 1985).

7.5 Techniques of Maintaining Genetic Purity of Seed Stocks

Various techniques are followed in breeding programs to maintain/enhance the purity of seed stocks. The choice of the methods depends on the quantity of seed required, the resources available, and the extent of genetic purity and trueness to type required for the given material. These methods are described briefly here.

7.5.1 Roguing

Before flowering, all odd plants or "rogues" are removed. The panicles, after trimming the tips (top quarter of the panicle), are harvested and bulked from the plots in breeding blocks. This method does not assure genuine purity of the stocks. Therefore, when a crop is raised from seed multiplied in this manner, roguing should be repeated.

7.5.2 Isolation

When one or two stocks need to be multiplied in large quantities, sowing in isolation, field plots away from other sorghum plots, with or without variable sowing times, is desirable to maintain a high level of genetic purity. Isolation with variable sowing times is risky as there is a chance of overlapping of flowering between different plots. Isolation that physically separates plots is considered more useful. A minimum distance of 200 m between sorghum plots is recommended for multiplying varieties or pure lines with the required standards of genetic purity. Generally, in India, seed certification standards call for 99.5% genetic purity. Roguing of "odds" or "rogues" before and during flowering is essential, and plants with trueness to type should be retained/harvested.

7.5.3 Selfing by Bagging

Sorghum is a perfect-flowered plant (i.e., it has both sexes in the same floret). Self-pollination or selfing is the process of ensuring the transfer of pollen of a floret to the stigma of the same floret or of another floret within the same panicle. This is usually

accomplished in a breeding program by using kraft paper bags. In addition to kraft paper bags, the other things required for selfing are a pair of scissors, paperclips or a stapler, watchmaker tags, and a marker. The steps involved in selfing are:

- Remove odd or off-type or rogue plants from the plot before they reach the boot leaf stage.
- When a few florets have opened at the tip of the panicle, snip off the
- flowered florets.
- Cut the flag leaf at the base.
- Record the date of selfing on the selfing bag.
- Put the bag (with the date) over the panicle, taking care to see that the whole of the panicle is covered by the bag and that the bag also covers about 5–8 cm of the peduncle.
- Make sure the peduncle stays in the center of the mouth of the bag wrapped over by the folded corners of the paper bag on either side.
- Either staple the folded corners of the paper bag or put a paper clip, taking care to see that the bag holds the peduncle tightly.

Ten to 15 days after bagging, the bags can be removed from the panicle. The same bags or watchmaker tags can be stapled around the peduncles to mark selfing. The number of plants to be bagged in a plot to ensure selfing depends on the purpose of developing materials (in case of segregating lines) and the quantity of seed needed (in case of near uniform lines). Care should be taken to harvest individual plants separately in case of segregating generations, and bulk harvest the true-to-type panicles only in case of uniform lines.

7.5.4 Precautions

Bags can be blown away by the wind or can be damaged by rain. Care must be taken to replace them immediately, recording the information originally written on the bags. Periodic inspection of the selfed plots is essential to detect damaged bags.

7.6 Molecular Cytogenetics and Breeding

Sorghum bicolor has a haploid chromosome number of 10, and it is classified as a diploid ($2n = 2x = 20$). Most species within *Sorghum* are diploid ($2n = 20$), but several species, most notably *Sorghum halepense*, are tetraploid ($2n = 4x = 40$). As the basic chromosome number in the Sorghastrae is 5, it has been hypothesized that sorghum may be of tetraploid origin. Earlier studies on the meiotic chromosome pairing analysis did not provide evidence for the tetraploid origin of *S. bicolor*, and the information on the existence of homologous segments in the chromosomes of *S. bicolor* is poor; therefore, the chromosomes were regarded as distinct. Recent studies provide limited evidence about tetraploid origin of sorghum (Lazarides

1991). Duplicated loci were found on the map, suggesting that sorghum has tetraploid origin. However, Subudhi et al. (2000) contended that these evidences of tetraploidy are not satisfactory. They argued that in both analyses, the duplicated loci found on the mapped genome are only to an extent of 8% and 11%, respectively. The cultivated sorghum could be considered a diploid from the perspective of genome organization (Subudhi et al. 2000; Rooney 2000; OECD 2017).

7.7 Interspecific Hybridization

The genus *Sorghum* consists of 25 species that are classified into five taxonomic subgenera or sections, Eu-sorghum, Chaetosorghum, Heterosorghum, Parasorghum, and Stiposorghum. Cultivated sorghum belongs to the Eu-sorghum section, and 19 sorghum species belonging to sections other than Eu-sorghum are distributed primarily in Australia, southern Asia, and Africa and comprise an untapped tertiary gene pool. Most of the undomesticated sorghum species having agronomically important genes fall within the tertiary genepool, making gene transfer to domesticated species very difficult due to strong sterility barriers (Harlan and de Wet 1972). Tertiary wild species of sorghum carry many desirable traits for resistance to pests, diseases, and drought. Pollen-pistil interactions are the primary reasons why *S. bicolor* will not hybridize with divergent *Sorghum* species. The *iap* (inhibition of alien pollen) allele was identified to allow hybridization; however, it is patented and is under restricted use. Interspecific hybridization in sorghum was initiated as early as 1930s, but was not successful due to pollen pistil inhibitions (Ayyangar and Ponnaiya 1937; Rooney 2000).

Compared with interspecific hybridization, intergeneric pollination is more difficult to accomplish because of the lack of homology between chromosomes of the two parental genotypes that causes irregular meiosis. Intergeneric pollination plays a key role to generate extensive stochastic genomic and epigenomic variations that can be translated into phenotypic novelties. Their progenies show various genomic, epigenomic, and transcriptomic differences compared with the parental genotypes. It was demonstrated that introgressive hybridization between rice *Oryza sativa* ssp. *japonica* cv. Matsumae and its incompatible counterpart, *Zizania latifolia* Griseb, has provoked genome-wide, extensive genomic changes in the rice genome, and some of which have resulted in important phenotypic novelties. Several genome-wide homozygous single nucleotide polymorphisms (SNPs) and insertion/deletions (InDels) were identified in a typical rice-*Zizania* introgression line RZ35. Pollination by pollen from an incompatible species causes massive activation of silent TEs, thus acting as a "genome shock," resulting in the transcriptional activation of the TE mPing. Introgression line RZ35 had several TEs transcriptionally activated or mobilized that led to enhance the resistance to blast fungus (Rooney 2000; OECD 2017).

7.8 Genetic Studies on Qualitative and Quantitative Traits

7.8.1 Maturity

Quinby (1967), who has pioneered research on maturity and height in sorghum, identified factors at four loci that influence maturity, Ma1, Ma2, Ma3, and Ma4. Generally tropical types are dominant (Ma-) at all four of these loci, and a recessive condition (mama) at any one of them will result in more temperate zone adaptation. In fact, the great bulk of lines used in the temperate zone have been found to be recessive at locus 1. Lines in a program to convert tropically adapted lines to temperate zone adaptation are likely recessive at locus 1 and dominant at the other three loci. Genes at these loci interact: when dominance occurs at the Ma1 locus, the dominant and recessive classes at the Ma2, Ma3, and Ma4 loci can be identified in an F2 population. When the recessive condition (ma1ma1) occurs, variation in time to flowering will diminish, making it difficult to separate genotypes (52.4–56.7 days' variation, compared to variation of 64.6–90.5 days when dominance occurs at locus 1). If Ma1 is dominant, Ma2, Ma3, and Ma4 demonstrate dominance (lateness), but if the gene at locus 1 is recessive (ma1ma1), then the recessive ma2ma2, ma3ma3, and ma4ma4 may express dominance. There is an exception: when the gene at locus 1 is heterozygous (Ma1ma1) and that at locus 2 is recessive (ma2ma2), maturity is later than if both alleles at the Ma2 locus are dominant (Ma2Ma2) (Quinby 1974). Most of the lines from the tropical-to-temperate conversion program are recessive (ma1) and dominant at the other loci. Yet the time to flowering varies from 60 to 85 days. Early-maturing tropical varieties tend to be early after conversion, and late-maturing tropical lines tend to be late.

7.8.2 Height

Genes at four loci in sorghum are important in the control of plant height. These genes are assigned the symbols Dw1, Dw2, Dw3, and DW4. Tallness is partially dominant to dwarfness. The dwarfing effect of recessive genes (dwdw) at any of those four loci is brachytic in nature (i.e., the length of the internode is reduced, but not the peduncle length, head size, or leaf number, and the maturity is not changed). The zero-dwarf type (dominant [DW-] at all loci) may reach a height of 4 m. The change from four to three dominant genes may result in a height change of 50 cm or more. If genes at one or more of the loci are recessive, the difference in height resulting from the recessive condition at an additional locus may have a smaller effect in reducing plant height. The difference between a 3-dwarf (recessive genes [dwdw] at three loci) and a 4-dwarf type may be only 10 or 15 cm. There is variation in height between different varieties with the same genotype. This is thought to be due to an allelic series at a particular locus and not to modifying factors at other loci. There is instability at the Dw3 locus; a dw3 allele mutates to the dominant allele at a high rate (one mutation in 1209 gametes). Thus, some sorghum fields may have a ragged appearance due to a greater frequency of tall plants. Some instability has also

been found at the Dw4 locus, but not at Dw1 or Dw2. Tall hybrids can be produced from shorter parents using complementary factors. For example, 2-dwarf hybrid (dw1Dw2Dw3dW4) can be produced from two 3-dwarf parents (dw1Dw2dw3dw4 and dw1dw2Dw3dw4).

7.8.3 Male Sterility

Genetic male sterility is caused by single recessive genes. Of these, ms3 is most widely used because its expression of male sterility is good and it is stable over many environments. Male sterility caused by the recessive condition for ms2 has been useful; also male sterility caused by the anther-less gene (al) has been found useful. Genetic male sterility is used primarily in composites to enhance the level of recombination.

Cytoplasmic genetic male sterility in sorghum makes possible the commercial production of hybrid seed. Male sterility results from an association of Milo cytoplasm with sterility genes found primarily among kafirs but also in some varieties of other races. The genetics involved is not completely clear, but two genes (msc1 and msc2, when recessive in the presence of Milo cytoplasm) result in male sterility. There are other factors that influence the sterility reaction, possibly having a modifying effect on the level of partial fertility. Most of the information in this section is adapted from Doggett (1970).

7.8.4 Disease Resistance

Kernel smut (*Sphacelotheca sorghi*)—three races of this disease are known, and resistance to each is controlled by an incomplete dominant, Ss1, Ss2, and Ss3. Head smut (*Sphacelotheca reiliana*)—in most varieties, resistance is dominant to susceptibility. Reaction to milo disease (*Periconia circinata*) is controlled by a single locus (Pc). Susceptibility is partially dominant; F1 is intermediate. Anthracnose (*Colletotrichum graminicola*) susceptibility on the leaf is controlled by a simple recessive gene (I). Susceptibility to the stalk rot phase of this organism is controlled by the simple recessive (Is). Rust (*Puccinia purpurea*) susceptibility is controlled by a simple recessive gene (pu). For leaf blight (*Exserohilum turcicum*), most grain sorghums are resistant. Susceptibility in sudan grass is inherited as a simple dominant. In charcoal rot (*Macrophomina phaseolina*), the inheritance of resistance is recognized but has not been analyzed completely; apparently, more than one gene is involved. Downy mildew (*Peronosclerospora sorghi*) resistance is inherited as a recessive character. A number of types, including most of the kafirs, are resistant. Sudan grass is susceptible. Maize dwarf mosaic virus resistance is dominant.

7.8.5 Insect Resistance

The genetics of insect resistance are not well understood. Generally, it appears to be multigenetic, and complete resistance is seldom found. Shoot fly (*Atherigona soccata*) resistance is found at a low but useful level. There are apparently three aspects: (1) non-preference for oviposition; (2) antibiosis (i.e., plant resistance per se); and (3) recovery resistance (tillers form after the main stem is destroyed and survive to make a crop). Recently, the presence of trichomes (microscopic hairs on the lower surface of leaves) has been found to contribute to oviposition non-preference. The presence of trichomes is controlled by a single recessive gene.

Stem borer (*Chilo partellus*): Variation in resistance is generally found at a relatively low level. Storage insects: small corneous grains store better than large soft ones.

7.8.6 Stalk Dryness and Sweetness

Dry stalks are controlled by a simple dominant gene, D; juiciness is recessive. Dry stalks have a white leaf midrib; juicy stalks have a dull green leaf midrib (possibly with a narrow white strip in the middle). An insipid stalk is controlled by a single dominant gene, ×, sweetness being recessive. There is apparently no linkage between the genes at loci controlling the dry juicy and insipid sweet characters. There is no clear evidence favoring either sweet or non-sweet stalks for forage; livestock eat both.

7.8.7 HCN Content

Sorghum produces HCN, which may be dangerous when the crop is used for feed. The problem is particularly acute in seedlings or on re-growth of a ratoon crop and is aggravated by drought and low temperatures. Inheritance seems to be controlled by more than one factor, and in many cases low HCN content shows partial dominance.

7.8.8 Plant Colors

Genes for plant color influence the green portions of the plant—leaves, stems, and glumes. The gene P produces a purple color, and recessive plants (pp) are tan. The shade of purple pigmentation is influenced by alleles at the Q locus; purplish-black is due to the allele q, and reddish-purple is due either to the Q or q′ alleles.

7.8.9 Glume Color

This is controlled by the same two loci (P and Q). Black and red glumes are dominant (P-), and mahogany or sienna glumes are recessive (pp). There appear to be inhibitors causing the glume colors in some plants to fade. A red pigment appears in dead leaves and sheaths in red-seeded varieties, but not in white-seeded ones. Genes R-yy are responsible for red pericarp color and for this effect in dead leaves. The Q gene influences color in plant sap (spots on white-seeded varieties are red in red-pigmented plants and purple black in purple-pigmented plants.)

7.8.10 Grain Color

This is determined by pigmentation of the pericarp, testa, and endosperm. Color in these tissues is controlled by different sets of genes. Genes for grain color are the following:

Ce. Plant color is present in the testa and the glume cup (Q is expressed).
B1, B2: Cause a brown testa (when both genes are found in presence of Ce).
B1b2, b1B2, B1B2, and Ce B1B2 have colorless testas.
S1: Testa spreader, in the presence of B1 and B2, results in brown color in the epicarp (outer layer of the pericarp).
Y: Epicarp is yellow color (in rrY-condition) versus white (yy condition).
R: Epicarp is red if Y is found; otherwise it is yellow or white.
I: Intensifies color of the pericarp (epi-carp).
Bw1 and Bw2 are complementary factors; when both are found, there is a brown wash on the seed.
M: Causes a colored wash on the seed.
Pb: Causes purple blotching of the seed.
Pt: Causes a purple tip on the grain.

7.8.11 Endosperm Characters

Wx (waxy) results in a starch with a normal amylose-amylopectin balance; when homozygous, the recessive allele wx results in a waxy endosperm, i.e., a predominance of amylopectin. Su (sugary) gives normal sugar content, while the recessive (su) condition results in high sugar content. Seeds are usually dimpled, and the stems are usually sweet. Z causes greater endosperm hardness than the homozygous recessive z, which produces a chalky condition. Yellow pigment in the endosperm is not well understood, but it appears that more than one major gene or modifier is involved. The yellow color is due to xanthophylls and carotene pigments.

7.9 Breeding Objectives

7.9.1 Yield, Exploitation of Heterosis, and Hybrid Development

Sorghum is one of the crops in which heterosis could be exploited. In sorghum, commercial exploitation of heterosis has been possible owing to (1) the availability of a stable and heritable CMS mechanism (Stephens and Holland 1954), enabling large-scale, economic hybrid seed production, and (2) sufficiently high magnitude of heterosis across a range of production environments for economic characters. The greater contribution of hybrids to yield, compared to improve and landrace varieties has been demonstrated in almost every situation/condition. The hybrids besides being superior for grain yield and other traits of interest are stable across environments. The adoption of the first commercial hybrid (CSH 1) in India over much of the rainy season sorghum area, while local varieties confining to fairly narrow specific environmental niches, is a testimony to the wide adaptability of hybrids over the varieties (Rao 1970, 1982).

Indian public sector agricultural research agencies have been breeding improved sorghum genotypes since the early part of the twentieth century. In this article, the status of research on grain sorghum hybrid development under All-India Coordinated Sorghum Improvement Project (AICSIP) is reviewed. Hybrid sorghum research in India started in the early 1960s, with the establishment of hybrid breeding programs at a number of agriculture research centers and the SAUs in Maharashtra, Karnataka, and Andhra Pradesh with Indian Institute of Millets Research (IIMR) (Formerly Directorate of Sorghum Research and National Research Centre for Sorghum) as the nodal agency dealing with all aspects of sorghum research and development including coordination and consultancy. The Indian Institute of Millets Research is mandated with organizing and coordinating sorghum research at all India levels through AICSIP with a network of 18 centers located in states having major sorghum-growing areas. Through these coordinated efforts, the Indian national program over the years released 41 hybrids and 42 open pollinated varieties under the kharif sorghum (rainy season), rabi sorghum (post-rainy season), sweet sorghum, and forage sorghum. Release of more than 40 hybrids at national level and several at state level is a standing testimony to the success of Indian sorghum improvement program (Prabhakar et al. 2015). So far, outstanding and significant progress has been achieved in case of kharif hybrids. But a great deal is yet to be done to exploit heterosis in breeding hybrids for rabi cultivation.

7.9.2 Kharif Sorghum

In spite of the decrease in the area of kharif cultivation, there has been impressive enhancement of productivity of sorghum from 560 kg/ha in 1970 TE to 1000 kg/hectae in 2000 TE which has actually kept production levels relatively stable despite constant erosion of area under kharif sorghum. This increase in productivity is due to introduction of short duration dwarf high yielding hybrids which are especially well

adopted in the state of Maharashtra, a major kharif-growing area in the country. Sorghum improvement program though initially followed wide adaptability as the basis for breeding strategy, as the time goes by, considering the demands of local conditions; it is now following specific adaptation as the breeding strategy. For kharif sorghum, the different zones have been identified.

The discovery of cytoplasmic genetic male sterility in sorghum and its use for hybrid-seed production made the commercial exploitation of heterosis possible. Though the Indian sources of cytoplasm such as Maldandi (M 31-2A and M 35-1), Vijayanagaram (VZM 2A), and Guntur (G1) are well-known, the milo cytoplasm discovered in the USA has been most extensively utilized in the entire hybrid program of our country. The hybrid program in India was initiated in the early 1960s by attempting crosses of Indian tall cultivars as well as temperate dwarf parents as male parents on exotic CMS lines. Combined Kafir (CK) 60A is one such CMS line, which was extensively utilized in developing two commercial hybrids (later released as CSH 1 using IS 84 as restorer in 1964 and as CSH 2 (1965) with IS 3691 as the male parent). The male parents of CSH 2 and CSH 3 were shorter than respective CMS lines. Therefore, they imposed restriction on the spread of these hybrids. The next commercial hybrid, CSH 4, was based on CMS 1036A and Swarna as a male parent.

With the breeding efforts in the Indian sorghum improvement program, improved and promising parental lines became available, and hybrids such as CSH 5 (2077A × CS 3541) and an early hybrid, CSH 6 (2219A × CS 3541), were developed. These hybrids released in 1975 (CSH 5) and 1976 (CSH 6) showed a quantum jump in grain yields. Both hybrids were widely adapted in the country with acceptable grain and fodder yields as well as tolerance to grain molds and leaf diseases (mainly attributed to the male parent CS 3541, converted *zerazera* line from Ethiopian germplasm) compared to CSH 1. The next breakthrough came through the release of CSH 9 (296A × CS 3541) based on improved parental lines. This hybrid maintained 18–20% higher yield than CSH 5 and CSH 6 and became the best-selling hybrid. CSH 10, a dual-purpose (grain + stover) hybrid, and CSH 11 could not find popularity with farmers due to seed production problem and/or small seed size.

Another potential hybrid based on 296A was released as CSH 13 (296A × RS 29). It is adapted to both kharif and rabi seasons and also as single-cut fodder hybrid and gives 45% higher dry fodder yield. As dependence on CMS 296 A increased, diversification of CMS lines became a high priority in the late 1980s in addition to genetic enhancement of R lines. These efforts helped to develop another set of four hybrids, i.e., CSH 14 (AKMS 14A × R 150), CSH 16 (27A × C 43), CSH 17 (AKMS 14A × RS 673), and CSH 18 (IMS 9A × Indore 12) on new CMS lines. The hybrids CSH 14 and CSH 17 are also known for earliness by 8–10 days with grain yield potential of CSH 9. CSH 16 has 9% higher grain yield than CSH 9 along with bold seed and is tolerant to grain molds. The hybrid CSH 18 released by Indore Center of AICSIP is 9% superior in grain yield than CSH 9; further it is a dual-purpose hybrid yielding good amount of stover.

CSH 23 is another early maturing hybrid released in 2005 for the states of Maharashtra, Karnataka, AP, MP, Gujarat, Rajasthan, and UP. It takes

101–103 days to mature and yields 43 q/ha of grain. One more medium maturing hybrid, CSH 25, was released for zone II in 2008. It is developed from the parents PMS 28A and C 43 and yields 43 q/ha of grain and 120 q/ha of fodder and was found to have good tolerance to shoot fly and grain molds.

Another medium maturing hybrid, CSH 27, was released recently in 2012 for zone I involving the states of Rajasthan, N. Gujarat, UP, AP, and Tamil Nadu. It is a dual-purpose hybrid with 39 q/ha of grain and 136 q/ha of fodder yield. It was developed based on the parents 279A × CB 11. It has better level of tolerance to grain molds. The latest hybrid which has been recommended for release in zone II comprising of the states of Maharashtra, Karnataka, MP, South Gujarat, and North AP was CSH 30, which is an early maturing hybrid with good level of tolerance to grain molds.

All these hybrids played a major role in pushing up productivity and production, particularly in the case of kharif sorghum (Table 7.2). Among the kharif hybrids, CSH 1, CSH 5, CSH 6, CSH 9, CSH 14, and CSH 16 need special mention as CSH 5 and CSH 6 had a yield potential of 34 q/ha which was raised to 40 q/ha in CSH 9 and further raised to 41.0 q/ha in CSH 16, CSH 23, CSH 25, and CSH 30 with distinct superiority in grain and fodder quality.

7.9.3 Rabi Sorghum

Focused breeding on rabi sorghum was initiated in the early 1970s. These breeding efforts led to the central release of varieties like Muguti. Heterosis breeding led to the central release of hybrids like CSH-7R, CSH-8R, and CSH-12R. These cultivars were notable in two respects: first, that none of these cultivars succeeded in gaining consistent acceptability from farmers at a scale to effect a discernible impact and second, there was problem of seed production of hybrids.

The second phase of *rabi* sorghum breeding with emphasis on hybrid cultivars was initiated in the late 1980s. During this period, 250 experimental hybrids were evaluated in the AICSIP trials. These trials resulted in the identification of two hybrids, SPH 504 and SPH 677, for central release as CSH-13R and CSH-15R. CSH-13R has significant yield superiority over M 35-1 but is highly vulnerable to shoot fly and low temperature and has inferior grain quality. The case of CSH-15R having a *rabi* based MS line (104A) developed at Mohol Centre is different from CSH-13R which has a marginal yield advantage over M 35-1. *Rabi* sorghum hybrids will have a tangible impact only when the male sterile and restorer lines having the season adaptability and desired combining ability are developed (Rao 1970) (Table 7.3).

7.9.4 Forage Sorghum

In the 1990s, multi-cut sorghum hybrid received much emphasis. At this juncture, the private sector also came forward to join the efforts. Since then, many hybrids like Punjab Sudex Chari, Pro-Agro Chari, MSFH3, Hara Sona, and Safed Moti and

Table 7.2 Nationally released kharif sorghum hybrids

S. No.	Hybrid/variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSH 1	1964	CK 60A × IS 84	NRCS (DSR), Hyd. (currently IIMR)	Maharashtra, Karnataka, MP, Gujarat, UP, Rajasthan, Tamil Nadu
2	CSH 2	1965	CK 60A × IS 3691	NRCS (DSR), Hyd.	– do –
3	CSH 3	1970	2219A × IS 3691	NRCS (DSR)	– do –
4	CSH 4	1973	1036A × Swarna	NRCS (DSR)	– do –
5	CSH 5	1975	2077A × CS3541	NRCS (DSR)	– do –
6	CSH 6	1977	2219A × CS3541	NRCS (DSR)	Maharashtra, Karnataka, AP, Gujarat, Rajasthan, Tamil Nadu
7	CSH 9 (SPH 61)	1983	296A × CS3541	NRCS (DSR)	Maharashtra, AP, MP, Gujarat, Rajasthan, UP.
8	CSH 10 (SPH 196)	1984	296A × SB 1085	Dharwad	Maharashtra, Karnataka, AP, MP, Rajasthan, UP
9	CSH 11 (SPH 221)	1986	296A × MR 750	ICRISAT	Maharashtra, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
10	CSH 14 (SPH 468)	1992	AKMS14A × AKR 150	Akola	Maharashtra, Karnataka, AP, UP, Rajasthan, Tamil Nadu
11	CSH 13K & R (SPH504)	1995	296A × RS 29	NRCS (DSR), Hyd.	Maharashtra, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu, Karnataka
12	CSH 16 (SPH 723)	1997	27A × C43	NRCS (DSR)	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
13	CSH 17 (SPH 660)	1998	AKMS 14A × RS 673	NRCS (DSR)	Karnataka, Gujarat, MP, Tamil Nadu
14	CSH 18 (SPH 960)	1999	IM 9A × Indore 12	Indore	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
15	CSH 21 (SPH 1342)	2005	MLSA 848 × MLR 34	Mahendra Hybrid Seeds, Jalna	For the zones I and II: Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Gujarat, Rajasthan, Uttar Pradesh

(continued)

Table 7.2 (continued)

S. No.	Hybrid/variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
16	CSH 23 (SPH 1290)	2005	MS 7A × RS 627	NRCS (DSR)	– do –
17	CSH 25 (SPH 1567)	2005	PMS 28 A × C43	Parbhani and DSR	All major kharif sorghum areas of Maharashtra, Karnataka for sole crop
18	CSH 26 (SPH 1629)	2011	MLSA 848 × R 400	Devgen Seeds & Crop Tech Pvt. Ltd, Secunderabad	Maharashtra, Karnataka, MP, South Gujarat, North AP, and Tamil Nadu
19	CSH 27 (SPH 1644)	2012	279 A × CB 11	DSR, Hyderabad	Rajasthan, north Gujarat, UP, AP, and Tamil Nadu
20	CSH 28 (SPH 1647)	2013	NS 516A × NS444R	Nuziveedu Seeds, Secunderabad	Maharashtra, Karnataka, MP, south Gujarat, and north AP (zone II)
21	CSH 29 (SPH 1648)	2013	501A and 606R	Mahodaya Hybrid Seeds Pvt. Ltd, Jalna	Maharashtra, Karnataka, MP, South Gujarat, and North AP (zone II)
22	CSH 30 (SPH 1655)	2012	415 A × CB33	DSR, Hyderabad	Maharashtra, Karnataka, MP, South Gujarat, North AP under rainfed kharif cultivation
23	CSH 32 (SPH 1674)	2014	MLA 55 and R 421	Devgen Seeds	Maharashtra, Karnataka, MP, South Gujarat, and north AP
24	CSH 33 (SPH 1703)	2015	NS 509A × NB 235 R	Nuziveedu Seeds	Rajasthan, UP, North Gujarat, South Andhra Pradesh, and TN
25	CSH 34 (SPH 1702)	2016	HTJP001 A × HTJP002 R	Hytech Seed	Maharashtra, Karnataka, MP, AP, Chhattisgarh Gujarat, and Rajasthan
26	CSH 35 (SPH 1705)	2016	AKMS 30 A × AKR 504	Akola	Maharashtra, Karnataka, MP, South Gujarat, and Telangana
27	CSH 37 (SPH 1778)	2017	HTJP004A × HTJP007R	Hytech Seed	All India

28	CSH 38 (SPH 1779)	2017	HTJP008A2X HTJP006R	Hytech Seed	All India
29	CSH 41 (JAICAR GOLD; SPH 1820)	2018	296-1A × C43	IIMR	All kharif sorghum-growing areas of MP, Rajasthan, Gujarat, Maharashtra, Karnataka, AP, TS, and TN
30	CSH 42 (SPH 1883)	2020	Single cross Hybrid: 2219A × SVD 1278 SVD-1278 is a R line developed by pedigree breeding from a NCB breeding material code 09R-GQ-02	UAS-Dharwad	Karnataka, Madhya Pradesh, Andhra Pradesh, and Gujarat

Table 7.3 Rabi sorghum hybrids released at national level

S. No.	Hybrid	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSH 7R	1977	36A × 168	NRCS(DSR) IIMR	Maharashtra, Karnataka, AP
2	CSH 8R	1977	36A × PD3-1-11	Parbhani	Maharashtra, Karnataka, Gujarat, AP
3	CSH 12R (SPH 218)	1986	296A × M148-138	Dharwad	Maharashtra, Karnataka, Gujarat, AP
4	CSH 13R (SPH 504)	1991	296A × RS29	NRCS (DSR)	Maharashtra, Karnataka, Tamil Nadu, Gujarat, AP
5	CSH 15R (SPH 677)	1995	104A × RS 585	NRCS (DSR)	Maharashtra, eastern parts of Karnataka, Tamil Nadu, Gujarat, AP
6	CSH 19R (SPH 1010) (AKMS 9601(R))	2000	104A × R354	Akola	Maharashtra, Karnataka, Gujarat, AP
7	CSH 31R (SPH 1666)	2013	MLSA 1426 × 6644R	Devgen Seeds, Secunderabad	All rabi sorghum-growing states of India
8	CSH 39 R (SPH 1801)	2017	AKRMS 66-2 XSPV 1359-3	PDKV Akola	All India—deep black soils

varieties like UPMCH 503 have been released. In addition to high green and dry fodder yields and wide adaptability, these varieties have high seed production, resistance to foliar diseases, and better-quality fodder. From an interspecific cross, a multi-cut variety, Co(FS)29, was released from Coimbatore in 2001. Multi-cut hybrids developed at Pantnagar, CSH 20MF and CSH 24MF, have improved fodder yield and quality (Table 7.4).

7.10 Major Constraints of Hybrid Adoption

7.10.1 Kharif Sorghum

The yield levels of kharif sorghum hybrids and varieties are showing a tendency toward plateauing, and critical studies are needed to get over this. This could be accomplished through (a) wider exploitation of genetic resources in line improvement, (b) refinement of breeding procedures, (c) diversification of male sterile parents including the use of diverse cytoplasmic sources and restorers, and

Table 7.4 Forage sorghum hybrids released at national level

S. No.	Hybrid	Year of release	Pedigree of the hybrid/ variety	Centre which developed	Area for which recommended
1	CSH 13	2000	296 A × RS 29	NRCS (DSR)	All India cultivation
2	PCH 106	1997	MS 2219 A × PC 23	IARI, New Delhi	All India cultivation
3	CSH 20MF (UPMCH 1101)	2005	2219 A × UPMC 503	Pantnagar	All India cultivation
4	CSH 24MF (UTMCH1302)	2009	467 A × UPMC 503	Pantnagar	All India cultivation
5	CSH 36 F (SPH 1752) (DFSH 109/Dairygreen)	2017	MLA0052 × MLSFR0179	Syngenta India Ltd. Pune	Single-cut forage for irrigated growing condition for medium to high soil fertility under normal sowing window for spring and kharif season for Delhi, Gujarat, Rajasthan, Uttarakhand, Haryana, Uttar Pradesh, and Punjab
6	CSH 40F (SPH 1797); (UTFSH 2)	2017	11 A2 × Pant Chari 5	G.B. Pant University of Agric. and Tech., Pantnagar	Delhi, Gujarat, Rajasthan, Uttarakhand, Haryana, Uttar Pradesh, and Punjab Maharashtra, Tamil Nadu, Karnataka, and Madhya Pradesh for cultivation during rainfed kharif season. Optimum temperature for good crop growth 28–350 °C
7	CSH 43 MF (SPH 1881)	2020	11A2 × Pant Chari 6	G.B. Pant University of Agric. and Tech., Pantnagar	Haryana, Punjab, Rajasthan, Gujarat, Uttarakhand, Uttar Pradesh, Maharashtra, Tamil Nadu, Telangana, and Karnataka

(d) incorporation of multiple resistance traits for insect pests, diseases, and striga. The major constraints for hybrid in kharif are discussed below.

7.10.1.1 Lack of Demand for Kharif Sorghum as Food

Kharif sorghum is not preferred as food because of quality concerns. However, the utilization of kharif sorghum grain as a raw material in various industries is increasing, given the limited prospects of rainy season (kharif) sorghum for human consumption. The main industries currently using sorghum in India are the poultry feed, animal feed, and alcohol distilleries.

7.10.1.2 Poor Grain Quality of Kharif Sorghum

The kharif seed is not as remunerative as that of rabi produce because the seed is not round and lustrous. Though grain yield was doubled by utilization of exotic breeding material in hybrid program, this resulted in relatively poor grain quality (in turn led to consumers' non-preference and price differences between locals and hybrids). The grain quality of the hybrids was inferior to that of local varieties as selection process among temperate × tropical crosses was more on heterotic lines rather than on quality of the grain. Efforts are underway to make kharif seed bold and lustrous by using diverse germplasm lines from world collection.

7.10.1.3 Susceptibility to Grain Molds

Development of high yielding cultivars with relatively early maturity resulted in coincidence of grain development with late rains during certain years. This increased the incidence of grain molds that largely nullified the advantage of increased productivity.

7.10.1.4 Susceptibility to Shoot Fly

High yielding cultivars of sorghum are also generally vulnerable to shoot fly particularly under late sowing.

7.10.1.5 Yield Plateau in Kharif Hybrids

Looking over the years, whenever there was a change in male sterile line, the yield increased. There is a need for developing new male sterile lines having better combing ability in comparison to that of available MS lines. Till now, a lot of germplasm of different botanical races have been utilized in the development of parental lines. The grain yield levels of rainy season hybrids have reached plateau, and there is a need to exploit unused germplasm. The yield genes are to be identified from unexplored photosensitive germplasm lines from world collection, and these lines are to be converted into photoinsensitive ones before proper use.

7.10.2 Rabi Sorghum

Difficulties in recombining grain and fodder yield, grain quality, and resistance to biotic and abiotic stresses equivalent to popular variety Maldandi restricted genetic

improvement to a few varieties and hybrids only. Multiple trait selection is coupled with low genetic advance in rabi sorghum. Both grain and fodder being of higher value than that of kharif, building high grain yield and increased harvest index at the expense of fodder yield could not be favored. This is one of the reasons why there is low magnitude of heterosis in rabi compared to kharif.

Contrary to kharif hybrids, the heterosis in rabi hybrids is insignificant because the landraces (which are low community performers) are used (mainly to maintain the consumer-preferred grain size and luster) in the development of both parents. It is envisaged that introduction of larger grain size and luster in the female parents of kharif hybrids by novel methods and hybridizing such female parents with rabi based R lines would increase the yield levels of rabi hybrids to fetch better farm incomes to farmers.

Other constraints restricting the yield improvement in rabi sorghum include:

- Growing crop over large area in medium to shallow soils where the occurrence of drought is much faster than the deep soils
- Susceptibility to shoot fly and charcoal rot
- Low temperature affecting growth
- Lack of appropriate hybrids with required traits of rabi adaptation and limited response to applied nutrients under moisture stress situation. Low heterosis because of using maldandi for the improvement of both parents

7.11 Biotic Stresses

In the early 1960s, the first organized sorghum improvement program was started in India when the Indian Council of Agricultural Research (ICAR) initiated an "Accelerated hybrid sorghum project" in 1962. The intensive research efforts of this project resulted in the development of the first commercial hybrid, CSH1, in 1964. Thereafter, the development and release of new sorghum hybrids and varieties marked a genetic breakthrough in the yield levels of sorghum in India. During the 1980s and 1990s, focus was shifted to insect and disease resistance breeding. Initially, it was mainly concentrated on the screening of germplasm for insect and disease resistance, identifying traits associated with resistance, inheritance of resistance, and development and evaluation of segregating populations. As of now, significant improvements have been made particularly in developing screening techniques for major pest and diseases. New resistance germplasm lines have been identified for shoot fly (*Atherigona soccata*), stem borer (*Chilo partellus*), midge (*Stenodiplosis sorghicola*), aphids (*Schizaphis graminum* and *Melanaphis sacchari*), shoot bug (*Peregrinus maidis*), and grain mold, charcoal rot, and foliar diseases (Das and Padmaja 2016).

7.11.1 Breeding for Resistance to Diseases

Among the diseases of sorghum, grain mold is the most important during *kharif* seasons followed by foliar diseases, viz., downy mildew, anthracnose, and ergot. Out of these diseases, grain mold is one of the principal reasons behind reduction in *kharif* sorghum area. Mechanism of resistance against grain mold and its inheritance are very complex with significant genotype-environment interaction. The Guinea and zera-zera races from West Africa have provided resistance against grain mold and other foliar diseases. Through introgression program using these sources, which were otherwise not agronomically desirable, 14 genetic stocks have been developed and registered with the National Bureau of Plant Genetic Resources. Converted MS lines with grain mold resistance helped in infusing resistance in the hybrids and cultivars. In all of India, testing moderate resistance among test entries remained a focused attention. Significant tolerance against grain mold was infused in CSH 16. Integration of tan pigmented plant types in *kharif* program further helped breed disease-resistant varieties and parental lines. Unlike *kharif* sorghum, grain mold or other foliar diseases are not of major concern in *rabi* sorghum. Charcoal rot is a major problem, which gets aggravated under water stress. Resistance among high yielding *rabi* cultivars is low. Incorporation of drought tolerance is likely to infuse charcoal rot tolerance, and this problem is handled accordingly.

7.11.1.1 Grain Mold

Grain mold is one of the most important biotic challenges to address in kharif (rainy season) sorghum-growing areas. Overall the sorghum grain per hectare has increased, but the total area under cultivation has been declining. There are several reasons for this decrease such as low demand for sorghum, limited commercialization, limited yield gains, and grain mold susceptibility. The grain molds are caused by complexes of fungi, predominantly by *Fusarium moniliforme*, *Curvularia lunata*, *F. semitectum*, and *Phomasorghina*. Breeding grain mold-resistant sorghum hybrids and varieties requires identification of useful resistance gene(s) in the germplasm or defining other sources of such genes. Highly significant correlations between measures of grain mold and seed hardness, seed phenol content in acid methanol extract, and glume color indicated that they strongly affected grain mold response. Harder grain, higher levels of seed phenols, and darker glumes contributed to grain mold resistance. Weaker and less consistent correlations between measures of grain mold and seed color, seed flavan-4-ol content, glume phenol and flavan-4-ol contents, and glume cover indicated relatively less effect of these traits on grain mold response.

Success in developing grain mold resistance in high yielding genotypes was limited because of the fact that grain mold resistance is governed by multiple mechanisms of resistance and many agronomically undesirable traits are associated with the resistance (Aruna and Audilakshmi 2004). Moreover, resistance should be incorporated in the genotype with acceptable grain quality, which is governed by several major and minor genes showing significant genotype (G) × environment (E) interactions. Due to this, breeding efforts in about three decades to develop grain

mold resistance in high yielding genotypes have not paid many dividends. The complex genetics of mold resistance is due to the presence of different mechanisms of inheritance from various sources. Evaluation of segregating population for resistance and selection for stable derivatives in advanced generations in different environments was found effective. The grain mold occurring before physiological maturity (PM) is influenced by genetics and to some extent by the environment. Efforts were made to assess the host plant resistance at pre-PM stage.

Along with conventional breeding, population breeding approach has been initiated during 2000 to tackle grain mold problem. There is an improvement of grain mold incidence over three cycles of random mating (personal communication). Through random mating population, an improvement to the tune of grain mold score 7 in cycle I and score of 6.4 in cycle II was realized in B lines from the base score of 9.0. Similarly, improvement of 6.5 score in cycle I and 5.4 in cycle II was realized over the base score of 8 in case of R lines.

7.11.1.2 Foliar Diseases

In sorghum, apart from grain mold, downy mildew, anthracnose, and ergot are the major diseases during kharif season. Sorghum crop is also infected by several foliar diseases like leaf spots, chlorotic stripe virus, and rust which are prevalent under warm humid conditions and are highly destructive. These diseases reduce the amount of green leaf area available for photosynthesis and affect the quality of fodder by reducing the protein, zinc, and IVDMD (in vitro dry matter digestibility). The use of host plant resistance is considered to be more practical and reliable for managing foliar diseases. To develop resistant cultivars, plant breeders require a detailed knowledge of the inheritance of resistance. Resistance can be complex, controlled by a single or several genes depending on the source of resistance, plant development stage, and the pathotype used. Recently, genes for anthracnose resistance in sorghum have been mapped to chromosomes SBI-05 and SBI-08 (Perumal et al. 2008). Mohan et al. (2010) reported QTLs (quantitative trait loci) conferring resistance to foliar diseases. This information generated will be useful for transferring resistance into elite susceptible cultivar through marker-assisted selection (MAS).

7.11.1.3 Charcoal Rot

Charcoal rot caused by *Macrophomina phaseolina* Tassi (Goid) is a stress-related disease. The disease is prevalent in entire rabi (post rainy) sorghum-growing tracts. Post-flowering lodging and poor grain filling indicate that the crop is infected by this disease. There are studies on inheritance of this disease. It was noticed that dominance of susceptibility governed by three major, one basic complementary, and two duplicate complementary genes. Reports of partial dominance, non-additive with a high degree of dominance (Indira et al. 1984), polygenic threshold character governed by partial dominance, duplicate epistasis, and low heritability among the crosses involving resistant and susceptible parents have been studied. Polygenic nature of susceptibility was reported with dominance in the F_1 generation. On further investigations it revealed non-allelic interactions, like additive $\times$ dominance and

dominance × dominance with a major role in an inheritance of this trait. Dominance gene action was observed for resistance with over-dominance and low heritability values for number of nodes crossed and length of stem infected by the pathogen (Garud and Borikar 1985). Phenolic compounds produced by sorghum plants also attribute resistance against pathogens by inhibiting spread of fungus during the dry period thus making sorghum plant tolerant to pathogen.

7.11.2 Breeding for Resistance to Insect Pests

Shoot fly is the most important insect pest of sorghum both in *kharif* and *rabi* seasons, followed by stem borer and midge. Large-scale screening of germplasm against shoot fly has led to identification of moderately resistant sources. However, transfer of shoot fly resistance to cultivars has still remained elusive due to complex nature of inheritance. IS 18551 is a proven source of resistance against shoot fly. Major QTLs governing shoot fly resistance have been identified, and currently efforts are being made to convert parental lines of popular hybrids through marker-assisted selection. Against stem borer efforts are being made to use identified sources of resistance like IS 2205 in breeding program and to screen the advanced lines at hot spots. In case of both shoot fly and stem borer, simultaneous screening of new cultivars under all India coordinated programme at the hot spots has led to identification and release of cultivars with moderate tolerance. Transgenic approaches are also being made to infuse resistance against stem borer using *Cry1b* gene, and initial field evaluation of advanced events showed much promise. Sources of resistance against midge have been identified. One midge-resistant cultivar, DSV 3, has been released for the state of Karnataka, which is a selection from ICSC 745. In recent past, aphid is emerging a major pest, particularly under *rabi* sorghum. Sources of resistance have been identified, and currently, efforts are being made to understand the genetics of aphid resistance for deciding effective breeding strategies.

7.11.2.1 Shoot Fly

The major task of screening 10,000 germplasm and variety world collections was systematically done at different locations. Initially, the "deadheart" was taken as the parameter for evaluating resistance. This work resulted in the identification of a number of varieties with comparatively less shoot fly damage. The identified lines were from the rabi-growing areas. The genetic studies revealed that the shoot fly resistance is a very complex trait and quantitative in nature with various mechanisms of resistance (Dhillon et al. 2006). Resistance attributing traits like leaf glossiness, trichome density on lower surface of leaves, and seedling vigor deter shoot fly from egg laying and confer resistance (Dhillon et al. 2005). The inheritance studies also revealed that the alleles for shoot fly resistance are contributed by both resistant and susceptible parents and thus require methodologies to bring together the favorable alleles present in the resistant and susceptible genotypes. Resistance exhibits partial dominance under low infestation but appears to be partially recessive under high infestation. Insect populations vary from location to location and season to season,

causing varying degrees of damage. In the absence of high levels of resistance, persistence or stability of even a low level of resistance is of considerable value. This can be handled by the application of advanced breeding technologies like marker-assisted breeding (Aruna et al. 2011a, b, c). Identification of varieties with such inherent genetic characteristics is useful for a resistance breeding program. Subsequent screening in the All India Coordinated Sorghum Improvement Project (AICSIP) has identified a number of resistant lines. During kharif 2009, 1321 experimental lines (1250 lines from National crossing Block, 26 advanced progenies from MAS program, 24 shoot fly nursery lines, and 21 germplasm accessions) were evaluated for shoot fly resistance. About 21 lines were selected that which showed on a par performance with resistant check IS 2312.

Recently, in one of the novel approaches, field observations have suggested that shoot fly-susceptible sorghum varieties emit attractive volatiles. Efforts are underway to identify plant-derived attractants for shoot fly in relation to breeding sorghum varieties less attractive to this pest (Padmaja et al. 2010).

7.11.2.2 Stem Borer

Sources for stem borer resistance were reported by Trehan and Butani (1949). About 3953 germplasm lines from world collection were systematically screened. Plant height, tassel percentage, stem thickness, number of leaves, leaf length, leaf width, leaf thickness, and leaf strength are negatively correlated with deadheart formation. Days to panicle initiation and shoot length are associated with resistance to stem borers. Genotypes with early panicle initiation (IS 12308 and IS 13100) escape deadheart formation due to inability of the larvae to reach the growing point. Faster internode elongation is also associated with borer resistance. Shoot length, moisture content, plant growth rate or seedling vigor, leaf glossiness, and ligular hairs are associated with resistance. Antibiosis is a major factor associated with stem borer resistance. A number of biochemical factors such as amino acids, sugars, tannins, phenols, neutral detergent fiber, acid detergent fiber, lignins, and silica content also contribute to resistance mechanism. Information on these factors is very important for any breeding program. On the basis of general (GCA) and specific combining ability (SCA), estimates revealed the additive type of gene action in governing resistance to spotted stem borer. Presence of both antixenosis and antibiosis to *C. partellus* in terms of reduced eggs per plant, larval survival, and development was reported in the advance breeding lines developed (Padmaja et al. 2012). Results indicate the transmission of characteristics responsible for resistance to the progeny from the resistant parent.

7.11.2.3 Sorghum Midge

Sorghum midge, *Contarinia sorghicola* (Coquillett), is probably the most common insect pest of sorghum in most of the sorghum-growing countries. Resistance sources have been reported from several countries. Midge resistance is a polygenically inherited trait and governed by both additive and non-additive gene action. Knowledge about the traits attributing resistance is important for developing screening techniques. Most of these traits are not present in single genotypes, so

there is need to club them in a common background to increase the resistance levels. Use of random-mating populations appears to be ideal for accomplishing this task. Patil and Thombre (1983) reported that both gca and sca effects were important for midge resistance. They found additive genetic variance greater than non-additive genetic variance. Also resistance has to be present in both parents of a potential hybrid.

7.12 Abiotic Stress Resistance Including Climate Change

7.12.1 Genetic Improvement Against Abiotic Stresses

Among biotic stresses, drought is the main production constraint in *rabi* sorghum. Though sorghum possesses excellent drought tolerance, post-flowering drought causes extensive yield losses to the crop. Stay greenness is considered as an important trait attributing drought tolerance in sorghum. Major QTLs contributing toward stay-green trait has been identified and are being attempted to be transferred to superior cultivars. Crossing between high yielding adapted lines and screening under stress situation has yielded release of several *rabi* adapted cultivars both at state and central level. The varieties are necessarily drought tolerant and were released based on the performance under specific soil situations.

7.12.2 Breeding Approaches for Drought Tolerance

The development and use of crop cultivars adapted to water-stressed conditions is a long-term solution for improving and stabilizing crop productivity. Sorghum will likely become much more important in arid and semi-arid regions of the world as the demand for limited fresh water and global warming trends increases. Drought resistance in sorghum is a complex trait affected by several interacting plant and environmental factors. The growth stage (GS) at which moisture stress occurs is very important in determining the response or reaction of sorghum to water stress. Strategies for genetic enhancement of crop plants for drought tolerance have been widely discussed. It has been postulated that the genes for yield and tolerance to stresses are different, at least at some of the loci, and therefore, drought tolerance can be improved without sacrificing substantial yield.

Four basic approaches to the breeding for drought tolerance/resistance have been proposed. The first is to breed for high yields under optimal conditions, i.e., to breed for yield potential, and then to assume that this will provide a yield advantage under suboptimal conditions. The second is to breed for maximum yield by empirical selection in the field in the target drought-prone environment. The success of this approach depends entirely on how variable the target environment is. It works well in the Indian post-rainy season environment, which is very predictable, but not in the rainy season environment, which is highly unpredictable. The third approach is to incorporate the selected physiological and/or morphological mechanisms conferring

drought tolerance into traditional breeding programs. The fourth breeding approach involves identifying key trait that confers drought tolerance at specific growth stages and its introgression into the high yielding background. This method involved selecting (through pedigree selection) breeding materials for specific traits such as (1) longer mesocotyl length for emergence under crust and grain yield under drought-prone and yield potential areas for early seedling stage drought; (2) for grain yield under drought-prone and yield potential areas alternatively for mid-season drought; and (3) for stay-green and non-lodging and grain yield under drought-prone and yield potential areas alternatively for terminal drought.

Crosses were made between high yielding adapted lines, and lines were selected for high yields under drought and/or with one or more drought-related traits. Selections from F_2 onward were made by evaluating the segregating material in alternate generations under specified drought (early, mid-season, and terminal stage) and in yield potential environments. The F_5/F_6 pure lines are evaluated for drought yields, potential yields, and specific drought-related traits. Testing for yield under mild stress was adequate as the rankings of genotypes for potential and drought yields were similar, since the drought-tolerant lines selected under mild stress had high yield potential in non-stress environments. However, breeding for drought tolerance has been slow due to G × E interaction effects (Sajjanar et al. 2011).

7.12.2.1 Heterosis Breeding for Drought

Rao and Khanna (1999) reported the superiority of sorghum hybrids over their parents for leaf area and dry matter production under both pre-flowering and post-flowering drought stress. Potential for hybrid breeding for drought tolerance in rabi sorghum was reported by Patil et al. (2013). The increased performance of hybrids than their parents is due to greater growth rates and greater total biomass production and higher harvest index with or without an apparent increase in leaf photosynthetic rates. Bhale et al. (1982) found that some sorghum hybrids showed heterosis for proline accumulation (known to confer drought tolerance) under moisture stress. Evidences suggest that wider adaptability of hybrids is due to their relative tolerance to a wide range of abiotic stresses including soil moisture stress and related factors than varieties. The improvement of per se performance and combining ability of parents for agronomic traits and grain yield under drought stress should be given strategic importance, considering that parental per se performance and general combining ability in sorghum are strongly correlated with hybrid performance (Bhavsar and Borikar 2002).

7.13 Quality Characters Including Biofortification

7.13.1 Genetic Improvement for Grain Quality

Physical quality of sorghum refers to color, size, texture, and luster of grain. Genetics of all these traits are quite complex. However, breeding efforts have succeeded in developing hybrids with bold and round grains by having at least

one parent with desirable trait(s). In *rabi* sorghum, the breeding efforts always revolved around grain quality in terms of physical appearance and roti-making qualities. Among recently released varieties, CSV 22, Phule Vasudha, Phule Chitra, and Parbhani Moti have very good *roti*-making qualities besides being high yielders. Besides *roti* quality, sorghum is also used for other preparations as well like pop sorghum, papad making, and hurda sorghum. Some such special sorghum varieties are RSSGV 46 (hurda purpose), RPASV 3 (papad making), SMJ 1, RSJ 1, PKV Ashwini (hurda purpose), AKJ 1 (flaking purpose), KMJ 1 (popping purpose), etc. In terms of biochemical quality, starch and protein quality, protein digestibility, and iron and zinc content are important characters. However, till recently, these traits were not the focus of breeding efforts due to complexity of the trait. Currently focused breeding efforts using biotechnological tools have been initiated at the national level. Efforts are also being made to identify specific cultivars for specific end uses. Grain sorghum can be processed to develop different end products, flours, semolina, alcoholic beverages, pet foods, packaging materials, etc. (Aruna et al. 2018). A wide range of end use products that can be made from sorghum demand different grain characteristics and altered crop ideotype. Lot of research is underway at the Indian Institute of Millets Research on suitability of sorghum for different end uses, and also a successful and sustainable value chain model has been furnished through innovations in sorghum food processing assuring sustainable food and nutritional security (Aruna et al. 2020a, b; Dayakar Rao 2018).

The rabi sorghum possess excellent grain quality and are characterized by bold and round grains and have a characteristic luster. Among various sorghum food preparations, the unleavened roti and bhakri are prepared and consumed traditionally. Compared to hybrids, landraces/varieties possess superior quality traits and are more suited for food purposes Dough and *roti* properties of hybrids were much inferior to those of the local cultivars (Anantharaman 1968). Viraktamath et al. in 1972 reported differences among varieties for culinary properties in sorghum and demonstrated that the traditionally grown cultivars possessed relatively superior culinary properties over recently developed cultivars. During the 1960s in India, several hybrid combinations superior for yield were developed; however, they were reported non-acceptable. Anantharaman (1968) observed considerable variation for *roti* quality among hybrids with pearly white grains. M 35-1 (*Maldandi*), a sorghum cultivar, is known for its good quality of *roti* due to having pearly white grain color, its flour having higher water-holding capacity and good organoleptic taste. However, this cultivar is low yielder. To evolve sorghum high yielding genotype coupled with these good roti qualities, systematic breeding program was planned and executed at MPKV, Rahuri.

Local landraces RSLG 428-1, RSLG 1238, and RSLG 1275 and the genotypes RSV 290, RSV 292, RSV 858, RSV 859, RSV 861, RSV 868, RSV 894, RSV 985, RSV 992, RSV 995, and RSV 999 were found to be promising for protein, sugar, water absorption, and soluble protein content and were suggested for further improvement in nutritional quality through breeding program. Among the latest cultivars, Phule Vasudha (RSV 423), CSV 22, and Phule Chitra (SPV 1546) were found to be the most promising for *roti* quality (Chavana et al. 2009). Bhakri

prepared from rabi sorghum is white and more palatable than that of kharif sorghum. Apart from roti, several other food preparations like popping are prepared and consumed in traditional sorghum-growing states. The popped sorghum grains were found to have much flavor and to be as nutritious as popcorn (Subramanian 1956). Popped sorghum grains are used in the preparation of sweet snacks, which are commonly consumed in Maharashtra. It was reported that popping varieties of sorghum belong to the *Talavirchina* group (*S. roxburghii* var. *hians*) characterized by small grain with a dense and corneous endosperm (Ayyangar and Ayyer 1936). *Pelalujonna* belonging to the Snowden species *S. membranaceum* was considered to be good for popping (Reddy 1957).

Apart from its major use as food, the crop finds its potential for alternative uses such as livestock and poultry feed, potable alcohol, and starch and ethanol production. In India, rainy season produce is mostly used for industrial purposes due to inferior-quality grains infected by molds. Mold-affected grains are generally not used for food purposes, but recent efforts are being made to remove mold-affected parts by decortication or pearling techniques and test its feasibility for food uses.

7.14 Breeding Approaches: Conventional and Non-conventional Including the Use of Genomic Tools

7.14.1 Conventional Approaches

Sorghum is grown in a wide range of physical conditions in locations ranging from the equator to over 50°N and 30°S. The crop is therefore subject to a wide variety of temperature, day length, and moisture regimes. Development of improved sorghum cultivars for a particular environment always involves breeding for adaptation to the specific climatic conditions found there. This is usually indicated by the appropriate crop duration for that environment and by acceptable and stable yield levels and appropriate grain qual

The type of cultivar required for a target location also influences the objectives of the plant breeder. For example, the height of a pureline variety for a specific environment and the heights of the parental lines of a hybrid for the same environment are likely to be different.

In addition, improved cultivars for specific locations must possess resistances to major constraints to production encountered and grain- and stover-quality factors appropriate for sorghum there. These constraints include biotic such as diseases, insects, and parasitic weeds and abiotic stresses, the requirements for which are usually quite different from one location to another. Resistances to these constraints are deliberately bred into cultivars by crossing resistant types with cultivars possessing other desirable traits and selecting plants with both resistance and desirable traits.

7.14.2 Trait-Based Approach

Trait-based approach for the genetic improvement of sorghum would make use of cutting-edge technologies of plant biotechnology and molecular biology to develop genotypes with improved performance under stress during crop growth and enhanced quality of the produce with extended shelf life of seed, grain, and novel sorghum products. Genomics has made rapid advances during the past decade. The sorghum genome has been sequenced, and important gene transcripts and regulatory mechanisms are being deciphered on a large scale worldwide. Our national program has already begun implementing precision breeding using molecular marker-based selection for traits under complex genetic control such as resistance to shoot fly, post-flowering drought, and grain mold.

Efforts are on to identify genes and alleles associated with abiotic stresses and quality using allele mining approach. The system has achieved a very high degree of success in producing genetically transformed plants for an array of genes of interest that add value to existing cultivars. In this background, it is necessary to explore and attempt the new technologies for improving relevant traits. The traits of interest to be addressed by new technologies include improving resistance to complex traits—biotic (shoot fly, grain mold, stem borer, aphids, etc.) and abiotic (drought, salinity)—improving quality (grain for food, poultry, and industry, fodder, stalk for ethanol production) and novel bio-products. In addition, research aimed at predicting heterosis and incorporation of apomixis needs to be pursued using new tools to help farmers realize the maximum yield potential at minimum cost.

7.14.3 Nutritional Quality

In the past, the sorghum project carried out several genetic studies to transfer quality protein/high lysine trait from Ethiopian sorghums with shriveled seeds and also a purdue mutant. From the late, photosensitive Ethiopian sources, early dwarf high lysine types with shriveled seeds and normal plump seed types were developed. The transfer of high lysine to plump seeds was partially successful. Studies need to be continued using modern biotechnology tools. β-carotene content in several yellow endosperm types was analyzed, and the stability of carotene was also studied. The studies need to be pursued.

7.14.4 Population Improvement

For incorporation of multiple traits and traits governed by quantitative genes and population improvement procedures are ideal. Using the Maldandi source of cytoplasm and allowing 4–5 years of inter-mating and crossing with diverse types, selfing was practiced, and fertile types were isolated. They were designated FR lines. Some of them were agronomically good. When some of the FR lines were crossed to male steriles, surprisingly, they did not restore fertility. We had a feeling

whether the restoration was apomictic. Development of improved populations for incorporating multiple traits is certainly a useful adjunct.

7.15 Emerging Challenges at National and International Level

Various factors leading to the decline of sorghum crop in Indian agriculture are a matter of concern. Sorghum-based agricultural systems need to withstand biotic and abiotic stresses because of their cultivation mostly in unfavorable soil and climatic conditions. Further, they also need to adjust to changing economic (prices and income) and policy-induced stresses as has been the case in India where subsidized wheat and rice are supplied through the public distribution system. With this background in view, there is a need to embark on future approaches for improvement. Promotion of genetic diversity, cropping system stability, and economic advantage or parity will become the major criteria. Genetic approaches should promote genetic diversity and cropping system performance and stability.

7.15.1 Kharif Sorghum

The higher order yield of the currently available hybrids and varieties is around 4–5 tons/ha. Average yields hover around 1 ton/ha only. The food use of kharif sorghum is declining. Marginal yield improvements at the experimental level without perceptible changes in grain quality or a major advance in insect resistance, etc. do not make much difference. Areas under kharif sorghum have been substantially reduced in the traditional sorghum areas due to the grain mold problem and competition from more remunerative crops (like cotton, maize, soybean, etc.), in spite of the fact that high yielding hybrids of sorghum were developed, and their replacement ratio had been very high (up to 90%). In view of the self-sufficiency in food grains in the country (from other cereals) and changing food habits of the people, demand for kharif sorghum grains as food has been substantially reduced. Hence, there is good scope to divert kharif sorghum grain to cattle and poultry feed, biofuel, and value-added food products as well as exports. Colored sorghums have good potential as feed and also for export purposes.

Future program on kharif sorghum improvement should concentrate on incorporation of resistance to grain mold by different tools, improvement of feed value, and improvement in starch and other attributes of sorghum grain for higher ethanol production and value addition for food products. Shoot fly resistance is being incorporated in sorghum cultivars of economic worth. Quality standards of both grain and forage to meet international standards need to be addressed Extension of sorghum cultivation to non-traditional areas (e.g., rice fallows) will produce mold-free grains, and hence it should be explored (e.g., in states like Andhra Pradesh, Odisha, Jharkhand, Bihar, and West Bengal and in all other states).

Exploration of non-conventional areas, for harnessing favorable environmental conditions for production of sorghum at highly cost-effective manner, is an avenue

for increasing sorghum production to compensate for lower production from kharif sorghum. Rabi/summer conditions in rice fallows do not experience limitations from biotic stresses like shoot fly and grain mold and therefore provide greater opportunity to realize higher yields from lower investment in terms of external inputs and natural resources (Prabhakar et al. 2015).

7.15.2 Rabi Sorghum

In view of the lack of significant progress in improving rabi sorghum yields, more basic and fundamental genetic studies are warranted to understand the genetic processes involved in the improvement of yield and the adaptation processes. Studies on heterosis in diverse crosses, temperature implications, lodging, and shoot fly and shoot bug resistance stability across early normal and delayed sowings in the respective regions under receding soil moisture situations need to be achieved.

Terminal drought is one of the major production constraints for higher rabi sorghum productivity and limits the use of purchased inputs like hybrid seeds and fertilizers. Therefore, research on drought tolerance involving development of early maturing rabi sorghum varieties and incorporation of stay-green and other drought QTL/genes needs to be taken up. Another important issue is the lack of substantial heterosis in rabi hybrids as landraces (which are low community performers) are used (mainly to maintain the consumer preferred grain size and luster) in development of both A/R parents. Breeding diversified A and R lines involving exotic durra and other sorghum races can be of much help. However, the basic requirements of grain quality, resistance to shoot fly, charcoal rot, and terminal drought, should be kept in mind.

Program to develop genotypes for mechanical harvesting is required in the light of non-availability, high cost of manual laborers, and difficulties due to crop loading. More employment of genomic tools to dissect the complex traits of rabi sorghum per se and its adaptability needs to be taken up in structured program involving all AICRP centers. Programs to implement genomic selections and genome editing may be planned as a long-term activity. In order to achieve higher genetic gains in rabi sorghum, new tools of speed breeding can be taken up (Prabhakar et al. 2015).

7.15.3 Forage Sorghum

Forage sorghum improvement programs are beset with lack of information on variability and useful genetic stocks for various traits. Therefore, concerted and planned efforts are needed to collect, evaluate, catalogue, and maintain germplasm exclusively for forage sorghum. Since sorghum is also important as a source of green forage in the country, and the diary and livestock industry is growing in a faster pace, strategies to develop efficient forage production systems are the need of the day. Research on evolving high yielding cultivars with good-quality, management practices to ensure fodder availability during lean seasons may be focused. More

hybrids and varieties with wider adaption are required for use across the country. Use of wide hybridization to diversify the genetic base may be explored to incorporate desired traits.

Major areas of improvement required for forage sorghum production in India include drought-tolerant high biomass single-cut types and high seed yielding multi-cut hybrid parents. Another important area is to augment the protein content in all types of forages, especially in the cultivars from public sector. The future emphasis should be to increase per day dry matter production in single-cut cultivars. Drought-tolerant types that can recover faster are preferred for both single- and multi-cut types.

To get a regular supply of green fodder for a longer period of time, multi-cut varieties having profuse tillering, quick regeneration, faster growth, and capability of giving a minimum of 4–5 cuttings should be developed. The high yield and multi-cut potential of Sudan grass should be further exploited in breeding program to develop highly adapted and high yielding stable multi-cut variety of forage sorghum. Attention should be paid to stability in production of biomass and nutrient content through resistance breeding. This can be achieved through tile incorporation of resistance to biotic factors, improved tillering capacity, and quick growth (Prabhakar et al. 2015).

7.15.4 Sweet Sorghum

In the context of climate change, sweet sorghum outperforms other crops as an attractive climate-resilient crop to produce ethanol, generate power, and reduce carbon emissions produced from fossil fuel utilization. One of the obstacles to this crop's expansion as a biofuel feedstock is the fact that sugarcane has established dominance over the production chain of sugar and ethanol, receiving the majority of the investments. A limiting factor for its widespread cultivation is the availability of varieties/hybrids adapted to different seasons and agroclimatic conditions with both biotic and abiotic stress tolerance, including cold tolerance. Consequently, research should address the optimization of sweet sorghum as an energy crop through breeding for enhanced productivity under limited available resources. Genetic improvement should focus on stalk sugar, biomass quantity and quality and general agronomic traits, and adaption of sweet sorghum to rabi conditions and arid saline and alkaline conditions.

Further improvement in brix juice volume and stalk yield ($\geq$45 tons/ha with hybrids) should be targeted in sweet sorghum to help improve the benefits to the industry and farmers without any detrimental effect on grain yield. Efforts may be made to improve the means of stabilizing the juice to minimize sugar loss during the storage. Production of high value-added products from sweet sorghum juice may be explored. Opportunities should be explored for the integration of sweet sorghum into cropping systems without compromising sustainability and disruption of crop production for other purposes including food. Sustainable value chains for sweet sorghum for ethanol should be developed and popularized by waking with sugar industry (Prabhakar et al. 2015).

Table 7.5 Sweet sorghum hybrids

S. No.	Hybrid	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSH 22 SS (NSSH 104)	2004	ICSA 38 × SSV 84	NRCS (DSR)	All India cultivation

The road blocks for large-scale cultivation of sweet sorghum are ethanol pricing, limited availability of genotypes suited to different agroclimatic conditions with all resistances, photoperiod sensitivity, and non-availability of required quantity of feedstock suited to off-season crushing in sugar industries. There is a need to develop sweet sorghum cultivars that produce high stalk yield per unit time, input, energy, and land area in different agroclimatic areas of the country. These cultivars should also be photo- and thermo-insensitive with desired levels of resistance/tolerance to various stresses and should be of different maturities to widen the harvest window which thereby ensures a continuous supply of feedstock to the industry. The predominant role of non-additive gene action for most of the sweet sorghum productivity traits like plant height, stem girth, total soluble solids, millable sweet-stalk yield, and extractable juice yield indicates the importance of heterosis breeding for genetic enhancement of sweet sorghum. The wide variability in germplasm and hybrid parents offers bright scope for the development of high stalk yielding sugar-rich varieties and hybrids. The introduction of the *bmr* trait in sweet sorghums would result in a dual-purpose bioenergy crop that address both first- and second-generation biofuel production issues. In sweet sorghum, a good effort has been made to release many varieties; however, one hybrid is developed already, and many are in pipeline (Table 7.5).

7.16 Need for Critical Analysis and Characterization of Germplasm Collection

Because of their evolution and adaptation to poorer soils and unfavorable agroclimatic conditions/environments, sorghum has developed unique attributes such as tolerance to drought and heat, adaptation to poorer soils and unfavorable conditions, nutritional and therapeutic values, processes of starch and vitamin bio-synthetics, etc. Some of these attributes have been discussed earlier. The germplasm collections of sorghum have been characterized for their morphological and physical attributes. In the present context, the germplasm collections should be analyzed for such specific physiological, nutritional, and therapeutic attributes and identify the genes controlling them and their bio-synthesis and gene markers characterizing such unique traits and processes. Such databases will be of immense value to the improvement of millet crops by traditional as well as biotechnological methods and marker-aided selection.

7.16.1 Public-Private Partnerships (PPP)

Public-private partnerships are described as collaborative efforts between public and private sectors in which each sector contributes to the planning, resources, knowledge, and capabilities needed to accomplish mutual objectives. Diversified use of sorghum for demand generation and improvement of income is market driven. They warrant collaborative research between agricultural research agencies, the concerned industry, or even industrial research laboratories. Some opportunities to be explored are in the areas as below.

Feed grains	Poultry feed and dairy feed manufacturing organizations with the private and cooperative sectors like NDDB
Ethanol	Sugar mills and ethanol manufacturing plants
Alcohol/beer	Breweries association
Starch and starch-based production	Starch industry
Exports	Export organizations
Straw	Straw board manufacturers

7.16.2 Other Important Issues

Government policy is bound to be in favor of support for promotion of sorghum due to increasing population growth rate and unmet demand for food consumption by rice and wheat. Thus, it is expected that government policies are going to be strengthened for millet promotion during different plan periods. Further, it is expected that creation of awareness is being given more importance so as to generate consumption demand owing to nutritional merits of sorghum. The increasing incidence of lifestyle diseases which are linked with relatively poor nutritive composition of fine cereals especially rice and the promotion of nutri-cereals such as sorghum will be more pronounced owing to their superior composition of nutrients and minerals. It is also expected that sorghum as a healthy food is being included in PDS which may gain some area under sorghum.

Overall, at least 88% increase in millet acreage over the current levels is expected to be attained by 2050 AD, if policy push and demand creation trend is going to be continued, even otherwise with current acreage, if continued with more productivity enhancement through R & D in place, and the increase in production will be doubled. Accordingly, the estimated production in sorghum with policy push and R & D enhancement will be 35.0 million tons, of which rabi production contribution alone will be 68% of total production.

Different genotypes suited to different growing conditions may be essential to bring in all-round increase in productivity. The economic gains that may be augmented by addressing envisaged benchmarks will result in significant improvement

in productivity, profitability, and even export earnings. All these are expected to translate sorghum farming into a healthy and prosperous proposition, justifying the public support for sorghum research in the country.

7.17 Breeding Progress/Varietal Development

7.17.1 Conventional Breeding

Sorghum improvement till the 1960s focused on selections from local landraces, which were tall with low harvest index, photosensitive, late maturing after seize of monsoon, and with localized adaptation. Notable varieties of this period are Saonar, Ramkel, Aispuri, PJ, Maldandi, and Dagdi selections from Maharashtra; Bilichigan, Fulgar white, Fulgar yellow, Kanvi, Nandhyal, Hagari, and Yanigar varieties from Karnataka; Nandyal (N), Guntur (G), and Anakapalle series from Andhra Pradesh; Co series from Tamil Nadu; BudhPerio (BP 53), Sundhia, and Chasation from Gujarat; Gwalior and Indore selections from Madhya Pradesh; and RS selections from Rajasthan. With the launching of the Accelerated Hybrid Sorghum Project through the Rockefeller Foundation, a wide range of germplasm was made available in India. This led to significant improvement principally through manipulation of plant height and maturity.

7.17.2 Kharif Varietal Improvement

Hybrid sorghum breeding and variety development programs continue simultaneously in India. First outcome of focused sorghum breeding in India was the release of early maturing variety, CSV 1, in 1968. The next decade witnessed the release of five varieties, viz., CSV 2 to CSV 6. Out of these, CSV 2 and CSV 3 were of early type, while CSV 4 and CSV 5 were dwarf, and CSV 6 was a relatively tall variety. Zera-zera landrace from Ethiopia was brought into use to incorporate resistance against biotic stresses. Further tan plant pigment got incorporated in all *kharif* nurseries to confer resistance against leaf diseases. During the 1980s, five varieties, like SPV 462 and CSV 9 to CSV 13 (K and R), were released. Further using SPV 462 and CSV 13 in crossing program, a high yielding dual-purpose variety, CSV 15, was released in 1996. Two new varieties were developed from the derivatives of crosses involving this variety, viz., early maturing CSV 17 for light soils and drought-prone areas and improved dual-purpose variety CSV 20. This was followed by the release of two more improved dual-purpose varieties, viz., CSV 23 and CSV 27. Besides these central releases, there are several varieties released for specific agro-ecologies of different states. These include Swati, PVK 801 (Parbhani Sweta), SPV 297, PKV 809, Pratap Jowar 1403, JJ 938, JJ 1041, GJ 36, GJ 37, GJ 38, GJ 40, DSV 1, PSV 1, NTJ 2, etc. (Prabhakar et al. 2015) (Tables 7.6 and 7.7).

Table 7.6 Nationally released kharif sorghum varieties

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSV 1	1968	Sel. from IS3924	NRCS (DSR) (currently IIMR)	All sorghum-growing areas in the country
2	CSV 2	1974	IS 3922 × Karad local	NRCS (DSR)	– do –
3	CSV 3	1974	IS 2954 × BP 53	NRCS (DSR)	– do –
4	CSV 4	1974	IS 3675 × IS 3541	NRCS (DSR)	– do –
5	CSV 5	1974	IS 3687 × Aispuri	NRCS (DSR)	– do –
6	CSV 6	1974	IS 3922 × Aispuri	NRCS (DSR)	– do –
7	CSV 7	1982	CS 3541 (Tall mutant)	NRCS (DSR)	– do –
8	CSV 10 (SPV 346) (SU 53)	1983	SB 1066 × CS 3541	Udaipur	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan
9	CSV 11 (SPV 351)	1985	(SC 108-3 × CS 3541)-11-1	ICRISAT	Maharashtra, Karnataka, AP, MP, UP
10	CO 26 (USV 24)	1985	(IS2947 × SPV232) × 1022	Coimbatore	Karnataka, Tamil Nadu, AP
11	CSV 13 (SPV 475)	1988	(IS12622 × 555) × IS 3612 × E 35-1-52	ICRISAT	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
12	CSV 15 (SPV 946)	1996	SPV 475 × SPV 462	NRCS (DSR)	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
13	CSV 17 (SPV 1489)	2002	SPV 946 × SPV 772	Udaipur	Low rainfall and drought-prone sorghum-growing regions of the country
14	CSV 20 (SPV 1616)	2006	SPV 946 × Kh 89-246	DSR, Hyderabad	All sorghum-growing areas of India
15	CSV 23 (SPV 1714)	2007	SPV 861 × SU 248	Udaipur	All sorghum-growing areas of India
16	CSV 27 (SPV 1870)	2012	(GJ 38 × Indore 12)-2-1-2-1; GJ 38 = GJ 35 × E 35-1	DSR, Hyderabad	All sorghum-growing areas of India
17	CSV 28 (SPV 1822)	2012	IRAT 204 × SPV 1134	MPUA & T, Udaipur	All sorghum-growing areas of India

(continued)

Table 7.6 (continued)

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
18	CSV 31 (SPV 2122)	2014	SPV 462 × SPV 1329	Palem	Andhra Pradesh, Tamil Nadu, Rajasthan and Gujarat
19	CSV 34 (SPV 2307)	2016	Sel from (AKMS 37 B × AKMS 60B)—3	PDKV, Akola	Maharashtra, Karnataka, Madhya Pradesh and Gujarat
20	CSV 36 (JAICAR HEERA; SPV 2301)	2017	SPV 1231 × NSV 13	IIMR-Hyderabad	Tamil Nadu, Telangana, Andhra Pradesh, Rajasthan, and Gujarat
21	CSV 39 (JAICAR SONA; SPV 2358)	2017	SPV 772 × SPV 1754	IIMR-Hyderabad	Maharashtra, Karnataka, Madhya Pradesh, Rajasthan, Gujarat, Tamil Nadu, and Telangana
22	CSV 43 BMR (JAICAR Nutrigraze; SPV 2018)	2019	SPV 2018 = (SPV 462 × IS 21891)-3-1-1-1	IIMR Hyderabad	Andhra Pradesh, Telangana, Karnataka, Maharashtra, Tamil Nadu, Gujarat, M.P, Rajasthan, UP, Uttarakhand, Haryana, and Jharkhand

Table 7.7 Nationally released dual-purpose sorghum varieties

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSV 15	1996	SPV 475 × SPV 462	NRCS (DSR)	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
2	CSV 20	2006	SPV 946 × KH 89-246	NRCS (DSR)	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
3	CSV 23	2007	SPV 861 × SU 248	Udaipur	Maharashtra, Karnataka, AP, MP, UP, Gujarat, Rajasthan, Tamil Nadu
4	CSV 27	2012	(GJ 38 × Indore 12)-2-1-2-1; GJ 38 = GJ 35 × E 35-1	DSR-Hyderabad	All sorghum-growing areas of India

Pedigree and origin of nationally released dual-purpose sorghum hybrids/varieties

7.17.3 Varietal Improvement in Rabi Sorghum

Focused *rabi* sorghum breeding was initiated in the early 1970s. The most popular *rabi* variety, M35-1, was released in 1969 from Mohol, Maharashtra. It has remained popular among farmers over the past five decades for its stable performance under rainfed situations with above average yield and bold lustrous grains. Most of the present-day improved varieties are the result of pureline selection practiced among the local/popular varieties and their crosses. The popular varieties have lustrous, bold, and globular grains. At the national level, the first *rabi* variety CSV 7R was released in 1974. Subsequent releases are CSV 8R, Swati, CSV 14R, Sel 3, Phule Yashoda (CSV 216R), CSV 18R, CSV 22R, CSV 26R, and CSV 29R. Out of these, the last five are released in this millennium. Besides these, there are several state varieties like for the state of Maharashtra are Phule Maulee, Phule Anuradha, Phule Revati, Phule Vasudha, Phule Chitra, and Phule Suchitra from Rahuri center, Mukti, Parbhani Moti (SPV 1411) from Parbhani center, and PKV Kranti from Akola. Similarly, popular varieties released for Karnataka state are DSV 4 and DSV 5. NTJ 2 and NTJ 3 were released from the Nandyal station for Andhra Pradesh state. Many of these varieties have yielding ability better than five-decade-old local variety M35-1, with roti making quality at par or even better. Soil depths play an important role in *rabi* sorghum-growing ecologies. In recent past, efforts have been made to develop varieties adapted to specific soil situations (shallow, medium, and deep). The variety Phule Maulee is released for shallow to medium soils, Phule Chitra and Phule Suchitra for medium soils, Phule Vasudha for deep soils, Phule Revati for medium to deep soils, and Phule Anuradha for shallow (Prabhakar et al. 2015) (Table 7.8).

Table 7.8 Nationally released rabi sorghum varieties

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSV 7R	1974	IS 2950 × M35-1	NRCS (DSR)IIMR	Maharashtra, Karnataka, Tamil Nadu, Gujarat, AP
2	CSV 8R	1979	R24 × R16	NRCS (DSR)	– do –
3	CSV 14R (SPV 839)	1992	(M35-1 × (CS -2947 × CS 2644) × M35-1	NRCS (DSR)	– do –
4	CSV 216R (SPV 1359) (RSV 56)	2000	Land race selection from rabi germplasm Dhulia	Rahuri	– do –
5	M35-1	1969	Land race selection from local Maldandi bulk	Mohal	Maharashtra
6	Swati (SPV 504)	1984	SPV86 × M 35-1	Rahuri	Maharashtra
7	Sel.3	1995	Sel. from Bidar rabi local	Rahuri	Maharashtra
8	CSV 18 (Parbhani Jyothi) (SPV 1595)	2005	A selection from cross (CR 4 × IS 18370)	Parbhani	For zone II
					Maharashtra, Karnataka, AP under irrigation
9	CSV 22 (SPV 1626)	2007	SPV 1359 × RSV 2	Rahuri	Maharashtra, Karnataka, Tamil Nadu, Gujarat, AP
10	CSV 26 R (SPV 1829)	2011	Selection from the cross SPV-655 × SPV-1538	DSR (IIMR)	Rabi season. Under shallow soils conditions of rabi sorghum-growing areas of India.
11	CSV 29R (SPV 2033)	2012	(CSV216R × DSV5) × CSV216R	Bijapur	Rabi sorghum-growing areas of Maharashtra, Karnataka and Andhra Pradesh under deep black soils

7.17.4 Forage Sorghum Improvement

Forage sorghum is cultivated in about 3.0 million ha area in India each year. Forage sorghums are principally cultivated in Punjab, Haryana, Delhi, western and central Uttar Pradesh, and adjoining areas of Madhya Pradesh. In these states, it is grown during *kharif* and summer seasons, either as single-cut (mostly in *kharif*, as rainfed) or as a multi-cut (summer and *kharif*) forage crop. The major objectives in forage sorghum breeding are to develop varieties both for single cut and multi-cut with high tonnage, better quality, good seed yield, and resistance to insect pests and diseases. The cultivar improvement before the 1970s was mostly through the straight selection on available varieties and landraces. During this period, T-8B, 5-Tall, No. 9, 30-C, and T-4 were released in Uttar Pradesh for grain as well as fodder production. JS 20, JS 29/1, and JS 263 were released exclusively for fodder purposes in the state of Punjab. Out of these, JS 263 was sweet and juicier. These varieties were early in flowering and possessed early vigor and high growth rate but were highly susceptible to foliar diseases and single tillered with poor seed potential.

Concerted breeding efforts for the improvement of forage sorghum were initiated in 1970 under AICSIP. During the 1970s, single-cut varieties, viz., Pusa Chari 1, Haryana Chari 73/53, and SL44, were bred at IARI, Hisar, and Ludhiana, respectively. In 1977, multi-cut forage sorghum variety SSG 59-3 developed at Haryana Agricultural University. Barring the problem of poor seed yield, it is now one of the most popular multi-cut varieties. Subsequently, in the 1980s, another multi-cut variety, Pusa Chari23, was released, but it was highly susceptible to foliar diseases. During this period, three single-cut forage sorghum varieties, PC 6 (from IARI), HC136 (from Hisar), and UP Chari1 (from Pantnagar), were released for general cultivation across India. Other single-cut varieties, PC 9, RC 1 and 2, and UP Chari 2, were also released in that decade. Later from Hisar, two more single-cut varieties, viz., HC171 and HC 260, were released for the whole country. Some of the recent single-cut forage sorghum varieties are CSV 30F and CSV 32F. The latest forage cultivars possess improvement in terms of resistance to leaf spot diseases, stem borer, and seed yield. Beside these, several private sector hybrids are also popular for multi-cut forage (Prabhakar et al. 2015) (Table 7.9).

7.17.5 Sweet Sorghum Improvement

Sweet sorghum, similar to grain sorghum except for its juice-rich sweet stalk, is considered to be a potential bioethanol feedstock and is expected to meet food, feed, fodder, fuel, and fiber demands. Some sweet sorghum lines contain 15–23% soluble fermentable sugar (by comparison, sugarcane has 14–16%). Most of the sugars are distributed in the stalk, making the crop particularly amenable to direct fermentable sugar extraction. Further, the silage from sweet sorghum after extraction of juice has higher biological value. Besides these, the whole plant biomass can also be used as a substrate for production of lignocellulosic ethanol, known as second-generation biofuel. Early efforts toward sweet sorghum improvement were made at Nimbkar

Table 7.9 Forage sorghum varieties

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	HaryanaChari (JS73/53)	1976	Sel. from local germplasm	HAU, Hisar	All India cultivation
2	PC 1	1976	Sel. from IS 609 (bicolor)	IARI, New Delhi	All India cultivation
3	MP Chari	1978		Jabalpur	All India cultivation
4	PC 6	1980	Sel. from IS 5977 (Durra)	IARI, New Delhi	All India cultivation
5	HC 136	1982	IS 3214 (bicolor) × PC 7R	HAU, Hisar	All India cultivation
6	UP Chari-I	1982	Sel. from IS 4870 (Durra)	Pantnagar	All India cultivation
7	UP Chari-II	1985	Vidisha 60-1 × IS 6953 (Guinea-caudatum)	Pantnagar	All India cultivation
8	PC 9	1985	Sel. from IS 4870 (Durra)	IARI, New Delhi	All India cultivation
9	Rajasthan Chari—1	1985	CSV 6 × NCL 3	Udaipur	All India cultivation
10	Rajasthan Chari—2	1985	Sel. from local of Udaipur	Udaipur	All India cultivation
11	HC 171	1987	SPV 8 × IS 4776 (Durra)	HAU, Hisar	All India cultivation
12	HC 260	1987	SPV 103 × PC 9	HAU, Hisar	All India cultivation
13	HC 308	1996	SPV 8 × IS 4776 (Durra)	HAU, Hisar	All India cultivation
14	Pant Chari—5	1999	–	Pantnagar	All India cultivation
15	CSV 21F	2006	GSSV 148 × SR 897	NAU, Surat	All India cultivation
15	SSG 59-3	1977	(Non-sweet sudan grass × JS 263)	HAU, Hisar	All India cultivation
16	Pusa Chari—23	1985	Exotic hybrid Martin × 99070 10 sudan grass	IARI, New Delhi	All India cultivation
17	Jadu Chari	1991	1) (PFF x PFB 2) x PFM 1	Pioneer Seed Company	All India cultivation
18	Hara Sona	1995	(PFS 5A × PFS 5C) × PFS 5R)	Proagro Seed Company	All India cultivation

(continued)

Table 7.9 (continued)

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
19	Safed Moti		PSA 93016 × FSR 93025	Proagro Seed Company	All India cultivation
20	CSV 30 F	2014	NSS 223 × NARI 111	Rahuri	All India cultivation
21	CSV 32 F (SPV 2128)	2014	(HC 260 × B 35)-5-3-1-1	DSR, Hyderabad	Maharashtra, Karnataka, Tamil Nadu, Madhya Pradesh, South Gujarat Telangana, Andhra Pradesh, and Tamil Nadu
22	CSV 33MF (SPV 2242)	2016	EMS Mutant of CO FS 29	TNAU, Tamil Nadu	Haryana, Punjab, Uttarakhand, Uttar Pradesh, Gujarat, Rajasthan, Tamil Nadu, Karnataka, Maharashtra
23	CSV 35F (SPV2317); (UTFSH 2)	2017	Pant Chari 5 (female) × IS 7002 (male)	G.B. Pant University of Agric. and Tech., Pantnagar	Delhi, Gujarat, Rajasthan, Uttarakhand, Haryana, Uttar Pradesh, and Punjab Maharashtra, Tamil Nadu, Karnataka and Madhya Pradesh for cultivation during rainfed kharif season. Optimum temperature for good crop growth 28–350 °C.
24	CSV 38F—(SPV 2316 JAICARHariyali)	2018	(CSV 20 × Pant Chari 5) × (CSV 20 × PVK 809)-11-1-1-2-1-1	ICAR-Indian Institute of Millets Research, Hyderabad	Rainfed kharif areas of Maharashtra, Tamil Nadu and Karnataka

(continued)

Table 7.9 (continued)

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
25	CSV 40 F (SPV 2387)	2019	PVK 809 × 1037 R	Vasantrao Naik Marathwada Krishi Vidyapeeth—Parbhani	Rainfed, timely sown conditions of kharif season in Maharashtra, Tamil Nadu, and Karnataka
26	CSV 44 F (SPV 2445)	2020	Selection from cross HC 308 × S 437-1-2	CCSHAU, Hisar	Zone II of India comprising states Maharashtra, Tamil Nadu, Karnataka

Table 7.10 Sweet sorghum varieties released at national level

S. No.	Variety	Year of release	Pedigree of the hybrid/variety	Centre which developed	Area for which recommended
1	CSV 19SS (RSSV 9)	2004	RSSV 2 × SPV 462	MPKV, Rahuri	Maharashtra, Karnataka, AP, MP, and Gujarat
2	CSV 24 SS (SPSSV 6)	2010	NSS 1005A × (SSV 84 × 401B)	NRCS (DSR)	All India cultivation

Agricultural Research Institute (NARI), Phaltan, Maharashtra. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, major attempts were made to evaluate and identify useful sweet sorghum high biomass germplasm from world collections. Sweet sorghum research under AICSIP started in 1989. Concerted research efforts during the last two decades have resulted in excellent sweet sorghum varieties and hybrids for use in ethanol production and for use as green/dry fodder. Promising nationally released varieties/hybrids are SSV 84 (high brix), CSV 19SS (High stalk yield, shoot fly tolerance), CSV 24SS (High stalk and sugar yields), and hybrid CSH 22 SS (High stalk and sugar yields) (Table 7.10). The productivity ranged between 40 and 50 tons/ha. Stalk yields obtained during *rabi* are 30–35% less with reduced sugar content than *kharif* and summer-grown crops (Prabhakar et al. 2015).

7.18 Genomics-Assisted Breeding

The genetic diversity in sorghum provides an opportunity to search for new genes and alleles that are responsible for conferring desirable phenotypes. Genome profiling using molecular markers would provide a large number of DNA markers. Association mapping methods, joint linkage and linkage disequilibrium mapping, genetic fingerprinting and diversity analyses, and genomic selection programs would pave way for effective utilization of sorghum germplasm for crop improvement. The development of large mutant population as a reverse genetic tool is envisaged to unravel the expression of battery of genes and the mechanisms of their regulation.

State-of-the-art technologies would be utilized to select for traits that are otherwise difficult to measure or that requires particular conditions for their expression. Further, they provide genetic fingerprints that measure relationships between different lines of sorghum and can be used to protect IPRs. Also, development of gene variant specific markers for major genes controlling adaptive traits would help in accomplishing requisite level of trait expression. It may be expected that genome-wide selection methods that incorporate marker technology into practical breeding processes would be routine in the future.

Another dimension of accomplishing traits of interest including novel ones in sorghum cultivars is the deployment of transformation technology to transfer the genes of interest or regulate the expression of host genes. The Bt transgenic sorghum already developed in the system not only holds promise as an important source of resistance to stem borer but exemplifies the possibilities of incorporating new genes into sorghum for innumerable end uses. A similar approach would be a major option for improving resistance to shoot fly, grain mold, aphids, etc. if suitable candidate genes are identified. Research in functional genomics of sorghum would pave way for identifying the sorghum candidate genes for such manipulations.

New avenues in understanding the genomics, evolution, and biology of sorghum crop were opened with the availability of whole genome sequence (Paterson et al. 2009). In addition, gene-level comparative analysis was also made possible with the sequencing of genomes of landraces, crop progenitors, and wild species. With the availability of the sorghum genomic sequence, development of molecular markers progressed steadily from development of genome-wide DNA markers such as simple sequence repeats (SSRs), intron length polymorphism (ILP), insertion deletions (indels), and single nucleotide polymorphisms (SNPs). Many high-density SNPs are being generated for sorghum due to the advances in high-throughput sequencing technologies, and these SNPs are valuable genomic resources for designing SNP chips/arrays and GWAS leading to the identification of key genomic regions associated with important target traits. This further helped in the identification of genomic regions/QTL associated with economically important target traits, which are the current targets for marker-assisted breeding in sorghum. Several studies on transcriptomes have identified differentially expressed genes (DEGs) related to abiotic stress tolerance paving way for better understanding of cellular and molecular responses of the plants to stress factors. The data/information on whole genome sequence, SNPs, gene expression, gene families, gene networks, and

annotation are available in the databases such as Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html), SorGSD (http://sorgsd.big.ac.cn), MOROKOSHI (http://matsui-lab.riken.jp/morokoshi/Home.html), Sorghum Transcription Factor Database (http://planttfdb.gao-lab.org/index.php?sp=Sbi), and SorghumFDB (http://structuralbiology.cau.edu.cn/sorghum/index.html).

Development and use of climate-resilient crops and genotypes therein are critical to safeguard against global food scarcity and malnutrition (Mickelbart et al. 2015). Sorghum is well-known for its ability to thrive under harsh growing conditions across major global agricultural production regions. Understanding genetic variation and underlying adaptive traits that are responsible for sorghum's ability to tolerate challenging growing environments would have implications for additional improvement in sorghum as well as other agronomically important crops (Boyles et al. 2019). Several traits that are critical for broadening adaptation and enhancing production have been the targets of genetic dissection and marker-assisted breeding approaches in sorghum. Application of DNA markers is gaining importance in the genetic improvement of sorghum crop. Several marker systems have been developed and used for tagging and mapping of major effect genes and quantitative traits (QTL) of economic importance like grain yield and its component traits; resistance to insect pests, diseases, and *Striga*; drought; salinity; cold and nutritional quality; etc.

Genetic improvement of grain yield is a challenging task as it involves accumulation of positive alleles involved in the expression of component traits like panicle length, panicle weight, number of primary branches per panicle, grains per primary branch, grain weight, etc. Several studies have identified QTL for grain yield and its component traits (Brown et al. 2006; Hart et al. 2001; Murray et al. 2008; Mace and Jordan 2011; Reddy et al. 2013; Ritter et al. 2008; Srinivas et al. 2009; Boyles et al. 2016; Zhang et al. 2015; Sukumaran et al. 2016; Bernardino et al. 2019). Sorghum gene Sobic.001G341700 (like grain length and weight protein), an orthologous of rice (GS3) and maize gene (ZmGS3) for grain size, has been reported in sorghum (Tao et al. 2017).

Plant height and grain yield usually have a positive relationship under favorable environment. Plant height in sorghum is a complex trait consisting of number and length of internodes and the peduncle length. Four major effect genes (Dw1, Dw2, Dw3, and Dw4) inherited independently have been described in sorghum (Karper and Quinby 1947). QTL with major effects for plant height have been consistently identified in different genetic backgrounds and were related to qualitative loci, Dw1 on SBI-09, Dw2 on SBI-06, and Dw3 on SBI-07 (Brown et al. 2008; Reddy et al. 2013; Higgins et al. 2014; Morris et al. 2013; Zhao et al. 2016; Lin et al. 1995; Zou et al. 2012). Of these major loci, Dw1 was proposed to encode a putative membrane protein of unknown function (Sobic.009g229800) that is highly conserved in plants (Hilley et al. 2016; Yamaguchi et al. 2016). Morris et al. (2013) proposed that Dw2 phenotype is a result of loss of function in a sorghum histone deacetylase gene (Sobic.006G067600) analogous to its function in controlling plant height in other crops like maize, rice, and *Arabidopsis*. Dw3 has been fine-mapped, and the gene (Sobic.007G163800) was cloned (Multani et al. 2003) coding for P-glycoprotein that regulates polar auxin transport and is orthologous to br2 in maize.

Another adaptive trait which determines the extent of distribution of a crop in diverse climatic conditions is flowering time (Bhosale et al. 2012; Craufurd et al. 1999). Though grain sorghum is a short-day plant and mostly photoperiod sensitive, there are genotypes which exhibit differential sensitivity to varying photoperiods and temperature regimes (Doggett 1988). For maturity, four major genes (Ma_1, Ma_2, Ma_3, and Ma_4) with qualitative effect have been described, with multiple alleles at each locus (Quinby 1967, 1974). Both Ma_1 and Ma_3 have been cloned. Ma_3 encodes a phytochrome B (Childs et al. 1997). The gene encoding pseudo-response regulator protein 37 (*PRR37*) was identified as a likely gene candidate for Ma_1 based on the known roles of PRR genes in flowering of *Arabidopsis* (Murphy et al. 2011). Sorghum Ma_6, a strong repressor of flowering in long days, was identified as the CONSTANS, CO-like, and TOC1 (CCT)-domain protein encoded by *SbGhd7* (Murphy et al. 2014). Sorghum *Ghd7* increases photoperiod sensitivity and delays flowering by inhibiting expression of the floral activator SbEhd1 and genes encoding FT. Sorghum germplasm, both photoperiod sensitive and photoperiod insensitive, remain important sources of new genes for the continued development of cultivars and hybrids in terms of improvement in yield and resistance to biotic and abiotic stresses.

Disease and insect pest management through host plant resistance has been an effective means of reducing economic losses in sorghum. Availability of DNA markers for biotic stress resistance would do away with the need for phenotypic screening, and undesirable plants can be removed before flowering by marker analysis even at the seedling stage. Satish et al. (2009) and Aruna et al. (2011a, b, c) reported QTL for leaf glossiness and seedling vigor, oviposition, deadhearts, adaxial trichome density, and abaxial trichome density. Leaf glossiness QTL on SBI-05 and SBI-03 was syntenic to maize LG 4 and LG3, respectively, and carries genes glossy3 and glossy9 for leaf glossiness and harbors long-chain Acyl-CoA synthetase and wax synthase genes involved in wax biosynthesis. Seedling vigor QTL on SBI-03 hosts a gene for indole-3 acetic acid-amino synthase GH3.5 that promotes plant growth and light and stress adaptation. Similarly, the QTL on SBI-10 where QTL for oviposition, deadhearts, and trichome density are co-located, genes viz., Cysteine protease Mir1, Homogentisate phytyl transferase vte2, Hydroxyproline-rich glycoprotein, NAC1, glossy15 and mh11 responsible for biotic and abiotic stress resistance have been identified.

For midge, two QTL on SBI-03 and SBI-09 were associated with antixenosis explaining 12% and 15% of variation in egg number per spikelet (Tao et al. 2003). One region on SBI-07 was significantly associated with antibiosis and explained 34.5% of the variation of the difference of egg and pupal counts. For green bug resistance, Punnuri et al. (2013) reported four major QTL regions on SBI-09 between Starssbnm 78 and Starssbnm 102 SSR markers collectively accounting for 34–82% variation. A genic marker for *Xa21*-binding protein 3 was tightly linked to greenbug resistance traits. Transcriptomic studies have shown the involvement of signalling compounds and defence-activated R genes in defence response to green-bug attack.

Grain mold is a major disease complex of sorghum that severely affects grain production and grain quality. A complex of fungal pathogens, mostly *Fusarium* and *Curvularia* are parasitic that can infect sorghum spikelet at anthesis itself. In a recent association-mapping study, two SNP loci linked to grain mold resistance have been identified (Upadhyaya et al. 2013). Among these, one contained a NB-ARCLRR class of R gene (Sb02g004900) that shares 37% identity and 57% similarity to the non-host resistance gene of maize, Rxo1. Recent genome-wide association mapping using Ethiopian sorghum landraces identified a major grain mold resistance QTL containing tightly linked transcription factors, YELLOW SEED1 (Y1), and a second R2R3 MYB gene, YELLOWSEED3 (Y3), defining a narrow genomic region that could be used for grain mold resistance selection.

A major QTL on SBI-06 between SSR markers Xtxp95 and Xtxp57 (Klein et al. 2001; Mohan et al. 2010) influencing resistance against various unrelated pathogens causing foliar diseases was consistently detected with the phenotypic variation ranging from 32% (bacterial leaf blight, zonate leaf spot) to 55% (anthracnose) indicating involvement of a key gene for disease resistance. Genes known to be involved in plant defense mechanisms like NB-ARC class of R genes, HR-related genes, a transcription factor that functions in the R gene pathway, a gene that functions in the non-specific host resistance, and a gene for antimicrobial compound production were identified as putative genes for anthracnose disease resistance in sorghum (Cuevas et al. 2014; Upadhyaya et al. 2013).

Charcoal rot disease is a root and stem disease caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. QTL for charcoal rot resistance were identified (Patil et al. 2012; Reddy et al. 2008a, b; Adeyanju et al. 2015). Ergot (sugary disease) is an endemic fungal disease and causes significant losses in seed production plots. It was also observed that the major QTL for percent ergot infection on SBI-06 was co-located with QTL for a number of diseases including grain mold, anthracnose, zonate leaf spot, and bacterial leaf spot (Klein et al. 2001; Mohan et al. 2010). Three other regions on SBI-07, SBI-10, and SBI-08 that are known to contain QTL for grain mold and rust resistance (Klein et al. 2001; Tao et al. 1998) also appear to contain a QTL for ergot resistance (Parh et al. 2008).

A single recessive gene controls low Striga germination stimulant (*lgs*) activity, a well-known resistance mechanism in sorghum (Hess and Ejeta 1992; Vogler et al. 1996). Satish et al. (2012) precisely mapped and tagged the *lgs* gene on SBI-05 between two tightly linked SSR markers SB3344 and SB3352. The use of molecular markers to breed for *Striga* resistance in sorghum has been shown to be possible (Mohamed et al. 2014).

Stay-green is the best-characterized component of drought tolerance and enhances the yield of sorghum grain under drought, by modifying canopy development, root growth, and crop-water usage. Several QTL associated with stay-green trait have been detected across genetic backgrounds.

Comparison of stay-green QTL (Xu et al. 2000; Subudhi et al. 2000; Tao et al. 2000; Tuinstra et al. 1997; Haussmann et al. 2002) introgressed lines (Kassahun et al. 2010) and near-isogenic lines (Harris et al. 2007) involving B35 consistently identified four major QTL, namely, *Stg1* (SBI-03), *Stg2* (SBI-03), *Stg3* (SBI-02),

and *Stg4* (SBI-05), which together accounted up to 53.5% phenotype variance. The potential use of stay-green QTL in improving transpiration efficiency and water extraction capacity in sorghum for terminal drought tolerance and grain yield particularly under low yield environments has been demonstrated. Co-location of stay-green and nodal root angle QTL in sorghum highlights the probable role of roots in retaining leaves green through higher water uptake. Transcriptomic analysis comparing stay-green and senescent lines identified a role for proline biosynthesis in the stay-green trait. Current studies at IIMR, Hyderabad, on marker-assisted introgression of stay-green QTL, Stg3a and Stg3b from B35 to Indian post-rainy sorghum lines, CRS4, and RSLG262 have shown some promise in imparting terminal drought tolerance. Some of the partial introgression lines (BC1F3 generations) of CRS4 and RSLG262 had higher green leaf area retention at maturity, grain yield, and stover yield under both stress and no-stress conditions. The introgression lines also showed significantly better drought tolerance in terms of their low drought susceptibility index compared to respective recurrent parents (R Madhusudhana Unpublished data).

7.18.1 Cold Tolerance

Genetic mapping of cold tolerance detected two QTL for germination-one on SBI-03 contributing 12–15% of variation under both cold and optimal temperatures, while the second QTL on SBI-07 showed greater significance only under cold temperature accounting to 10% trait variation (Knoll et al. 2008). A major QTL with 8–27% trait contribution was identified on SBI-01, which showed strong associations with seedling emergence and seedling vigor under early as well as late field plantings. Similarly, one QTL for both early and late seedling emergence was identified on SBI-02, explaining 8–10% of trait variation. A new source of cold tolerance, PI610727, was used to tag the genomic regions exhibiting significant contributions to traits for early-season cold tolerance.

Some of the important QTL regions reported in sorghum are given here (Table 7.11).

7.18.2 Modernization of Crop Improvement Program

The current genetic gains in several important crops are inadequate to meet future food demand. This slow improvement rate is attributed partly to the long generation times of crop plants. Recently, "speed breeding," a method to greatly shorten generation time and accelerate breeding and research programs, is introduced (Watson et al. 2018). This method uses regulated environmental conditions and prolonged photoperiods to achieve between four and six generations per year of long duration crops (i.e., wheat, barley, and canola). The method has great potential for integrating speed breeding with other modern crop breeding technologies, including high-throughput genotyping, genome editing, and genomic selection, accelerating

Table 7.11 Important QTL in sorghum with their associated markers

Trait/genes/QTL	LG	LOD	R2	Linked markers	Reference
I. Agronomic traits					
Plant height (Dw1)	9	6	20	isu140/PIO100016	Pereira and Lee (1995)
Plant height (Dw2)	6	16	27	AG/CTG9	Ritter et al. (2008)
Plant height (Dw3)	7	8	29	isu123/isu116	Pereira and Lee (1995)
Maturity	6	91	86	pSB189/pSB580	Lin et al. (1995)
Maturity	1	6	15	txp58/Dsenhsbm63	Srinivas et al. (2009)
Maturity	6	11	36	psb521/psb708	Kebede et al. (2001)
Grain yield	2	4	18	AAG/CAA1	Ritter et al. (2008)
Grain yield	6	5	15	GlumeT/Xtxp145	Srinivas et al. (2009)
Grain yield	10	3	14	AAG/CTT2	Ritter et al. (2008)
Grain yield	10	5	11	Xcup67/txa3777	Murray et al. (2008)
Seed mass	1	13	20	TS138/rio72	Murray et al. (2008)
Seed mass	1	–	11	bnl6.25/umc84	Rami et al. (1998)
Seed mass	1	5	15	Dsenhsbm64/Xcup24	Srinivas et al. (2009)
Seed mass	1	3	11	isu027/npi209	Pereira and Lee (1995)
Seed mass	2	–	19	umc122/bnl16.06	Rami et al. (1998)
Seed mass	3	6	10	psB443a/pSB614	Feltus et al. (2006)
Seed mass	3	–	12	umc152/umc10	Rami et al. (1998)
Seed mass	4	4	10	Xtxp12/Dsenhsbm39	Srinivas et al. (2009)
Seed mass	4	4	16	txs604/cdo516.1	Feltus et al. (2006)
Seed mass	4	5	16	Xtxp51/txa6257	Brown et al. (2006)
Seed mass	6	7	10	pSB521a/pSB428a	Feltus et al. (2006)
Seed mass	6	8	15	txa2873/txa2067	Murray et al. (2008)
Seed mass	7	–	31	umc23/sscir88	Rami et al. (1998)

(continued)

Table 7.11 (continued)

Trait/genes/QTL	LG	LOD	R2	Linked markers	Reference
Seed mass	8	6	11	rio65/rio37	Murray et al. (2008)
Seed mass	8	5	12	isu145.2/txa558	Brown et al. (2006)
Seed mass	9	6	18	txs1703/cdo580	Brown et al. (2006)
Seed mass	10	4	14	txs1106/bnl5.04	Feltus et al. (2006)
Seed mass	10	5	16	isu156/isu034	Pereira and Lee (1995)
II. Insect resistance					
Shoot fly (glossiness)	5	3	17	Xtxp65/ XnhsbmSFC61	Satish et al. (2012)
Shoot fly (deadhearts)	10	7	23	XnhsbmSFC34/ Xnhsbm1039	Satish et al. (2012)
Shoot fly (trichome density)	10	9	20	XnhsbmSFC34/ Xnhsbm1039	Satish et al. (2012)
Shoot fly (trichome density)	10	10	24	Xgap1/Xnhsbm1011	Satish et al. (2012)
Midge	3	3	12	rz543/st698	Tao et al. (2003)
Midge	7	11	34	txs1931/sg37	Tao et al. (2003)
Midge	9	5	15	ST1017/SG14	Tao et al. (2003)
Green bug (biotype I)	1	2	15	Xtxp43/Xtxp85	Nagaraj et al. (2005)
Biotype I	4	4	20	Sb1-10	Nagaraj et al. (2005)
Biotype I	7	–	10	bdc098/csu61	Katsar et al. (2002)
Biotype K	1	2	16	Xtxp335/Xtxp204	Nagaraj et al. (2005)
Biotype K	4	3	13	Xtxp12/Xcup20	Nagaraj et al. (2005)
Biotype K	10	–	15	psb0106/rz144	Katsar et al. (2002)
III. Disease resistance					
Anthracnose	6	13	40	Xtxp95-Plcor	Mohan et al. (2010)
Zonate leaf spot	6	5	14	Xtxp95-Plcor	Mohan et al. (2010)
Zonate leaf spot	6	5	17	Fdnhsbm107-Fdnhsbm24	Mohan et al. (2010)
Zonate leaf spot	3	4	13	Xtxp228-Drenhsbm103	Mohan et al. (2010)
Target leaf spot	6	20	50	Xtxp95-Plcor	Mohan et al. (2010)
Rust	1	3	26	bnl5.09/txs1625	Tao et al. (1998)

(continued)

Table 7.11 (continued)

Trait/genes/QTL	LG	LOD	R2	Linked markers	Reference
Rust	2	3	17	sscir51/txs2042	Tao et al. (1998)
Rust	3	4	24	rz323/isu102	Tao et al. (1998)
Rust	8	9	43	psb47/txs422	Tao et al. (1998)
Rust	6	8	24	Xtxp95-Plcor	Mohan et al. (2010)
Ergot (% infection)	1	5	12	sPb-8261	Parh et al. (2008)
Ergot (% infection)	6	6	14	sPb-1543	Parh et al. (2008)
Ergot (% infection)	7	4	10	Xtxp168	Parh et al. (2008)
Ergot (% infection)	8	4	11	AGG + CAG6	Parh et al. (2008)
Ergot (% infection)	9	3	20	Sb4-32	Parh et al. (2008)
Ergot (pollen viability)	7	3	13	sPb-5594	Parh et al. (2008)
Ergot (pollen viability)	8	3	10	Xtxp273	Parh et al. (2008)
Ergot (pollen viability)	6	7	20	AAG + CTT6	Parh et al. (2008)
Charcoal rot (internodes crossed)	2	4	19	Xtxp297	Reddy et al. (2008a, b)
Charcoal rot (% lodging)	4	4	15	Xtxp343	Reddy et al. (2008a, b)
IV. Weed					
Striga (lgs)	5			SB3344, SB3343, SB3346	Satish et al. (2012)
V. Abiotic resistance					
Drought-stay-green (Stg1)	3	5	20	Xtxp442/Xtxp38	Xu et al. (2000)
Stg2	3	6	30	Xtxp2/Xtxp503	Xu et al. (2000)
Stg3	2	3	16	Xtxp430/Xtxp1	Xu et al. (2000)
Stg4	5	2	11	Xtxp225/Xtxp15	Xu et al. (2000)
Cold tolerance (late emergence %)	1	–	21	PeriCol/OPK18	Knoll et al. (2008)
Cold tolerance (late emergence %)	2	–	11	Xtxp201/Sb110	Knoll et al. (2008)
Cold tolerance (early vigor)	1	–	20	PeriCol/OPK18	Knoll et al. (2008)
Cold tolerance (early vigor)	4	–	12	Xtxp51/Xtxp21	Knoll et al. (2008)
Cold tolerance (late vigor)	1	–	28	PeriCol/OPK18	Knoll et al. (2008)
Cold tolerance (cold germination)	3	–	13	ubc171/SbAGE01	Knoll et al. (2008)
Cold tolerance (optimal germination)	3	–	15	umc60/ubc171	Knoll et al. (2008)
VI. Agronomic traits					
Plant height (Dw1)	9	6	20	isu140/PIO100016	Pereira and Lee (1995)
Plant height (Dw2)	6	16	27	AG/CTG9	Ritter et al. (2008)

(continued)

Table 7.11 (continued)

Trait/genes/QTL	LG	LOD	R2	Linked markers	Reference
Plant height (Dw3)	7	8	29	isu123/isu116	Pereira and Lee (1995)
Maturity	6	91	86	pSB189/pSB580	Lin et al. (1995)
Maturity	1	6	15	txp58/Dsenhsbm63	Srinivas et al. (2009)
Maturity	6	11	36	psb521/psb708	Kebede et al. (2001)
Grain yield	2	4	18	AAG/CAA1	Ritter et al. (2008)
Grain yield	6	5	15	GlumeT/Xtxp145	Srinivas et al. (2009)
Grain yield	10	3	14	AAG/CTT2	Ritter et al. (2008)
Grain yield	10	5	11	Xcup67/txa3777	Murray et al. (2008)
Seed mass	1	13	20	TS138/rio72	Murray et al. (2008)
Seed mass	1	–	11	bnl6.25/umc84	Rami et al. (1998)
Seed mass	1	5	15	Dsenhsbm64/Xcup24	Srinivas et al. (2009)
Seed mass	1	3	11	isu027/npi209	Pereira and Lee (1995)
Seed mass	2	–	19	umc122/bnl16.06	Rami et al. (1998)
Seed mass	3	6	10	psB443a/pSB614	Feltus et al. (2006)
Seed mass	3	–	12	umc152/umc10	Rami et al. (1998)
Seed mass	4	4	10	Xtxp12/Dsenhsbm39	Srinivas et al. (2009)
Seed mass	4	4	16	txs604/cdo516.1	Feltus et al. (2006)
Seed mass	4	5	16	Xtxp51/txa6257	Brown et al. (2006)
Seed mass	6	7	10	pSB521a/pSB428a	Feltus et al. (2006)
Seed mass	6	8	15	txa2873/txa2067	Murray et al. (2008)
Seed mass	7	–	31	umc23/sscir88	Rami et al. (1998)
Seed mass	8	6	11	rio65/rio37	Murray et al. (2008)
Seed mass	8	5	12	isu145.2/txa558	Brown et al. (2006)

(continued)

Table 7.11 (continued)

Trait/genes/QTL	LG	LOD	R2	Linked markers	Reference
Seed mass	9	6	18	txs1703/cdo580	Brown et al. (2006)
Seed mass	10	4	14	txs1106/bnl5.04	Feltus et al. (2006)
Seed mass	10	5	16	isu156/isu034	Pereira and Lee (1995)
VII. Insect resistance					
Shoot fly (glossiness)	5	3	17	Xtxp65/ XnhsbmSFC61	Satish et al. (2012)
Shoot fly (deadhearts)	10	7	23	XnhsbmSFC34/ Xnhsbm1039	Satish et al. (2012)
Shoot fly (trichome density)	10	9	20	XnhsbmSFC34/ Xnhsbm1039	Satish et al. (2012)
Shoot fly (trichome density)	10	10	24	Xgap1/Xnhsbm1011	Satish et al. (2012)
Midge	3	3	12	rz543/st698	Tao et al. (2003)
Midge	7	11	34	txs1931/sg37	Tao et al. (2003)
Midge	9	5	15	ST1017/SG14	Tao et al. (2003)
Green bug (biotype I)	1	2	15	Xtxp43/Xtxp85	Nagaraj et al. (2005)
Biotype I	4	4	20	Sb1-10	Nagaraj et al. (2005)
Biotype I	7	–	10	bdc098/csu61	Katsar et al. (2002)
Biotype K	1	2	16	Xtxp335/Xtxp204	Nagaraj et al. (2005)
Biotype K	4	3	13	Xtxp12/Xcup20	Nagaraj et al. (2005)
Biotype K	10	–	15	psb0106/rz144	Katsar et al. (2002)
VIII. Disease resistance					
Anthracnose	6	13	40	Xtxp95-Plcor	Mohan et al. (2010)
Zonate leaf spot	6	5	14	Xtxp95-Plcor	Mohan et al. (2010)
Zonate leaf spot	6	5	17	Fdnhsbm107-Fdnhsbm24	Mohan et al. (2010)
Zonate leaf spot	3	4	13	Xtxp228-Drenhsbm103	Mohan et al. (2010)
Target leaf spot	6	20	50	Xtxp95-Plcor	Mohan et al. (2010)
Rust	1	3	26	bnl5.09/txs1625	Tao et al. (1998)
Rust	2	3	17	sscir51/txs2042	Tao et al. (1998)
Rust	3	4	24	rz323/isu102	Tao et al. (1998)
Rust	8	9	43	psb47/txs422	Tao et al. (1998)

(continued)

Table 7.11 (continued)

Trait/genes/QTL	LG	LOD	R2	Linked markers	Reference
Rust	6	8	24	Xtxp95-Plcor	Mohan et al. (2010)
Ergot (% infection)	1	5	12	sPb-8261	Parh et al. (2008)
Ergot (% infection)	6	6	14	sPb-1543	Parh et al. (2008)
Ergot (% infection)	7	4	10	Xtxp168	Parh et al. (2008)
Ergot (% infection)	8	4	11	AGG + CAG6	Parh et al. (2008)
Ergot (% infection)	9	3	20	Sb4-32	Parh et al. (2008)
Ergot (pollen viability)	7	3	13	sPb-5594	Parh et al. (2008)
Ergot (pollen viability)	8	3	10	Xtxp273	Parh et al. (2008)
Ergot (pollen viability)	6	7	20	AAG + CTT6	Parh et al. (2008)
Charcoal rot (internodes crossed)	2	4	19	Xtxp297	Reddy et al. (2008a, b)
Charcoal rot (% lodging)	4	4	15	Xtxp343	Reddy et al. (2008a, b)
IX. Weed					
Striga (lgs)	5			SB3344, SB3343, SB3346	Satish et al. (2012)
X. Abiotic resistance					
Drought-stay-green (Stg1)	3	5	20	Xtxp442/Xtxp38	Xu et al. (2000)
Stg2	3	6	30	Xtxp2/Xtxp503	Xu et al. (2000)
Stg3	2	3	16	Xtxp430/Xtxp1	Xu et al. (2000)
Stg4	5	2	11	Xtxp225/Xtxp15	Xu et al. (2000)
Cold tolerance (late emergence %)	1	–	21	PeriCol/OPK18	Knoll et al. (2008)
Cold tolerance (late emergence %)	2	–	11	Xtxp201/Sb110	Knoll et al. (2008)
Cold tolerance (early vigor)	1	–	20	PeriCol/OPK18	Knoll et al. (2008)
Cold tolerance (early vigor)	4	–	12	Xtxp51/Xtxp21	Knoll et al. (2008)
Cold tolerance (late vigor)	1	–	28	PeriCol/OPK18	Knoll et al. (2008)
Cold tolerance (cold germination)	3	–	13	ubc171/SbAGE01	Knoll et al. (2008)
Cold tolerance (optimal germination)	3	–	15	umc60/ubc171	Knoll et al. (2008)

the rate of crop improvement. A method to shorten breeding cycle from the regular 17–11 week by embryo rescue was demonstrated in sorghum (Rizal et al. 2014).

Modern trends in digital agriculture have seen a shift toward artificial intelligence for crop quality assessment and yield estimation (Mosley et al. 2020). Reliable, automatic, multifunctional, and high-throughput phenotypic technologies are increasingly considered important tools for rapid advancement of genetic gain in breeding programs (Zhao et al. 2019). Phenomics platforms have allowed scientists

to use fast and simple quantitative and qualitative methods to assess plant growth and development. This facilitates the detailed observation and measurement of the different traits resulting from the expression of genetic characteristics of plants, both physical and environmental factors. Several approaches to field-based plant phenomics have been proposed of which aerial drones are promising. Aerial drones, by contrast, can cover large areas quickly, allowing all genotypes in a study to be measured simultaneously, and are not impeded by plant height, which allows them to capture data throughout the entire growth season (Liebisch et al. 2015).

7.19 Status of Varietal Development and Maintenance Breeding

Developing improved cultivars of sorghum requires considerable expenditure of time and effort to put together the specific combinations of traits needed to achieve high and stable production of grain and/or stover in a particular environment. These traits include the correct phenology for the environment, the necessary resistances to the biotic and abiotic constraints prevalent there, and the quality traits preferred by farmers.

If improved cultivars are not maintained systematically, they are likely to deteriorate in yield and quality due to outcrossing with the unadapted cultivars lacking one or more of the component traits. Deliberate and systematic maintenance of cultivars and multiplication of their seed is, therefore, required to ensure that the genetic package assembled by the plant breeder is kept together and delivered to farmers. Similarly, attention should be given to the crop health of seed production plots to ensure that the seed delivered to farmers is in good condition to germinate and establish the intended crop.

A detailed information on varieties released in kharif, rabi, forage, and sweet sorghum has already earlier appeared in the chapter, and only maintenance aspects are given here.

7.20 Maintenance of Genetic Purity in Sorghum Varieties

7.20.1 Land Requirement

Land should be free from volunteer plants, Johnson grass, Sudan grass, and other forage types. The same crop should not be grown on the same piece of land in the previous one season unless it is the same variety and certified by certification agency for its purity.

7.20.2 Isolation Requirement

Sorghum is a self-pollinated crop, but cross-pollination up to 8–10% may occur. In some of the varieties with loose or lax panicle types, the extent of natural cross-pollination may go up to 50%. Hence, the seed fields must be isolated from other varieties of grain and dual-purpose sorghum and same variety not confirming to varietal purity by 200 m for foundation seed class and 100 m for certified seed class. An isolation of 400 m is required from Johnson grass (*Sorghum halepense*) and other forage sorghums with high tillering and grassy panicles. Differential blooming for modifying isolation distance is not permitted (i.e., time isolation is not permitted).

7.20.3 Brief Cultural Practices

Obtain appropriate class of the seed from the source approved by seed certification agency. The seed rate required is 12–15 kg/ha, and the spacing adopted is 45 cm between the rows and 15 cm between the plants. Other cultural practices are similar to raising a commercial crop. Necessary prophylactic measures should be taken so as to raise a good crop.

7.20.4 Rouging

Remove all the offtypes and volunteer plants before they start shedding pollen. The rouged plants must be cut from the bottom or uprooted to prevent re-growth. Offtypes can be identified based on morphological characters like plant height, leaf shape, leaf color, stem pigmentation, days to flowering, etc. Rogue out other related plants like Johnson grass, Sudan grass, forage plants, and plants affected by kernel smut and head smut from time to time.

7.20.5 Number of Field Inspections

A minimum of three field inspection should be done. First inspection should be done during vegetative stage to determine isolation, volunteer plants and designated diseases, etc. Second inspection shall be made during flowering to check isolation, offtypes, and other relevant factors. Third inspection shall be made at maturity prior to harvest to verify designated diseases true nature of plants, head, and seed.

7.20.6 Harvesting and Threshing

The seed crop must be harvested when it is fully ripe. The harvested heads should be sorted out to remove the diseased or otherwise undesirable. The heads should be dried on the threshing floor or tarpaulin for a couple of days before threshing.

Threshing can be done by threshers or manually. The seed should be thoroughly cleaned and dried to 10% moisture before storage.

7.20.7 Seed Yield

Depending up on the potentiality of the variety and the management practices adopted, seed yield may be in the range of 35–40 q/ha.

7.21 Maintenance of Genetic Purity in Sorghum Hybrids

In sorghum hybrid seed is produced by utilizing cytoplasmic genetic male sterile system. The source of male sterile cytoplasm used is combined kafir. Hybrid seed production involves two steps:

- Maintenance of parental lines (A-line, B-line, and R-line)
- Commercial hybrid seed production (A × R)

Maintenance of parental lines is generally referred to as foundation seed production and hybrid seed production as certified seed class. The A-line can be maintained by crossing with B-line in an isolated plot, while in hybrid seed production, A-line crosses with R-line or fertility restorer line. The B-line and R-line can be maintained just like normal varieties by following the required isolation and field standards.

7.21.1 Seed Production of B-line and R-line

The seed is produced in an isolated plot, and it is similar to seed production of open pollinated varieties. However, the isolation distance required and the fields' standards are similar to that of maintenance of A-line.

7.21.2 Maintenance of A-line or Hybrid Seed Production (A × R)

7.21.2.1 Land Requirement

Land should be free from volunteer plants, Johnson grass, Sudan grass, and other forage types. The same crop should not be grown on the same piece of land in the previous one season unless it is the same variety and certified by certification agency for its purity.

7.21.2.2 Isolation Requirement

The isolation distance for maintenance of A-line (A × B) is 300 m from fields of other varieties of grain and dual-purpose sorghum and same variety not confirming to varietal purity and 400 m from Johnson grass, Sudan grass, and other forage types.

For commercial hybrid seed production of (A × R), the isolation distance required is 200 m from fields of other varieties of grain and dual-purpose sorghum, and same hybrid not confirming to varietal purity requirements of certification, 5 m from other hybrid seed production plot having the same male parent and 400 m from Johnson grass, Sudan grass, and other forage types. Differential blooming dates for modification of isolation distance are not permitted.

7.21.2.3 Planting Ratio

The planting ratio of female to male plants is 4:2 with two rows of male parent all around the field.

7.21.2.4 Brief Cultural Practices

The success in hybrid seed production depends on synchronization of flowering between the male and female parent. For maintenance of A-line, synchronization of flowering will not be a problem as both A- and B-lines are isogenic lines and come to flowering at the same time, while in hybrid seed production, synchronization will be a problem as A-line and R-line have different genetic constitutions. If there is any difference between the male and female parent for days to flowering, the sowing dates should be adjusted for proper synchronization of flowering. The seed rate required is 8.0 kg/ha of A-line and 4.0 kg/ha of B or R-line. Other cultural practices similar to commercial crop production should be adopted for raising a good crop.

7.21.2.5 Cultural Manipulation for Nicking

Proper synchronization of flowering between Aline and R-line is a common problem. In spite of taking the precautions like adjusting the sowing dates, sometimes, synchronization may be a problem. If the difference between the male and female parent is less than a week, it can be manipulated by cultural practices. The parent which is lagging should be sprayed with 1% urea solution 2–3 times at an interval of 2–3 days, or additional irrigation should be given to the lagging parent. Blowing air by operating empty duster with the mouth directed horizontally to the male ears will help disseminate pollen.

7.21.2.6 Rouging

Before flowering, remove all offtypes from both seed parent and pollen rows based on morphological characters. Some of the precautions to be taken while rouging are:

- Start rouging before offtypes, volunteers, and pollen shedders in female rows start shedding pollen.
- Outcrosses can be easily identified because of their greater height and more vigorous growth and should be removed.
- At flowering, rouging should be done every day to remove pollen shedders from female parent rows. The sterile types have only stigma or pale aborted anthers without pollen, while the fertile ones have yellow colored plumpy anthers which shed large amount of residual pollen. Remove all plants out of their place (i.e., plants in between the lines) and male plants in female rows and vice versa. Special

attention should be given at the ends where there is a chance of male seed falling in female rows.
- Remove other sorghum-related plants like Johnson grass, Sudan grass, and other forage types from the seed plot and from within the isolation distance.
- Remove the plants affected by kernel bunt and head smut.
- Pre-harvest rouging may be done based on grain and ear characters.

7.21.2.7 Number of Field Inspections

A minimum of four field inspections should be conducted. The first field inspection should be conducted before flowering stage, second and third during flowering stage, and fourth before harvesting. During the first field inspection, verification should be done for volunteer plants, isolation requirement, errors in planting, and the actual acreage sown. During the second and third field inspection, verification should be done for isolation requirement, offtypes, diseased plants, pollen shedders, and objectionable weed plants. Actual counts should be taken during second or third field inspection. Fourth or final field inspection should be done to verify for all the above factors, and the offtypes can be identified based on panicle or seed characters.

7.21.2.8 Harvesting and Threshing

Harvest the male rows first, and keep their heads separate to avoid mixture male and female seed. Then harvest the female parental line and thresh it separately. Precautions may be taken while harvesting and threshing to avoid mechanical mixtures.

7.21.2.9 Seed Yield

The seed yield may be in the range of 4–6 q/ha depending on the parent line and the cultural practices adopted.

7.22 Coordinated System of Testing

Focused sorghum research in India started with the establishment of the Project for Intensification of Regional Research on Cotton, Oilseeds and Millets (PIRRCOM) in 1958. Under the PIRRCOM sorghum research was led from the Indian Agricultural Research Institute (IARI), New Delhi. In 1966, sorghum research was shifted from New Delhi to Hyderabad as a part of IARI Regional Research. Realizing the success of hybrid sorghum in the United States in 1962, the Indian Council of Agricultural Research (ICAR) launched the Accelerated Hybrid Sorghum Project. In December 1969, All India Coordinated Sorghum Improvement Project (AICSIP) was launched from the existing IARI RRS in Hyderabad. Subsequently, in 1987, a full-fledged "National Research Centre for Sorghum (NRCS)" was established which has evolved into the Indian Institute of Millets in 2015. Currently, AICSIP functions with a total of 24 centers spread across nine states. Sorghum improvement efforts since the 1960s were focused on improving grain and fodder yields. However, with

demands of sorghum as forage crop and in recent past as sweet sorghum, an alternate source of bioethanol, intensive efforts toward these ends have also been initiated.

Detailed information on varieties and hybrids released in kharif, rabi, forage, and sweet sorghum in the coordinated has already earlier appeared in the chapter.

7.23 Future Thrust Area

Sorghum improvement efforts have succeeded in increasing productivity of *kharif* sorghum but could not impact much in *rabi* sorghum. Productivity of *kharif* sorghum may further be improved through diversification of genetic base not only through use of caudatum races but other races like guinea and kafir. In *rabi* sorghum efforts need to improve parental lines with better combining ability, desired levels of resistance to biotic and abiotic stresses, and acceptable grain qualities. Though hybrids have not gained much popularity in *rabi* sorghum, to enhance productivity of *rabi* sorghum, concerted efforts need to be focused on development of hybrids with acceptable grain quality. Alternate use is another area of focus needed in *rabi* sorghum breeding program particularly to breed cultivars suitable to specific end uses. In forage sorghum, attention needs to be given toward improvement of digestibility and resistance against foliar diseases. Use of *bmr* mutants has opened up a new area of opportunity. Seed production of forage cultivars is a major concern. Production of second-generation biofuels using lignocellulose components opens up the new area of research in sweet sorghums.

Sorghum as health food is another area which needs concerted efforts to diversify sorghum uses. Sorghum production can only be enhanced with appropriate policy support by the government. With inclusion of sorghum and other millets in national food security mission, there is a ray of hope that sorghum will regain a new place in the food basket of the country.

In addition to the biotic and abiotic challenges, climate change is expected to influence the sorghum area and its importance globally. With the current level of greenhouse gas emissions and the associated temperature rise, the areas suitable for sorghum are likely to increase by 9% globally, but many areas currently suitable for sorghum will be lost. Increased temperature makes sorghum crops mature early. Considering all these points, crop improvement research in sorghum needs to be oriented toward genetic and cytoplasmic diversification for high yield and large grain, shoot fly and grain mold resistance, drought and salinity tolerance, post-rainy season adaptation, sweet stalk traits, and grain micronutrient density. Grain and stover quality needs special attention to enhance the market value.

7.24 Conclusions

Climate change is expected to influence the sorghum area and its importance globally, in addition to the biotic and abiotic challenges. With the current level of greenhouse gas emissions and the associated temperature rise, the areas suitable for

sorghum are likely to increase by 9% globally, but many areas currently suitable for sorghum will be lost. Increased temperature makes sorghum crops mature early. Considering all these points, crop improvement research in sorghum needs to be oriented toward genetic and cytoplasmic diversification for high yield and large grain, shoot fly and grain mold resistance, drought and salinity tolerance, post-rainy season adaptation, sweet stalk traits, and grain micronutrient density. Grain and stover quality needs special attention to enhance the market value.

Various factors leading to the decline of sorghum crop in Indian agriculture are a matter of concern. Sorghum-based agricultural systems need to withstand biotic and abiotic stresses because of their cultivation mostly in unfavorable soil and climatic conditions. Further, they also need to adjust to changing economic (prices and income) and policy-induced stresses as has been the case in India where subsidized wheat and rice are supplied through the public distribution system. With this background in view, there is a need to embark on future approaches for improvement. Promotion of genetic diversity, cropping system stability, and economic advantage or parity will become the major criteria. The genetic approaches should promote genetic diversity and cropping system performance and stability.

Concerted efforts to diversify sorghum as health food are another area which needs attention. Sorghum production can only be enhanced with appropriate policy support by the government. In India, with inclusion of sorghum and other millets in national food security mission, there is a ray of hope that sorghum will regain a new place in the food basket of the country.

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Small Millets Breeding

8

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Abstract

Millets are small-grained ancient cereal crops and one of the earliest sources of food known in the prehistoric period. It is believed that they were under cultivation since 8000 BC. These crops adapted well to the marginal and dryland ecosystem due to their climate resilience, biotic stress tolerance and ability to grow in the wild besides providing nutritious grains. Among the millets, small millets form a distinct group and comprise of finger millet (*Eleusine coracana*), little millet (*Panicum sumatrense*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), barnyard millet (*Echinochloa frumentacea*) and brown top millet (*Brachiaria ramosa* (L.)) in India along with a few more cereals like teff, fonio, job's tears and guinea millet in some other parts of the world. Except finger millet, other small millets received very limited attention with respect to crop improvement and crop husbandry. Early efforts in finger millet and other crops mainly concentrated on introduction and domestication of indigenous accessions and selection among the available variability. This led to release of some cultivars prior to independence which were major staples before green revolution. Post 1960s, the area and importance of these crops started declining with the emergence of fine cereals. Realizing the importance of small millets and lack of organized research efforts, the Indian Council of Agricultural Research established the All India Coordinated Small Millets Improvement Project in 1986 with its headquarters in University of Agricultural Sciences, Bangalore. Since then, concerted efforts in germplasm collection, recombination breeding and crop production and protection have

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_8

resulted in development and release of many improved cultivars for commercial cultivation by the dryland farmers along with improved package of practices. Development of early maturing, high yielding and drought-resistant varieties with emphasis on both grain and biomass continue as important breeding objectives in all the small millets. Breeding methodologies aiming at recombination breeding slowly started gaining momentum in all the small millets though the progress is hampered by typical floral biology and anthesis behaviour. In the last three decades, more than 200 improved cultivars have been released by adopting different breeding approaches to suit the location-specific requirements of the farmers. This chapter describes the breeding efforts made in different small millets crops and progress so far in India in other countries.

Keywords

Little millet · Barnyard millet · Foxtail millet · Proso millet · Minor millets

8.1 Introduction

Millets and sorghum comprise of an important group of cereal crops known for their nutritional values. Small millets, also known as minor millets, are a group of small-seeded cereal crops of the grass family Poaceae. Small millets are hardy and climate resilient suitable for range of soil, environmental conditions and are prone to drought and high temperature. Small millets are adapted to a range of temperatures, moisture-regimes and input conditions and are perhaps the only cereal crops that can grow in arid lands with only 350–400 mm annual rainfall. Millets, with their ability to tolerate and survive under conditions of continuous or intermittent drought periods, are the major crops successfully cultivated in dry regions where fine cereals such as rice and wheat cannot be grown. The area under millet production has been on the decline since green revolution, more so in case of small millets (80% for small millets other than finger millet, 53% for finger millet). The period between 1961 and 2015 saw a dramatic decrease in cultivated area under millets. The area under all small millets other than finger millet has declined drastically in all states, and the total production of small millets has declined by more than 75% (Table 8.1). Low productivity under marginally grown conditions, declining support has significantly contributed to the fall of millets in Indian agriculture, which is gradually reversing. However, the production has increased over time through productivity improvement by crop breeding efforts (Ganapathy et al. 2021a, b).

Small millets are important crops of rainfed areas in semi-arid regions. Presently, they are cultivated on a limited area globally mainly due to the shift from traditional crops to more remunerative crops. Small millets serve as major food components in various traditional foods and are generally eaten as rice apart from its use in various value-added products and also for beverages and are ingredients in a variety of multigrain and gluten-free cereal products. Due to sedentary lifestyle conditions and its associated health concerns, there is a growing awareness among the consumers

Table 8.1 All India area, production and productivity of small millets

Year/crop	Element	1951–1952	1961–1962	1971–1972	1981–1982	1991–1992	2001–2002	2011–2012	2015–2016	2016–2017	2017–2018	2018–2019[a]
Ragi	Area	2.19	2.51	2.43	2.61	2.13	1.65	1.18	1.14	1.03	1.19	0.92
	Prodn.	1.31	2.03	2.21	2.96	2.58	2.37	1.93	1.82	1.39	1.99	1.22
	Yield	599	808	911	1134	1212	1442	1641	1601	1363	1662	1332
Other small millets	Area	4.76	4.87	4.48	3.79	2.09	1.31	0.80	0.65	0.62	0.55	0.50
	Prodn.	1.92	2.05	1.67	1.64	0.88	0.58	0.45	0.39	0.44	0.44	0.37
	Yield	402	421	373	433	423	440	565	601	714	804	747
Total millets	Area	32.41	36.91	35.44	34.78	26.61	22.28	17.01	15.01	14.26	14.24	12.25
	Prodn.	11.64	15.76	16.92	22.20	16.23	18.80	18.64	14.52	16.2	16.44	13.97
	Yield	359	427	491	638	610	844	1096	968	1136	1154	1140

Area, million ha; Prodn, million tonnes; Yield, kg/ha
Source: DMD, GoI and Agricultural Statistics at a glance 2018; https://eands.dacnet.nic.in/Advance_Estimates.htm
[a] Fourth Advance Estimates

and are seeking more diversified diets that are both tasty and healthy (Ganapathy et al. 2021a, b). Small millet fit in the diversified food system as a healthy food choice because they provide high energy, high dietary fibre, quality protein and balanced amino acid, essential minerals, vitamins and antioxidants, and many of them have low glycaemic index (GI). Due to these inherent features of small millets, they are popularly known as nutri-cereals. The nutrient content of grains varies among different small millets. Finger millet grains contain high calcium (~350 mg/100 g). Nutrient contents like grain iron, zinc, calcium, protein and crude fibre among small millets are presented in Table 8.2. Small millets play a strategic role as a staple food for the poor and, lately, as a healthy food for those in urban areas. It underlines the necessity of directing more research and development towards these crops.

8.2 Genetic Improvement in Small Millets

Diversity in crop cultivars is very important for sustaining global food production. Germplasm provides the required variability for genetic improvement of crops. Small millets germplasm possess larger genetic variation for morpho-agronomic traits, grain quality and stress tolerance traits, and promising germplasm sources have been reported and are being utilized in the improvement programmes. Various breeding methods such as pureline selection, pedigree selection, mass selection and mutation breeding, which are applicable to self-pollinating crops, are followed in small millets as well. Reports on small millets cultivars released over a period of time shown that a majority of them were released following selection from local landraces/cultivars, followed by pedigree selection in the early phase of crop improvement. Recombination breeding has not been exploited to its fullest extent as in other crops like sorghum, pearl millets and other fine cereals. The major reasons are difficulty in hybridization due to small sized florets and irregular flowering behaviour. Mutation breeding is one of the viable approach in different breeding crops and has been successfully demonstrated in finger millet (Ganapathy et al. 2021a, b). Hybridization to create variability followed by selection in segregating population has been an important breeding method in finger millet and comparatively less in other small millets like little millet, barnyard millet, foxtail millet and proso millet. The diversity in small millets in the form of genetic resources, floral biology, breeding methods, constraints and strategies for small improvement in each of the seven small millets are discussed hereunder.

8.3 Finger Millet

Finger millet (*Eleusine coracana* (L.) Gaertn.) is an important food staple of Africa and Southern Asia. Finger millet cropping area globally is estimated to be around 4.0–4.5 million ha. In India, the crop is estimated to be cultivated on an area of 1.2 million ha with an estimated production of 2.0 million tonnes. The major finger millet-growing countries in Africa are the sub-humid regions of Ethiopia, Malawi,

Table 8.2 Nutritive composition of millets vis-a-vis fine cereals (values per 100 g)

Crop	Protein (g)	Carbohydrate (g)	Fat (g)	Crude fibre (g)	Mineral Matter (g)	Calcium (mg)	Phosphorous (mg)
Sorghum	10.4	72.6	1.9	1.6	1.6	25	222
Pearl millet	11.6	67.5	5.0	1.2	2.3	42	296
Finger millet	7.3	72.0	1.3	3.6	2.7	344	283
Proso millet	12.5	70.4	1.1	2.2	1.9	14	206
Foxtail millet	12.3	60.9	4.3	8.0	3.3	31	290
Kodo millet	8.3	65.9	1.4	9.0	2.6	27	188
Little millet	8.7	75.7	5.3	8.6	1.7	17	220
Barnyard millet	11.6	74.3	5.8	14.7	4.7	14	121
Barley	11.5	69.6	1.3	3.9	1.2	26	215
Maize	11.5	66.2	3.6	2.7	1.5	20	348
Wheat	11.8	71.2	1.5	1.2	1.5	41	306
Rice	6.8	78.2	0.5	0.2	0.6	10	160

Source: National Institute of Nutrition (NIN), Hyderabad

Tanzania, Kenya, Uganda, Zambia, Zaire and Zimbabwe. Similarly, in South Asia, the crop is mainly grown in Southern India followed by Nepal and to a certain extent reported in Bhutan and Sri Lanka. In India, the crop is mostly grown in Karnataka followed by other states like Tamil Nadu, Uttarakhand, Orissa, Maharashtra and Andhra Pradesh. Cultivation of this crop extends from mean sea level to hilly regions of Himalayas and is adapted to wide range of soil and environmental conditions but performs well under well-drained, loamy or clay loamy soils. The grains of finger millet are known for its highest amount of calcium and also with contents of iron, zinc, dietary fibre and essential amino acids (Shobana et al. 2013). The grains are resistant to storage infestation by pests, and with minimum attention, the grain can be stored for up to 50 years (Iyengar et al. 1945). The stover after harvest of grains is a source of nutritive fodder to animals and is highly preferred due to sweet-smelled stalks. Comparing other small millets, genetic gain in yield is much pronounced in finger millet but not exploited to the extent observed in other major cereals. The reason is mainly due to irregular flowering behaviour, small-sized florets owing to difficulties in hybridization. However, the diversity existing for grain yield, nutritional superiority and its ability to tolerate range of environmental conditions makes it a promising crop for the future. Research efforts aims at using a combination of approaches for genetic improvement of grain and forage yield, nutritional parameters, biotic and abiotic stresses, identification of end-use specific genotypes and exploring possibilities of exploitation of heterosis.

8.3.1 Gene Pool of *Eleusine coracana*

The cultivated *Eleusine coracana* is highly variable in their centres of origin both in Africa and the Indian subcontinent. The species *E. coracana* is classified into subspecies *africana* and *coracana*. The subspecies *africana* is a wild type and consists of race *africana* and *spontanea*. The subspecies *coracana* is a cultivated type and are classified into four different races based on inflorescence morphology, viz. *elongata*, *plana*, *compacta* and *vulgaris* (Prasada Rao et al. 1993). Both wild (subspecies *africana*) and cultivated finger millet (subspecies *coracana*) are being collected conserved in various gene banks. *africana* subspecies occasionally crosses with the subspecies *coracana* to produce fertile hybrids. Derivatives of such crosses are aggressive colonizers and are grouped under the race *spontanea* (De Wet et al. 1984). Wild finger millet is native to Africa and believed to have been migrated to warmer parts of Asia and America. The diploid wild species *E. indica*, *E. floccifolia* and *E. tristachya* are believed to form the secondary gene pool, while the species *E. intermedia*, *E. jaegeri*, *E. kigeziensis*, *E. multiflora* and *E. semisterlis* (*E. compressa*) form the tertiary gene pool (Guarino 2012).

8.3.2 Floral Biology and Breeding Behaviour of Finger Millet

The floral biology has been described by Rachie and Peters (1977) and recently by Gupta et al. (2011). Finger millet is predominantly self-pollinated, and extent of outcrossing is reported to be less than 1%. The inflorescence consists of terminal

digitate spikelets, borne on a long peduncle from the end of 4–5 spikes which radiate in a whorl called fingers with one finger a little lower the whorl referred to as thumb. Rachis of the spikes is flat. Spikelets are sessile arranged in two rows alternatively attached to one side of the rachis. Each spikelet consists of 3–7 flowers averaging to 5 florets and enclosed by common glumes. Androecium consists of three stamens with long filaments and short oblong anthers. Ovary has two styles with plumose stigma. The terminal floret is sterile. Anthesis proceeds from top spikelets and progresses downwards. The maximum number of flowers opens on the third to fourth day. Flower opening also depends on the earhead shape; the compact types open during 2–3 am, fisty 3–5 am and open types during 1–2 am. Flower opening varies from place to place depending upon the temperature and humidity. Flowering takes place simultaneously in all fingers. Pollen viability is short, 10–15 min. Complete opening of the inflorescence requires 7–8 days. Anthers require about 45 min for dehiscence after emergence. The stigma is receptive for about 5 min after emergence from the glumes. Self-pollination is the general rule because the period of anthesis is very short. Cross fertilization by wind and insects is not a rarity, but less than 1%.

8.3.3 Germplasm Conservation and Utilization of Finger Millet

Gene bank assumes greater role and serves as reservoirs of diversity and source of alleles for genetic enhancement of crop plants. In India, the National Active Germplasm Collection Site (NAGS) located at All India Coordinated Small Millets Improvement Project (AICSMIP), Bangalore, made efforts to assemble large collections of germplasm at the global level with a total collection of 7070 germplasm. Similarly, the National Bureau of Plant Genetic Resources based in New Delhi, India, maintains 11,170 accessions of finger millet under long-term conservation. Most of these collections are indigenous with about 117 accessions of exotic origin. These Indian collections include six wild relatives, 154 advanced improved varieties and 64 breeding/research materials. ICRISAT, Patancheru, India, maintain a total of 7519 accessions. The Agricultural Research Station, USDA, Griffin, Georgia, maintains about 766 accessions of which 17 are wild relatives belonging to *E. floccifolia*, *E. indica*, *E. jaegeri*, *E. multiflora* and *E. tristachya*. The major germplasm conservation centres in small millets are given in Table 8.3.

The Indian and African germplasm are highly diverse, and a very few systematic studies have been conducted to compare the diversity among the accessions available in the gene banks. In general, the Indian germplasm are reported to be diverse for grain and dry stover yield. Early maturing varieties combining high grain yield, quality and stover yield are ideally preferred by farmers. The African germplasm exhibited larger variation for plant height, stout plant stature, long narrow finger, higher number of spikelets, poor threshability, late maturing and poor harvest index.

Table 8.3 Major germplasm conservation centres in small millets

Crop	Gene bank	Number of germplasm (Approx)
Finger millet	National Active Germplasm Collection Site (NAGS), Bangalore. India	7070
	ICAR-National Bureau of Plant Genetic Resources, New Delhi	11,170
	International Crops Research Institute for Semi-Arid Tropics, Hyderabad	7519
Proso millet	Russian Federation	8778
	Chinese Academy of Agricultural Sciences	8451
	ICAR-National Bureau of Plant Genetic Resources, New Delhi	1005
	National Active Germplasm Collection Site (NAGS), Bangalore, India	920
	International Crops Research Institute for Semi-Arid Tropics, Hyderabad, India	842
Foxtail millet	Chinese National Gene Bank	26,670
	ICAR-National Bureau of Plant Genetic Resources, New Delhi	4667
	National Active Germplasm Collection Site (NAGS), Bangalore, India	2821
	ORSTOM-MONTP, France	3500
	Svalbard Global Seed Vault, Norway	2505
	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru	1542
Little millet	National Active Germplasm Collection Site (NAGS), Bangalore, India	2000
	International Crops Research Institute for Semi-Arid Tropics, Hyderabad, India	1000
	ICAR-National Bureau of Plant Genetic Resources, New Delhi	1799
Barnyard millet	National Institute of Agro-Biological Sciences, Tsukuba, Japan	3671
	ICAR-National Bureau of Plant Genetic Resources, New Delhi	1953
	National Active Germplasm Collection Site (NAGS), Bangalore, India	2000
Kodo millet	ICAR-National Bureau of Plant Genetic Resources, New Delhi	2362
	National Active Germplasm Collection Site (NAGS), Bangalore, India	1537

The African germplasm are said to possess higher level of resistance to blast, the most devastating disease in finger millet (Kiran Babu et al. 2013).

For effective utilization of the germplasm for genetic improvement programme, Upadhyaya et al. (2006) established core collection of 622 genotypes representing

geographical regions and biological races from the entire collection. The African (58.7%) and Asian (35.8%) collections were predominant, while those from America and Europe were represented by 0.8–1.1%, respectively. The cultivated subspecies *coracana* occupied 97.4% of the core, while African accessions represented only 2.6%. Among the *coracana* subspecies, race *vulgaris* were predominant (62.5%) followed by *plana* (16.8%) *compacta* (12.4%) and *elongata* (8.3%). The core collections were evaluated for 15 quantitative and five qualitative traits, and a mini-core collection of 80 accessions was constituted. Wide variability was reported among the mini-core collections for economically important traits like days to flowering (51.24–93.73), plant height (72.66–113.31 cm), length of longest finger (49.79–139.73 mm), finger per ear (6.13–9.41) and grain yield (691–2430 kg/ha). Upadhyaya et al. (2007) reported diversity in 909 accessions introduced from southern and eastern African region from ICRISAT gene bank and observed large variability for plant pigmentation, growth characters, flowering, plant height and inflorescence length and width and grain colour. Their study also characterized the variability among different finger millet races. Daba and Keneni (2010) reported little or low effect of geographical origin on the pattern of diversity studied from native and exotic collections in Ethiopia. Their study revealed biomass, earweight and grain weight contributing more towards the observed diversity.

8.3.4 Varietal Improvement of Finger Millet in India

Breeding methods such as pureline selection, recombination breeding and mutation breeding are the widely used approaches for genetic improvement in finger millet. Mass selection has been used for purification of the landraces and varieties developed by pureline or pedigree systems. Pureline selection has also been extensively used in finger millet improvement. Single plants selections were made from landraces (germplasm) and improved farmers' varieties, and the promising lines for earliness, pest and disease resistance and grain yield were evaluated under multilocational trials and released as varieties (Ganapathy 2017a).

Hybridization was used extensively in finger millet compared to other small millets. Until the 1950s, the improvement aimed at improving locally adapted lines by centres. Subsequently millet improvement took place after establishment of the Project for Intensification of Regional Research on Cotton, Oilseeds and Millets (PIRRCOM) during the late 1950s and later formulation of the All India Coordinated Millets Improvement Project during 1965. Genetic resources of finger millet and small millets were assembled during the 1960s. The exotic lines from Africa were introduced to India during the 1970s. The lines from Africa had greater vigour, high finger length and good grain filling ability and thick robust stems with broad dark green leaves (Seetharam 1982). Recombination breeding took place between indigenous, exotic and indigenous with exotic lines. Selections were

followed from crosses involving Indian and exotic lines in various combinations for early, medium and late duration (more than 110 days).

Varieties developed from Indian × Indian crosses such as Udaya, K 7, Purna, Annapurna, Cauvery, Shakti and HPB 7-6 had moderate productivity. Hybridization among the Indian × exotic cross led to breakthrough in bringing greater variability and improving productivity of finger millet. This led to 50–60% increased productivity in Karnataka and Tamil Nadu (Seetharam 1982; Nagarajan and Raveendran 1985).

The period of finger millet improvement during 1964–1986 witnessed a revolution due to the introduction of Indo-African crosses of finger millet by the late Dr. CH Lakshmanaiah, who is known popularly as "Ragi Brahma" for his pioneering work that resulted in the release of 16 varieties designated as "Indaf" series. The yield levels of these varieties ranged from 3000 to 4000 kg/ha. In a significant move, the All India Coordinated Small Millets Improvement Project (AICSMIP) was launched in 1986 by the Indian Council of Agricultural Research with its headquarters in GKVK, UAS, Bangalore. Following this, during the period between 1986 and 2000, the yield potential has further improved ranging up to 4500 kg/ha with varieties which are resistant to blast disease. GPU 28 is one such medium-duration variety maturing in 110–115 days released during 1996. The variety is suited for delayed sowing under terminal drought conditions. It is also resistant to neck and finger blast which is a major constraint to finger millet production. The potential yield of the variety is 3500–4000 kg/ha. Presently the variety is grown on a larger area (about 70% area) in Karnataka. Joint efforts were made by the Department of Agriculture in each of the finger millet growing states, state seed corporation, state agricultural universities and private seed sectors for augmenting the availability of quality seed of GPU 28. During 2000–2012, upon establishment of AICSMIP, efforts were laid on developing productive lines with elite background through hybridization to improve high grain and straw yield suitable for *kharif* and also *rabi* seasons. The yield levels further increased subsequently ranging up to 5000 kg/ha. Currently, research efforts are underway to develop long-, medium- and short-duration varieties with high grain yield, resistant to blast disease, and to address the challenges like drought, saline and alkaline soils, cold season and hilly areas and mechanical harvesting. Some of the latest finger millet varieties released for cultivation are given in Annexure.

8.3.5 Strategies for Enhancing Productivity and Utilization of Finger Millet

8.3.5.1 Germplasm Evaluation

There is need for systematic evaluation of indigenous and exotic germplasm available with the national gene bank under multilocation germplasm for early maturity, photo insensitivity, drought tolerance, blast and other disease and pest resistance,

nutritional (protein, calcium, iron, zinc and essential amino acids) and antioxidants of therapeutic value.

8.3.5.2 Participatory Selection and Varietal Development

Farmers grow finger millet under marginal fertile soil conditions, and the performance of these varieties largely depends upon edaphic and climatic conditions which is least represented in research stations. This warrants participation of the farmers in selection of desired genotypes/varieties. Appropriate selection and breeding efforts involving farmers participatory approach is required to be taken up to develop high yielding finger millet varieties for different production systems under varying environmental conditions.

8.3.5.3 Breeding for Blast Resistance and Other Pests

Blast disease is major problem in finger millet affecting the crop at all stages of crop growth, and number of varieties are susceptible. Understanding the pathogen diversity in the geographical area, screening germplasm collections, identification of resistant germplasm and development of blast resistant varieties based on knowledge of the strains are desirable for development of durable resistance.

8.3.5.4 Interspecific Hybridization and Search for Novel Traits

There is need for search of novel traits in wild species especially from primary and secondary gene pool for disease resistance, cytoplasmic genic male sterility and other novel traits. The search and discovery of genetic or cytoplasmic male sterility would be a substantial breakthrough in the improvement of this crop by opening up the possibilities for effective population improvement.

8.3.5.5 Development of Early Maturing Varieties

Development of early maturing superior genotypes which can fit in different cropping systems as well as provide substantial yield under water stress is one of the important breeding objectives.

8.3.5.6 Genetic Improvement for Drought Tolerance

Systematic research to identify diverse drought tolerant genotypes, understand mechanisms of drought tolerance from multilocational screening, as well as identify key surrogate/adaptive traits needs to initiated. The identified lines should be used in introgression breeding for development of improved drought tolerant finger millet genotypes.

8.3.5.7 Stover Yield and Quality Improvement

Although finger millet crop is predominantly grown for grain/food purpose, the stover after harvest of crop is an excellent source of nutritive fodder to cattle. There is need to identify superior non-lodging tall types with superior grain and fodder yield. The African types are known to possess more plant height, and there is need to utilize these types for improvement of fodder yield and quality.

8.3.5.8 High Yielding White Finger Millet Varieties

Few white grain types are rich in protein (~12%) compared to coloured types (~8%) but are low yielding. Therefore, there is need to develop improved white grained finger millet genotypes for malting purposes as well as other end uses such as weaning foods, infant foods and malted milk foods, etc.

8.3.5.9 Nutritional Improvement

Finger millet is highly nutritious and thus calls for intensive evaluation of germplasm to assess its nutritional qualities. Finger millet grains besides providing energy are also a rich source of calcium and iron, and its proteins are a good source of essential amino acids and can greatly contribute to micronutrient and protein malnutrition affecting women and children in African and south-east Asian countries. The most cost-effective approach for mitigating micronutrient and protein malnutrition is to introduce varieties bred for iron, zinc and protein content in grains. Attempts to breed finger millet for enhanced grain nutrients are still in its infancy. Evaluation of finger millet core germplasm for grain nutrients and agronomic traits revealed a substantial genetic variability for grain iron, zinc, calcium and protein contents. Therefore, there is need to use the identified accessions in strategic research for development of nutritionally rich cultivars of finger millet.

8.4 Foxtail Millet

Foxtail millet [*Setaria italica* (L.) Beauv.] is one of the oldest of the cultivated millets in the world and is grown in about 23 countries in Asia, Africa and America. It is a self-pollinating species ($2n = 2x = 18$), belonging to family *Poaeceae* and subfamily *Panicoideae*. It is good as food, feed and fodder crop, which matures in a short duration. It is cultivated mainly on poor or marginal soils in southern Europe and in temperate, subtropical and tropical Asia (Marathee 1993). Its grain is used for human consumption and as feed for poultry and cage birds. The total world area is estimated to be about 10.5 lakh ha with a grain production of about 22.9 lakh tonnes. The major growing countries are China, the USA and India, with a contribution of only 2.4% to the total millets production in the world. In China, foxtail millet is next to rice and wheat in importance. In India, because of the drought tolerance, it was once an indispensable crop of vast rainfed areas in semi-arid regions, especially the Deccan plateau. But the area under foxtail millet has come down drastically during the 1990s mainly due to introduction of more remunerative crops like sunflower and soybean in blacksoils (Hariprasanna 2017). At present, foxtail millet is cultivated on a very limited area of about 70,000 ha mostly in Andhra Pradesh, Karnataka, Telangana, Tamil Nadu, Maharashtra, Rajasthan, Madhya Pradesh and north-eastern states.

Foxtail millet is adapted to a wide range of elevations, soils and temperatures and can grow from mean sea level to up to 2000 m altitude. It is drought tolerant and has a low water requirement, but does not recover well from drought conditions because of shallow root system. It is mostly grown to meet the domestic needs of the rural

people and is widely used as an energy source for pregnant and lactating women and also for sick people and children, and especially for diabetics (Sema and Sarita 2002; Hariprasanna 2017). The grains are rich in protein (12.3%) and crude fibre (8%) and gaining importance as a diabetic food due to relatively low-to-medium glycaemic index (Janani et al. 2016; Dayakar Rao et al. 2017; Wahlang et al. 2018). It has been suggested that foxtail millet protein be used as a food component to fight type 2 diabetes and cardiovascular diseases (Choi et al. 2005).

8.4.1 Origin and Taxonomy of Foxtail Millet

Foxtail millet has originated in China and has the longest history of cultivation among the millets, having been grown in China since sixth millennium BC. Carbonized foxtail millet has been identified in archaeological sites in China. The cultivation has been mentioned in Chinese records as early as 2700 BC (Vinall 1924). Its domestication and cultivation were the earliest identifiable manifestation of neolithic culture, the beginning of which has been estimated at over 4000 years ago (Chang 1968). The principal centre of diversity for foxtail millet is East Asia, including China and Japan (Vavilov 1926). A multiple domestication hypothesis (de Wet et al. 1979) is widely accepted though several hypotheses concerning the origin and domestication of foxtail millet have been proposed. From Central Asia, it spread to India and European countries (Oelke et al. 1990).

The genus *Setaria* consists of approximately 125 species (Dwivedi et al. 2012), widely distributed in warm and temperate areas. The genus includes a large number of valuable perennial forage grasses and grain crops (Chennaveeraiah and Hiremath 1991). Foxtail millet is the most economically valuable of the genus (Baltensperger 1996). Malm and Rachie (1971) thoroughly reviewed the domestication of foxtail millets and the taxonomy. The geographical origin of foxtail millet based on cytological studies indicated that wild ancestor of foxtail millet is *S. viridis* (Li et al. 1945). Three main groups of cultivated foxtail millet gene pool were suggested, namely, Chinese (from China, Japan and Korea), tropical (from Taiwan, India and Kenya) and European group, on the basis of isozyme studies in accessions of *S. italica* and *S. viridis*, respectively (Jusuf and Pernes 1985; Panaud 2006).

On the basis of inflorescence morphology, foxtail millet is classified into two species, *S. pumila* and *S. italica*. The species *S. italica* is divided into two subspecies *viridis* and *italica*. The subspecies *italica* is classified into three races and ten sub-races (Prasada Rao et al. 1987). The race *moharia* (common in Europe, south-east Russia, Afghanistan and Pakistan) is divided into sub-races *aristata*, *fusiformis* and *glabra*; race *maxima* (common in eastern China, Georgia, Japan, Korea, Nepal and northern India) is divided into sub-races *compacta*, *spongiosa* and *assamense*; and race *indica* (remaining parts of India and Sri Lanka) is divided into sub-races *erecta*, *glabra*, *nana* and *profusa*. Race *maxima* has also been introduced in the USA.

8.4.2 Germplasm Resources and Utilization of Foxtail Millet

Wide genetic diversity is available in foxtail millet, and more than 44,000 germplasm have been conserved in different gene banks all over the world (Vetriventhan et al. 2020). Major collections include Chinese National Gene Bank (CNGB, 26,670 accessions); ICAR-National Bureau of Plant Genetic Resources, New Delhi (4667); ORSTOM-MONTP, France (3500); Svalbard Global Seed Vault, Norway (2505); International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (1542); National Institute of Agro-biological Sciences (NIAS), Japan (1299); and North Central Regional Plant Introduction Station, USDA-ARS, USA (1000).

The ICAR-Indian Institute of Millets Research, Hyderabad, holds about 4554 accessions in its medium-term storage module. The National Active Germplasm Site (NAGS) for small millets was established at University of Agricultural Sciences, Bengaluru, in 1992. It holds about 2559 accessions, most of which have been characterized and catalogued. At Tamil Nadu Agricultural University, Coimbatore, considerable diversity was observed among 741 accessions maintained for all the agronomic characters (Nirmalakumari and Vetriventhan 2010). The Regional Agricultural Research Station, Acharya NG Ranga Agricultural University, Nandyal, Andhra Pradesh, also maintains an active collection of more than 1000 accessions, which has been used in varietal development programmes. The ICRISAT has constituted a core collection comprising 155 accessions using the taxonomic and qualitative traits data (Upadhyaya et al. 2008). Multi-location evaluation of core collection has resulted in identification of a number of diverse germplasm accessions with agronomically and nutritionally (high seed protein, calcium, iron and zinc) superior traits. Further, a mini core comprising of 35 accessions has been developed (Upadhyaya et al. 2011), which is an ideal resource for studying population structure and diversity and identifying new sources of variation for use in breeding and genomics studies in foxtail millet.

8.4.3 Genetics and Cytogenetics of Foxtail Millet

The most common basic chromosome number in genus *Setaria* is $x = 9$ (Singh and Gupta 1977), although numbers like $x = 7$, 8 and 10 are also found rarely. Cultivated millet *S. italica* (L.) Beauv. and closely related species *S. viridis* L. are both diploids with $2n = 2x = 18$. Different levels of polyploidy ($3x$, $4x$, $5x$, $6x$, $7x$, $8x$ and $12x$) are also known in different species of *Setaria*. Intraspecific polyploidy has been reported in many *Setaria* species like *S. sphacelata* showing $2x$, $4x$ and $6x$ (Singh and Gupta 1977). Studies on chromosome structure revealed four pairs of metacentric, four pairs of submetacentric and one pair of acrocentric chromosomes in foxtail millet (Chandola 1959). Chikara and Gupta (1979) observed one pair of SAT-chromosomes each in six varieties, which differed in the number of metacentric, submetacentric and acrocentric chromosomes. These differences were attributed to structural changes. However, meiosis was normal showing nine bivalents

(Chandola 1959; Singh and Gupta 1977). A complete set of nine primary trisomics ($2n + 1$) (cv. Yugu No. 1) of foxtail millet was identified cytologically from progenies derived from crosses between autotriploids ($2n = 2x = 27$) and their diploid counterparts (Wang et al. 1999). In a *S. italica* × *S. viridis* cross regular nine bivalent formation was noticed. Regular chromosome pairing in the hybrid and its partial fertility suggests that genomes of foxtail millet (*S. italica*) and *S. viridis* are similar and that foxtail millet originated from wild *S. viridis* through selection and further cultivation (Li et al. 1945).

Genetic studies in foxtail millet have been conducted mostly on morphological characters and disease response (Malm and Rachie 1971). Most of the previous works have focused on estimating heritability and realized genetic gains, with little attention directed to measuring levels of heterosis or to assessing the relative importance of different types of gene action due to highly self-pollinated nature (Athwal and Singh 1966; Gill and Randhawa 1975; Vishwanatha et al. 1981; Gurunadha Rao et al. 1984; Prasada Rao et al. 1985; Nirmalakumari and Vetriventhan 2010). Agronomic traits like days to 50% flowering, plant height, total number of tillers, number of productive tillers, panicle length and days to maturity exhibited highly significant positive correlations with grain yield. Characters such as flag leaf area and 1000-grain weight were also observed to influence yield. High heritability was observed for all the quantitative traits like flowering duration, plant height, inflorescence length and test weight (Hariprasanna et al. 2015, 2017, 2018, 2019). Negative association of protein content and calcium content with grain yield has been reported along with negative association of carotene with grain yield (Prasanna et al. 2013). So simultaneous improvement of these traits along with grain yield may not be possible.

8.4.4 Reproductive Biology of Foxtail Millet

Foxtail millet matures in 60–90 days depending on genotype. It forms a slender, erect, leafy stem varying in height from 40 to 150 cm. The stem produces an inflorescence terminally. There is a very quick transition period from vegetative growth to flower development (Baltensperger 1996). The inflorescence is a dense, cylindrical terminal spike borne on a thin and very short pedicel (Sundararaj and Thulasidas 1976) with short side branches bearing spikelets and bristles. The shortened side branches are called secondary clusters or lobes. The compressed hairy panicle 5–30 cm long resembles yellow foxtail, green foxtail or giant foxtail. Each spikelet consists of a pair of glumes that embrace two minute flowers (about 1 mm in length), the lower one sterile and the upper one bisexual, with three stamens and a long oval smooth ovary with two long styles, which terminate in a brush-like stigma (Hector 1936). The spikelets are glabrous, elliptic to obovate and about 2 cm long. One to three bristles develop at the base of each spikelet (Vinall 1924). Anthesis in foxtail millet generally takes place near midnight and in the morning, but varies significantly with the environment (Malm and Rachie 1971). The rate of anthesis is generally favoured by low temperature and high humidity.

The small seeds, around 2 mm in diameter, are encased in a thin, papery hull which is easily removed after threshing. The seed coat and husk of foxtail are generally of single entity with glossy appearance. The grains are very similar to paddy rice in grain structure with outer husk, which needs to be removed in order to be used. Seed colour varies greatly between genotypes and is usually yellowish, cream to orange or yellow brown to black in colour (Seetharam et al. 2003). Seed colour varies greatly between genotypes and is usually yellowish in colour. Morphology and anthesis behaviour make foxtail millet one of the most difficult species to cross pollinate.

8.4.5 Breeding Objectives of Foxtail Millet

The principal breeding objectives in foxtail millet include development of early maturing, high yielding and drought-resistant varieties like any other small millets. Maximization of biomass and the harvest index are also the target traits. Genotypes have to be tailored for early, mid-late or late maturity, depending on the location-specific requirements of soil, rainfall, temperature, humidity, day length and cropping patterns (Harinarayana 1989). Dwarf plant stature to reduce lodging, water-use efficiency and nitrogen-use efficiency, uniform maturity, etc. are other breeding objectives.

8.4.6 Breeding Methods of Foxtail Millet

Pureline selection from the local germplasm remained the prime breeding method for improving performance, particularly grain yield of foxtail millet. As foxtail millet is largely self-pollinated and out-crossing is very much limited, and because of the anthesis behaviour, hybridization followed by selection was a difficult breeding procedure to adopt and could not gather much research efforts. Difficulties in emasculation and pollination in small millets in general, identification of true hybrids, limited heterosis in inter-varietal crosses (Srivastava and Yadava 1977) and the non-availability of diverse germplasm resources to the breeders acted as major constraints in varietal improvement in foxtail millet (Hariprasanna 2017). Some of the latest foxtail millet varieties released for cultivation are given in Annexure.

8.4.7 Production Constraints of Foxtail Millet

Though foxtail millet is not severely affected by any major biotic stress factors, blast disease caused by pathogenic fungus, *Magnaporthe grisea*, is one of the major diseases affecting the production, especially in northern China and India (Nakayama et al. 2005). Blast affects both grain and forage production in foxtail millet. Symptoms of the disease appear as circular spots with straw-coloured centres on

leafblades. Other frequently occurring diseases are sheath blight, downy mildew and rust. Among the insect pests, shoot fly (*Atherigona atripalpis* Wiede) is one of the important pests in foxtail millet. Shoot fly do not prefer the foxtail millet genotypes possessing lower moisture, crude protein and total sugar content for its oviposition and dead heart infestation (Sanjeev et al. 2011).

Drought stress and high temperature are important abiotic factors affecting grain yield and biomass. Prolonged moisture stress severely affects crop establishment and biomass yield. High temperature above 35 °C coinciding with flowering affects seed set. Lodging is another important yield and quality-reducing factor depending on the environmental conditions. Thin stem, tall plant height and poor root anchoring due to light soils often lead to lodging during maturity.

8.4.8 Future Prospects of Foxtail Millet

The foxtail millet genome has been sequenced, and the first assembled reference genome of foxtail millet and green foxtail was released in the year 2012 by two independent groups (Zhang et al. 2012; Bennetzen et al. 2012). The Joint Genome Institute of the US Department of Energy and the Center for Integrative Genomics had constructed a public domain database PHYTOZOME (http://phytozome.jgi.doe.gov/pz/portal.html) to provide online resources for foxtail millet genomics with unrestricted public access. The genome data generated by Beijing Genomics Institute (Zhang et al. 2012) are available in Foxtail millet Database (http://foxtailmillet.genomics.org.cn/). A large volume of information on genomic, genic and ILP marker resources are available in the Foxtail Millet Marker Database developed by National Institute for Plant Genome Research, New Delhi (https://59.163.192.91/foxtail/markers.html). Availability of genomic information and resources has now provided numerous scientific leads to proceed further towards crop improvement of millets, cereals and bioenergy grasses. The major research areas that need to be further explored are structural genomics, genome-wide studies of gene families involved in abiotic stress tolerance, epigenetics and gene expression regulation and understanding the genetics and genomics of nutritional traits (Muthamilarasan and Prasad 2015). More high yielding and nutritious varieties need to be developed to popularize the crop and regain the lost area. Promotion of foxtail millet cultivation through appropriate policy interventions can ensure food, nutrition, fodder and livelihood security in the dryland agriculture.

8.5 Barnyard Millet

Barnyard millet (*Echinochloa frumentacea* (RoxB). Link), being a climate-resilient crop, also produces multiple securities like food, nutrition, fodder, fibre, health, livelihood and ecology. Globally, India is the largest producer of barnyard millet, both in terms of area (0.146 million ha) and production (0.147 million tonnes) with average productivity of 1034 kg/ha during the last 3 years (IIMR 2018). It is widely

cultivated as minor cereal for food as well as fodder in India, China, Japan, Korea, Pakistan, Nepal and Africa. Due to its remarkable ability to withstand erratic rainfall and varying weather conditions, it has been classified as one of the drought stresses, hardy crops which is largely cultivated in harsh and fragile environments. It is a regular crop up to 2700 m above MSL during *kharif* season in Uttarakhand and Tamil Nadu and forms a mainstay of agricultural diet and cultural system of hill people. Diversity in this crop is eroded fast due to several reasons like cultivation on poor, shallow and marginal soils under rainfed conditions, reduction in area under cultivation and changing social, economic and cultural dimensions of farming community in India (Maikhuri et al. 2001). Barnyard millet with rich nutritional profile is one of the best choices for patients with dietary-based health defects like diabetics, heart-related diseases and celiac diseases. Millets are free from gluten; hence, gluten-allergic patients require these minor millets for health improvement. Traditional foods prepared from barnyard millet such as roti, payasam, pongal, pulav, idli, dosa and muruku are very popular in southern India. In addition to human food, it has an important place in dairy due to high palatability of its fodder, which can also be used for making hay or silage.

8.5.1 Origin and Taxonomy of Barnyard Millet

The barnyard millet belongs to the genus *Echinochloa*, the family *Poaceae* and subfamily *Panicoideae* (Clayton and Renvoize 2006). The genus *Echinochloa* comprises about 250 related species of annual or perennial grasses widely distributed in temperate and warmer parts of the world (Bajwa et al. 2015). Xiaoyan et al. (2015) conducted microfossil studies on both lithic implements and sediment samples from previously studied levels of occupation, and they found evidence for the exploitation of nuts, such as acorns (*Lithocarpus*/*Quercus sensulato*), and water chestnuts (*Trapa*), as well as extensive evidence for the processing of another wetland grass, barnyard grass (*Echinochloa* spp.), indicating that this wild millet was an important resource harvested and processed alongside rice and only abandoned in favour of rice at a later stage of the domestication process. The two species of barnyard millet are grown as cereals. *Echinochloa crus-galli* is native to temperate Eurasia and was domesticated in Japan some 4000 years ago. *E. oryzoides*, the most aggressive weed, is recognized taxonomically as *E. crus-galli* var. *oryzicola*. It differs from *E. crus-galli* in having $2n = 36$ rather than $2n = 54$ chromosomes (De Wet et al. 1983). *Echinochloa colona* is widely distributed in the tropics and subtropics of the Old World. It was domesticated in India. *Echinochloa colona* is morphologically allied to *E. crus-galli*. Four morphological races are recognized, although these do not have geographical, ecological or ethnological unity. Race *laxa* is confined to Sikkim, while races *robusta*, *intermedia* and *stolonifera* are also grown in India (De Wet et al. 1983). The two cultivated species under genus *Echinochloa*, *E. frumentacea* (Indian barnyard millet) and *E. esculenta* (Japanese barnyard millet) are cultivated for food and fodder by hilly and tribal communities in Asia particularly in India, China, Africa and Japan.

8.5.2 Germplasm Resources and Utilization of Barnyard Millet

The most of the germplasm accessions of barnyard millet are housed in India and Japan. ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora, India; ICAR-National Bureau of Plant Genetic Resources, New Delhi; ICAR-Indian Institute of Millets Research (IIMR), India; National Institute of Agrobiological Sciences (NIAS), Japan; and International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India, are actively involved in collection, conservation and utilization of germplasm lines in barnyard millet. Germplasm characterization for morphological traits is the preliminary step; this not only provides information on heritability of the traits but also increases the germplasm utilization. Gowda et al. (2008) evaluated 729 germplasm accessions of barnyard millet for yield and related traits over the years. Correlation and path coefficient analysis for morphological traits in barnyard millet showed significant direct effect of heat units at maturity, photothermal units at 50% flowering, plant height, days to 50% flowering and weight of panicle on grain yield, and they could serve as a useful criterion in the development of short high yielding cultivars (Mehta et al. 2007).

Wallace et al. (2015) have genotyped the core collection (89 accessions) following genotyping-by-sequencing (GBS) approach and identified several thousand SNPs and found four populations within *E. colona* and three in *E. crus-galli*, which match with phylogenetic relationships. ISSR (Nozawa et al. 2004) and RAPD (Deepti et al. 2012) markers were identified and used in the diversity analysis in the germplasm accessions of Indian and Japanese barnyard millet accessions. Lal and Maloo (2006) studied the path coefficient analysis in Indian barnyard millet and found that main panicle weight, plant height and primaries had high effect on seed yield. The 95 germplasm lines representing the global collection were evaluated for qualitative and quantitative traits, and cluster analysis showed that Indian and origin unknown accessions grouped in *E. frumentacea* group, Japanese accessions grouped in *E. esculenta* group and third group contained the accessions from Russia, Japan, Cameroon and Egypt (Sood et al. 2015). Gupta et al. (2009) evaluated 194 accessions collected from different eco-geographical regions of India for 14 quantitative traits. Five groups were formed based on location of collection; group C with unknown origin exhibited maximum diversity with 17.67% coefficient of variation. The other groups recorded mean coefficient of variation between 12% and 13%. Correlation studies showed leaf width and number of racemes are useful to carry out selections in segregating populations.

8.5.3 Reproductive Biology of Barnyard Millet

In barnyard millet, the inflorescence is seen in varying shapes (pyramidal, cylindrical, globose and elliptic), colours (green, light green, light purple and dark purple) and compactness (open, intermediate and compact) (Gupta et al. 2009; Sood et al. 2015). Raceme number varies from 22 to 64 per inflorescence (Renganathan et al. 2017) and varies in arrangement from one side, two sides or around the rachis. The

position of florets varies from one side of raceme to around the rachis in which spikelets will be arranged in 3–4 rows (florets positioned on one side of raceme) and irregularly arranged (florets positioned around the raceme). Each spikelet contains two florets; upper fertile floret contains two unequal glumes, lemma and palea, three stamens (white, yellow and dark purple coloured) and plumose type of bifid stigma (white, pink and purple coloured). Anthesis and pollination are basipetal in nature where flower opening which starts at 5 am reaches maximum at 7–8 am and closes by 10 am (Sundararaj and Thulasidas 1976: Jayaraman et al. 1997).

8.5.4 Breeding Objectives of Barnyard Millet

Barnyard millet is known for its high biomass production apart from grain yield; hence, it is cultivated as a dual-purpose crop. The breeding goal is to improve its biomass yielding ability (green fodder and dry fodder yield) along with grain yield. The quality of the fodder for its easy digestibility and preference by the cattle is also good in barnyard millet when compared to other millets. Breeding methodologies focussing on the use of physiological traits-based phenotyping in millets will be one of the potential areas to exploit for crop improvement. Development of cultivars with dual-purpose types (early maturing quick growing and high biomass yielding lines) is another breeding objective. The grain size in the developed cultivars is small, and dehulling efficiency directly depends upon the grain size of the crop. Hence, increasing the grain size in barnyard millet is another area of research to increase the grain yield as well as dehulling percentage. The length of the panicle and number of productive tillers are having high correlation with grain yield. Developing lines with more spikes and long panicles is another targeted area. Grain smut is the major biotic constraint causing yield loss up to 60%. Japanese barnyard millet and *E. colona* are immune to the disease. Japanese type and Indian type are not easily crossable because of hybrid sterility. However, *E. colona*, wild relative of Indian barnyard millets, is crossable with cultivated type and may be used as a donor source for resistant genes to grain smut. In barnyard millet, male sterile lines are not available and hand emasculation and pollination are very difficult because of the large number of tiny flowers in single inflorescence. Identification/development of male sterile lines is of prime importance for recombination breeding. Barnyard millet is rich in dietary fibre, iron and zinc minerals; targeting the nutritional traits and developing varieties with high nutrient content are of great advantage in developing naturally fortified cultivars.

8.5.5 Breeding Methods of Barnyard Millet

Barnyard millet is a self-pollinated crop with 5–10% of outcrossing. In kharif season because of high relative humidity and less difference between day and night temperatures, the flowers will open in less number, and pollination is mostly cleistogamous. In rabi season because of low relative humidity, more difference

between day and night temperature (night cold temperature followed by early morning exposure of florets to sunlight) leads to opening of a greater number of florets and pollination after opening of the floret. Hence, in rabi season, the outcrossing percentage is higher than in kharif season. The breeding objectives are based upon the pollination type in the crop. Barnyard millet being a self-pollinated crop uses breeding methods like selection, pedigree method, single seed descent method and mutation breeding. Most of the varieties released in barnyard millet are based on selection method from germplasm lines. In recent years, pedigree method is in practice to combine the favourable traits of two parents with more understanding of the traits and emasculation and pollination techniques.

Use of chemical hybridizing agents and mutation with physical and chemical means are the other methods of creating variation for selection and also to identify male sterile lines if any produced in the population. Improvement/modification over the SSD method is single panicle descent method which is in practice in barnyard millet for the development of improved cultivars. The information on molecular marker development and use in breeding programs is lacking in this crop. Recently, the Japanese barnyard millet *E. crus-galli* (STB08) genome is sequenced (Guo et al. 2017), and the draft genome sequence is available for use in development of markers and identification of candidate genes for particular traits. Availability of the molecular markers (SSRs, SNPs) and the genome sequence information boosts the breeding work through the application of advanced breeding tools like MABB and MARS for the development of cultivars with specific defect correction or for multiple trait improvement.

8.5.6 Production Constraints of Barnyard Millet

The developed cultivars in barnyard millet are based on selection from germplasm collections without much intervention of advanced breeding methodologies. Lack of clear information on physiological traits, biochemical traits and nutritional traits in barnyard millet is hindering the progress in improving the crop for high yield other end specific cultivar development. Negative correlation of earliness, high grain yield and biomass yield is also limiting the development of superior varieties. Fodder yield is one of the most important components nowadays along with grain yield; development of high biomass lines hinders high grain production as photosynthates translocate more to biomass production and less to reproductive parts hence lowering the grain yield. Recombination breeding combines the favourable traits of two or more parents and releases the variation in the segregating populations, but recombination breeding is difficult in barnyard millet because of many tiny flowers arranged closely in the inflorescence. Emasculation and hybridization are difficult in this millet like other millets because of early hours of flower opening, non-abundance of pollen grains, short viability of pollens, short opening time of flowers and tightly attached lemma and palea around the stigma and anthers (Sood et al. 2015). Genomic resources are helpful for the progress of any crop species, and they assist in effective characterization of germplasm resources and subsequent use in the discovery of

QTL/gene(s) for the crop improvement program. However, genome research in barnyard millet is still in the early stage and far behind the other minor millets (Renganathan et al. 2020). Very limited attempts have been made to discover the genomic structure and associated downstream processes due to its genome complexity and lack of research funding on this orphan crop. Some of the latest barnyard millet varieties released for cultivation are given in Annexure.

8.5.7 Future Prospects of Barnyard Millet

Understanding the genetic base and inheritance pattern of traits governing the yield and biomass is of prime importance in this crop. Usefulness of physiological trait-based phenotyping to unravel the stress tolerance mechanisms and to identify the suitable cultivars for stress-prone areas is urgently required to combat the changing climatic conditions. The nutritional superiority available in barnyard millet germplasm will help the breeders to develop suitable cultivarsnaturally enriched with nutrients and minerals gives added advantage for popularization and also for nutritional security. Widening the genetic base specifically for the traits like grain size, panicle length, high biomass and number of productive tillers through recombination breeding and mutational breeding is the way forward to bring improvement in both pre-harvest and post-harvest processing aspects in barnyard millet. Use of wild relatives and progenitor species for transferring the resistant genes for biotic stresses may fetch rewards in the near future by avoiding the yield losses both in grain and fodder.

The fertility barriers between Indian and Japanese types may be analysed, and advanced biotechnological tools may be used to overcome the barriers. Overcoming the barriers will help in many ways of combining the favourable traits of both types in one background. Molecular sequence data generation and its use in breeding are completely lacking in barnyard millet specifically in Indian barnyard millet when compared to other millets like foxtail millet, proso millet, finger millet, pearl millet and sorghum. Generation of genome sequence data, genome structure studies, evolution pattern of the genome and relatedness of barnyard millet genome with other millets, unraveling of nutrient accumulation pathways, identification of genes/QTLs and tagging of genes with particular traits on particular linkage groups are the niche areas of research in barnyard millet to bring suitable improvement in the development of high yielding cultivars.

8.6 Little Millet

Little millet (*Panicum sumatrense* Roth. ex. Roem and Schultz) is indigenous to the Indian subcontinent and also has wild ancestor *Panicum psilopodium* present throughout India. It is widely cultivated as minor cereal across India and to a certain extent in Nepal and western Burma. In India, the crop is majorly grown in Madhya Pradesh, Chhattisgarh, Karnataka, Tamil Nadu, Orissa, Andhra Pradesh, Jharkhand

and Bihar. The crop is hardy and moisture stress tolerant and provides reasonable harvest even in degraded soils and unfavourable weather conditions (Selvi et al. 2014). The crop is cultivated both in the tropics and subtropics even up to an altitude of 2000 m above mean sea level. The crop is known for its drought tolerance and is considered as one of the least water demanding crops. Other advantage is that the grains can be stored for up to 10 years or more without much loss due to deterioration (Selvi et al. 2014). Consequently, it has traditionally played an important role as a reserve food crop. The crop is also grown in the eastern parts of India where it formed part of tribal agriculture. Cultivated area under crop has drastically declined from about 5 lakh ha during 2001–2002 to presently about 2–2.5 lakh ha and production of about 1 lakh tonnes. However, exact figures on current area, production and productivity are not available. Madhya Pradesh state occupies about 40–50% of the area under little millet followed by Chhattisgarh, Tamil Nadu, Karnataka, Maharashtra, Orissa, Andhra Pradesh and Jharkhand. The average productivity of the crop is around 500–600 kg/ha.

Nutritionally the grain of little millet is comparable or even superior to some of the major cereals. In general, little millet is a disease-free crop, but occurrence of grain smut (*Macalpino mycessharmae*) can lead to economic losses at times. Among insect pests, incidence of shoot fly is widely reported and known to cause economic losses. The crop is known for high content of crude fibre in its grains. It is also rich source of protein (~7.7%) and fat (~4.8%), minerals and vitamins and requires consideration as essential food for nutritional security. The crop matures in 80–120 days depending on the type of cultivar grown. Yield levels in this crop may reach close to 3 tonnes/ha under favourable conditions.

8.6.1 Morphological Variation in Little Millet

Panicum sumatrense subsp. *sumatrense* is a morphological variable cereal largely cultivated in India followed by Sri Lanka, Nepal and Burma. The species include wild and cultivated forms. The species is divided into *P. sumatrense* subsp. *sumatrense* to include cultivated one and subsp. *psilopodium* (Trin.) de Wet comb. nov. to include the wild progenitor. These two subspecies cross where they are sympatric to produce fertile hybrids, derivatives of which are often weedy in little millet fields. Weedy types are derived between wild and cultivated types and escape from cultivation due to the ability of efficient natural seed dispersal. *P. sumatrense* is extensively variable with regard to growth habit and inflorescence morphology. Based on the morphological variation and distribution, two races of cultivated little millet are recognized, namely, *nana* and *robusta* (de Wet et al. 1983). Race *nana* resemble wild subspecies of *P. sumatrense* with regard to inflorescence morphology. Race *nana* includes plants with decumbent to almost prostrate culms that become erect at time of flowering. Inflorescences are large and open with the upper branches sometimes clumped and curved at time of maturity. Height of the plants ranges from 50 to 100 cm. Terminal inflorescences size range from 14 to 50 cm long and are erect, open and strongly branched, and sometimes branches get clumped at time of

maturity. Race *robusta* are erect plants with large strongly branched open or compact inflorescence. Flowering culms grow tall and range between 120 and 190 cm and robust. Terminal inflorescence is in range of 20–46 cm long, open or compact and strongly branched. Open inflorescences are essentially erect, while compact inflorescences are curved at maturity. This race is grown in northwestern Andhra Pradesh and adjacent Orissa where it crosses with race *nana* (de Wet et al. 1983).

8.6.2 Genetic Improvement of Little Millet

Major work reported so far is on screening of the germplasm for improved genotypes for yield and other important traits, germplasm diversity studies, identification of resistant sources for smut resistance, testing association among different traits influencing yield, proximate and mineral composition in grains, mutation studies and creation of new variation and identification of surrogate/adaptive traits and genotypes for drought tolerance (Ganapathy 2017b). The race *nana* matures faster and generates less biomass than *robusta* (de Wet et al. 1983). In the tribal area of the Indian Kolli Hills, diversity among locally grown landraces of little millet was found to be high for all morphological traits measured both within and between landraces (Arunachalam et al. 2005). High diversity and heritability and genetic advancement was reported for yield and productive tillers in 109 landraces indicating selections for varietal development (Nirmalakumari et al. 2010). Evaluation of about 460 collections of little millet held by ICRISAT revealed high genetic variation for most of the quantitative traits tested (Upadhyaya et al. 2014). A core collection of 56 genotypes was constituted which represents seed bank collections. Increased heritable lodging resistance has been introduced to a population of little millet with γ-ray mutational breeding (Nirmalakumari et al. 2007). Channappagoudar et al. (2007) identified traits influencing grain yield in the crop. Taller genotypes were found to be high yielding, and shorter genotypes are medium to low yielding. Other parameters are number of leaves and tillers per plant positively contributing towards higher grain yield. Leaf area index and leaf area duration were also important growth parameters for improving the yield and total dry matter as it indicates the efficiency of the photosynthetic system. Overall, the study reported TNAU 63 (20.2 q/ha) and DPI 1869 (18.3 q/ha) and TNAU 18 (16.9 q/ha) were high yielding with high values for most morpho-physiological parameters. Gollagi et al. (2013) analysed the biophysical basis of yield enhancement in little millet and observed high transpiration rate in low yielding genotypes and low rate in high yielding genotypes. They also observed that higher yielding types had higher stomatal conductance (60 DAS) which could be because of higher stomatal frequency on abaxial surface leading to enhanced canopy photosynthesis. Nirmalakumari and Ulaganathan (2013) followed farmers' participatory approach to identify trait-specific genotypes. Twelve genotypes were evaluated by farmers on community plots managed by them at several sites in agro-ecological areas. Farmers evaluated for panicle type, yield traits, seed size and lodging resistance and rated the varieties based on their preferences. Farmers showed more interests towards

compact panicles possessing bold seeds, pest and disease-free genotypes and non-lodging characteristics.

Salini et al. (2014) evaluated 105 germplasm of little millet for various traits and selected 12 promising lines as parents and reported several cross combinations. Gene action was studied for 11 characters, and they reported non-additive gene action for all the characters except plant height where additive and non-additive played equal role. High coefficient of variation was observed for grain yield per plant and number of basal tillers per plant. Heritability estimates were high for all the characters except flag leaf sheath length. Inheritance of qualitative characters indicated monogenic simple dominance inheritance for most traits except grain colour. Stability analysis was carried out for 12 parents in four environments for eight characters and identified IPmr 1046 and IPmr 889 as stable genotypes for grain yield.

Girish et al. (2013) evaluated six improved types, RLM 40, BL 4, RLM 186, DLM 14, GV-2-1 and RLM 141, to identify high yielding types with checks JK 8 and OLM 203. They identified two lines, BL 4 (1141 kg/ha grain yield and 5 tonnes/ha fodder yield) and DLM 14 (grain yield of 1292 kg/ha and fodder yield of 5 tonnes/ha), as promising compared to check JK 8 which yielded 994 kg/ha with 6 tonnes/ha fodder yield. DLM 44 was reported to be early with height of about 100 cm and suited for intercrop with redgram. Sasamala et al. (2012) studied the genetic diversity among 22 little millet lines evaluated over 12 environments. The study identified KCM 42, KCM 102D, Sabar and Co 2 most divergent with KCM 594 and RCM 4 indicating that hybridization between these genotypes likely to give better recombinants in segregating generations.

M.S. Swaminathan Research Foundation (MSSRF), Chennai, has taken initiatives to collect, evaluate and conserve little millet in Tamil Nadu to improve cultivation of the crop. In Kolli Hills of Tamil Nadu, little millets were preferred by tribal farmers as they provided sustainable benefits. MSSRF attempted to bring back cultivation of the small millets and also to revitalize their conservation of local landraces and cultivars. The participatory rural appraisal conducted with farmers of the regions revealed interest of the farmers in millets but are getting eroded due to low productivity of landraces that are under traditional cultivation. Ideal approaches are to introduce scientific steps to optimize their cultivation practices under site-specific constraints. Arunachalam et al. (2005) from MSSRF studied the stability of genetic diversity among landraces of little millet in south India at the ecological level. Genetic divergence studies revealed high diversity among set of landraces analysed at two locations for two seasons. Days to maturity and flowering contributed most to the genetic differentiation. The study confirmed the sustained availability of the divergence in little millet in Kolli Hills of Tamil Nadu. They observed location specific expression of traits among the landraces and further suggested that breeding and selection to be followed as per the needs of the region.

8.6.3 Varietal Development of Little Millet

Varietal development has received less attention as the case in other small millets. Most of the varieties released were developed through mass selection or pureline selection methods. Breeding for new varieties using hybridization techniques is limited owing to difficulties encountered in crossing due to tiny spikelets on brittle pedicels (Nirmalakumari et al. 2007). Mutation breeding was used as complement to conventional breeding methods for genetic improvement of little millet. A number of high yielding varieties have been developed and released for cultivation in different little millet-growing states. The improved varieties are able to meet the specific requirements of different regions. Although yield has been the main criterion for development of new varieties, the varieties OLM 20 possess drought tolerance, OLM 36 for brown spot and sheath blight and OLM 203 blast and grain smut. Birsa Gundli 1, selection from local developed from BAU, Ranchi, is very early and matures in 55–60 days with reasonable grain yield. The latest varieties released in different states and popular in different little millet growing states are given in Annexure.

8.6.4 Future Prospects of Little Millet

For valuing the genetic diversity in the germplasm, systematic evaluation and unlocking the genetic diversity in the germplasm should be the major objective for genetic improvement. Grain yield has always been an important trait for genetic improvement. Efforts towards improvement for yield have led to marginal improvement. Selection for component traits such as compact panicle types and large seed size should be done. Knowledge on floral biology and hybridization techniques requires attention. Moreover, the small-sized florets in this crop have hindered in the genetic improvement of this crop through hybridization techniques. Although millets are grown on poor soils under dry conditions, genotypes/cultivars responsive to high input conditions should also be identified. Little millet is well-known for its drought tolerance and is considered as one of the least water-demanding crops. Few isolated studies have indicated less loss in grain yield under water stress compared to proso millet and foxtail millet.

Cultivars possessing drought tolerance and better regenerative capacity on reversal of dry spell for harsh conditions should be focus of genetic improvement. Farmers' participatory approach should be followed for deriving improved varieties. The dry fodder after harvest of the crop is source of nutritious fodder to animals. It is possible that the variability in the quality stover could be exploited to develop improved varieties with better nutritive value. Pests and diseases infecting little millet are less. However, earlier studies have indicated shoot fly and grain smut causing economic losses. Host plant resistance should be a major strategy for pest and disease resistance. Lodging and grain shattering are the other important agronomic traits which should receive due attention while improving for grain yield. Breeding for specific end uses should receive priority as this attracts the private

sector industries to invest in millets. Little millet is known to mature in 60–80 days. However, early maturing genotypes with substantial high yield and photo-insensitive genotypes suiting different cropping systems should be developed.

8.7 Kodo Millet

Kodo millet (*Paspalum scrobiculatum* L.) is widely distributed in damp habitats in the tropics and subtropics. It is grown in arid regions of Asia, New Zealand and the USA as a pasture crop. It is an indigenous cereal of India largely grown in Madhya Pradesh, Chhattisgarh, Uttar Pradesh, Tamil Nadu, Maharashtra, Karnataka and some parts of Andhra Pradesh (Yadava and Jain 2006). It is popularly known as kodo (Hindi), Varagu (Tamil), Arika (Telugu), Harka (Kannada), Kodra (Gujarati, Marathi and Punjab) and Kodua (Oriya) in India (Ayyangar and Rao 1934; Deshpande et al. 2015). It has several names in different parts of the world like bastard millet, creeping paspalum, ditch millet, Indian paspalum, koda grass, scrobic, water couch and Kodohirse (German) (Knees and Gupta 2013). In India, kodo millet is widespread and grown in about 1.5–2.0 lakh ha with a productivity of about 400–500 kg/ha depending on environmental conditions. Madhya Pradesh and Chhattisgarh account for nearly 70–80% area under this crop, and other important states are Tamil Nadu, Maharashtra, Uttar Pradesh and Gujarat.

8.7.1 Floral Biology of Kodo Millet

Kodo millet is a highly self-pollinated crop with cleistogamous flowers, but several researchers observed opening of the flowers at varying time between 2:30 am and 11:30 am at various growing regions and also protogynous lines (Youngman and Roy 1923; Ayyangar and Rao 1934; Verma 1989; Yadava 1997). Inflorescences are composed of 3–5 racemes alternately arranged on a short to elongated primary axis. Racemes are up to 13 cm long, with sub-sessile spikelets arranged in 2–3 rows along one side of flattened rachis. Spikelets are glabrous, orbicular or broadly elliptic, conspicuously plano-convex, 1.8–3.5 mm long. The lower glume is absent and upper glume is as long as the spikelet. The lower lemma is flat more or less membranaceous and without palea. The upper lemma is crustaceous, often brown and shiny when grains are mature and embraces the crustaceous palea. The grain is elliptic-orbicular in outline and 1.5–2.5 mm long (de Wet et al. 1983). Gynoecium is monocarpellary, ovary is superior, one cell with one ovule, two stigmas, feathery with distinct style. The grains are elliptic, convex in front and flat on back of palea; scutellum is up to half the length of the grain. The grain is enclosed in hard, corneous, persistent husks that are difficult to remove.

Three races of kodo millet were recognized based on arrangement of spikelets on the raceme. They are *regularis*, where the spikelets arranged in the two rows on the one side of flattened rachis; *irregularis*, where the spikelets are arranged in 2–4 irregular rows along the rachis; *variabilis*, where spikelets in the lower part are

irregularly arranged and in the upper part the spikelets are in two regular rows on the raceme. In all three types of racemes, spikelets are arranged on flattened rachis. The grain may vary in colour from light red to dark grey (de Wet et al. 1983). The crop matures in 3–4 months with average yield varying from 250 to 1000 kg/ha (Hulse et al. 1980), and crop has a potential yield of 2000 kg/ha (Harinarayana 1989).

8.7.2 Germplasm and Core Collection Status

About 8000 accessions of kodo millet have been conserved in main ex situ gene banks around the world. In Indian gene banks, around 3956 accessions have been conserved at AICSMIP, Bangalore (1537); ICSRISAT, Hyderabad (665); and NBPGR, New Delhi (2362) (Upadhyaya et al. 2014). National Active Germplasm Site (NAGS), AICSMIP, published a catalogue on evaluation of 1038 accessions for 16 and 11 qualitative and quantitative descriptors, respectively, and at TNAU, Coimbatore, 427 accessions have been maintained. ICRISAT established a core collection comprising 75 accessions belonging to 53 distinct clusters of 656 kodo millet germplasm collections (Upadhyaya et al. 2014). These genotypes could be utilized in breeding programme aimed at development of new genetic variants and recombinants.

8.7.3 Varietal Development of Kodo Millet

Kodo millet has received very less priority like other small millets in the agricultural research though the genetic improvement work started before the independence in the country. Some of the early efforts in crop improvement of kodo millet have resulted in the release of improved varieties as early as the 1940s. The first improved variety PLR 1 was released in 1942 for the rainfed areas of Tamil Nadu. Another improved variety, T2, was released in 1949, and Co 1 was released from TNAU, Coimbatore, during 1953. Post independence, the genetic improvement work was initiated in Madhya Pradesh during 1964 with the financial assistance from the state government. Niwas 1, another improved variety, was released in 1971 as the outcome of this work for general cultivation in Madhya Pradesh (Yadava and Jain 2006). Since 1978, the centre of excellence for the improvement of small millets established at Dindori (JNKVV) by ICAR with the assistance of International Development Research Centre (IDRC), Canada, is devotedly working towards the improvement of kodo millet.

With the establishment of AICSMIP in 1986, the IDRC centre became part of AICSMIP, and varietal development gained momentum. The AICSMIP centres located at Tribal Agricultural Research Station, JNKVV, Dindori, and Agricultural College, JNKVV, Rewa, are exclusively working on kodo and little millets. Presently emphasis is being given to the development of high yielding varieties with resistance to biotic and abiotic stresses; enrichment of germplasm; their critical evaluation for morphological, physiological and biotic and abiotic resistance traits;

and enhancement in available genetic variability through hybridization/mutagenesis for identification of ideotypes suitable for different farming situations (Yadava and Jain 2006; Hariprasanna 2017).

8.7.3.1 Pureline Selection in Kodo Millet

The varieties released in kodo millet so far are mainly developed through selection from landraces or germplasm. Some of the kodo millet varieties, namely, APK 1, GK 2 and KMV 20, were the product of selection from germplasm introduced in different agro-ecosystem. The pureline selection remained the prime breeding method for improving performance, particularly grain yield. Single plant selection from landraces and cultivated varieties and their evaluation for economic characters like earliness, resistance to biotic stresses and high yield have resulted in the development and release of many varieties in kodo millet. During the period from 1942 to 2020, about 36 varieties have been released, of which 11 were released before establishment of AICSMIP (1986) and rest after 1986. Among these, pureline selection has resulted in the development and release of 22 varieties of kodo millet.

8.7.3.2 Recombination Breeding

The difficulties in emasculation and pollination due to small and delicate spikelets combined with brittleness of flattened rachis have resulted in a slow progress in recombination breeding in kodo millet. However, the methods of hybridization and selection have been standardized and extensively used in the recent past for the creation of variability and selection of transgressive segregants from advanced generations. The hand emasculation technique developed by Verma (1989) and modified by Yadava (1997) has been found effective in kodo millet. The controlled pollination just after the emasculation helps in development of hybrids. The contact method as suggested for finger millet is successfully used in kodo millet with slight modifications. In this method, the panicles of selected plants are tied to other panicle in which crossing is to be attempted before flowering to enhance the chances of natural cross pollination. It resulted in low frequency of true hybrids which can be identified with the help of marker characteristics of the parents.

8.7.3.3 Mutation Breeding

In kodo millet, Mishra et al. (1985) were first to introduce quantitative variation through gamma irradiation. The sensitivity to gamma irradiation varied from genotype to genotype. Yadava (1997) found maximum mutation frequency and effectiveness at 25 kR dose of gamma irradiation. The mutants having auricle pigmentation, late maturity, complete panicle emergence and dark brown seed have been developed (Yadava et al. 2003). A protogynous mutant having two rows of spikelets on rachis was also identified from 5 kR dose of gamma irradiation in JK 76. Among the mutants identified, KM 86 and KM 99 have high yield potential coupled with early maturity. There is thus ample possibility for improvement following physical and chemical mutagenesis in kodo millet (Yadava and Jain

2006). Some of the latest kodo millet varieties released for cultivation are given in Annexure.

8.8 Proso Millet

Proso millet (*Panicum miliaceum*) is an important minor millet belonging to the family Gramineae. The crop is a short-duration millet variety and grown in India, the USA and other countries. It is specially adapted to tropics and high altitudes, where the growing season is short and the soil is marginal and poor in fertility. Among grain crops, proso millet requires less soil moisture. The crop is well adapted to high elevations and cultivated even in the Himalayan region up to an altitude of 2700 m. The dehusked grain (about 70% of the whole grain) is nutritious and is generally cooked like rice. Sometimes, it is ground to make roti and eaten. Green plants are fodder for cattle and horses, also used as hay. The crop is ready for harvest in 70–80 days. The average grain yield in India varies between 500 and 700 kg/ha in drylands and 1500 and 2000 kg/ha under irrigated/favourable conditions. The dry stover is normally three times higher than grain yield and is used as cattle feed.

8.8.1 Morphology and Reproductive Biology

The plant grows to a height of 30–100 cm, and stem is hollow, hairy or glabrous with swollen internodes and a shallow root system. It is a short-day, short-duration (60–90 days) crop. The crop is harvested at its physiological maturity to avoid shattering of grain. The flowering takes place usually between 10:00 am and 12:00 noon. However, flowering is influenced by environmental conditions especially humidity and temperature. The inflorescence is a drooping panicle and looks like a broom with basipetal opening of florets, i.e., from top to bottom. The spikelet consists of two glumes and two lemmas. The lower lemma has a sterile floret, and upper lemma has a fertile floret. The stamen possesses three anthers and two feathery stigmas. The anther dehiscence coincides with stigma receptivity, and anthers appear dry within a few minutes of flower opening. It usually takes 10–12 days for complete flowering within an inflorescence. Though self-pollination is the rule, up to 10% cross-pollination may occur (Popov 1970). Nelson (1984) reported crossing techniques in proso millet using hand emasculation. Cold spray technique for emasculation and crossing was reported by Nandini et al. in 2019. Seeds of proso millet are oval and 3 mm long, and colour varies from white, golden yellow, orange, red, brown to black (Baltensperger 2002).

8.8.2 Germplasm Resources of Proso Millet

The extensive collection of proso millet germplasm (8778) is maintained in Russia followed by Chinese academy of agricultural sciences (8451). Other major gene

banks conserving the crop's genetic variability are in Ukraine, India, and the USA (Upadhyaya et al. 2014). In India, the AICSMIP, NBPGR and ICRISAT are involved in plant genetic resource in collection, distribution and utilization. AICSMIP is a national active germplasm site and maintains a collection of about 920 accessions of proso millet. NBPGR is maintaining about 1005 germplasm accessions. ICRISAT with about 842 accessions is involved in germplasm characterization and evaluation of proso millet. ICRISAT developed a core collection of 106 accessions from 842 proso millet accessions of 30 countries based on 20 qualitative and quantitative traits.

8.8.3 Genetic Improvement of Proso Millet

In India, AICSMIP centres located in different states are involved in screening the germplasm lines since inception for grain yield and components. In proso millet, crop improvement programmes are focused on improving traits like yield, lodging resistance, non-shattering, early maturity, panicle type, waxiness, etc. Through conventional methods like pureline selection and pedigree breeding, improved varieties have been developed in proso millet in China, India, the USA, Russia and Kenya. In India, K2 is a variety developed through pureline selection, which is non-lodging and non-shattering (http://agritech.tnau.ac.in). The varieties TNAU 202 and ATL 1 are high yielding varieties developed by hybridization. Genetics and inheritance of waxy traits have been carried out. Waxy trait is reported to be controlled by duplicate recessive alleles. GBSSI gene (with two loci—S, L) mutations are identified to result in waxiness, while the GBSSI-S locus mainly contributes to the trait (Graybosch and Baltensperger 2009; Hunt et al. 2013; Rose and Santra 2013; Santra et al. 2015). Rajput et al. (2014) used molecular breeding tools to identify 18 quantitative trait loci (QTL) for phenotypic traits like heading date, test weight, grains per panicle, lodging, plant height, peduncle length, grain shattering and panicle length which may be validated and used for marker-assisted selection. Waxy forms of grains are preferred in the food industry for glutinous nature suited for beverage industry for their high fermentation efficiency.

Increasing proso millet production with declining area was the major challenge and was overcome with the development of modern varieties with greater adaptability to soil and environmental conditions and high yield potential of more than 4 tonnes/ha. Proso millet breeding programmes in Russia aimed at increasing productivity, smut disease resistance and grain quality like uniform size and shape and yellow endosperm with high carotenoid content. The major breeding method employed in Russia is intra-specific hybridization. A number of varieties were developed, and the notable cultivars are "Bistrove" and "Krupnoskoroe" (ssp. subcoccineum), which recorded up to 5 tonnes/ha grain yield with very short duration. Identification of genes for resistance, the improved varieties "Sputnik" (ssp. coccineum) and "Slavjanskoe" (spp. subflavum) were developed during 2006. The varieties were medium maturing and gave grain yield as high as 7 tonnes/ha. Russian variety Alba is reported as non-shattering grain variety. During 2006–2010,

the new selection technologies based on mutant forms, dihaploid plants production, genotype identification with the use of storage proteins electrophoresis and DNA markers were developed. As an outcome of this, a new variety "Regent" was developed during 2011 using anther culture technique, and selection was done keeping in mind the productivity and grain quality parameters. The variety had high yield, medium maturity (95–105 days) and resistance to lodging and shattering.

8.8.4 Future Prospects of Proso Millet

The genetic and genomic resources development in proso millet lags behind most cereals. There is a need for an extensive trait-specific germplasm evaluation or donors for traits like biotic and abiotic stress tolerance, non-lodging, non-shattering, yield, compact panicle, bold grains and genetic male sterility systems. Precision phenotyping for major traits is a prerequisite. The identified germplasm can be used for introgression of genes into popular cultivars using modern breeding methods like genomics assisted breeding (marker assisted selection, marker assisted backcrossing, haplotype breeding, speed breeding, etc.) and transgenic approaches. Functional markers and gene introgression for traits like genetic male sterility, non-lodging, etc. can also be attempted from the model grass species foxtail millet in which genetic dissection of traits occurs at a faster pace.

8.9 Browntop Millet

Browntop millet (*Brachiaria ramosa* (L.) Stapf Nguyen) is an annual/perennial warm-season grass originated in Southeast Asia, often used in forage/pasture management systems. It is a minor millet mostly confined to a few thousands of hectares in South East Asia and parts of the USA. In India, it is grown mostly in southern India (Bhat et al. 2018) where it is locally known as pedda sama or korle. Though presently it is restricted to remote parts of Andhra Pradesh, Karnataka and Tamil Nadu states in southern India, it appears to have been a major staple crop in the late prehistory of the wider region of the Deccan. Domestic and wild/weedy forms of browntop millet are found in agricultural systems, often within the same field. Browntop millet grows in rocky, shallow soils from sea level up to 8000 ft above MSL. It is adaptable to almost all upland soil (Mitchell and Tomlinson 1989), but does not grow well in water-restricted, drought conditions. It will not survive in temperature less than 52 °F.

Browntop millet is used as both a human food crop and fodder. In some parts of the USA, it is grown as a fodder crop and bird feed and was introduced from India around 1915. Morphotypes or races are not known in browntop millet. Mulay and Leelamma (1956) have reported $2n = 36$ chromosomes in this species from India. The somatic chromosomes are small in size ranging in length between 1 and 2.5μ. This species was found to show two cytological races, which are morphologically indistinguishable. Diploid, tetraploid and hexaploid status has been reported in

browntop millet with basic chromosome number of 9 ($2n = 2x = 18$; $2n = 4x = 36$, 72) (Vetriventhan et al. 2020).

Very limited genetic variability has been observed among the accessions assembled at ICAR-Indian Institute of Millets Research, Hyderabad. Two different panicle types have been found—open and bunchy. The florets are very minute, 1–2 mm in size. There are few reports on floral characteristics and anthesis behaviour. Artificial hybridization through hand emasculation and pollination has not been standardized so far, and hence, recombination breeding has not taken up shape as a breeding methodology. ICAR-Indian Institute of Millets Research maintains about 30 germplasm of browntop millet which are used for assessing the variability and for its use in further improvement programme. Pureline selection from the available germplasm appears to be a feasible strategy for improvement and release of cultivars in this crop as a short-term measure.

Annexure: Improved Varieties Developed and Released in Small Millets in India (2005–2020)

S. No.	Variety	Pedigree	Institute where developed	Year of release	Maturity (days)	Avg. yield (q/ha)	Area of adaptation
Finger millet							
1	GPU 48	GPU 26 × L 5	PC Unit, Bengaluru	2005	95–100	28–30	Karnataka
2	PRM 1	Selection from Ekeshwar of Pauri Garhwal Region	Hill Campus, GBPUA&T, Ranichauri	2006	110–115	20–25	Hills of Uttarakhand
3	Bharathi (VR 762)	Pureline selection from VMEC 134	ANGRAU, Vizianagaram	2006	110–115	26–30	Andhra Pradesh
4	GPU 66	PR 202 × GPU 28	PC Unit, Bengaluru	2009	112–115	35–40	Karnataka
5	GPU 67	Selection from germplasm accession GE 5331	PC Unit, Bengaluru	2009	114–118	30–35	National
6	Srichaitanya (VR 847)	GPU 26 × L 5	ANGRAU, Vizianagaram	2009	110–115	26–28	Andhra Pradesh
7	KMR 301	MR 1 × GE 1409	VC Farm, Mandya, UAS, Bengaluru	2009	120–125	35–40	Southern Dry zone of Karnataka
8	KOPN 235	Selection from local germplasm	MPKVV, Rahuri	2011	115–120	25–26	Sub-mountain and Ghat zone of Maharashtra
9	OEB 526	SDFM 30 × PE 244	OAUT, Bhubaneswar	2011	110–115	25–26	Odisha, Bihar, Chhattisgarh, Karnataka, Tamil Nadu
10	OEB 532	GPU-26 × L-5	OAUT, Bhubaneswar	2012	110–115	22–25	Odisha, Bihar, Chhattisgarh, Karnataka, Tamil Nadu
11	KMR 204	GPU 26 × GE-1409	VC Farm, Mandya, UAS, Bengaluru	2012	95–100	30–35	Karnataka
12	VR 936	IE 2695 × PR 202	ANGRAU, Vizianagaram	2012	115–120	28–30	Andhra Pradesh

13	PPR 2700 (Vakula)	KM 55 × U22/B	ARS Perumallapalle, A.P.	2012	105–110	25–30	Andhra Pradesh
14	Indira Ragi 1	HR 911 × GE 669	Jagdalpur, IGKVV	2012	120–125	25–26	Chhattisgarh
15	VL 352	VR 708 × VL-149	ICAR-VPKAS, Almora	2012	95–100	33–35	All Ragi growing areas of country
16	Chhattisgarh Ragi-2	PR 202 × GE 669	Jagdalpur, IGKVV	2012	115–118	32–35	Chhattisgarh
17	VL 376	GE 4172 × VL Ragi 149	ICAR-VPKAS, Almora	2016	103–109	29–31	All Ragi growing areas of country
18	GNN-6	Selection from local germplasm WN-259	Navsari Agricultural University, Navsari	2016	120–130	28–30	Gujarat
19	GN-5	Selection from local germplasm WWN-20	Navsari Agricultural University, Navsari	2016	120–130	25–27	Gujarat
20	VL Mandua-348	VL Ragi 146 × VL Ragi 149	ICAR-VPKAS, Almora	2016	104–112	18–20	Uttarakhand
21	KMR 340	OUAT-2 × WRT-4	VC Farm, Mandya, UAS, Bengaluru	2016	90–95	35–40	Karnataka
22	Dapoli-2 (SCN-6)	Soma-clone of Dapoli-1	Dr. BSKKV, Dapoli	2017	118–120	25–27	Konkan region of Maharashtra
23	CO 15	CO 11 × PR 202	Centre on Excellence of Millets, TNAU, Athiyandal, Tamil Nadu	2017	115–120	29.0 under rainfed and 34.0 under irrigated	Tamil Nadu
24	GNN-7	Pureline selection from white type landrace of Nagli collected from Ahwa-Dang District, State: Gujarat	NavsariAgril. Univ., Gujarat	2017	123–128	25.0 q/ha	Gujarat

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S. No.	Variety	Pedigree	Institute where developed	Year of release	Maturity (days)	Avg. yield (q/ha)	Area of adaptation
25	VL-379	GE-440 × VL-149	ICAR-VPKAS, Almora	2017	105–107	30–32	Recommended for Uttarakhand, Bihar, Jharkhand, Madhya Pradesh and North eastern states
26	Chhattisgarh Ragi-2 (BR-36)	PR-202 × GE-669	ZARS, Jagdalpur, IGKVV	2018	115–118	34–36	Chhattisgarh
27	DHFM-78-3	GE 1219 × Indaf 8	ARS, Hanumanamatti, UAS, Dharwad	2018	114–116		Recommended for cultivation in agro-climatic Zone—3 and 8 of Karnataka state
28	VL Mandua-380	GEC 440 × VL 149	ICAR-VPKAS, Almora	2019	115–116	18–19	Uttarakhand
29	Vegavathi (VR 929)	GE-3076 × VR-854	ARS, ANGRAU, Vizianagaram	2019	115–120	Grain yield is 36.1 q/ha and fodder yield is 7.2 tonnes/ha	National
30	Tirumala (PPR 1012)	AE 3077 × Ratnagiri	ARS, Perumallapalle, ANGRAU, Guntur	2019	115–120	Grain 35–37 q/ha fodder yield 7–8 tonnes/ha	Andhra Pradesh
31	GN 8	Pureline selection from local collection of Waghai Tal. Dist. Dang.	Navsari Agricultural University, Navsari	2019	105–110	31–32	Gujarat
32	FMV-1102	VR-708 × GPU-48	ZARS, Jagdalpur, IGKVV	2019	110–115	30–32 q/ha (grain) and 11–12 tonnes/ha (fodder)	Assam, Bihar, Chhattisgarh, Jharkhand,

							Uttarakhand, Madhya Pradesh
33	KMR-630	PR-202X GE1409	VC Farm, Mandya, UAS, Bangalore	2020	95–100	28–30	Karnataka
34	VR-988	GE 3076 × VR 855	ARS, ANGRAU, Vizianagaram	2020	110–115	28–30	Andhra Pradesh
35	PR-10-45 (Gowthami)	GPU 28 × GE 4931	ARS, ANGRAU, Peddapuram	2020	122–125	35–37	Andhra Pradesh
36	CFMV-1 (Indravathi)	VL 330 × GE 532	ARS, ANGRAU, Vizianagaram	2020	110–115	30–32	Andhra Pradesh, Karnataka, Tamil Nadu, Puducherry, and Odisha
37	CFMV-2	Pureline selection from local collections made under Dang District of Gujarat	Navsari Agricultural University, Navsari	2020	119–121	29–31	Andhra Pradesh, Chhattisgarh, Gujarat, Maharashtra and Odisha
38	VL-378	GEC 440 × VL 149	ICAR-VPKAS, Almora	2020	110–114	22–24	Rainfed organic conditions of Uttarakhand hills
39	VL-382	WR 2 × VL 201	ICAR-VPKAS, Almora	2020	106–108	11–13	Rainfed organic conditions of Uttarakhand hills
Foxtail millet							
1	Co 7 (TNAU 196)	Co 6 × ISe 247	TNAU, Coimbatore	2005	85–90	18–19	Tamil Nadu
2	HMT 100-1	RS 118 × PS 3	ARS, Hanumanamatti, UAS, Dharwad	2008	90–95	20–25	Karnataka
3	SiA 3085	Pure line from SiA 2644	RARS, Nandyal, ANRAU	2011	80–85	20–30	All foxtail millet growing areas of the country

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S. No.	Variety	Pedigree	Institute where developed	Year of release	Maturity (days)	Avg. yield (q/ha)	Area of adaptation
4	Surya Nandi (SiA 3088)	Pure line from SiA 1244	RARS, Nandyal, ANRAU	2012	70–75	20–25	All foxtail millet growing areas of the country
5	SiA 3156	Pure line from 2871	RARS, Nandyal, ANRAU	2012	85–90	20–25	Andhra Pradesh, Bihar, Gujarat, Karnataka, Madhya Pradesh, Tamil Nadu and Uttarakhand
6	RAU (Rajendra Kauni 1-2)	Selection from Local germplasm of Laukaria, Raxaul, East Champaran	Rajendra Agricultural University, Bihar, Pusa, Samastipur	2017	80–83	23–25	Irrigated and rainfed upland of Bihar
7	DHFt-109-3	Co-5 × GPUS-30	ARS, Hanumanamatti, UAS, Dharwad	2018	86–88	Grain yield 29 q/ha Fodder yield 5.23 tonnes/ha	Recommended for cultivation in agro-climatic Zone 3 and 8 of Karnataka state
8	Hagari Navane-46	Sia 2644 × PS-4	Agricultural Research Station, Hagari, Ballari, University of Agricultural Sciences, Raichur	2019	85–90	16–20 q/ha (rainfed) 20–25 q/ha (irrigated)	Zones 1, 2 and 3 of Karnataka
9	SiA 3222 (Garuda)	SiA 3075 × SiA 326	Agricultural Research Station, ANGRAU, Nandyal, AP	2020	60	19–20	Andhra Pradesh
10	SiA 3223 (Renadu)	Developed from GS 96 population through MDPLS breeding	Agricultural Research Station, ANGRAU, Nandyal, AP	2020	86–90	34–35	Andhra Pradesh
11	ATL-1 (TNSi-331)	PS 4 × Ise 198	Centre for Excellence in Millets, Athiyandal, Tiruvannamalai, TNAU	2020	80–85	20–22	Tamil Nadu

Kodo millet							
1	JK 13	Selection from mutant JK 76	Rewa, JNKVV, Jabalpur	2007	95–100	22–23	National
2	JK 106	Selection from Sidhi dist. germplasm	Rewa, JNKVV, Jabalpur	2009	100–105	19–20	M.P. State
3	JK 65	Selection from Sidhi dist. germplasm	Rewa, JNKVV, Jabalpur	2009	105–110	23–25	National
4	JK 98	Selection from GPLM 317	Rewa, JNKVV, Jabalpur	2010	100–105	25–30	National
5	DPS 9-1	Selection from local land race	Dindori, JNKVV, Jabalpur	2011	105–110	27–30	National
6	Indira Kodo 1	Pureline selection	Jagdalpur IGKVV	2012	100–105	22–25	Chhattisgarh
7	Chhattisgarh kodo-2	Mutant Line of CO 3	Jagdalpur IGKVV	2014	95–100 days	25–26	Chhattisgarh
8	TNAU-86	Pureline selection from IPS 85	TNAU Coimbatore	2012	95–110	27–30	National
9	RK 390-25	Mutant of RK-390	Rewa, JNKVV	2012	100–105	25–28	National
10	Jawahar Kodo 137	Mutant of RK-390	Rewa, JNKVV	2016	100–105	26–29	Rainfed areas of Madhya Pradesh
11	KMV-543	Mutant of CO-3	ZARS, Jagdalpur, IGKVV	2019	105–110	25–27	Andhra Pradesh, Chhattisgarh, Gujarat, Jharkhand, Karnataka, Madhya Pradesh and Tamil Nadu
12	Kodo millet variety KMV 545 (TNPsc 262)	Pureline selection from DPS 63/58	Centre for Excellence in Millets, Athiyandal, Tiruvannamalai, TNAU	2020	105–110	28–30	Andhra Pradesh, Chhattisgarh, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Tamil Nadu and Telangana

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S. No.	Variety	Pedigree	Institute where developed	Year of release	Maturity (days)	Avg. yield (q/ha)	Area of adaptation
14	GAK-3	Pureline selection from locally collected germplasm of Hilly regions of Rewa district of Madhya Pradesh	Hill Millet Research Station Anand Agricultural University Dahod 389 151	2020	105–110	23–25	Dry lands, hilly and tribal region of Dahod and Panchmahal districts of Gujarat
Barnyard millet							
1	CO(KV) 2	Pureline selection from EF 79	TNAU, Coimbatore	2008	95–100	21–22	Tamil Nadu state
2	DHBM 93-3	VL-13XIEC-566	ARS, Hanumanamatti, UAS, Dharwad	2016	90–95	22–24	National
3	DHB-93-2	EF-8 × IEC-566	ARS, Hanumanamatti, UAS, Dharwad	2018	86–88	Grain yield 27.6 q/ha and fodder yield 6.19 tonnes/ha	Recommended for cultivation in agro-climatic Zone—3 and 8 of Karnataka state
4	MDU-1	Pureline selection from Aruppukottai local	Agricultural Engineering College and Research Institute, TNAU, Madurai	2018	95–100	Grain yield of 15–17 q/ha (rainfed) and 22–25 q/ha (irrigated) Fodder yield of 30–33 q/ha	Suitable for southern districts of Tamil Nadu
5	DHBM-23-3	VL-13 × IEC-566	ARS, Hanumanamatti, UAS, Dharwad	2019	88–100	20–21	Andhra Pradesh, Karnataka, Madhya Pradesh and Tamil Nadu

Little millet							
1	OLM 208	Selection from Lajigada local	OUAT, Berhampur	2009	100–105	12–15	National
2	OLM 217	Selection from Udayagiri local	OUAT, Berhampur	2009	105–110	15–16	National
3	Co 4	Co 2 × MS 1684	TNAU, Coimbatore	2005	75–80	16–20	Tamil Nadu
4	JK 36	Selection from local Shahdol germplasm	Rewa, JNKVV, Jabalpur	2009	75–80	10–12	M.P. state
5	BL 6	Paiyur 1 × OLM 29	Jagdalpur, IGKVV, Raipur	2016	90–95	12–14	National
6	DHLM 36-3	Co-4 × Paiyur—2	ARS, Hanumanamatti, UAS, Dharwad	2018	95–100	14–16	Karnataka
7	Chhattisgarh Kutki-2 (BL-4)	CO-2 × TNAU 97	Jagdalpur, IGKVV, Raipur	2016	90–95	10–12	Chhattisgarh
8	GV-2	Derivative from mutant of released variety 'Gujarat Vari-1'	Waghai, NAU, Navsari	2016	115–125	26–28	Gujarat
9	Phule Ekadashi (KOPLM 83)	Selection from local germplasm	ZARS, Kolhapur, MPKV Rahuri	2016	120–130	12–14	Sub-montane and Ghat zone of Maharashtra
10	Jawahar Kutki 4 (JK 4)	DLM 42 × Kutki1	Rewa JNKVV Jabalpur	2016	75–80	13–15	Rainfed areas of Madhya Pradesh
11	DHLM-14-1	CO-2 × TNAU-110	ARS, Hanumanamatti, UAS, Dharwad	2018	97–99	Grain yield 16.0 q/ha and fodder yield 6.10 tonnes/ha	Recommended for Tamil Nadu, Karnataka, Gujarat, Maharashtra and Orissa

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S. No.	Variety	Pedigree	Institute where developed	Year of release	Maturity (days)	Avg. yield (q/ha)	Area of adaptation
12	GNV-3	Pureline selection from local land races collected from the Dang district of Gujarat	Waghai, NAU, Navsari	2018	110–115	28–29	Gujarat agro-climatic Zone I, II and III (dry lands/hilly/tribal region of Dang, Valsad, Navsari and Panchmahal districts of Gujarat)
13	ATL-1 (TNPsu 177)	CO (Samai) 4 × TNAU 141	Agriculture Research Station, Tamil Nadu Agricultural University	2019	85–90		Tamil Nadu
14	LMV513	CO-2 × TNAU-26	ARS, Hanumanamatti, UAS, Dharwad	2019	93–96	17–19	Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Chhattisgarh, Odisha and Jharkhand.
15	BL-41-3	Paiyur 2 × TNAU 97.	ZARS, Jagdalpur, IGKVV	2019	95–100	16–19	Chhattisgarh
16	Jaicar Sama 1 (LMV-518)	Pureline selection from indigenous germplasm collection GPmr-1153	Indian Institute of Millets Research, Hyderabad	2020	98–102	15–17	Maharashtra, Andhra Pradesh, Telangana, Tamil Nadu, Puducherry
Proso millet							
1	TNAU 145	PV 1454 × TNAU 96	TNAU, Coimbatore	2007	70–72	18–20	Tamil Nadu
2	CO(PV) 5 (TNAU 143)	PV 1403 × GPUP 21	TNAU, Coimbatore	2007	70–75	23–25	National
3	TNAU 151	TNAU 96 × PV 1673	TNAU, Coimbatore	2008	72–75	18–20	National

4	TNAU 164	TNAU 137 × CO 4	TNAU, Coimbatore	2009	70–75	18–20	National
5	PratapCheena-1 (PR-18)	Pure line selection	MPUA&T, Udaipur	2006	65–70	15–17	National
6	PRC 1	Selection from GPMS 519	Ranichauri, GBPUA&T, Pantnagar	2008	70–75	10–12	Uttarakhand hills
7	TNAU 202	PV 1453 × GPUP 16	TNAU, Coimbatore	2011	70–75	18–20	National
8	TNPm-230	TNAU-164 × IPM-19	TNAU, Coimbatore	2017	70–75	21–23	National
9	DHPM-2769	Selection from IPM-2769	ARS, Hanumanamatti, UAS, Dharwad	2018	70–72	Grain yield 24.6 q/ha and fodder yield 4.16 tonnes/ha	Recommended for cultivation in agro-climatic Zone—3 and 8 of Karnataka state
10	PMV-442	GPMS 109 × GPMS 908	Project Coordinating Unit, UAS, Bengaluru	2019	70–75	14–16	Andhra Pradesh, Karnataka, Madhya Pradesh and Tamil Nadu, Bihar, Telangana, Puducherry

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Sugarcane Breeding

9

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Abstract

Sugarcane breeding in India started at the ICAR-Sugarcane Breeding Institute (SBI), Coimbatore, in 1912. A major breakthrough in sugarcane breeding was the use of wild species, viz. *S. spontaneum*, which led to the development of the first sugarcane interspecific hybrid variety Co 205. About 78% of the total sugarcane area is covered by 'Co' varieties alone, whereas Co and Co-allied varieties covered about 99% of the total sugarcane area in the country during the season 2020–2021. Co 0238 (53.42%), Co 86032 (17.06%), CoM 0265 (6.90%), Co 0118 (2.45%) and CoLk 94184 (2.33%) were the top five sugarcane varieties in cultivation during the season 2020–2021. There have been about 50 tonnes/ha and 2.69% improvement in cane yield and sugar recovery, respectively, from 1930 to 2020 due to cultivation of varieties released by the ICAR-SBI. With new challenges on sugarcane cultivation, it is essential that all future breeding programmes focused on ecologically viable, environment-friendly and resource matching technologies. Bioethanol and electricity production from sugarcane will be the major focus in the coming years to meet the energy demands of India. Research on true seed as sugarcane propagule is being pursued to develop homozygous lines to bring in a paradigm shift to sugarcane agriculture. Recent advancements in genome sequencing have provided opportunities to better apply genomic selection (GS) to improve major traits and for more precise breeding of this complex crop. Harnessing genetic diversity of the '*Saccharum* Complex' is progressing with the support of molecular cytogenetics and molecular markers. Emerging tools like genome editing to improve an otherwise promising sugarcane variety is challenging but potentially very rewarding. India will continue to

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_9

develop improved varieties to achieve profitable and sustainable sugarcane production for food, fuel, fibre, fodder and high-value products.

This chapter is intended to provide basic and applied aspects of sugarcane breeding useful to agriculture/botany students, plant breeders, sugarcane researchers, cane development officials and sugar industry personnel. The origin and movement of sugarcane; floral biology; species of *Saccharum* and allied genera; hybridization techniques; progress made on varietal development in India and other major sugarcane-growing countries such as Brazil, Australia, the USA, West Indies, Java, South Africa, Thailand, etc. since time immemorial to present day; and advances made on molecular breeding are covered in this book.

Keywords

Sugarcane · Saccharum · Wild species · Gene pool · Varietal development · Breeding strategies · Germplasm · Hybridization

9.1 Introduction

Sugarcane (*Saccharum officinarum* L.) is an important crop worldwide used for producing sugar, jaggery (gur or panela) and ethanol as primary products and molasses, bagasse and pressmud as co-products. Green leaves and tops of sugarcane are used as cattle fodder. Sugarcane is grown over 26.47 million ha spread across 103 countries, situated mostly in the tropical region (FAOSTAT 2020). Sugarcane contributes 78% of the world's crystal sugar production, while sugar beet contributes 22%. Brazil is the largest sugarcane-growing country in the world with 9.90 million ha of cane area followed by India with 4.85 million ha (2020–2021). Six countries, namely, Brazil, India, China, Thailand, Pakistan and Mexico, together accounted for three-fourths (76.41%) of the world's sugarcane acreage in 2019–2020. Brazil, India, China, Thailand, the USA, Pakistan, Mexico, Australia, the Philippines, Indonesia, South Africa, Colombia and Argentina are the major sugarcane-producing countries in the world. These countries accounted for three-fourths of the world sugar production.

India was the largest sugar producer in the world during 2018–2019 (33.07 million tonnes) which accounts for nearly 17.57% of the total production (Anonymous 2019). In spite of poor rainfall in some of the tropical sugarcane-growing states of India, the country had managed to produce surplus sugar from 2015 to 2020 except 2016–2017 and also exported sugar to more than 80 countries during 2011–2019. The country emerged as the third largest sugar exporter (9.1%) in the world during 2019 by exporting worth 1.8 billion US dollars (Anonymous 2020a). It ranked seventh among India's Export of Principal Agricultural and Allied Commodities (Anonymous 2020b). India by contributing 18.10% in area and 19.81% in production ranks second among sugarcane-growing countries of the world for both area and production of sugarcane during 2018 (FAOSTAT 2020). The turnover of sugar and other related economic activities (which includes sugarcane, gur and others) was

approximately Rs. 123,372 thousand crores per annum during 2018–2019 at the current price, out of which, nearly Rs. 86,277 thousand crores were paid to the sugarcane farmers by the sugar mill as prices for its supply during 2018–2019.

It is estimated that about 5.568 million sugarcane farmers and 6.769 million sugarcane labourers (total of about 12.337 million sugarcane workers) are engaged in the cultivation of sugarcane in India, which accounts for 1.427% of the total rural population of India (Kumar et al. 2016). Out of the 6.26 million sugarcane landholdings of the country, 82.82% are marginal and small (<2.0 ha) which occupy nearly 54.44% sugarcane area. Five lakhs skilled and unskilled workers including highly qualified and trained technologists are engaged in the manufacturing of sugar. Sugarcane is more important than other crops as it is more remunerative. It contributed nearly 0.616% in GDP of the country in the year 2018–2019 at current prices with an area of nearly 5 million ha, and the same trend is expected to follow in the coming years also. During 2020–2021, India produced 397.65 million tonnes of sugarcane from 4.857 million ha area and produced 31.10 million tonnes of sugar and 12.00 million tonnes of molasses (Anonymous 2021).

Sugarcane is a tropical plant in the grass family. It has C4 photosynthetic apparatus (Kranz leaf anatomy) and is highly efficient in converting solar energy into chemical energy. The photosynthetic rate is estimated up to 100 mg CO_2 fixed per dm^2 leaf area per hour (Santos and Diola 2015). The theoretical maximum yield of sugarcane estimated based on photosynthetic rate, energy content and energy storage in sugarcane plant is 381 tonnes/ha/year equivalent to dry matter (biomass) yield of 48.5 g/square metre/day (Waclawovsky et al. 2010). The maximum stalk yield achieved in research stations is 212 tonnes/ha/year, and world average yield in farmer's field is 70.6 tonnes/ha (FAOSTAT 2020) with sucrose levels in the commercial varieties ranging from 15% to 22%. Sugarcane distributes one-third of their carbon into sucrose and two-thirds into tops and stems. The crop can accumulate large amounts of sucrose in the stem parenchyma cells, up to ~650 mM in the vacuoles (Welbaum and Meinzer 1990).

More than 30 products are produced from crushing sugarcane at sugar mills. It includes raw sugar, refined sugar, molasses, alcohol, rum, bagasse, syrups, dextran, confectionary, crude wax, glucose, etc. (Perez 1997). Hundred tonnes of sugarcane is estimated to produce 14.3 tonnes of raw sugar, 27.2 tonnes of bagasse, 5.2 tonnes of filter cake, 2.6 tonnes of molasses and 50.7 tonnes of waste water (Allen et al. 1997). The annual production of white sugar in 2019–2020 sugar season (October–September) globally is 171.57 million metric tonnes (ISO Quarterly, November 2020). Brazil is the largest sugar-producing country in the world (29.43 million tonnes or 17% of world sugar output in 2019–2020 sugar season). India is the second largest sugar producer (27.38 million tonnes or 16% of world sugar output in 2019–2020 sugar season). Other countries with largest sugar production (from sugarcane and sugar beet) are the European Union (10.6% of the world sugar production), China (6.0%), Thailand (4.8%), Russia (4.6%), the USA (4.3%), Pakistan (3.2%), Mexico (3.0%) and Australia (2.5%). About 55–65 million tonnes of sugar is traded annually in the international markets; the rest are consumed domestically in the cane-growing countries.

9.2 Progress Made in the Last 25 Years in Sugarcane Agriculture

During the colonial period, sugar was considered as an expensive commodity, consumed by rich and royals. The British called sugar as 'white gold'. Now sugar is an essential commodity for billions of common persons. Scientific advances made in Australia, Brazil, India, Indonesia, the USA and elsewhere have fuelled progress in sugarcane agriculture and the sugar sector. Hybridization with wild species has given faster and desired genetic improvement in sugarcane; hence, interspecific hybridization or 'nobilization' in sugarcane in the early 1990s has been heralded as the top five achievement in the annals of plant breeding. Improved cane varieties, crop production technologies and sugar-processing technologies have increased sugarcane and sugar production in Brazil, India, Australia, Thailand, etc. The conventional and molecular breeding tools have helped in evolving superior sugarcane varieties. In India, through conventional breeding varieties combining early maturity, high sugar and high yield (they are negatively correlated traits) such as Co 0238 and Co 86032 evolved which in the 2019–2020 season occupied 54% and 13% sugarcane area in the country. 'Double-high' (high in sugar content and yield) varieties were bred in China (Zhang and Govindaraju 2018).

Another milestone in the conventional breeding is the development of short-duration sugarcane varieties such as Co 11015 in India which attain sucrose maturity at 8 months (with >20% brix, >18% sucrose and >85% purity with acceptable yield level at 8 months) compared to 10–18 months in normal varieties. Short-duration varieties enable farmers to take three crops in a span of 2 years (one plant + two ratoon) instead of two crops in 2 years. Development of energy canes is yet another progress in sugarcane research. In Coimbatore, Louisiana, Barbados, Mauritius, Vignis (Brazil) and Puerto Rico, breeding programmes on Type I and Type II energy canes (Tew and Cobil 2008) were developed. Type I energy canes are the genotypes with relatively low sucrose (>15% brix) but moderate-to-high fibre content (>20%). They are the dual-purpose canes, the juice can be used in distilleries for direct fermentation and fibre for cogeneration. Type II energy canes are specifically bred for high biomass yield with high fibre (>25%) and low brix (<15%) and are exclusively used for energy generation and lingo-cellulosic-based second-generation ethanol production (Alexander 1985; Matsuoka et al. 2014; https://sugarcane.icar.gov.in).

Sugarcane varieties cultivated in the world are unusual among the Poaceae crops in that they are polyploid interspecific hybrids (with approximately 80% chromosomes from *S. officinarum*, 10–15% chromosomes from the wild species *S. spontaneum* and 5–10% recombinant chromosomes), with singularly complex genomes. And because of its huge complexity, it was the last among the major cultivated plants to have its genome sequenced. Genetic maps of sugarcane have been produced mainly based on single-dose markers that are the most informative markers in this high polyploidy crop (Zhang et al. 2014). The genome size of polyploidy sugarcane cultivar was determined to be about 10 Gb, while the monoploid genome size is about 800–900 Mb, close to that of sorghum (750 Mb) (D'Hont

and Glaszmann 2001). A reference genome sequence is a representative example of the set of genes in one idealized individual of a species. It is a good approximation of the DNA sequence of any single individual hence viewed as key platform for genetic analysis and molecular breeding. The development of mosaic monoploid reference genome sequence for the sugarcane commercial variety R570 (382 Mb single tiling path) (Garsmeur et al. 2018), assembly of the 373k genes and their putative regulatory region in the variety SP80-3280 (Souza et al. 2019) and haploid assembly of *S. spontaneum* clone AP85-441 (3.13 Gb) (Zhang et al. 2018) etc. are the large steps toward a whole-genome assembly of a highly complex genome of sugarcane cultivars.

Transgenic sugarcane varieties were evolved in Indonesia, Brazil, India and Australia. In 2013, three drought-resistant transgenic events (NXI-1T, 4T, 6T) were approved for field trial in Indonesia, but commercial cultivation has not yet taken place (www.isaaa.org). In 2017, the CTNBio (National Biosafety Technical Commission), Brazil, has given approval for cultivation of CTC175-A event, the first GM sugarcane variety (CTC20 Bt) developed by the the Brazilian company Centro de Tecnologia Canavieira (CTC) for the management of sugarcane borer *Diatraea saccharalis*. In 2018, another transgenic variety (transgenic event CTC91087-6) carrying the Cry1Ac gene which confers resistance to *Diatraea* was approved for cultivation in Brazil (Cheavegatti-Gianottoa et al. 2019; CTNBio 2019). Transgenic sugarcane (in the cultivars Co 86032, Co 0238), carrying drought-tolerant genes EaDREB2, EaHSP70 and PDH45 were developed at ICAR-SBI, Coimbatore, India.

In the conventional method, sugarcane planting requires 7–9 tonnes of seed cane per hectare, and this is the main reason for the slow rate of seed and varietal replacement. Sugarcane being a long-duration crop and heavy biomass producer requires more water, i.e. about 1200–1800 mm in subtropical region and 1600–2700 mm in tropical region. The availability of water is declining at a faster rate. The problem is further aggravated by the variability of rainfall influenced by climate change. So, unless sugarcane farmers are provided with technological options of obtaining high yields using much less water, India will find it difficult to meet its growing demand of sugar. Hence, there is a need to adopt water-saving technologies for sustainable sugarcane production. The ICAR-SBI has developed a sugarcane cultivation model comprising of integrated approach, keeping in mind the likely problem to be faced in the future, for sugarcane agriculture (ICAR-SBI 2019). Components of the model are (1) high yielding and better-quality varieties, (2) raising and transplanting of settlings raised from single-budded setts/bud chips, (3) subsurface drip irrigation and fertigation, (4) wider row planting, (5) intercropping, (6) trash mulching, (7) multiple ratooning and (8) mechanization. The objective is to increase the productivity of sugarcane and sugar, reduction in preparatory tillage and seed costs (multiple ratooning), reduction in input costs (settling transplanting and drip irrigation/fertigation, trash mulching), increased water and nutrient use efficiencies (drip irrigation with fertigation and trash mulching), reduction in labour requirement (wider row planting and mechanization of cultural operations from planting to harvesting) and intermittent and additional income to farmers (intercropping).

Table 9.1 Decade-wise average area, sugarcane production, sugarcane productivity, sugar production and sugar recovery in India

Years	Area (000' ha)	Sugarcane production (000' tonnes)	Sugarcane productivity (tonnes/ha)	Sugar recovery (%)	Sugar production (000' tonnes)
1989–1990 to 1998–1999	3814	258,074	67.59	9.92	12,928
1999–1920 to 2008–2009	4389	292,073	66.41	10.27	19,015
2009–2010 to 2018–2019	4837	348,297	71.98	10.41	25,834

In India, importance is given to produce disease-free planting materials. The standardization of aerated steam therapy (AST) and moist heat aerated steam therapy (MHAT) for elimination of sugarcane sett borne pathogens of sugarcane has been a success story of ICAR-Sugarcane Breeding Institute and adopted worldwide to generate pathogen-free sugarcane crop. The ICAR-Sugarcane Breeding Institute, Coimbatore, India, was the first to develop sugarcane tissue culture (TC) and micro-propagation protocols in India. Use of disease-free and vigorous tissue culture-derived sugarcane plantlets is becoming popular in India. Besides healthy seeds, the yields are improved by using TC plants. Significant progress has been made on the mechanization front. Machineries and implements for planting sugarcane setts, settlings at closure and wider spacing, for intercultural operation including earthing up, combine harvester have been developed in the last 25 years and put into use in grower's field.

The decade-wise average area, sugarcane production, sugarcane productivity, sugar production and sugar recovery in India is given in Table 9.1. Between the decade 1989–1999 to 2009–2019, sugarcane productivity has increased by 4.39 tonnes/ha, whereas increase in sugar recovery is 0.49 unit. This improvement could be due to research efforts, in terms of improved varieties and agronomic practices. The increase in sugarcane productivity (5.57 tonnes/ha) and sugar recovery (0.14%) during the decade 2009–2010 to 2018–2019 over the decade 1999–2020 to 2008–2009 has been mainly due to released and adoption of sugarcane variety Co 0238 in subtropical India. Increasing area, cane yield and sugar recovery led to increase in sugarcane and sugar production over the decades.

9.3 Origin, Evolution and Distribution of Species of Sugarcane

9.3.1 Species of Sugarcane

The genus *Saccharum* belongs to the grass family Poaceae, tribe Andropogoneae. Six species of sugarcane (*Saccharum* spp.) are recognized by classical taxonomist. A detailed description can be found in Jeswiet (1925), Grassl (1946), Mukherjee

(1954), Brandes (1958), Rao (1989), Heinze (1987) and Amalaraj and Balasundaram (2014).

9.3.2 Wild *Saccharum* Species

9.3.2.1 *Saccharum spontaneum* L. (2*n* = 40–128)

This is the most primitive species which has its centre of origin and diversity in India (Mukherjee 1957). This wild species has no sucrose but produces numerous thin shoots. It has wider adaptability ranging from severe drought and prolonged water-logged environments, so it was used in sugarcane breeding programmes in the early years. It is speculated that the primitive form of cultivated sugarcane might have evolved from *S. spontaneum* in the foothills of Himalaya in North India (Stevenson 1965). Natural occurrence of 31 cytotypes with somatic chromosome number ranging from 2n=40 to 2n=128, i.e. 2n=40, 48, 50, 52, 54, 56, 58, 60, 61, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 90, 93, 96, 100, 104, 112, 116, 120, 124, 126 and 128 was reported (Panje and Babu 1960).

9.3.2.2 *Saccharum robustum* Brandes and Jeswiet ex Grassl (2*n* = 60, 80)

This is another wild species discovered on mud flats on the banks of the Laloki River near Port Moresby in the 1928 Sugarcane Expedition to Papua New Guinea. This species has hardy, thin-to-thick exposed stalk without rhizome (like *S. officinarum*) but has no sweet juice (like *S. spontaneum*). *Saccharum robustum* probably evolved in New Guinea by natural hybridization between *S. spontaneum* × *Erianthus*, *Sclerostachya* and *Miscanthus* species (Roach and Daniels 1987).

9.3.2.3 *Saccharum edule* (2*n* = 60–80)

This is a wild (or semi-wild) species which closely resembles *S. robustum* except that the inflorescence is compacted and remains unopened and enclosed inside the leaf sheaths. The species is cultivated as vegetable in small pockets in the islands of the Pacific, Papua New Guinea and Fiji for its edible aborted inflorescence (hence the name 'edule'). It is known as 'navisco' in Vanuatu, 'pitpit' in Papua New Guinea and 'duruka' in Fiji and Java (Grivet et al. 2004; Mudhaliar 2007). This species possibly originated in the Polynesia islands by mutation of *S. robustum* (Brandes et al. 1939) or by natural crossing between *S. robustum* and *Miscanthus* (New Guinea form of *S. edule*) or *S. officinarum* and *Miscanthus* (Fiji form of *S. edule*) (Grassl 1967; Roach and Daniels 1987). Some taxonomists do not recognize *S. edule* as separate species and treat it under *S. robustum*.

9.3.3 Cultivated *Saccharum* Species

Three species of sugarcane were cultivated and used for chewing and production of jaggery (gur) and crystal sugar from ancient time. They may be grouped into two categories viz., (1) thick-stalked, soft rind, juicy, low-fibre, high-sugar-type

sugarcane belong to *S. officinarum* L. (Dutch scientists in Java named this species as 'noble cane' on account of its majestic appearance and quality), and (2) thin-stalked, hardy, high-fibre, low-juice and low-sugar-type sugarcane belongs to the species *S. barberi* Jeswiet and *S. sinense* Roxb.

9.3.3.1 *Saccharum officinarum* L. (2*n* = 80)

It is the cultivated sugarcane of the world. Dutch scientists in Java named this species as 'noble cane' because of its majestic appearance and quality. Its accepted centre of origin is Papua New Guinea (Polynesia), but there are two centres of diversity, namely, (1) Papua New Guinea and (2) the Indonesian (Java) archipelago. There were two views regarding the origin of *S. officinarum*: (a) that it originated from *S. robustum* due to natural and human selection in New Guinea/Polynesia (Grassl 1977) and (b) that it evolved from the complex introgression between *S. spontaneum*, *Miscanthus sinensis* and *Erianthus arundinaceus* (Roach and Daniels 1987) probably in India but could not survive in India because of red rot disease and established well in New Guinea where the disease was not a problem (Parthasarathy 1946; Rao et al. 1957). But, the widely accepted hypothesis is that the noble cane evolved from *S. robustum* in New Guinea and then dispersed to the Pacific Islands during 4500 BC and mainland Asia along human migrations. In mainland Asia, it hybridized with local *S. spontaneum* giving rise to North Indian and Chinese canes (Brandes 1958).

9.3.3.2 *Saccharum barberi* Jeswiet (2*n* = 111–120)

This species is called as North Indian cane. The canes are shorter, cylindrical internodes, hard rind, high fibre but with low-to-medium juice and sucrose content, and leaves are dark green. Together with *S. sinense* (the Chinese cane), these indigenous canes were in cultivation in North India from prehistoric time. Barber (1922) classified the indigenous canes that were in cultivation in North India into five groups, namely, Mungo, Nargori, Saretha, Sunnabile and Pansahi. Later, Jeswiet classified these clones as separate species. Clones in the first four groups were treated as *S. barberi* (named in memory of Dr. C. A Barber and then the Director of SBI Coimbatore), and clones in the Pansahi group were treated as *S. sinense*. Both species may have originated from natural hybridization, undoubtly in North-East India (erstwhile Assam-Bengal-Orissa region) about 1000 years BC. These species exhibited better adaptability to cold weather (winter) climate of North India and China. According to Parthasarathy (1946), in ancient India, thick noble cane (*S. officinarum*) had existed, and *S. barberi* may have evolved from natural hybridization between *S. spontaneum* and *S. officinarum*, but Grassl (1977) disputed this view and suggested that *Erianthus procerus*, *Sclerostachya* and *S. spontaneum* could have contributed to the origin of North India canes.

9.3.3.3 *Saccharum sinense* Roxb. (2*n* = 81–124)

This species is called as Chinese cane. It is morphologically similar to *S. barberi* (hard rind, high fibre but its with low-to-medium juice and sucrose content), but the internode shape is mostly bobbin, and leaves are light green or yellowish and taller

than *S. barberi*. It was in cultivation in South of China, Indo-Burma region (along with *S. barberi*) from ancient time. Consequently, these species are thought to be ancient intergeneric hybrids (Roach and Daniels 1987). For the origin of Chinese cane, Grassl (1977) suggested that *Miscanthus sacchariflorus* initially introgressed with *S. officinarum*, and later introgression was with *S. spontaneum*.

9.3.4 Allied Genera and *Saccharum* Complex

The genus *Saccharum* is amenable to hybridization with eight genera such as *Eccoilopus*, *Erianthus*, *Miscanthidium*, *Miscanthus*, *Narenga*, *Ripidium*, *Sclerostachya* and *Sorghum* (Grassl 1963, 1977). Mukherjee (1954) reported that the four genera *Saccharum* L., *Erianthus* Michx., *Sclerostachya* (Andersson ex Hack) A. Camus and *Narenga* Bor. constituted a closely inter-breeding group and concerned with the origin of *Saccharum* hence termed these large breeding pool as '*Saccharum* Complex'. Later, Daniels et al. (1975) included *Miscanthus* section *Diandra* to '*Saccharum* complex' as it was thought to be involved in the origin of *Saccharum*. Detailed description on the allied genera of *Saccharum* may be found in Heinze (1987) and Amalaraj and Balasundaram (2014).

9.4 Spread of Sugarcane Across the World

Two centres of origin for sugarcane were recognized, namely, (1) Polynesia Islands of the South Pacific particularly Papua New Guinea as the home for the species *S. officinarum* (proposed by Deerr 1911) and (2) India as the home for *S. barberi* (proposed by Ritter 1841). The indigenous people of Papua New Guinea knew sugarcane since 8000–6000 BC (Brandes and Sartoris 1936; Ming et al. 2006). Over 1060 clones of *S. officinarum* and intermediate wild forms like *S. robustum* collected from these Islands in different expeditions supported the origin of sugarcane in Papua New Guinea (Deerr 1949; Berding and Koike 1980). The natives in these Islands did not prepare sugar from the cane, but doubtlessly they chewed the stalks perhaps as source of food and for the sweet taste. From the South Pacific, sugarcane might have first spread westward to Solomon, Hebrides and New Caledonia Islands during 8000 BC and then eastward to Java, the Philippines and India during 6000 BC. Irian Jaya, Kalimantan, Java and Sumatra (Indonesian Islands) exhibited considerable diversity of *S. officinarum*; hence, Artschwager and Brandes (1958) described Java as shifting or satellite centre of diversity for sugarcane.

In India, *S. officinarum* might be naturally hybridized with indigenous canes grown here (*Saccharum barberi* or *S. sinense*), and the hybrids spread further into Western Eurasia and the Mediterranean (Daniels et al. 1974). According to Geerligs (1912), Deerr (1949), Parthasarathy (1946) and Chaturvedi (1951) sugarcane and sugar (*gur* in Sanskrit) were known to India from the prehistoric period. From India,

the commercially grown sugarcane along with the art and skill of growing sugarcane and making of 'gur' or jaggery from it gradually spread to other countries.

All the evidences now show that it was the Indian variety of sugarcane that was taken from India to Persia after the Persian invasion into India (Alexander the Great invaded India in 325 BC). After the return of Alexander the Great from India (325–324 BC), the Indian cane and *Sarkara* were known to the Greeks, Persians, Egyptians and Arabs (Deerr 1949). The region extending from the undivided Punjab in the West to greater Assam in the East was the cradle of sugarcane cultivation and *gur* manufacturing using indigenous technical know-how. Perhaps during fourth century BC, the indigenous clones of *S. barberi* and later during fourteenth century AD the 'Puri cane' or 'Creole cane', i.e., a naturally introgressed form of *S. officinarum* × *S. barberi*, were taken from India to Babylon (Iraq) and Persia (Iran) through Arab/Persian traders and travellers (Geerligs 1912; Deerr 1949; Galloway 1989). Arabs played a great role in the cultivation and spread of sugarcane in and around Iran, Iraq, Egypt and West Africa. From the Mediterranean region, sugarcane spread to Africa through the Arabs and to Mauritius, Reunion (Bourbon) and Europe through Portuguese and Spanish navigators.

When the Arabs conquered Persia, in the Middle East, they spread sugarcane to their newly conquered territories: Syria and islands in the Mediterranean sea, namely, Cyprus, Crete and Egypt (introduced in 641 AD). In 703 AD, the Arabs introduced sugarcane to Sicily (island in Mediterranean sea, now under Italy; it was an important sugar trade centre during the 1570s) and then to North Africa (in 900 AD), Morocco and Spain (introduced in 714 AD especially on the south coast of Andalusia). During that period (or around 400 AD), two important events have happened: (1) the Arab innovations in irrigation (particularly the *qanat* system) played a large part in making the arid Mediterranean region suitable for sugarcane cultivation, and (2) probably, the Egyptian chemists perfected processing sugarcane juice and began to make refined or white sugar or crystal sugar. The Indian cultivar Creole cane (a soft yellow natural interspecific hybrid) was an important variety for more than two centuries both in the Old World and in the New World before the introduction of noble cane.

In the fifteenth century (1420 AD), the Portuguese brought sugarcane to Madeira (an archipelago off the northwest coast of Africa in the North Atlantic Ocean) where it grew up luxuriantly and spread fast. The Island soon produced unheard of quantities of sugar. In 1444, the Azores (an archipelago in the North Atlantic Ocean) and between 1456 and 1462 the Cape Verde Isles (small islands off the northwest coast of Africa in the North Atlantic) were captured and colonized by the Portuguese. In 1496, the Spanish colonized the Canary Islands (off the northwest coast of Africa in the North Atlantic). Columbus during his second voyage (in 1493) took sugarcane from Canary Islands and introduced into the New World/Caribbean Islands particularly in Hispaniola (Haiti and Dominican Republic). Hispaniola acted as a distribution centre from where sugarcane was taken to Cuba, Puerto Rico and the other islands in the West Indies. From Hispaniola, sugarcane was introduced into Mexico in 1520. In the year 1500, Brazil was discovered by Portuguese Navigator Pedro Álvares Cabral. The Portuguese imported sugarcane from Madeira and Cape

Verde and introduced it into the northeast coast of Brazil in 1532 (Geerligs 1912; Edel 1969). The early production system in Brazil was like those techniques and system developed in Madeira, where the owner (engenho) leased his land to a number of smaller planters in return for a portion of the sugar produced (Edel 1969).

Historians mentioned that up to the first half of the fifteenth century, hardly any slavery existed in Christian countries; it only occurred in Mohammedan lands (Geerligs 1912). The Portuguese and Spanish followed Arabian examples and introduced sugarcane in the islands captured by them and used African slaves for cultivation. Sugarcane grew luxuriantly in the New World Islands (West Indies or Caribbean Islands) due to mild and moist climate. Attempts to use convicted labourers and African Negro slaves and the coercion of colonized natives to work in sugar plantations encouraged expansion of sugarcane cultivation in the colonized lands. In 1481 the Portuguese built three fortresses in Africa, namely, on the Gold Coast, on an island in the Gulf of Guinea and at Loango, and from these sent slaves to the New World colonies. Spanish, French, British, Dutch and Danes also imported slaves from the Gulf of Guinea and Africa to their colonized American and West Indies lands. In the New World, North Atlantic slave trade flourished, and African Negro slaves were engaged in sugarcane cultivation and sugar manufacturing. After Brazil, sugarcane spread to the new European colonies in Africa and in the Pacific (Fiji). In the 1560s, Mauritius, Natal and Queensland in Australia started growing sugarcane. Today, sugarcane is cultivated in all continents, except the Antarctic. The Indian variety Puri/Creole which was in cultivation for more than 250 years was the basis for development of sugar industries in Iberia (Portuguese and Spanish colonies). It was the only variety in the Americas when the sugar industry was set up (Heinze et al. 1994). Although the true name of Indian sugarcane variety was not known (Watt 1893), it may be a natural interspecific hybrid between *S. officinarum* and *S. barberi* and was called differently as Puri (India), Cana del pais (in Port Rico), Country cane (in Jamaica) and Yellow Egyptian (in Java germplasm collection), more popularly as Creole (in French), Criola (in Spanish) or Crioula (in Portuguese) (Earle 1928).

9.5 Sugarcane Genetic Resources

The gene pool of sugarcane consists of three cultivated species (*Saccharum officinarum*, *S. barberi* and *S. sinense*), one semi-wild species (*S. edule*), two wild *Saccharum* species (*S. spontaneum*, *S. robustum*), allied genera (*Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya*) and commercial cultivars (which are mostly interspecific hybrids). Based on the genetic relatedness among the species/clones, easy with which the hybrids are produced and the fertility/sterility of the hybrid, and number of generations required to evolve superior clones, the cultivars of sugarcane and species of '*Saccharum* complex' may be grouped into the four categories of gene pool.

9.5.1 Gene Pool-1

It includes commercial cultivars and near-commercial clones/genetic stocks. They are the products of the biparental crosses or the products of the cross/backcross (*S. officinarum*/*S. barberi*/*S. sinense*) × *S. spontaneum*. In this group, the gene(s) are fixed in the first sexual generation itself.

9.5.2 Gene Pool-2

It includes the clones of the basic (cultivated) species such as *S. officinarum*, *S. barberi* and *S. sinense*. Utilization of these species in varietal improvement programmes may necessitate few generations of crossing or backcrossing.

9.5.3 Gene Pool-3

It includes wild species *S. spontaneum* and *S. robustum* and the semi-wild *S. edule*. If these species are utilized in the varietal development programmes, several generations of breeding are required to eliminate the undesirable effects of these wild species.

9.5.4 Gene Pool-4

It includes allied genera *Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya*. Species like *Sorghum*, *Zea* and *Bambusa* may be included in the category, as successful intergeneric hybrids were reported (Janaki-Ammal 1941; Sreenivasan and Sreenivasan 2000; Nair et al. 2016; Karuppaiyan et al. 2021a).

9.6 Germplasm Collection

Collection, conservation, evaluation, characterization and utilization of the '*Saccharum* complex' germplasm formed part of sugarcane improvement programmes in the major sugarcane-growing countries. Germplasm expeditions to the centres of origin (south of the Pacific islands) were mainly focused and promoted by colonial governments, sugar industries and of late the International Society of Sugar Cane Technologists (ISSCT) and the International Board for Plant Genetic Resources (IBPGR). Expeditions were made to collect primarily *S. officinarum*, *S. robustum*, *S. edule* plus related wild species from Polynesian-Melanesian islands where greater diversity for these species exists and *S. barberi*, *S. sinense*, *S. spontaneum*, *Erianthus*, *Miscanthus* and other species from Indo-Burma-South China and adjoining region where these species exhibited greater diversity.

9.6.1 World Collection of Sugarcane Germplasm

Saccharum and allied genera germplasm collections made by several institutions/agency prior to 1956 from the centres of origin were held primarily in Florida (USA) and Java (then the Dutch East Indies). These became known in the sugarcane world as the 'ISSCT World Collections'. Initially, the collection was maintained at the US Department of Agriculture, Agricultural Research Service (USDA-ARS), Sugarcane Field Station at Canal Point, Florida. Later it was shifted to Beltsville, Maryland, and maintained in greenhouses. Since 1976, the ISSCT world collection (except *S. spontaneum*) is maintained at the USDA-ARS, Subtropical Horticultural Research Station, Miami, Florida. It is now known as *World Germplasm Collection of Sugarcane and Related Grasses*. *Saccharum spontaneum* clones of the World Collection were moved to the USDA-ARS Sugarcane Field Station, Canal Point, Florida. In the USA, wild species *S. spontaneum* is classified as noxious weed and is closely regulated. Therefore, individual clones are grown in containers to prevent their spread by rhizomatous growth (Comstock et al. 1996).

The ISSCT World Collection at Java was lost in the 1940s during the World War II. By the 1950s, plant breeders and research organizations across the globe showed concern on the narrow genetic base of commercial sugarcane and the possible loss of valuable wild germplasm. This issue was addressed at the eighth ISSCT Congress held in 1953, and it was resolved to have another (duplicate) collection of sugarcane germplasm of the world that lost in Java. As the Sugarcane Breeding Institute, Coimbatore had undertaken the collection of *S. barberi*, *S. sinense* and *S. spontaneum* and related genera from tropical Asia and Africa, the ninth ISSCT Congress held in India in 1956 resolved that the Coimbatore (India) collection should be recognized as the 'Second world collection of sugarcane germplasm' (Dutt 1956). One set of the world collection maintained at Canal Point was brought to India during 1956–1958 to augment the Second World Collection of sugarcane germplasm at Coimbatore. As mosaic disease was widespread in Coimbatore, only clones of *S. spontaneum* and *Erianthus* spp. were retained at Coimbatore, while all other clones were sent to the Agriculture Research Station, Taliparamba, Kannur District (Kerala) in 1956 (Balasundaram et al. 1980). Then, the Research Centre of the Sugarcane Breeding Institute was established at Cannanore (now known as Kannur), Kerala, in 1962 exclusively for the maintenance of the World Collection. Soon, the collection maintained at Taliparamba was shifted to Kannur.

In the 1992 hurricane (named 'Andrew'), the World Collection at Miami was severely affected. About 568 clones of *S. officinarum* and many clones of *S. spontaneum* had lost, and identification tags of the germplasm were blown over by the hurricane. Re-establishment of the *S. officinarum* from the collection already shared to Copersucar, Brazil, and *S. spontaneum* clones from the breeding collection maintained at Canal Point, Florida, and Houma, Louisiana, was carried out (Comstock et al. 1996). As of 2014, 1427 accessions were maintained at Miami as against 1787 accessions reported in 1987 (Heinze 1987; Todd et al. 2014; Fickett et al. 2020). The Kannur Centre of SBI holds (as of January 2021), 1813 accessions of ISSCT World Collection and 1550 accessions of Indian Collection, which is the largest now (Table 9.2). The ISSCT World Collections of Sugarcane and Related

Table 9.2 World collection of sugarcane germplasm and related grasses maintained at the USDA-ARS-Subtropical Horticulture Research Station, Miami, and ICAR-Sugarcane Breeding Institute, Research Station, Kannur

S. No.	Species/genera	Miami, Florida, USA (2014)	Kannur, Kerala, India (2020)
1	*S. officinarum*	254 (546[a])	757
2	*S. robustum*	51 (97)	129
3	*S. edule*	6 (18)	16
4	*S. barberi*	38 (6)	42
5	*S. sinense*	33 (84)	30
6	*S. spontaneum*	316 (266)	384
7	*S. brevibarbe*	1	–
8	*Erianthus arundinaceus*	8	121
9	*E. bengalensis*	5	19
10	*E. elephantinus*	–	1
11	*E. longisetosis*	–	7
12	*E. ravennae*	3	–
13	*S. kanashiroi* (Syn: *E. kanashiroi*)	2	3
14	*S. rufipilum* (Syn: *E. rufipilus*)	2	–
15	*S. procerum* (Syn: *E. procerus*)	1	35
16	*Erianthus* spp	20 (172)	–
17	*Miscanthus floridulus*	1	–
18	*M. sinensis*	4	–
19	*Miscanthus* hybrid	6	–
20	*Miscanthus* spp.	5 (8)	2
21	*Miscanthidium*	0 (8)	
22	*Narenga porphyrocoma*	1 (11)	6
23	*Sclerostachya fusca*	–	5
24	*Imperata* sp.	1	–
25	*Eccoilopus*	0 (2)	–
26	*Coix gigantea*	1	–
27	*Pennisetum* spp.	–	4
28	*Sorghum plumosum*	1	–
29	*Sorghum arundinaceum*	1 (5)	
30	*Vettiveria*		11
31	Others + new collections	238 + 250 (381)	9
32	Indian hybrids/commercial cultivars		1031
33	Indo-American clones		130
34	Man-made/historical/natural *Saccharum* hybrids	176 (174)	621
	Total	1427 (1787)	3363

Source: Todd et al. 2014; ICAR-SBI (2020)

[a] Number within bracket indicates maintenance at Miami in 1987 (Heinze 1987)

Grasses maintained in Miami and Kannur includes accessions representing different species of *Saccharum*, allied genera and man-made historical and commercial hybrids, collected from over 45 countries (Todd et al. 2017; Balasundaram et al. 1980).

The IBPGR Working Group on the Genetic Resources of Sugarcane (1982) has recognized the existing ISSCT world collections at Miami and Kannur as primary collections as well as global field gene bank of sugarcane. In addition, 11 'secondary collections' in different countries having unique materials have been recognized (Anonymous 1982). Besides the world collections, some countries are maintaining working collection of *Saccharum* and related species for utilization in their breeding programmes. The National Plant Germplasm System, USDA and the National Institute of Agrobiological Resources (NIAR, now NIAS), Tsukuba, Japan were designated as base seed collections for sugarcane by the IBPGR (Croft et al. 1996)

9.7 Floral Biology

Sugarcane inflorescence is known as 'Arrow' by virtue of its shape, but botanically it is an open branched panicle or tassel (25–50 cm long) with 5000–8000 small florets. The apical meristem, which is surrounded by a leaf sheath, ceases to form leaves and develops into an inflorescence primordia about 3 months before the actual flower emerges (Van Dillewijn 1952). The first sign of flowering is when the distance between leaf triangle becomes greater as the successive leaf sheaths and internodes become longer and leaf blades become shorter. This stage is called the 'symptom stage'. Then the distinctive flag leaf appears. The last leaf sheath is about 3 ft longer fully enclosing the young panicle, while its leaf blade is only about ½ ft long and shaped like a pennant. This stage is called 'booting stage' or 'short blade stage'. Then, the stalk elongates, and the panicle emerges out from the enclosed leaf sheath ('tip emergence stage'). The main inflorescence axis or rachis arises from the terminal internode of 10–12-month-old crop. The primary branches (rachilla) arise from main rachis. The secondary branches arise in two rows, alternatively along the primary branches. The secondary branches may often bear tertiary branches, and the ultimate branches bear a pair of spikelets; one is sessile, and the other is pedicellate.

Both spikelets have two florets: one is sterile and is represented by a delicate pointed small lemma (shorter than glumes). The upper floret of each spikelet is larger and hermaphrodite but do not have lemma (except *S. spontaneum* which bears scaly lemma). Therefore, only one floret in each spikelet is visible to the naked eye. The parts of florets from the outermost portion to inward are numbered as G1 (outer or lower glumes or prophylletum), G2 (inner or upper glumes), G3 (a sterile lemma or third glume) and in few species G4 (a fertile lemma or fourth glume) (Kruger 1899; Rumke 1934; Grassl 1956). The whorl G4 is not present in *S. officinarum* but present in *S. spontaneum* and its hybrids. The upper florets of each spikelet as mentioned earlier are fertile and contain one palea, three stamens, one ovary and two long styles

with brush-like feathery stigma. Lemma is absent. Anthers are bi-lobed. The indehiscent anthers are usually yellow or pale yellow, while dehiscent anthers are brown or purple. Ovary is round with single anatropous ovule. Two short wedge-shaped lodicules are present inside the palea near the base of the ovary. They absorb water to swell and force apart the glumes for the exertion of anthers and stigma during anthesis. At the base of each spikelet in the pair, there are longer (than spikelet) silken or callus or pappus hairs in whorl known as 'fuzz' or 'fluff' which gives the arrow its characteristic silky appearance and helps disperse the seeds. The arrow is longer in *S. officinarum* but shorter in *S. spontaneum* and *S. barberi* and intermediate in *S. sinense* (Artschwager et al. 1929).

9.7.1 Flowering

Sugarcane is a short day plant and flowers when the day length is gradually reduced during its grand growth phase. In the northern hemisphere, sugarcane flowers during October to early December, whereas in the southern hemisphere, flowering occurs from April to early June. The duration of flowering is generally longer as the equator is approached (in equator flowering is throughout the year because of adequate day length and temperature). If a clone is shifted from one latitude to another, there will be a shift in flowering date by 2.4 days for each degree of latitude. Generally, ratoon crop flowers earlier than the plant crop. The wild species *Saccharum spontaneum* and *S. robustum* flower profusely in their original habitats and other established locations (Ethirajan 1987), whereas *S. officinarum* is a shy flowering species. Many clones of *S. officinarum* do not flower at Coimbatore condition. Flowering in *S. barberi* and *S. sinense* is generally sparse and late at Coimbatore condition due to their subtropical origin.

9.7.2 Anthesis and Pollination

Opening of florets or anthesis takes place in the early morning between 5:00 and 9:00 AM, and stigma emerges out from the florets little ahead of the anther dehiscence (around 6:30–8:30 AM). Protogyny leads to cross pollination in sugarcane. The opening of flowers commences from the top and proceeds downward. An arrow takes about 7–10 days to complete anthesis.

Pollen fertility may vary from 0% to 100% depending on the clone and environment. The percentage of pollen fertility in a clone is the deciding factor for its use as a male or female parent in the crossing programme. Fresh pollen of suspected clone is collected in the early morning, stained with 1% acetocarmine solution and observed under the microscope for pollen fertility. Fertile pollen will be deep red in colour. Those clones with low pollen fertility are used as the female parent. Heinz

and Tew (1987) suggested clone with <8% pollen fertility as safe female parent, but at Coimbatore, the following categorization is adopted:

Pollen fertility %	Classification of parental clone
1–30	Safe female
31–70	Both male and female
71–100	Strong males

Pollen grains are viable for a short time after anthesis, but stigmas are receptive for longer hours and persistent. After pollination, it takes 21–25 days for the seed to fill and mature. Sugarcane has poor seed set and seed viability. The seed set under Coimbatore condition ranged from 3.1% to 22.7% (Rao 1980). The matured seed is a one-seeded fruit or caryopsis, yellowish brown, very small (1 mm long), ovate with withered stigma persists at the tip, and at the base are whorls of silky hairs for wind dispersal. The seeds along with its appendages are collectively called 'fuzz' (due to fuzzy appearance) or 'fluff' or 'true seeds'. The weight of a defuzzed seeds is 0.4–0.5 mg. The viability of the fuzz is low (30–35 viable seeds per gram fluff), falls rapidly; therefore, it is stored in a freeze dryer. A gram of fuzz may contain 2013 seeds, and about 450–550 seedlings may be obtained from it. Good seed set and fuzz germination is observed at Coimbatore condition. Therefore, in India, the National Sugarcane Hybridization Garden (NHG) was set up at ICAR-Sugarcane Breeding Institute, Coimbatore.

9.8 Hybridization Techniques

The miniature nature of spikelets creates problem for emasculation and is not adopted in crosses meant for commercial breeding. The general practice is to use the entire inflorescence as a unit, and clones with low pollen fertility are designated as female parents in the crossing programmes. Heat treatments of inflorescence at 50 °C for 5 min (Heinz and Tew 1987), hot water dipping at 50 °C for 10 min (Krishnamoorthy 1977) and dipping of tassel in 63–70% alcohol for 9 min have been reported to provide complete male sterility, but these practices are not widely adopted in commercial cane breeding. Breeders across the world take the advantage of protogyny in making crosses. The hybridization techniques followed by breeders may be grouped under two categories: (1) hybridization done with both parents kept in situ and (2) hybridization done with the arrows of one or both the parents isolated.

9.8.1 Hybridization Techniques Where Both Parents Are Grown In Situ

9.8.1.1 Open Field Crosses

When the female arrows are not covered to exclude contamination from foreign pollen, the crossing method is designated as open crossing. Sugarcane is naturally a

cross-pollinated crop. Moreover, due to its small floret size, controlled hybridization was not performed in the past. In the early period of sugarcane improvement, i.e. 1900s, fluffs of open pollinated arrows were collected and sown. This seeds were called GC (**General Crosses**). Even today, GC collection is practised in many countries including India and Brazil. In this method, there is no control on pollination or choice of male parent. In India, Venkatraman (1925, 1927) planted parents for hybridization separately in isolation and called it as 'arrowing plot'. In the arrowing plot, a row of female parent was flanked on both sides by rows of male parent. At the time of flowering, the arrows of adjacent rows were brought together, covered with cloth bags, and in situ crossing was allowed to take place. The bags with arrow are secured in position by erecting a bamboo pole in the field. This kind of field crosses with minor modifications is used in Java, Barbados, Mauritius and Taiwan (Stevenson 1965). **Polycross** employed at ICAR-SBI, Coimbatore, India, is a modification of general crosses and is different from the Hawaii method. Polycross is defined as a cross between a female parent surrounded by more than one male parent. In polycross nursery, selected female parents with low pollen fertility are planted in one row, and flanked on both sides are a large number of selected male parents. The arrows of female rows are not covered. Natural (wind or insect) pollination is allowed to take place. Fluff from the female row alone is harvested and is designated as PC of that particular female parent. In polycross, the source of specific male parent is not known, but the group of male parent is known.

9.8.2 Hybridization Techniques Where One or Both Parents Are Isolated

9.8.2.1 Covered Field Crosses

Covered crossing was once widely used in Java, India, Hawaii, Formosa, Barbados, Australia and Mauritius. But due to poor seed set of tassel enclosed in lanterns than those allowed in the open, this method has been discontinued in Java and Hawaii but continued in India and other countries.

9.8.2.2 Lantern Method

Controlled field crosses followed in India are described below. In the National Hybridization Garden of ICAR-SBI, Coimbatore, about 500–600 diverse parental clones, which originated from different geographical regions in the world, are planted every year in the month of December. Flowering symptoms in early flowering clones are seen during the second week of September and short blade during the last week of September. Sugarcane breeders from different parts of the country arrive at Coimbatore, and crossing programme generally begins in the second fortnight of October. A day prior to crossing, selected female parent is covered with cloth bag supported by aluminium cages. The cages are hung from the bamboo poles erected close to the canes; hence, this method is called the lantern method of crossing. A portion of inflorescence from the male parent that is likely to open on the same day is identified with the aid of torch light and clipped off by

pressing between thumb and forefinger at 5:00 AM. The excess moisture on the clipped florets is removed by blotting paper and then kept under warm light (500 W electric bulbs) for half-an hour to force dehiscence of anthers artificially in a specially devised room called 'pollen chamber'. After an hour, i.e. around 6: 30 AM, the dehisced pollen together with inflorescence is placed in a butter paper, wrapped and gently dusted onto the female florets/inflorescent of female parent (after lifting the enclosed cloth bag) before its own pollen dehisce and self-fertilize. Dusting of pollen is continued for 6–7 days until all the florets are pollinated. The hybridized arrows are severed from the mother stalk after a minimum maturity period of 21 days, shade dried and sown immediately or stored in −20 °C for long-term storage.

9.8.2.3 Scaffolding: An Improved Platform for Easy Field Crossing

In the lantern method of field crossing, difficulties are encountered such as reaching the female arrows positioned at 3–6 m from the ground level. Pollination is possible difficult and possible only by using ladders. Ladders have to be shifted by two labourers after every pollination, and several ladders have to be used simultaneously to complete the targeted crosses within crossing time of 1–2 h (6:30–8:30 AM) under Coimbatore condition. To overcome these difficulties and to simplify the pollen collection and dusting operations, a robust structure called 'Elevated Hybridization Runways (EHR) or Scafolding' was developed at ICAR-SBI Coimbatore (Nair et al. 2013). The EHR structure consists of two tiers of GI platforms. These platforms are supported by H-shaped 48 × 2.65 mm G.I. pipe frames braced with 27 × 2.65 mm GI pipes. The H-shaped GI pipe frames are assembled on top of each other with interlocking arrangement to make the height to 4.5 m. Each unit has 42 H-shaped GI pipe poles interconnected by cross bracing at an interval of 2.2 m. The width of the structure is 1.0 m. The structure is positioned between two rows of female parents with the row spacing of 1.2 m. Since the parental clones are planted in field on either side of the platform, arrows are accessed easily for crossing. The height at which the flowers are positioned on the sugarcane varies with genotypes; the unit is fabricated in two different heights, i.e. the platforms are placed at 2.85 and 4.5 m height above the ground to facilitate crossing of the flowers situated at different heights. The unit is divided into two equal subunits each running a length of 13.2 m (span on either side of the central platform), thus covering a total width of 26.4 m. If parental clones are planted at 1.2 m row spacing, 11 main units are needed to cover an area of 700 m^2. A ladder made of M.S. pipe enables the breeders to reach the platforms for making crosses. The researcher can move freely from one platform to the other, effect dusting on the female arrows while standing on the platform and collect matured fluff.

9.8.2.4 Free Crossings

This is an open crossing, used mostly in Java and Hawaii before the development of the Hawaiian crossing method. In this method, the female arrows remain attached to the plant grown in field, while the male arrows are cut, placed in vases of water and

fastened in position around the female arrows. The male arrows are replaced daily. Pollination is aided by wind.

9.8.3 Hybridization Techniques Where Both Parents Are Isolated

9.8.3.1 Marcotting or Rooted Stalk Technique

A marcot is an air-layered rooted stalk. Way back in 1926, Venkataraman and Thomas of SBI Coimbatore standardized a technique for inducing rooting and then isolating flowering stalk from a standing cane for effecting crosses in shed instead of in the field. This method was known as 'tile pot technique'. In this technique, during advanced symptom stage, roots are induced on standing sugarcane stalk in the field by covering two to three internodes in the middle of the stalk (or leaving top 7–8 internodes) with soil which is held around the stalk tightly by placing two halves of a tile pot along the length of stalk and then tied by jute rope. Water is poured from the top portion of tile pot daily to induce roots. After the root formation, which takes place about 15–20 days, they are severed from mother cane by cutting at bottom (just below the rooted node) and brought to the crossing shed. In the hybridization shed, the arrow of male marcotted canes is kept slightly above the arrow of female marcotted cane, and both are enclosed in an aluminium cage covered outer with cotton cloth bags.

Everyday morning arrow pairs are tapped gently to ensure the pollen fall vis-à-vis fertilization. The rooted marcots are watered periodically to prevent it from desiccation. The fluffs from female arrows are collected 21 days after pollination. Later Dutt and Hussainy (1956) improved upon the method using alkathene or plastic sheets in place of the tile pot. A polythene tube is inserted from the cane top and is positionedat at middle of the stalk. The tube is filled with soil mixtures, bottom portion of the polythene tube is tied around the stalk tightly to prevent leakage of soil and water. The tube is watered from the top daily to induce roots. Premature drying of marcots is often encountered which affects seed set and viability of hybridized fuzz. Providing an environment with high humidity of >80% and root temperature at 22 °C for the isolated marcots was reported to prevent premature spikelet abscission (Narasimhan et al. 1963). Nagarajan et al. (1996) suggested marcotting at the top of the internodes so as to reduce the height for easy crossing. As of today, in India, this method is not practised for commercial breeding, but countries like Brazil, the USA, South Africa, Mauritius, Taiwan and Puerto Rico are following this technique.

9.8.3.2 Hawaiian Solution Technique

A solution for keeping the severed flowering stalks of sugarcane afresh for crossing purpose was developed in Hawaii, which initially consisted of a diluted solution of sulphurous acid. A small portion of stalk with arrows kept in the solution continued to flower normally and produce viable seeds so long as the solution is kept fresh. Later, phosphoric acid was added to improve the efficiency and seed set. The standard Hawaiian solution contained 0.03% sulphurous acid and 0.01% phosphoric acid. This solution was improved further by Mangelsdorf, with a composition of

150–180 ppm sulphurous acid (SO_2), 75 ppm phosphoric acid (H_3PO_4), 37.5 ppm nitric acid (HNO_3) and 37.5 ppm sulphuric acid (H_2SO_4) (Machado et al. 1987). In this solution, arrows maintain viability for a period of 8 weeks, but the solution requires renewal once in 3 days (Heinz and Tew 1987). The pH of the fresh solution is 2.3, and it should be measured daily. If the pH has reached to 3.0, the solution is changed. If not changed, then SO_2 is replenished daily through the addition of measured quantity of sulphurous acid. The hybridization procedure followed in Hawaii and Brazil using solution technique is described below.

Arrowed stalks intended for the biparental or polycrosses are identified. Generally tassel with light coloured or yellow anthers are used as females, and those with brown anthers are classified as male parent. Arrows to be used in the crossing are cut from field at 8 AM with 1.5 m stalk attached to it, and the bases of the stalk cut diagonally. Male tassels are marked with black label and female arrow with red label. The leaves of stalk are stripped off, the tassel is sprayed with fungicide and the arrows in upright position are transported to crossing house. Arrows indented for biparental crosses are bundled in such a way that six to eight female arrows from one parent are surrounded by eight male arrows of another clone, and the height of male arrows is kept 1 ft above the female arrows. In case of polycross, five to six male arrows from different clones are kept along with one or two female arrows in one bucket. Every morning, the arrows are slightly shaked to facilitate pollen dispersal and pollination. After 3 days, the arrows are removed, the stalk bases are recut, the solution is changed and the arrows are kept in the new solution. Success of Hawaiian solution technique is dependent on the use of mineral free water. In India, the solution rapidly turned toxic and panicle gets dried and falls off. Therefore, this technique is not used in India's commercial cane breeding programmes.

Solution technique is also used in combination with field crosses. The classical 'Barbados lantern' first used for field crosses in that country was big ($3' \times 3' \times 3'$), made of timber glazed on top and three sides and with a cloth shield on the fourth side. A smaller version of the same was in use in Mauritius. Subsequently when practice of using cut cane in crossing became popular, lanterns were modified suitably. Finally, in Barbados, tail lantern batteries made of iron angles and fitted with cross ties and horizontal bars were fixed permanently in the glass house itself. They were provided with lantern skirts made of light, closely woven, pollen proof cloth which when fully extended reached 2 ft from the floor. Just before the spikelet opening, arrows of female parents are cut with a large portion of stalk, placed in preservative solution and set up in lantern with minimum loss of time. The male arrows are introduced in similar way and placed slightly above the female arrows. The acid solution is changed twice a week. The canes are properly labelled and tapped lightly every day to ensure proper shedding of pollen. After crossing is completed, the arrows whose seeds are to be collected are transferred to seed ripening shed, keeping them in solution. Paper cones are placed around and below the arrows to collect the dried panicle branches and fuzz as they fall (Stevenson 1965). In South Africa, crosses are made both in field and glass house. In field, a glass lantern is suspended over the female arrows on long bamboo poles. The male arrows are then inserted into the lantern, and the cut stalks are placed in preservative

solution. The lantern is closed at the bottom with linen cloth tied to stalks. Male flowers are changed daily or alternate days. After crossing is over, male arrows are removed. A muslin bag is then put on the female arrows till seed is matured.

9.8.3.3 Melting Pot Technique or Modified Polycross

The dictionary meaning of melting pot is a place where many different people and ideas exist together, often mixing and producing something new. During World War II, the Hawaiian Sugar Planters' Association (HSPA) Experiment Station faced labour shortage. They employed a modified polycross technique known as melting pot (MP) technique (Mangelsdorf 1953). A large number of seedlings are produced at minimal cost, but a disadvantage of this method is the loss of pedigree information hence inability to repeat the crosses giving higher selection rate. Establishing MP crosses is a 1-day operation per crossing date in contrast to 2 days for biparental crosses. Stalks with arrows in early anthesis are cut and tagged in the field and then brought to the MP crossing shelters where a female arrow is interspersed (kept in Hawaiian solution) with large number of male arrows collected from diverse clones. The arrows (both male and female) in the MP shelter are moved around periodically to achieve wide distribution of pollen from each tassel. In many sugarcane breeding stations where the Hawaiian method is used, the male arrows are thrown away after pollination. Fluffs from female arrows alone are harvested; hence, the female parent of the seedling produced in the melting pot alone is known (Warner 1953). A modification of the melting pot is the 'area crosses' (Warner 1953) in which tassels from a single male parent were used to pollinate a number of female varieties. In this case, both the parents of the progeny are known, and this method enables screening a large number of genetic stocks for general combining ability.

9.9 Molecular Cytogenetics and Breeding

Cytogenetic manipulations of the chromosome complements are one of the most important methods available for introducing new variations into the crop plants. Classical cytological studies such as changes in chromosome number, morphology and chromosome behaviour in mitosis and meiosis played a pivotal role in establishing a classification of the *Saccharum* genus and, to some extent, revealed the process of 'nobilization' in varietal development. Molecular cytogenetic analysis is an advanced technology in cytogenetics by incorporating applications of molecular biology to understand crop evolution, genetics, genetic recombination and karyotype stability. Advent of these technologies has widened the scope of applications of classical karyotype analysis for exploring the location of a gene in the chromosome. The use of molecular cytogenetic studies in sugarcane research provides a precise karyotype analysis to introgression of alien genome in interspecific and intergeneric hybrids.

Recent molecular cytogenetic techniques such as fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) together have extensive use in identification of specific genomes, individual chromosomes or

chromosomal segments. The basic chromosome numbers of 10 for *S. officinarum* and *S. robustum* and 8 for *S. spontaneum* were established through FISH by physical mapping of ribosomal RNA genes (D'Hont et al. 1996, 1998). In addition, in situ hybridization studies using genomic DNA as probe have shown that the genomes of modern hybrids are composed of 70–80% *S. officinarum* chromosomes, 10–20% *S. spontaneum* chromosomes and 5–17% recombinant chromosomes containing part of *S. officinarum* and part of *S. spontaneum* chromosomes (Piperidis and D'Hont 2001; D'Hont 2005). A major drawback in sugarcane cytogentics is the lack of information on genome and chromosomal morphology which hampers clear identification of individual chromosomes from each genome.

The genome complexity in sugarcane has encouraged researchers to elucidate the aspects of genome constitution and architecture through molecular studies. Critical information on the behaviour of chromosomes during meiosis in modern cultivars is lacking. Vieira et al. (2018) explored the meiotic process and chromosome association at diakinesis using FISH. The use of centromeric probes confirmed the predominance of bivalent associations, avoiding possible errors due to the small size and high number of sugarcane chromosomes, and provided a model for analysing meiotic behaviour in other canes with possible implications for sugarcane breeding programmes.

GISH is a powerful technique with enormous potential for the identification of parental genomes in interspecific and intergeneric hybridization by using the genomic DNA from one species as the labelled probe. GISH allows monitoring the introgression of alien chromosomes in the wide hybrids. GISH was first demonstrated in synthetic *Hordeum chilense* × *Secale africanum* (Schwarzacher et al. 1989) and *Triticum aestivum* (wheat) × *S. cereale* (rye) (Le et al. 1989). Since then, many researchers could efficiently monitor chromosome transmission in interspecific and intergeneric crosses. Another huge advantage of the GISH is the ability to visualize the sites of recombination on the physical chromosome (Khrustaleva et al. 2005). For the first time, D'Hont et al. (1996) demonstrated that GISH can be used to differentiate chromosomes in interspecific hybrids between BNS 3066 (*S. officinarum*) and SES 14 (*S. spontaneum*). In addition, they identified $n + n$ transmission of parental chromosomes in the interspecific F_1 hybrid between *S. officinarum* and *S. spontaneum*.

Using GISH, the typical *S. officinarum* with 80 chromosomes and atypical with more than 80 were distinguished (Yu et al. 2018), and the results could be very well used in selection of pure *S. officinarum* for sugarcane improvement. Of late, GISH technique has been effectively used in elucidation of inheritance pattern of alien chromosomes in intergeneric hybrids involving *Erianthus*. Pachakkil et al. (2019) analysed the intergeneric hybrids of *Erianthus* and showed that 54–56 chromosomes were transmitted from sugarcane cultivar, while 18–29 chromosomes were from *E. arundinaceus*. These results could show that '$n + n$' parental chromosome transmission occurred during hybridization, with elimination of *E. arundinaceus* chromosomes in varying degrees. In another study, GISH analysis showed unexpected number of *E. arundinaceus* chromosomes in the BC_1 progeny which was more than in their F_1 female parents. This is the first cytogenetic evidence for an

unexpected inheritance pattern of *E. arundinaceus* chromosomes in sugarcane (Wu et al. 2014). The chromosome composition of *E. procerus* × *S. officinarum* hybrids at F_1, BC_1 and BC_2 stages via GISH was demonstrated, and the F_1 resulted from $2n + n$ chromosome transmission, while BC_1 and BC_2 from $n + n$ transmission (Sobhakumari et al. 2020).

For the first time, the chromosome composition and condensation behaviour of sorghum chromosomes in hybrids involving *Saccharum* and sorghum were demonstrated in two intergeneric hybrids of sugarcane, Co 86032 ($2n = 112$) × Sorghum ($2n = 20$) and Sorghum ($2n = 20$) × *S. officinarum* ($2n = 112$), through molecular cytological analysis. GISH using *Sorghum* genomic DNA as the labelled probe revealed $n + n$ chromosome segregation and introgression of ten *Sorghum* chromosomes in both hybrids (Sobhakumari et al. 2018). These molecular cytogenetic tools provide a wider opportunity to analyse the genomic structure and function, chromosome constituents, alien chromosome transfer, recombination, genome evolution, aneuploidy and polyploidy in sugarcane.

9.10 Genetic Studies on Qualitative and Quantitative Traits

Quantitative genetic studies are very important and of great use if they can indicate the relative importance of additive and non-additive genetic variance and of genetic and environmental variances for a particular character. Many designs proposed for studying the inheritance of quantitative traits are not suitable for sugarcane because of high polyploidy, irregular meiosis, cross fertilization, heterozygosity, self-sterility and incompatibility (of some varieties) and male sterility or low pollen viability of many varieties. However, the effect of the violation of these assumptions did not have serious effects on estimates of genetic parameters (Hogarth 1977).

Maternal effect has also been reported in sugarcane (Loh and Tseng 1950). Natarajan et al. (1967) reported maternal effects for stalk number, stalk length and hand refractometer brix. Rai et al. (1991) reported higher contribution of females than that of female × male to the total variance for number of millable stalk (NMS) and stool weight, which might be due to some self-pollination (unless the maternal parent is pollen sterile) as the maternal parent of a cross is not emasculated (Hogarth 1973). Bhagyalakshmi et al. (1986) observed higher contribution of female × male for NMS, stalk diameter, stalk length, Brix % and stalk yield. High contribution of females to the total genetic variance for commercial cane sugar percent (CCS%), Brix %, NMS, stalk diameter and stalk length indicates high *gca* (general combining ability) variance, whereas contribution of variances due to *sca* were higher for sugar yield, stalk yield, sucrose %, juice extraction % and single stalk weight (SSW). Contrarily, Ram et al. (2005) reported the highest contribution (41.83%) of males followed by that of female × male (34.26%) and females (23.93%) for red rot disease index.

The mid-parent offspring covariance, which allows estimation of additive genetic variance, requires fewer genetic assumptions than most other estimates and has the additional attraction of being a meaningful statistical parameter as well as genetic

parameter. Offspring-parent regression has been reported by Hogarth (1977), Hogarth et al. (1981) and Ram and Hemaprabha (1998a). The additive and dominance genetic variances were higher in commercial hybrids × first nobilized progenies (N_1) for sugar yield and stalk yield. The dominance variance was higher in progenies of noble × N_1 for stalk diameter and sucrose content (Ram and Hemaprabha 1998a). They also reported higher additive genetic variance for NMS, sucrose % and stalk diameter and negligible additive genetic variance for stalk and sugar yields, sucrose %, juice extraction % and SSW. On the basis of combining ability analysis, Ram and Hemaprabha (2000) found the importance of non-additive effects for both sugar content and stalk yield. They also reported above 1.5 ratio of additive genetic variance to dominance variance for NMS and near unity for stalk diameter and stalk length. Dominance variance was important for sugar yield, stalk yield, CCS %, sucrose %, Brix %, juice extraction % and SSW.

Earlier, Hogarth (1980) reported that most genetic variance for Brix was additive, but non-additive genetic variance was important for stalk yield and its components. Hogarth (1977) reported that additive genetic variance was more important for Brix and number of NMS per stool in the Australian population, whereas in the Hawaiian population, dominance variance was more important for stalk number, and both additive and dominance variances were important for Brix (Hogarth et al. 1981). Based on combining ability studies, Yang and Chu (1962), Miller (1977) and Rao and Ethirajan (1983) found that specific combining ability (*sca*) was more important than general combining ability (*gca*) for stalk yield and Brix. Rai et al. (1991) reported a predominant role of non-additive gene action in the inheritance of NMS, stalk length, stalk diameter and stalk density and additive gene action for SSW and stalk yield. They also reported that the degree of dominance was in the range of over-dominance for NMS, stalk length, stalk diameter and stalk density.

Narrow-sense heritabilities for stalk and sugar yields were low, whereas it was moderate to high for component traits. Data on additive dominance variance and heritability suggest that yield component breeding may be an effective way to indirectly increase sugar yield and stalk yield (Ram and Hemaprabha 1998b). In another study, Ram and Hemaprabha (2000) reported negligible heritability in narrow sense for cane yield. Cane yield and sucrose content are negatively associated in sugarcane. The degree of negative correlation increases with improvement in any of these two components above the present commercial status. However, this negative association is not absolute. Even a high negative correlation of −0.8 between cane yield and sucrose % meant 36% ($1 - r^2$) independent variability, which indicated the scope for simultaneous improvement in sugarcane yield and sucrose content (Ram and Hemaprabha 1992). Based on experiments conducted under water stress, waterlogging, salinity and non-stressed conditions, Ram et al. (2001a) concluded that number of millable stalks (NMS) had the highest genotypic coefficient of variation, heritability, expected genetic advance, higher correlations and direct effects with sugar yield, higher inter-environmental correlations and near unity relative response values. Hence, NMS was the most effective selection criterion for selecting better ratooning and high sugar yielding clones in sugarcane under different abiotic stresses.

The best way to manage the disease is to develop resistant varieties, and all new releases must be resistant to red rot. Study of large numbers of progenies involving resistant and susceptible parents established Mendelian segregation for red rot resistance (conferring vertical resistance) in many of the crosses (Ram et al. 2001b). Both additive and dominance variances were equally important, and heritability (in narrow sense) was 0.51 for red rot resistance in sugarcane. A high (0.97) broad sense heritability for red rot resistance indicates a high level of repeatability across different environments for red rot disease development in sugarcane (Ram et al. 2005). A detailed investigation involving interspecific and intervarietal genetic stocks revealed the presence of both vertical (race specific) and horizontal (race nonspecific) components for red rot resistance. It was also established that the level of horizontal resistance decreased with a decrease in the *S. spontaneum* chromosome complement present in the material (Natarajan et al. 2001).

9.11 Breeding Objectives

The objectives of sugarcane breeding are more or less the same throughout the world: the production of high sugared and high yielding disease-resistant varieties giving the maximum returns under the conditions for which they are intended although different means are used in different places to attain the same end. With the extension of sugarcane cultivation in non-conventional areas, the need was felt for breeding for tolerance to adverse climatic conditions such as drought, frost, waterlogging and salinity and emerging diseases such as red rot, smut, borer pests, etc. The recent awareness on the advantages of using green fuel for generation of electricity and use of bioethanol in automobiles to reduce greenhouse gas emission have resulted in setting up of a number of cogeneration plants and distilleries in various sugar mills. To achieve this dual goal of increased alcohol and cogeneration, sugar industries need special varieties to meet their specific requirement of raw materials. Sugarcane improvement, is therefore should be a continuous and dynamic programmes and by taking into the need of end users, more and more specific traits/gene(s) are to be introgressed now and then in the commercial cultivars. The most important objectives of cane breeding across the world are highlighted here.

9.11.1 High Cane Yield and Sugar Content

Initially the breeding work was concentrated on the two important economic characters: the yield of cane and sucrose content in juice. The theoretical possible yield of sugarcane is 374–381 tonnes/ha/year (Moore 2009; Waclawovsky et al. 2010). The maximum stalk yield achieved in research stations in Brazil, Australia and Columbia were in the range of 236–280 tonnes/ha (Duke 1983), and world average cane yield in farmer's field is 70.64 tonnes/ha (FAOSTAT 2020). The most productive nations in the world with respect to sugarcane yield (>100 tonnes/ha) are Peru (123 tonnes/ha), Senegal (114 tonnes/ha), Guatemala (112 tonnes/ha), Egypt

and Nicaragua (109 tonnes/ha), Malawi (108 tonnes/ha), Zambia (103 tonnes/ha) and Burkina Faso (102 tonnes/ha) (FAOSTAT 2020). Sugarcane productivity in India and Brazil has been at 80.50 tonnes/ha and 76.13 tonnes/ha in 2019–2020, respectively. There is tremendous increase in cane yield of improved modern-day bi- and tri-specific hybrids in comparison to the yield of natural hybrids/species clones cultivated during the 1890s.

During 1900s, the average cane yield of Badilla, a natural clone of *S. officinarum*, was 40 tonnes/ha in Queensland and yield of varieties evolved in 1904–1908 such as HQ 409 and Q 813 was 40–60 tonnes/ha (Roach and Daniels 1987). The average cane yield of modern hybrids presently cultivated in Australia was 80–90 tonnes/ha. Breaux (1984) reported 25% increase in sugar content in all CP varieties which evolved during 1970–1971 in comparison to varieties grown in Louisiana in 1958. In India prior to 1912, i.e. before the development of improved Co varieties, indigenous sugarcane varieties such as Agauli, Chin, Dhaul, Dickchan, Hemja, Katha, Lalri, Mungo, Matna, Nargori, Pathri, Rakhra, Kuswar, Saretha and Pansahi were in cultivation. Their cane yields were in the range of 18–35 tonnes/ha, and sucrose yield was 10–15.0%. However, with recent varieties such as Co 0238 and Co 86032, few farmers in India are harvesting 252 tonnes/ha under subtropical condition and 300 tonnes/ha in ring pit planting in tropical region (The Hindu, 13 Feb 2012, Business line, dt 14 Nov 2016). The mean cane yield and sucrose% of India's leading cane varieties Co 86032 is 105.33 t/ha and 19.28%, respectively and of Co 0238 is 81.08 t/ha and 17.99%, respectively. An analysis of cane yield of improved sugarcane clones tested under the All India Coordianted Research Project (AICRP) on Sugarcane was made zone-wise in comparison with the standards over a period of 6 years, from 2010–2011 to 2015–2016 (Ram and Karuppaiyan 2017). It showed that the mean cane yield of improved clones across the zones in India was 82.93 tonnes/ha which was 4.6% improvement over the yield of standards (79.28 tonnes/ha).

The data shows improved varieties are better in terms of cane yield and sugar yield and contributing to the livelihood security of farmers. There are many successful cases in India, Australia, Brazil and other countries on the improvement of sucrose content in sugarcane through selecting right parental combinations (Legendre 1995; Lo and Chen 1995). In 2012, the Federal University of Alagoas, Brazil, released the RB99395 variety with 15.88% pol % in cane (Barbosa et al. 2015) which was 1.1 unit higher than the standard variety SP79-1011 (14.77%). The sugarcane variety GT35, with very highest sucrose content (top, 19% or higher), was recently bred by the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, Nanning, China (Huang et al. 2016). In West Indies Central Sugarcane Breeding Station, Barbados, a high sugar variety B 4362 with 24.1% sucrose was reported. In Louisiana, where the growing condition is limited to 9 months as opposed to 12 month elsewhere, clones with 14.9% sucrose were achieved through recurrent selection cycle (Breaux 1984).

9.11.2 Earliness

Sugarcane crop is said to be matured if the juice brix% is 18% and above, Pol% in juice is 16% and above and purity of juice >85% (Sahi and Sundara 1986). Varieties attaining such level of juice quality at 10 months after planting are called early varieties, and those attaining sucrose maturity at 11–12 months are called mid-late varieties. At the start of crushing season, if late maturing or low sugar varieties are harvested, it will affect the sugar recovery (crush-to-kill period). Therefore, early maturing high sugared varieties are preferred for crushing in the initial phase of crushing season. For higher sugar recovery, it is always desirable to have one-third of cane area in each sugar mill zone with early maturing varieties. Breeding for high sugar early maturing varieties has been a major objective in India, Mauritius, the USA, etc. (Cuenya and Mariotti 1986; Domaingue et al. 1990; Cox et al. 1990; Das et al. 1997). Early varieties elongate earlier, but that final stalk height at harvest was lesser than that of late varieties. The partitioning of photosynthates into sucrose was higher and earlier in early varieties.

In Mauritius, the ratio of reducing sugars to sucrose is being used as a screening criteria at the preliminary phase (stage 2) of selection in the plant cane crop of 6–8 months of age with a view to detecting early/high sucrose varieties at this stage of selection (Mamet et al. 1996). Until the 1980s, relatively few early ripening varieties were available for commercial cultivation in many countries, and earliness appeared to be a difficult characteristic to attain in the breeding and selection programmes due to negative correlation between earliness and high sucrose. CoJ 64, Co 89003 and CoS 8436 were the well-known early varieties in the subtropical region of India, whereas CoC 671, Co 6806 and Co 997 were the known early maturing variety in the tropical states of India. The negative relationship between high sugar or high cane yield and red rot resistance is a bottleneck in sugarcane improvement. Despite of it, Dr. BakshiRam, sugarcane breeder in India succeeded in evolving high sugar high yield and red rot-resistant varieties such as Co 98014, Co 0118, Co 0237, Co 0238, Co 0239 and Co 05009 by suitably changing the selection criteria in the ground nursery. Early selection during October in seedling ratoon nursery is more efficient in identifying better quality clones for crushing during early in the season (Ram et al. 1997).

9.11.3 Short-Duration Varieties

Short-duration varieties (SDV) are those that attain >18% brix, >16% sucrose and >85% juice purity with acceptable cane yield at eighth month after planting (Sahi and Sundara 1986). The age of sugarcane crop is 10–12 months, but the objective of SDV is to harvest three crops (one plant and two ratoon crops, at 8 month interval) in 2 years instead of two crops in 2 years with conventional varieties. Short-duration varieties are capable of bringing marked improvement in recovery and ultimately in sugar production. The cropping intensity can be increased, as SDV can be profitably fitted in a multiple cropping system. Development of short-duration sugarcane

varieties has been a breeding objective at the ICAR-Sugarcane Breeding Institute, Coimbatore. To reduce the crop duration of sugarcane from 12 to 8 months, Dr. T.S. Venkatraman made crosses between sugarcane and sorghum, and hybrids were produced.

The recent sugarcane × sorghum hybrids evolved at the institute showed HR brix (hand refractometer Brix) up to 19.90% at 6 months as against 15.6% brix in the popular cultivar (Nair 2012). Parental clones with high sucrose (>16%) such as Co 0237, Co 09004, Co 13001, Co 13006, Co 0237, Co 16001, Co 11015, CP 96-1662 and CoC 671 were identified (Karuppaiyan et al. 2021b). In India, two varieties, viz. Co 8338 and Co 11015, were released as SDV. Co 11015 evolved from the cross CoC 671 × Co 86011 and was released in 2020 for commercial cultivation in Tamil Nadu (Hemaprabha et al. 2019). The average sucrose % in Co 11015 at 240 DAP was 17.2 which was 13.96% higher than that of the standard Co 86032 (15.09% sucrose at 240 days after planting). Within a span of 2 years from its release, this variety spread over 9500 ha in Tamil Nadu in the 2020–2021 crop season.

9.11.4 High Biomass, Bioethanol and Bioenergy

With the growing need for alternative sources of energy other than the currently predominant petroleum matrix, there has been resurgence in interest in biomass crops as a renewable energy source. Sugarcane is being viewed as an important bioenergy crop and source of low cost raw material for the production of bioethanol and bagasse-based electricity. The survival and profitability of sugar industry in the future would rely on how far and fast they switch to diversification from the monotonous sugar manufacturing. Sugar industries in Brazil, Columbia, Thailand and India have already built cogeneration plants to generate electricity from bagasse and distilleries to distill ethanol from molasses and/or raw sugarcane juice. Redefining breeding objectives and utilization of new genetic stocks with high biomass, total sugars and fibre content in breeding programmes in Brazil and India are discernible but in slow phase. A few clones in *Saccharum*, *Erianthus* and *Miscanthus* germplasm collections and in the prebreeding materials with high biomass yield have been reported in India, Brazil, Puerto Rico, Texas and Japan (Matsuoka et al. 2014). The recently made sugarcane × *S. spontaneum* and sugarcane × *Erianthus arundinaceus* crosses in India are focused to evolve Type I and II energy canes. Energy cane is distinct from normal sugarcane in that it is selected for total biomass and fibre production rather than for sucrose.

Type I energy canes are the sugarcane hybrids with relatively low sucrose (>15% brix) but moderate-to-high fibre content (<25%). They are the dual-purpose canes, its juice can be used in distilleries for direct fermentation and fibre for cogeneration. Type II energy canes are specifically bred for high biomass yield with high fibre (>25%) and low brix (<15%) and are exclusively used for energy generation and lignocellulosic-based second-generation ethanol production (Alexander 1985; Matsuoka et al. 2014; https://sugarcane.icar.gov.in). In the breeding programmes at Coimbatore, Louisiana, Barbados, Mauritius, Vignis (Brazil) and

Puerto Rico, identification of Type I and Type II energy canes were reported (Tew and Cobil 2008). Govindaraj (2017, 2020) developed fibre-rich, high biomass yielding dual-purpose energy canes (<20% fibre and 15% juice brix) such as SBIEC 11001, SBIEC 11002, etc. from the cross between commercial sugarcane varieties × *S. spontaneum*/*Erianthus*. SBIEC 11002 (Co 1148 × *S. spontaneum* SES 404) was registered with ICAR-NBPGR for high harvestable biomass yield (247.53 tonnes/ha/year), dry matter production (85.23 tonnes/ha) and fibre content (22.58%) (Govindraj and Suganya 2012).

Molasses is the main source for ethanol production. Ethanol yielding ability of sugarcane varieties depends on stalk yield and juice sugar content. To understand the genetic variability for ethanol production, a study was undertaken in a set of commercial varieties at ICAR-SBI, Coimbatore, during 1992. Wide variation was observed for total sugars, fermentable sugars and alcohol yield. Total sugars ranged from 16.4% (Co 1158 and Co 1305) to 22.2% (Co 8153). In general, varieties with high total sugars also recorded high fermentable sugars with a significant positive correlation ($r = 0.974$). The highest alcohol yield of 13,619 L/ha was recorded in Co 8145, and the lowest alcohol yield of 5579 L/ha was recorded in Co 1305. A high positive correlation between cane yield and alcohol yield was also observed ($r = 0.890$). Hence, a variety meant for ethanol production should have high total sugars, cane yield and extraction percent (Rakkiyappan and Pandiyan 1992). Under the subtropical condition of India, the estimated alcohol yield (L/tonne of cane) ranged between 59.08 and 71.66 in plant crop and between 63.49 and 70.44 in ratoon crop. The variety CoJ 88 recorded the highest alcohol yield 4791 L/ha in comparison to 3071 L/ha in Co 1148 (Uppal et al. 2006).

The species *Erianthus arundinaceus* (Rez.) Jeswiet shows considerable potential as breeding material due to its high biomass yield, high tillering, high fibre, better ratooning ability, multiple pest resistance, drought and waterlogging resistance and low nutrient requirement (Jackson and Henry 2011). In India, clones of *Erianthus arundinaceus* were evaluated with view to using them as raw materials in paper and cogeneration industries (Amalraj et al. 2008). Since 1928, hybrids between sugarcane × *Erianthus* spp. were reported (Rumke 1934; Janaki-Ammal 1941; Rao et al. 1963; Sreenivasan and Sreenivasan 2000; Aitken et al. 2006; Piperidis et al. 2000; Nair et al. 2017; Babil et al. 2019). Shanmughasundaram et al. (2010) reported high stalk yield in *Saccharum* × *E. arundinaceus* hybrids over the parents. The juice brix in the hybrids was in the range of 14.00–16.30%, which is relatively high for hybrids involving the wild species.

Miscanthus is a C_4 perennial grass with strong, thick, long stem and high biomass yield hence viewed as potential source of high cellulosic biomass and high degree of chilling tolerance in temperate environment (Beale et al. 1996; Long and Spence 2013). Two species, i.e. *M. sacchariflorus* and *M. sinensis*, are considered superior source to *S. spontaneum* for improving chilling tolerance of commercial sugarcane varieties and energy canes. The intergeneric hybrids between sugarcane × *Miscanthus* are often termed 'Miscanes' (Kar et al. 2019). Miscanes have been studied since the late 1940s for their biomass production and adaptive traits

especially under subtropical and temperate regions (Burner et al. 2015). Natural hybrids between *Saccharum* and *Mischanthus* were reported by Price (1965).

Each tonne of sugarcane processed by a sugar mill generates approximately 270–280 kg of bagasse which has a net calorific value of 7300 kJ/kg. Bagasse has been used as fuel in boilers to generate electricity (called cogen unit) that inturn is used to run sugar industries. The excess electricity produced in sugar mills is sold to the electricity grid. In 2016, Brazil produced 666 million tonnes of sugarcane and in the same year produced 35,236 GWh of electricity from bagasse. In India, the installed cogeneration capacity is estimated to be around 34 TWh, i.e. about 5575 MW in terms of the plant capacity (Mane 2016). The lignocellulosic biomass yield (dry matter including bagasse) of sugarcane is 22.9 tonnes/ha/year (Van Der Weijde et al. 2013). The bioethanol yield from bagasse is estimated at about 3000 L/ha in a total yield of 9950 L/ha from sugar and bagasse (Somerville et al. 2010). The ethanol output to input ratio is high from sugarcane bagasse (8–10) compared with maize (1.6) (Lam et al. 2009; Waclawovsky et al. 2010; Loureiro et al. 2011). The available lignocellulosic biomass from sugarcane worldwide is estimated at 584 million dry tonnes/year. Bagasse has huge potential to yield bioethanol (Hoang et al. 2015) hence, it has been increasingly exploited as raw material for production second-generation biofuel (ethanol). Future breeding programmes in leading sugar-producing countries would focus on evolving high bioenergy and high biofuel yielding sugarcane varieties.

9.11.5 Biotic and Abiotic Stresses

Biotic and abiotic factors have been the main limiting factors of crop productivity. In the recent years, increasing concentration of greenhouse gases and increase in global surface air temperatures caused through climate change create new environmental conditions which may result in increased crop failure and frequent incidences of abiotic and biotic stresses. Long growth cycle of sugarcane crop, for 10–12 months and more, exposes it to several abiotic and biotic stresses, which get prolonged and intensified under changing climatic conditions (Table 9.3). These factors necessitate incorporation of new and diverse genetic resources into future sugarcane varieties.

9.11.5.1 Drought/Salinity/Waterlogging

Among the abiotic stresses, drought is the most serious threat throughout the world. Drought coupled with waterlogging, i.e. early drought and subsequent waterlogging in Bihar, Eastern Uttar Pradesh (UP) and Odisha, is a serious productivity constraint affecting more than 50% of area under the crop. Waterlogging is experienced in Tarai region in UP, Bihar and Kolhapur region in Maharashtra. Sugarcane is basically a drought-tolerant species. However increasing intensities and duration of water deficit stress not only cause yield reduction between the mean yield and the potential yield but also cause yield instability. Recent occurrence of drought has necessitated the need to evolve drought-tolerant varieties to sustain sugarcane cultivation. In sugarcane, the formative phase (60–150 days after planting) was

Table 9.3 Major biotic and abiotic factors to sugarcane productivity in different agroclimatic zones of India

Zone	Peninsular	East coast	North central	North west	North east
A. Biotic factors					
1. Diseases					
(a) Red rot	✓	✓	✓	✓	✓
(b) Smut	✓	✓	✓	✓	✓
(c) Wilt					
2. Pests					
(a) Borers	✓	✓	✓	✓	✓
(b) Whit grub	✓	✓	✓		✓
(c) SWWA	✓	✓			
B. Abiotic factors					
1. Drought	✓	✓	✓	✓	✓
2. Waterlogging	✓	✓	✓	✓	
3. Salinity and Alkalinity	✓		✓	✓	
4. High temperature with water stress			✓	✓	
5. Cold				✓	

✓, indicates presence of pest, diseases and environmental stress in the region

identified as the critical water demand period, and any amount of water stress at this early growth phase had a direct influence on growth, dry matter accumulation, cane yield and juice quality. Hence screening for drought tolerance is carried out by withdrawing irrigation during the critical water demanding period. Number of millable canes, cane height, juice extraction % and sucrose % in juice are the most dominant parameters for yield build-up under stress, and these traits are used for identifying resistant varieties. Development of stress-tolerant genotypes is the most common approach in enhancing drought stress tolerance in sugarcane. A large number of elite clones of commercial status having drought tolerance have been identified through screening under natural drought conditions in addition to potential parents for breeding for drought tolerance (Hemaprabha et al. 2006). Cane yield has invariably remained the core selection index along with total sugar productivity under drought conditions. The selection efficiency for development of drought tolerant cultivars can be further improved by using a particular physiological and/or morphological trait related to yield as selection criteria (Clarke and Townley-Smith 1984). Almost all the clones of wild species *S. spontaneum* and some clones of *S. barberi* such as *Hemja*, *Khari*, *Katha* and *Ikhri* provide drought tolerance. Similarly, related genera such as *Erianthus* spp. (especially *E. arundinaceus*) and *Narenga* spp. have inherent capability towards drought tolerance (Sreenivasan et al. 2001).

Efforts to introgress *E. arundinaceus*, which has wide distribution in India, China, Myanmar, Thailand, the Philippines, Japan and Malaysia, into the sugarcane varieties were challenging due to hybrid sterility, difficulty in identifying the

true hybrids using the morphological traits, chromosome elimination during the successive back crossing and lack of recombination between the chromosomes of the two genera (D'Hont et al. 1996). In such cases, bridge crosses were found to be effective. *S. spontaneum* was used as a bridge cross, and *S. spontaneum* × *E. arundinaceus* hybrid when crossed with sugarcane resulted in fertile hybrids which could be further backcrossed to sugarcane (Lalitha and Premachandran 2007). Apart from sturdiness and yield improvement, hybridization of *Erianthus* with sugarcane resulted in introgression of genes for cold tolerance and red rot resistance (Ram et al. 2001c). Another major source is *E. procerus* ($2n = 40$), which is distributed mostly in India, Burma, Indochina, China, Indonesia and New Guinea. Attempts were made to introgress desirable traits of *E. procerus* in sugarcane and diversify the genetic base. The BC_1 hybrids showed substantial improvement over F_1 in terms of cane and juice quality traits (Nair et al. 2017). These intergeneric hybrids could be the potential source for diversifying the genetic base of sugarcane and combining resistance to drought and red rot. Characterization of intergeneric hybrids of *Erianthus rockii* and *Saccharum* as well as estimation of hybrid diversity were made possible with 400 amplified fragment length polymorphisms (AFLP) markers (Aitken et al. 2006).

Lack cytoplasmic diversity can turn out to be a major limitation in the future though we still are in the dark as to whether the cytoplasm from related wild species may be able to provide more plasticity apart from higher yields and more stable disease resistance (Mangelsdorf 1983). Premachandran et al. (2011) reported successful development of new cytoplasm substitution lines in sugarcane with the cytoplasm from *S. spontaneum* and *E. arundinaceus*. Apart from several genetically diverse intergeneric hybrid derivatives both for nuclear and cytoplasmic genomes under CYM series, two commercial varieties, Co 15015 and Co 16018, were developed at ICAR-SBI with proven hybridity and novel cytoplasm. Co 15015, which is a third-generation hybrid with $2n = 106$, revealed two *E. arundinaceus* chromosomes through GISH (Lekshmi et al. 2017). Development of multiparent advanced generation intercross (MAGIC) population in sugarcane is in progress at ICAR SBI by using the best pre-bred clones which are highly diverse and derivatives of different basic species of *Saccharum* and *Erianthus* (*S. officinarum*, *S. robustum*, *S. spontaneum*, *S. barberi*/*S. sinense* and *E. arundinaceus*, *E. bengalense*, *E. procerus*). Once developed, these hybrids with a mosaic of different genomes would be the valuable novel and diverse genetic resources for genetic enhancement for biotic and abiotic stresses apart from serving as a panel for mapping key traits (Mohanraj et al. 2017).

Salinity and alkalinity stresses are experienced in India in about one-fourth of the area under sugarcane. It is caused by the chlorides and sulphates of sodium, calcium, magnesium and potassium. The electrical conductivity of these soils is more than 4 dS/m, while alkalinity is imparted mainly by sodium carbonate. High osmotic pressure of the soil water restricts absorption of water and nutrients in adequate quantities. Sugarcane is ranked moderately susceptible to salinity with a threshold value of 1.4 dS/m (Maas 1986). The symptoms of salt damage are pale green or yellow leaves, scorched tips and margins, reduced leaf area and stunted canopy. In

addition to reduction in cane yield characters, sucrose content has also showed reduction with increased accumulation of Na, K and Cl ions. Salt-resistant varieties showed marginal accumulation of Na and hence lesser reduction. Though there is no directed breeding for salinity tolerance programme at ICAR- Sugarcane Breeding Institute, screening of elite selections at the AICRP(S) programmes has identified several salinity-tolerant clones.

Waterlogging stress is caused by the deficiency of oxygen (required for root respiration) following the replacement of air in the soil with water. Though sugarcane can tolerate long period of waterlogging, juice quality is drastically affected. Reduced stalk elongation, formation of aerial roots, drying of lower leaves and yellowing of younger leaves, shoot mortality and profuse flowering are the effects associated with waterlogging stress. Cane yield loss occurs due to high tiller or stalk mortality, reduced crop growth due to lack of nutrition and water uptake, lodging, cane breakage, etc. *Saccharum* species clones and hybrids shows differential response to waterlogging (Sreenivasan and Batcha 1963). About 15–20% yield reduction, in certain cases up to 70%, was reported due to waterlogging (Gosal et al. 2009; Singh et al. 2016; Swami et al. 2018).

Reduction in cane yield @ 0.5 t/ha for every day the water-table stagnating upto 50 cm of the soil surface was reported from Australia (Salter et al. 2018). Many clones of *S. spontaneum*, *S. robustum*, *E. arundinaceus* and *Narenga* are flood tolerant. Indian hybrids Co 513, Co 805, Co 815, Co 900, Co 958, Co 1290, Co 62100, Co 62136 and Co 62197 and foreign hybrids B 54-142, CB 40-13, CP 49-50, CP 63-361, H 63-361, H 50-7209, H 52-3689, H 53-263 and Q 61 were reported to be tolerant to waterlogging. The clones of *S. spontaneum* and *E. arundinaceus* and Co 513, Co 785, Co 805, Co 62100, Co 62136, Co 8231, B 54142, CB 4013, Co 99006 and Q232A were suggested as better sources for waterlogging resistance. Breeding for waterlogging is a continuous activity at the Kannur Centre of ICAR-SBI, where waterlogging is a perennial problem. Anakapalle, Kolhapur, Pusa and Thirvalla are other centres from where waterlogging-tolerant clones are emerging.

9.11.5.2 Cold/Frost/Winter Ratooning Ability

Sugarcane is a cold-sensitive plant (Tai and Lentini 1998). Sugarcane grown in subtropical states of India, Northern China and Louisiana suffer from extreme cold, and sprouting of stubbles is a major limiting factor. Chilling (low temperatures above 0 °C) and freezing (temperatures below 0 °C inducing extracellular ice formation) also limit the geographical distribution and growing season of sugarcane and cause significant crop losses (Xin and Browse 2000). In 1932, the ICAR, New Delhi has established a Regional Centre (of ICAR-SBI) at Karnal, Haryana, to evolve sugarcane varieties suitable for the subtropical climate of North India (now crop improvement work is restricted to the north-west zone of India). A good deal of work was done at this institute. The breeding and selection programme at ICAR-SBI, Regional Centre, Karnal, was modified to fit for selecting high sugared genotype combined with better winter ratoonability. The seedlings raised from fluffs are

ratooned during the last week of December to the first week of January, coinciding with peak winter. Selection is made in seedling ratoon nursery instead of seedling nursery. The time of selection in seedling ratoon is shifted from February to March to October, and traits like NMC, stalk diameter, stalk height and HR Brix are used as selection criteria (Ram and Sahi 2007). Varieties like Co 98014, Co 0118, Co 0237, Co 0238, Co 05009, Co 05011, Co 06034, Co 09022, Co 12029, Co 13035 and Co 15023 are the outcome of modified breeding programme.

To assess winter ratooning potential of sugarcane clones, Ram et al. (2017) proposed an index called 'winter sprouting index'. Significant and positive correlation between winter sprouting index (WSI) and tillers in plant crop ($r = 0.45$) and ratoon ($r = 0.41$), WSI vs. number of millable canes in plant crop ($r = 0.37$) and ratoon ($r = 0.42$), was reported. On the basis of WSI, sugarcane genotypes were classified into four categories, namely, excellent winter sprouting (WSI > 3.0), good sprouting (WSI = 2.01–2.99), poor sprouting (0.10–2.00) and low-temperature-sensitive (LTS) clones (WSI < 0.10). Ram et al. (2017) screened 632 genetically diverse sugarcane germplasm during 2009 to 2015 for winter sprouting. Fourty three clones were reported as excellent winter sprouters (winter sprouting index >3.00). They were: Co canes such as Co 06035, Co 12026 and Co 12027; commercial varieties such as CoP 9302, CoPant 96219, CoS 02264, CoS 109, CoS 797, CoS 94270, CoS 95222, CoS 97258, CoS 97264 and CoSe 95422; exotic genotypes such as BO 348, BM 368, B 33-65, BM 555, BM 61/1, CP 11-61, F 133, L 62-37, LF 64-2815, LF 65-3661, Mali, PR 1013, SP 80-1816 and TUC 472; and ISH and IGH clones derived from crosses involving *S. spontaneum*, *S. barberi* and *Erianthus* as one of the parents such as 20-200, 97-12, 99-304, 99-316, 99-356, 99-488, CYM 06-935, CYM 06-1144, CYM 07-284, GU 07-1841, GU 07-3704, GU 07-3730, GU 07-3774, KGS 99-1109, KGS 2004-72 and KGS 2004-20. Utilization of these clones in breeding programmes is suggested to concentrate genes for winter ratoonabilty in sugarcane varieties suitable for growing in subtropical region.

9.11.6 Biotic Stresses

Sugarcane diseases are constraints to crop production all over the world, and more than 125 diseases caused by fungi, bacteria, viruses, cytoplasma, etc. have been reported (Rott et al. 2000). The major diseases of sugarcane in India are red rot, smut, wilt, ratoon stunting disease, grassy shoot disease, mosaic, yellow leaf disease and pineapple disease.

9.11.6.1 Red Rot

Red rot caused by *Colletotrichum falcatum* Went is one of the major biotic factors limiting cane yield and quality (Chona 1980) both in the tropical and subtropical region of India. Barber in 1901 made the first recorded report of red rot occurrence in India. Since then a number of red rot epidemics have been reported, especially in eastern Uttar Pradesh, northern Bihar and pockets of Punjab, Haryana and Coastal Andhra Pradesh. The infected stalks become unfit for milling due to inversion of

sugar to reducing sugars. The stem becomes red and forms hollow cavity, and the canes perish completely causing heavy losses. The disease is primarily transmitted through infected setts.

The best and the most effective way of managing red rot epidemic is through growing resistant varieties. Resistance sources were identified in genotypes of *Saccharum officinarum*, *S. barberi*, *S. sinense*, *S. robustum* and *S. spontaneum* as well as related genera such as *Erianthus*, *Sclerostachya*, etc. Several clones of *S. spontaneum* are highly resistant to red rot and constitute suitable donors of red rot resistance. Biparental crossing, wherein one parent is red rot resistant, has been generally adopted for development of clones with disease resistance. Two popular methods of screening sugarcane clones to assess red rot resistance are plug and nodal methods of inoculation. In the plug method, the clones are inoculated with the pathogen culture when the crop is about 8 months old. The reaction of the clones is classified into resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS) to red rot based on a 0–9 scale. The resistance assessed by this method is referred to as protoplasmic resistance or physiological resistance. In the nodal method of disease evaluation, the pathogen inoculum is applied at the nodal region between the stalk and leaf sheath.

To overcome environment-induced variability in red rot disease reaction and to rapidly screen a large number of sugarcane clones for red rot, a controlled condition testing (CCT) was developed at ICAR-SBI. In this method, the pathogen is applied by nodal swabbing in the cane tops of 6- to 8-month-old cane and kept in a temperature- and humidity-controlled chamber. A new 0–9 scale was developed to assess disease resistance as R, MR, MS, S and HS in the clones. The CCT method has been aiding in screening a large number of selections emanating from breeding experiments. Clones selected from the first and second clonal nurseries, pre-release clones in Zonal Varietal Trials, are regularly screened against new isolates of *C. falcatum*. As a policy matter, clones showing MR or R reaction to red rot alone are approved for release in India.

9.11.6.2 Smut

This disease is of cosmopolitan distribution (particularly India, Pakistan, Brazil and Australia) and is caused by the fungus *Sporisorium scitamineum* (Syd.), and the fungus has no alternate host. Disease-affected clumps show profuse tillering with occasional formation of whips. The disease is favoured by hot dry conditions. Similar to red rot, smut disease severity also increases with increase in the levels of sett-borne inoculum. Breeding for smut resistance yielded many disease-resistant varieties. The resistance to smut appeared to be favoured by two dominant genes (S1 and S2), whose action was greatly modified by inhibitor and anti-inhibitor genes. Sources of resistance for sugarcane smut have been identified in *S. spontaneum* and *S. officinarum* clones. To screen sugarcane clones for smut resistance, artificial inoculation of the pathogen is being done in the field. The screening procedure consists of dipping the seed setts in heavy spore suspension of smut fungus and

planting in the field. Periodical observations of smut incidence is recorded, and on the basis of cumulative final percentage of disease incidence, varieties are graded as R, MR, MS, S and HS. A simple technique was developed to inoculate sugarcane setts using dikaryotic cultures. Smut pathogen colonization was assessed by trypan blue staining. This method ensures rapid screening for smut disease, and diseases escapes can be minimized.

9.11.6.3 Wilt

Wilt is common in locations where conducive environment and susceptible hosts are available (Viswanathan 2013). *Fusarium sacchari* (E.J. Butler) is the causative fungus. Abiotic factors like drought predisposes the plant for wilt infection. Wilt incidence is higher in ratoon crops and leads to reduction in yield and juice extraction.

9.11.6.4 Sugarcane Pests

About a dozen of insect and non-insect pests are recorded in sugarcane crop in India. Borers and sucking pests are the major aerial pests, whereas termites and white grubs are subterranean in nature. Among borers, early shoot borer is the key pest throughout the country. Internode borer is prevalent in southern states and stalk borer, top borer and root borer in subtropical states. Sugarcane white woolly aphid (SWWA) appeared in an epidemic form in Maharashtra and Karnataka in 2002. Scale insects, mealy bugs, mites, rodents and wild boar are other pests of sugarcane. The first potential source of resistance to be examined is the commonly grown and adapted varieties in the area where the experiments are being conducted. If resistance can be found among such varieties, the problem of breeding a satisfactory variety is greatly simplified.

9.12 Other Objectives

During 1918, a sugarcane station was established at Canal Point, Florida, with the objective of breeding for mosaic resistance varieties in Louisiana. One of the best varieties released for Louisiana was CP44-101 which occupied the State for a long period of time. Sereh disease was a major threat to sugarcane cultivation in Java and Fiji during the early 1900s, and it was addressed through breeding. Rust in Brazil and yellow leaf syndrome in Australia are considered important biotic stresses limiting cane yield. They are the objectives of sugarcane improvement in those countries. Nowadays, mechanization in sugarcane harvest is picking up fast, and to make it suitable for machine harvest, sugarcane is to be planted at a minimum row spacing of 4 ft. So far, selection of varieties in countries like India, Pakistan, China, Thailand and Indonesia has been practised for 3-ft row spacing. In the future, sugarcane breeders may have to include varieties suitable for mechanical harvest

as one of the breeding objectives besides yield, quality and resistance to biotic and abiotic stresses.

9.13 Exploitation of Heterosis and Hybrid Development

Sugarcane parental pool is highly polyploid (octaploid) and heterogeneous. Crosses are made between two diverse parents with desired complementary traits. In general, one parent is the cultivar, whereas the other one is a resistant source of major disease of the zone. The progenies segregate in the first generation itself, and hence desired clones are selected for further evaluation in subsequent clonal selection stages. Being an asexually propagated crop, the variability is fixed in the first generation itself.

A number of quantitative inheritance studies have been conducted on sugarcane using various statistical designs (Hogarth 1971, 1977; Hogarth et al. 1981; Miller 1977; Yang and Chu 1962). The higher yields in F_1 may be partially due to additive or non-additive gene actions, or both. Hogarth (1977) reported non-additive variance for sugar content, which was equal to additive genetic variance for stalk yield. Miller (1977) and Yang and Chu (1962) reported importance of non-additive effects for both sugar content and stalk yield. Rai et al. (1991) reported that the degree of dominance was in the range of over-dominance for NMS, stalk length, stalk diameter and stalk density. The proportion of additive genetic variance in relation to dominance variance was above 1.5 for NMS and near unity for stalk diameter and stalk length, whereas dominance variance was predominant for sugar yield, stalk yield, commercial cane sugar % (CCS%), sucrose %, Brix %, juice extraction % and single stalk weight (SSW) (Ram and Hemaprabha 2000). Ram et al. (2005) reported equal importance of additive and non-additive genetic variance for disease index of red rot. A study on 15 crosses indicated variation in heterosis for different crosses as well as for ten traits studied (Ram and Hemaprabha 2000). The authors reported positive heterosis over midparent value and over nobilized first generation (N_1) for stalk and sugar yields in 12 mating groups, whereas for sucrose %, positive heterosis was observed in (OB)H, (OR)H and (OS)O mating groups only (where O is *S. officinarum*; B is *S. barberi*; H is commercial hybrids; R is *S. robustum*; S is *S. spontaneum*).

In order to meet the future demands from the presently available area, there is need to exploit the dominance variance/heterosis to the maximum possible level. Research on developing hybrid varieties and propagation through true seed through developing homozygous lines was initiated at ICAR-SBI during 2015. The success of the programme will lead to paradigm shift to sugarcane agriculture. By adopting the seedling transplanting method in combination with true seed hybrids, it would be possible to change the mode of transportation of sugarcane-seed from truck to pocket (ICAR-SBI 2050).

9.14 Breeding Approaches

9.14.1 Conventional

The varietal development process in India, spanning over a century, has brought in refinements to the entire process of hybridization and selection and improved precision of sugarcane breeding. The major steps and significant findings which facilitated accelerated varietal development are mentioned below.

9.14.2 Hybridization

In India, hybridization is carried out coinciding with flowering during the months of October–December. The National Hybridization Garden accommodates about 600 parents and regularly adds new parents to maximize geographical and genetic diversity and elimination of poor parents based on progeny performance. Similarly, an arrowing plot with about 300 parents of broad genetic base is utilized by the ICAR-SBI breeders for specific breeding programmes, such as resistance breeding, quality improvement, development of short-duration clones and breeding for abiotic stresses especially drought. On an average, about 1000 crosses are made in both parental gardens.

The common hybridization methods adopted are biparental crossing (50–60%), polycrosses (1–5%) and open pollination (30–50%). However, breeders concentrate mostly on biparental crossing through careful choice of two parents guided by their per se performance, complementarity of characters, specific combining ability and genetic diversity measured based on pedigree, biometry or molecular methods. In contrast, polycrosses and open pollination are based on general combining ability. The merit of biparental crossing was evident from the pedigree analysis of Co canes developed over a century (1918–2017). Out of 1454 Co canes produced during this period, and maintained in the variety garden, 1241 (85.4%) were bred through biparental mating, while 81 (5.6%) were from open pollination and 31 (2.1%) from polycrosses, in addition to a few somaclones, mutants and selfs.

The large proportion of Co canes from biparental mating gives ample evidence of the importance of specific combining ability as well as genetic diversity of the parental combinations in choosing parents (Hemaprabha et al. 2019). Several biparental crosses with greater progeny performance index have been designated as proven crosses for raising large populations over 1000 seedlings per cross. These proven crosses facilitate adoption of family selection. Ethirajan in 1987 explained the concept of zonal crosses as a rational approach in the selection of parents. Accordingly, promising varieties identified from AICRP(S) trials of a zone are chosen as parents and crossed with genotypes with established excellence for a target trait. The fluffs from such crosses are distributed to all centres for further selection under respective location-specific conditions in the zone.

9.14.3 Selection

In total, over 200,000 seedlings are raised in ground nurseries in the country every year. The selection starts with screening in the ground nursery. Though the practice varies across the research centres, selection of seedlings in the ratoon crop has been found to be beneficial for selecting the best progeny. However, wide disparity in the performance of progeny at single stool seedling stage and subsequent clonal stages has been noticed, as evidenced by non-uniformity of correlation estimates for economic characters over locations and years. Ram et al. (1997) concluded that selection in seedlings and seedling ratoon nurseries was not effective because of low inter-stage correlations, non-significant regression coefficients and the number of significantly superior clones common at selection and evaluation stages. Considering both the effectiveness of selection methods and the costs involved, visual selection for superior seedlings based on the number of millable canes per plant, thickness of stalks, stalk height and HR Brix seems to be the best over the methods of selection through stalk yield and brix yield which are labour intensive, time-consuming and hence costly (Ram et al. 1997). However, realizing the importance of ratoonability trait in sugarcane, selection in seedling ratoon nursery has been adopted since 1998 at ICAR-SBI, Regional Centre, Karnal (subtropical India) and since 2016 at ICAR-SBI, Coimbatore (tropical India). Realizing the success of varieties evolved at Regional Centre, Karnal, a few State Sugarcane Research Stations also started following selection in seedling ratoon nursery stage.

9.14.3.1 Sample Size and Selection Criteria

Bhagyalakshmi and Ethirajan (1987) reported that a sample size of 40–50 seedlings was minimum for estimating family means for stalk diameter, stalk length, internode number, internode length and hand refractometer Brix (HR Brix), whereas samples of more than 100 were needed for yield, stalk number and single cane weight.

Studies on time of selection in subtropical India indicated that HR Brix of sugarcane clones recorded during August (7–8 months crop age) gives a good indication of HR Brix during October (9–10 moths). However, pol % in juice during January (12 months) was not correlated with HR Brix during August, but it was associated with HR Brix during October. The rate of sugar accumulation varied among sugarcane clones from August to January. The earliest possible period to classify sugarcane clones on the basis of HR Brix, as low-, medium- and high-quality types, is during October. However, if selection is delayed to beyond 12 months' crop age (spring season), selection based on pol % was effective as differences among clones for HR Brix narrowed down.

Genotypic and phenotypic coefficients of variation along with heritability and genetic advance of all yield and quality characters have been studied extensively to identify suitable selection criteria. Based on experiments conducted under water stress, waterlogging, salinity and normal conditions, Ram et al. (2001a) identified number of millable stalks (NMS) as the most effective selection criterion for selecting better ratooning and high sugar yielding clones as this character recorded the highest genotypic coefficient of variation (GCV), heritability, expected genetic

advance, higher correlations and direct effects with sugar yield, higher inter-environmental correlations and relative response values near unity. Ram (2007) reported that selection for juice quality traits would be easier if selection was practised at an early stage of crop age as higher GCV, heritability and genetic advance values were observed at 8 months of crop age (October). Improvement in sugar yield will also be faster if sugarcane clones are selected for stalk yield traits, namely, NMS, single stalk weight and stalk diameter, in comparison to selection for juice quality traits.

The maximum gains from selection may be achieved by selection based on several traits simultaneously than selection based on single trait. The extent of change in a character due to index based selection will depend upon the heritability, the economic weightage assigned and the magnitude and nature of genetic correlation with other traits. In practice, selection indices are not widely used in sugarcane, although they provide the most effective selection method. Selection indices are often inefficient when costs as well as results are considered. There are a few reports on selection indices for selection at clonal stages in sugarcane. Miller (1977) constructed various selection indices for each of the four populations of sugarcane. They reported 89% genetic advance in stalk yield and 92.2% in metric tonnes per hectare of sucrose. Another selection index based on internode length, Brix %, purity %, CCS % and sucrose % gave 43.54% expected genetic gain in sucrose content. Kang et al. (1983) calculated indices for tonnes per hectare of stalk and tonnes per hectare of sugar from ratoon crop data and correlated with actual performance in the final selection stage and reported low but significant correlation coefficients. Selection based on index can lead to selection efficiency in succeeding clonal stages.

When specific and general indices were applied in different open pollinated populations, it was found that specific selection indices were most effective only in their own source population (Ram et al. 1997). General selection index, when used in different populations, was better in terms of mean of selected clones and number of clones significantly superior to the best check, in comparison with specific indices.

9.14.4 Recurrent Selection

Recurrent selection programmes for improving yield and quality separately or simultaneously have been practised. Initial efforts substantially improved cane yield through improving cane diameter and single cane weight (Balasundaram 2002). These were consistent with the results of Bressiani et al. (2006), who reported gains through recurrent selection that were continuous and progressive with increase in the frequency of desirable genes for the target trait. In order to generate genetic stocks for high sucrose content, a simple recurrent selection scheme was employed with a base population of 25 Co canes and 12 foreign hybrids with juice sucrose of $\geq$19.0% at 12 months. After two cycles of selection, the progress made for sucrose content in comparison with the base population was significant with cycle II progenies recording an average juice sucrose of 22.8% which was a 13.40%

improvement over the base population (Shanthi and Alarmelu 2012). Another recurrent selection programme for simultaneously improving both cane yield and juice quality resulted in identifying several elite clones after three cycles of selection while maintaining sufficient genetic variability in cycle 1 and cycle 2 for selection in cycle 3 (Alarmelu et al. 2015).

Intraspecific improvement of major *Saccharum* species prior to interspecific hybridization had been suggested to achieve faster gains in interspecific hybridization (Walker 1987). Attempts were made during the 1980s at ICAR-SBI to develop improved populations of *S. officinarum* and *S. robustum* through repeated cycles of intraspecific hybridization and selection (Nair et al. 1998). In *S. robustum*, significant improvement in brix% (20.53%) and sucrose% (26.24%) was achieved after three cycles of selection and in *S. officinarum*, improvement in NMC/ha (37.18%), single cane weight (16.67%) and cane yield (61.51%) was achieved in two cycles of selection (Karuppaiyan et al. 2020a, b).

9.15 Specific Breeding and Research Programmes

In addition to the main mandate of developing high sugar high yielding disease resistant varieties for the country, specific time-bound programmes are conducted to develop short-duration varieties suitable for harvesting from 8 months onwards, use of foreign commercial varieties in breeding, recurrent selection programmes, trait-specific breeding programmes for high sucrose, red rot resistance, drought tolerance, pests including top borer tolerance, varieties suited for ethanol, paper and biomass production, deployment of advanced pre-bred material derived from diverse germplasm with respect to nuclear and cytoplasmic genomes, molecular diversity-based selection of parents in breeding programmes for sucrose, red rot, water stress and salinity, improving selection efficiency, crossing techniques, stability analysis. Testing of new statistical methods and experimental designs has contributed to improved methods for breeding and selecting new varieties. Specific investigations in breeding and biotechnology are in progress to develop homozygous lines (Annadurai and Hemaprabha 2016) for true seed production in sugarcane that could revolutionize sugarcane planting through substantial reduction in seed, which is about 6 tonnes of canes to about 32 g for planting a hectare (ICAR-SBI 2015).

9.16 Breeding for Disease Resistance

Disease resistance breeding has been an integral part of sugarcane improvement. In India, the most important diseases requiring varietal resistance are red rot caused by *Colletotrichum falcatum* and smut caused by *Sporisorium scitamineum*. Red rot has wiped off many popular varieties out of cultivation both in the subtropical and tropical regions. The first report of red rot in India was by Barber in 1901 from Godavari Delta in Andhra Pradesh and later in 1906 by Butler from West Champaran in Bihar (Alexander 1989). The best way to manage the disease is to

develop resistant varieties, and all new releases must be resistant to red rot. Study of large numbers of progenies involving resistant and susceptible parents established Mendelian segregation for red rot resistance (conferring vertical resistance) in many of the crosses (Ram et al. 2001b). Ram et al. (2005) reported that both additive and dominant variances were equally important and heritability (in narrow sense) was 0.51 for red rot resistance in sugarcane. A high (0.97) broad sense heritability for red rot resistance indicates a high level of repeatability across different environments for red rot disease development in sugarcane. The level of horizontal resistance decreased with a decrease in the *S. spontaneum* chromosome complement present in the material (Natarajan et al. 2001). In general, the proportion of resistant/moderately resistant (R/MR) progeny is more when both parents are resistant in comparison to one parent with R/MR reaction. However, a few resistant progenies were also observed when both parents were susceptible to red rot.

9.17 Precise and High-Throughput Phenotyping Protocols for Key Traits

Phenotyping is a challenging area of plant breeding and is labour intensive as it requires manual harvesting and assessing plants for particular criteria or visual ratings. Breeding programmes worldwide are exploring the possibility of implementing high-throughput phenotyping as an integral component of variety selection process. Rapid developments in phenomics technologies could be utilized to improve early-stage selection in sugarcane breeding programmes. Sugarcane lags behind in phenomics research. Sugarcane phenotyping for red rot and smut diseases is carried out under controlled conditions testing facility and field screening, respectively. Phenotyping for salinity is done using microplot facility, while screening for drought is done at seedling stage using pots and at tillering phase under field conditions.

An unmanned aerial vehicle (UAV)-based high-throughput phenotyping approach was tested for capturing valuable phenotypes in early stages of sugarcane breeding trials for improving clonal selection and assessing the potential new varieties for different yield and stress-related parameters (Basanayage 2017). The rapid and dense growing nature of sugarcane can be effectively captured from the air using drones fitted with a range of cameras and sensors, and integrated software is expected to enhance this process. The aerial platform required 15–17 min to survey 7 ha of sugarcane crop and to take measurements. This cutting-edge technology is being used to assess how potential new sugarcane varieties perform through the growing cycle, with the aim of delivering better varieties sooner for the Australian sugarcane industry. To add further credit to this technology, two crucial parameters such as canopy temperature (a proxy for canopy conductance) and crop vigour, if measured at the right time, could be associated with yield. Based on a single-row early-stage clonal assessment trial involving 2134 progeny derived from diverse crosses, and a multi-row experiment with an unrelated population, screening was done at several stages using visual, multispectral and thermal sensors mounted on a

UAV for indirect traits, including canopy cover, canopy height, canopy temperature and normalised difference vegetation index (NDVI). The results highlighted the potential of high-throughput phenotyping of indirect traits and developed a selection index based on indirect traits which are correlated with yield to improve clonal assessment in early stages of sugarcane breeding (Natarajan et al. 2019).

The application of UAV was also demonstrated on predicting the leaf nitrogen content and biomass following UAV optical imaging approaches, namely, LiDAR and surface from motion (SfM) photogrammetry in precision agriculture (Shendryk et al. 2020), and for monitoring growth response in sugarcane, measured in terms of height. The study also examined the possible correlation between sugarcane biophysical parameters of stalk population, total fresh biomass and cane yield.

9.17.1 Non-conventional Breeding Approaches Including Use of Genomic Tools

The non-conventional or advanced plant breeding is complementing the conventional breeding in improving the potential of crop plant by inducing gene(s) of interest in crop plants. Production of interspecific and intergeneric hybrids is useful for transfer of desirable genes from wild species into cultivated species (Nair et al. 2006, 2017; Pachakkil et al. 2019; Meena et al. 2020). Some varieties developed through mutagenic approach were also released for commercial cultivation (Jalaja et al. 2006). In sugarcane suitable explants for in vitro culture and plant regeneration have already been standardized (Lakshmanan 2006). The callus induction from leaf whorl independent of genotype is well established in sugarcane. Compared to other cereal crops, callus induction and plant regeneration are challenging.

Molecular marker technology has been evolving more than a quarter century to address these issues, and notable among them are the advances in molecular markers, structural and functional genomics and transgenics. Application of these approaches in sugarcane improvement is largely in the area of assisting plant breeding programmes for the evolution of better varieties through selection of parents for crossing, use as an efficient selection tool in screening progenies and for characterization of germplasm and introgression of wild germplasm, DNA fingerprinting, varietal identification and characterizing genes of economic importance and their selective transfer to the desired genetic background. The comparative analysis of molecular diversity and agronomic diversity in hybrid populations has increased the chance of relating markers to agronomic traits. The molecular marker techniques involving RAPD, AFLP, TRAP, ISSR, STMS, SSCP, SSR, ESTs are being employed for genetic diversity studies, gene mapping and quantitative trait loci (QTL) identification, DNA fingerprinting of varieties, functional genomics and marker-assisted selection and development of transgenics for important agronomic traits in sugarcane in India.

AFLP profiles of commercial cultivars with that of their progenitor species, viz. *S. officinarum* and *S. spontaneum*, revealed the presence of 78.8% of *S. officinarum*-specific DNA fragments and 28.85% of *S. spontaneum*-specific fragments (Selvi

et al. 2006). Several studies have used different markers to discriminate elite hybrids, commercial varieties, inbreds, induced mutants and somaclones in micropropagated plants of sugarcane (Hemaprabha et al. 2006; Sindhu et al. 2011). Using sugarcane and sorghum microsatellite markers, different combinations of *Saccharum* complex hybrids were successfully identified (Selvi et al. 2010). The markers identified have already been integrated in the breeding programmes as diagnostic tools to identify intergeneric and interspecific hybrids.

A considerable and remarkable success in genetic transformation was for genetic improvement for biotic and abiotic stresses (Lakshmanan et al. 2005; Babu et al. 2020). Sugarcane transgenics developed with different genes from various sources have showed better phenotypic performance under water deficit stress conditions (Augustine et al. 2015a, b, c; Mohanan et al. 2020; Narayan et al. 2021). Genetically modified sugarcane has been approved for commercial cultivation in Indonesia and Brazil, and in other countries, transgenic products are in different stages of field trials and/or commercialization. These include transgenics with genes conferring resistance to diseases and pests, salt and drought tolerance and high sucrose or herbicide tolerance (Babu et al. 2020). Sugarcane is also considered as a 'biofactory' for the production of high-value bioactive compounds due to high biomass production potential (Palaniswamy et al. 2016). Molecular breeding could be used to make improvement involving marker-assisted selection, genomic selection techniques and genome engineering approach. Recent advances in biotechnology of sugarcane highlight that the crop is on the threshold of genetic revolution as potential applications and benefits of molecular technology are being realized. Precision genome editing by homology directed repair has been reported in sugarcane for genetic improvement (Zhao et al. 2021).

9.17.2 Other Supporting Research and Development

The national sugarcane improvement programme is supported by different departments of research and development, viz. genetics and cytogenetics, tissue culture, crop production, crop protection, healthy seed nursery and agriculture extension. Mutation breeding was implemented in 1959 (Panje and Jagadesan 1959). Physical and chemical mutagens were used for improvement of many sugarcane varieties. Different varieties, viz. Co 8152 of Co 527, Co 8153 of Co 775, Co 8517 and Co 85035 of Co 740 and CoLk 8901 of CoJ 64, were subjected to mutagenesis, but none attained commercial status. Exploiting somaclonal variations, Co 85001, Co 85003, Co 85006, Co 85007, Co 85008, Co 85011, Co 85015, Co 85033 (Parent variety (Pv) Co 7704), Co 85035 (Pv Co 740), Co 85032, 85038 (Pv Co 7707), CoPant 93227 (Pv CoS 8118), VSI 434 (Pv CoC 671) and Co 94012 (Pv CoC 671), were also tried by different sugarcane research stations in India with focus on defect elimination in otherwise good commercial varieties. Presently, VSI 434 and Co 94012, two somaclones with high sugar content, are in cultivation in a limited area of Maharashtra.

9.18 Emerging Challenges at National and International Level

Sugarcane being a long-duration crop (12 plus months) faces many challenges. The following challenges are foreseen with regard to sugarcane agriculture. Sugarcane needs 1500–2000 mm water annually. Due to climate change, there is uneven distribution of rains in different states/countries. Scarcity of water for sugarcane agriculture is the biggest challenge. Water stress leads to lesser tillering and growth and reduction in juice quality and cane yields. Most of the sugarcane area is irrigated. Continuous exploitation of underground water coupled with lesser rainfall results in salinity problem. Therefore, water stress and salinity are going to be the major emerging abiotic stress challenges (Ram and Karuppaiyan 2018). Due to climate change, the minor insect pests and diseases of the past are emerging as the major ones, e.g. incidences of pokkah boeng and wilt are on the rise in North India. Similarly, there are increased incidences of white fly and mealy bugs in South India. For the new diseases and insect pests, screening techniques are not available.

In general, plateau in cane yield and sugar recovery is observed throughout the world (Walker 1987). However, there is improvement in average cane productivity and sugar recovery in India due to large-scale adoption of sugarcane variety Co 0238 in subtropical and central India. Due to increasing prices of inputs used in sugarcane cultivation coupled with disproportionate increase in cane price, the cost of production of sugarcane and hence sugar is increasing. This makes Indian sugar incompetent in the international market. Further, with improvement in cane productivity and sugar recovery due to increase in area of Co 0238, sugar is produced in excess in the country. Many sugar factories are finding it difficult to store the excess sugar, particularly in subtropical India.

Availability of labourers in sufficient number at the required time is another major challenge in sugarcane agriculture. Most of the medium and large farmers are finding it difficult to complete the different cultural practices in time. Complete mechanization, from planting to harvesting, of sugarcane agriculture is the solution to this problem. The varieties with erect habit, self-detrashing and lesser canopy are the traits of importance for mechanization. Breeders need to incorporate these traits in their selection criteria. Further, as For the evolution of a new sugarcane varieties it takes about 12–14 years. This long perido is to be shortened. There is need to identify the genetic stocks from the germplasm, genes for different traits and the screening techniques for evaluating the progenies for desired traits. All these emerging challenges need to be addressed in the near future.

9.19 Breeding Progress/Varietal Development

9.19.1 Conventional Breeding

Professionally directed sugarcane research in India was started in 1907 at Pratapgarh (Uttar Pradesh) by George Clarke, who was the Agricultural Chemist of United Provinces. This work gained impetus with the simultaneous establishment of

Sugarcane Breeding Station at Coimbatore and Sugarcane Research Station at Shahjahanpur in 1912 and the appointments of Dr. C. A. Barber as the Imperial Sugarcane Expert at Coimbatore and George Clarke at Shahjahanpur. In order to strengthen sugarcane improvement activities and production of cane and sugar in the then British India, a network of research stations were created across the country. Apart from the Imperial Council of Agricultural Research, Pusa (Bihar), other stations established for sugarcne research were Peshawar, Raisalwala (Punjab-now in Pakistan), Shahjahanpur (UP), Mushari (Bihar and Orissa), Dacca, Jorhat (Assam), Padegaon (Maharashtra), Anakapalle (Andhra Pradesh) and Tarnab (Kashmir).

Dr. Charles Alfred Barber initiated sugarcane varietal improvement based on the principles of plant breeding and developed an interest in the systematic crop improvement in all provinces in the country. The city of Coimbatore was endowed with climatic conditions favourable for good flowering and seed setting in sugarcane and thus chosen as suitable base for hybridization. It was also noticed that at Coimbatore the canes not only produced flowers every year but also shed pollen under natural conditions. Accordingly, Barber assembled a collection of sugarcane varieties from all parts of India in Coimbatore and produced sugarcane seedlings in 1912, a rare feat in those days. The first batch of seedlings was produced from the fluffs harvested in 1912. From these seedlings 1912, Dr. Barber and Dr. T.S. Venkatraman developed the first batch of 16 sugarcane varieties or Co canes (Co 201 to Co 216) at Coimbatore from the breeding efforts from 1912 to 1918 (Table 9.4) which was sent to different sugarcane-growing states of North India such as Punjab, Bihar, United Provinces, etc. for cultivation. Research stations at Lyallpur, Gurdaspur, Shahjahanpur and Pusa did much of the evaluation,

Table 9.4 Details of first batch of Co Canes

Name	Parentage	Name	Parentage
Co 201	Pansahi seedling	Co 209	Khelia seedling
Co 202	Chittan seedling	Co 210	POJ 213 seedling
Co 203	Saretha seedling	Co 211	Green Sports of Stripped Mauritius × Saretha
Co 204	Chittan seedling	Co 212	POJ 213 × M2 (Unbagged Cross)
Co 205	*S. officinarum* (Vellai) × *S. spontaneum* (Coimbatore form) (Bagged Cross)	Co 213	POJ 213 × Kansar (Unbagged Cross)
Co 206	Ashy Mauritius seedling	Co 214	Stripped Mauritius × (Saretha × *S. spontaneum*)
Co 207	POJ 213 × Saretha (Unbagged Cross)	Co 215	Stripped Mauritius × (Saretha × *S. spontaneum*)
Co 208	POJ 213 seedling	Co 216	Green Sports of Stripped Mauritius × Saretha

Source: Agricultural Journal of India, 1922

multiplication, distribution and cultivation of these clones. From this work, the famous clone Co 205, suited to the harsh subtropical environment of India, was identified. Co 205 emerged as the best variety, and cane yield improvement was remarkably higher in Punjab, about 50% more than the indigenous varieties, mainly Katha. The pedigree chart of Co 205 is given below.

Saccharum officinarum (Vellai) (2n=80) x *S. spontaneum* (Coimbatore) (2n=64)

↓

Co 205 (2n=112) (2n+n chromosome transmission)

The development of Co 205 was an important milestone in the history of sugarcane breeding because crossing the cultivated species *S. officinarum* with a wild species of grassy nature directly produced a successful hybrid of commercial value. Among the available thick-stalked canes (*S. officinarum*), Vellai was the first to flower which made it easy to cross with *S. spontaneum* canes characterized by early flowering. It may be noted that only 60 seedlings were obtained from which Co 205 was selected.

Along with Co 205, varieties such as Co 210, Co 213 and Co 214 were also popular among growers of North India and led to significant increases in cane and sugar production. Co 214 was the first early maturing variety bred at Coimbatore. Facilitated by the improved methods of sugarcane cultivation developed by George Clarke at Shahjahanpur Station, these varieties brought in a spectacular and revolutionary surge in cane and sugar production within a couple of years. This transformational research of Indian scientists attracted global attention, and Coimbatore received worldwide recognition for developing many world famous and highly productive Co varieties starting with Co 205. The impact of this program was enormous: the ancestry of almost all modern commercial sugarcane varieties in the world traces to early Co varieties. Howard (1940) attributed three factors, viz. sugarcane varieties bred at Coimbatore, sugarcane research work done at Shahjahanpur and protection policies of the governments, which made India self-sufficient in sugar production during 1940s.

Co 205 also spread to many countries including Cuba, the USA, Australia, South Africa, Argentina and Brazil. In Natal, a field trial conducted with Co 205, Co 210, Cuban Selection CH 64/21, Uba, Agaul, Kavangire, Oshima, Merthi, Townsend's selection, POJ 213 and SC 12/4 demonstrated the advantage of two Coimbatore varieties, viz. Co 205 and Co 210, which yielded 18% and 14%, respectively, more sugar per acre than the standard (Uba) (Todd 1939).

The SBI continued to supply Co canes after preliminary evaluation at Coimbatore to State Sugarcane Research Stations in the country for further evaluation under local conditions. Fluff was also supplied to these State Sugarcane Research Stations. Important commercial varieties identified were Co 285, Co 290, Co 312, Co 313, Co 419, Co 421, Co 449, Co 453, Co 467, Co 617, Co 1148, Co 1158 and Co 7717. These varieties had nearly 35% more sucrose content than their predecessors and also matured quickly, tolerated waterlogging and drought and

were resistant to red rot and Sereh diseases. They were cultivated in poor soils and were widely used as parents in breeding programmes of many countries (Roach 1989). Co 281 became a success in South Africa, while Co 419 was widely adopted in the tropics worldwide. Realizing these major scientific contributions and services, the Viceroy of British India Lord Irwin in 1930 praised Sugarcane Breeding Institute (SBI) at Coimbatore as the *Mecca of Sugarcane Research*. Further details are provided under coordinated system of testing.

9.19.2 Genomics-Assisted Breeding

Deploying genome-assisted breeding in sugarcane genetic improvement has always been challenging on account of complex nature of chromosomal pairing and assortment during meiosis, gametic sterility and hybrid inviability/sterility which hinder allele segregation in a Mendelian pattern. The lack of detailed genome information further reduces the quantitative trait loci (QTL) mapping resolution. A major breakthrough in marker development was utilization of the high-throughput sequencing methods that had already been developed for diploid crops. Bundock et al. (2012) identified 280,000 SNPs from sorghum coding regions and the sugarcane EST collections within a genotype, while Song et al. (2016) identified 1.1 m SNPs from 12 accessions from the *Saccharum* complex. The two main genotyping methods followed were array-based SNP genotyping and genotype by sequencing (GBS). The first fixed array developed was an Affymetric Axiom array (Aitken et al. 2017) which was specifically developed for use within breeding germplasm, and from the sequence information from Song et al. (2016), a second 100K Affymetric Axiom array was developed. Relatively high cost of screening large numbers of clones has been a drawback of the Affymetric Axiom array.

GBS method has been used to genotype many other crops (Scheben et al. 2017) but has not been very successful in sugarcane to discover single-dose SNP variants due to the complexity of sugarcane genome. However, encouraging progress is being made to implement markers in breeding programmes. The two approaches currently being adopted are marker-assisted selection making use of single genes or QTLs with large effect and a genomic selection. Both these methods have been developed in sugarcane. For marker-assisted selections, single candidate gene markers are used. A typical example is the brown rust resistance gene, *Bru*I, which was first identified by Daugrois et al. (1996). However, *P. melanocephala* race evolution has broken down the resistance conferred by *Bru*1 gene and necessitates more sources of resistance to combine with *Bru*1 for use in MAS. At ICAR Sugarcane Breeding Institute, already identified and validated candidate genes for drought and red rot are being used to explore the possibility of MAS for these two traits.

Genomic selection (GS) is a relatively new powerful breeding tool which is an upgraded form of marker-assisted selection. Individuals are selected based on their predicted breeding values that are estimated from genome-wide markers. Gouy et al. (2013) first tested the genomic selection approach in sugarcane, and predictions of

genetic values were carried out on two independent panels, each composed of 167 cultivars and breeding materials covering the worldwide diversity. Seven predictive models were used, and the accuracies of predictions were assessed through correlations between observed and predicted genetic values. Depending on the trait considered, the average GS accuracy values related to within-panel prediction ranged from 0.29 to 0.61 in the Reunion panel and from 0.13 to 0.5 in the Guadeloupe panel. In another study, three different populations of clones in early and advanced stage selection were evaluated for cane yield and sugar content in field trials and genotyped using a SNP array. The levels of prediction accuracy obtained in most datasets (0.25–0.45) are encouraging for developing applications of genomic prediction to predict breeding values of yield and sugar content in sugarcane breeding programmes (Deomano et al. 2020). A joint collaborative work between ICAR Sugarcane Breeding Institute and Sugar Research, Australia, is into developing a suitable genomic selection approach for traits such as sugar content, drought, red rot resistance and cane yield. Axiom array developed by Aitken et al. (2017) was used to develop genomic predictions for sugar content and cane yield in sugarcane clones in different stages of selection in a breeding programme (Deomano et al. 2020). The emerging results on red rot resistance are also encouraging to predict the breeding value.

9.20 Modernization of Crop Improvement Programme

With new challenges on sugarcane cultivation, it is essential that all future breeding programmes focused on ecologically viable, environment-friendly and resource matching technologies aimed at enhancing the production and productivity of sugarcane per unit time in the country. Sugarcane is now looked upon as a sugar and energy crop. Bioethanol production from sugarcane is a major focus in the coming years to meet the fuel demands of the country which warrants focus on prebreeding activities of germaplasm resources. Sugarcane is also emerging as a practical and viable platform in biopharming for high-value biomolecules.

The traditional varietal development programmes in most of the sugarcane research stations are mainly based on phenotypic selection which is very tedious and time-consuming. It takes around 12–14 years for development and release of new cultivar. Along with long breeding cycles and large populations, synchrony of flowering, insufficient planting materials for early generation trials, experimental errors, competition between adjacent plots and GxE interaction effects were typically causing problems and affecting the selection process. Maximizing the rate of genetic gain by reducing cycle length through rapid generation advancement, effective utilization of data-driven information management systems, advanced high-throughput phenotyping technologies and molecular tools would be a major step forward in improving sugarcane breeding programmes in the future.

9.20.1 Rapid Generation Advancement Through Speed Breeding

Rapid generation advancement in light-, temperature- and humidity-controlled conditions can significantly reduce the breeding cycle, thereby resulting in reduction of time and cost of crop varietal development. Recognizing the potential of speed breeding for accelerating crop improvement, many countries have initiated to use speed breeding platforms in crops such as wheat, barley, groundnut, chickpea, etc. The ability to induce flowering faster in sugarcane would significantly reduce the generation-to-generation cycle time and therefore speed up prebreeding activities such as introgression breeding, production of inbreds and transfer of GM events from a single cultivar into a range of backgrounds. Integration of speed breeding with other modern technologies like gene editing and genomic selection would maximize the genetic gain.

9.20.2 Advanced High-Throughput Phenotyping

One of the biggest bottlenecks in conventional sugarcane breeding is the ability to rapidly phenotype larger populations throughout the growing season. Current techniques are time and labour intensive and often introduce variation in collected data. Field-based high-throughput plant phenotyping (FB-HTPP) when applied to breeding programmes can contribute toward improving selection intensity with larger field trials, increasing selection accuracy by reducing human error and identifying novel genetic variation by capturing multiple phenotypes over time (Thompson et al. 2020). High-throughput phenotyping using unmanned aerial vehicles also provides an opportunity to improve the efficiency and effectiveness of clonal selection in early stages of sugarcane improvement programme (Natarajan et al. 2019). In sugarcane, the use of UAV-based high-throughput phenotyping approaches has been demonstrated for capturing valuable phenotypes in early stages of sugarcane breeding trials.

9.20.3 Molecular Tools for Effective Selection

The availability of a whole genome-sequencing (WGS) platform, genotyping by sequencing and advanced genetic models, such as genome-wide association studies (GWAS) and genomic prediction, presents opportunities to develop comprehensive datasets in sugarcane to accelerate genetic gains in the sugarcane breeding programmes. Genomic selection appears to be a promising approach in sugarcane as large numbers of markers that cover the genome are simultaneously fitted in GS models to predict the performance of new lines. With the anticipated publication of the complete commercial sugarcane genome, opportunities for high-throughput genotyping technologies for marker-trait associations and genomic selection would improve precision and reduce the varietal generation time tremendously. Considering the achievements done in the crop so far, there is all possibility of having

different cost-effective genotyping methods for varying breeding applications to reform sugarcane breeding.

9.20.4 True Seed as Sugarcane Propagule

Transportation of sugarcane seed is a difficult task as a truckload of sugarcane material (7–8 tonnes) is required as seed for planting 1-ha area. At the modest estimate, approximately 14.42 million tonnes of sugarcane is used as seed to plant 2.22 million ha of area in the country. The Settling Transplanting Technology (STT) will be advantageous to reduce the seed rate to 1/6th, 10–20% higher cane yield, faster multiplication and replacement of desired varieties and development of small entrepreneurs. In order to meet the future demands from the presently available area, there is need to exploit the dominance variance/heterosis to the maximum possible level. Research on developing hybrid varieties and propagation through true seed by developing homozygous lines needs to be strengthened. The success of the programme will lead to paradigm shift to sugarcane agriculture. By adopting the STT method combined with true seed hybrids, it would be possible to change the mode of transportation of sugarcane seed from truck to pocket (ICAR-SBI 2015).

9.20.5 Plant Breeding Data Management Platforms

Information management is essential for every plant breeding programme, and large amount of data will be generated, and decision-making becomes difficult. Development and utilization of advanced plant breeding data management platforms would help sugarcane breeders decide better crosses and further selection. At Sugar Research Australia, Brisbane Sugarcane Plant Improvement Database System (SPIDS) is currently used for all sugarcane research and selection activities (https://sugarresearch.com.au).

9.21 Status of Varietal Development and Maintenance Breeding

The ICAR-SBI has been catering sugarcane varieties in India since the release of the first interspecific variety Co 205. A list of popular sugarcane varieties in cultivation in tropical (Table 9.5) and subtropical region of (Table 9.6) India during different decades is given below. The five top varieties, in terms of area occupation, during 2020–2021, are given in Table 9.7. Co 0238 was occupying the maximum area, i.e. 2.78 million ha, which was 53% of the total sugarcane area in the country followed by Co 86032 (0.89 million ha, 17.1%). Other varieties were CoM 0265 (6.9%), Co 0118 (2.4%) and CoLk 94184 (2.3%). Co 0238, Co 0118 and CoLk 94184 are the subtropical varieties, whereas Co 86032 and CoM 0265 are the tropical varieties.

Table 9.5 Popular usgarcane varieties in cultivation in the tropical region of India

Decade	Varieties
1920s	Co 213
1930s	Co 213, Co 243, Co 281, Co 290, Co 313
1940s	Co 213, Co 419
1950s	Co 419, Co 449, Co 527
1960s	Co 419, Co 527, Co 658, Co 740, Co 853, Co 975, Co 997
1970s	Co 419, Co 527, Co 658, Co 740, Co 975, Co 997, Co 853, Co 62175, Co 6304, Co 6806, Co 6415
1980s	Co 419, Co 740, Co 975, Co 62175, Co 6304, Co 6907, Co 7219, CoC 671
1990s	Co 62175, Co 6304, Co 7219, Co 7508, Co 7504, Co 8011, Co 8014, Co 8021, Co 8208, Co 8362, Co 8371, Co 8338, Co 85004, Co 86032, Co 86249, Co 97009, CoC 671
2000s	Co 86032, Co 7219, Co 8371, Co 97009, CoM 0265, Co 94012, Co 91010, Co 8011, Co 62175, Co 2001-15, Co 86002, Co 8338, Co 86249, CoV 09356, CoC 23, CoC 24, Si 6, PI 1110, CoV 92102, CoA 95081, CoA 96081, CoA 93081
2010s	Co 86032, CoM 0265, Co 91010, CoV 09356, Co 11015

Table 9.6 Popular sugarcane varieties in cultivation in the subtropical region of India

Decade	Varieties
1920s	Co 205 Co 210 Co 213 Co 214 Co 223 Co 281 Co 290
1930s	Co 205 Co 213 Co 223 Co 244 Co 281 Co 285 Co 290 Co 312 Co 313
1940s	Co 213 Co 312 Co 313 Co 331 Co 356 Co 453
1950s	Co 312 Co 313 Co 453 Co 951 CoS 245 CoS 510
1960s	Co 312, Co 975, Co 1107, Co 1148, BO 17, CoS 510
1970s	Co 312, Co 1148, Co 1158, BO 17, CoS 510
1980s	Co 1148, Co 1158, Co 7717, BO 91, BO 99, CoJ 64, CoS 687, CoS 767, CoLk 8001
1990s	Co 89003, Co 7717, Co 1148, CoS 767, BO 120, BO 128, Co 87263, Co 87268, CoH 92, CoPt 84211, CoLk 8102
2000s	Co 89003, Co 98014, Co 1148, CoJ 64, CoS 767, CoS 97261, CoPant 97222, CoPant 90223, CoSe 92423
2010s	Co 98014, Co 0238, Co 0118, CoS 8432, CoS 8436, CoS 97261, CoSe 96436, CoPant 97222, CoPant 90223, CoSe 92423, CoSe 96234, CoPant 99214, CoPant 03320, CoSe 01434, CoLk 94184, Co 05009, Co 05011, Co 06034, Co 09022, Co 12029, Co 13035, Co 15023

Table 9.7 Top five varieties in cultivation in India during 2020–2021

S. No.	Varieties	Region	Area	
			In million ha	In percentage
1.	Co 0238	Subtropical	2.794	53.42
2.	Co 86032	Tropical	0.892	17.06
3.	CoM 0265	Tropical	0.361	6.90
4.	Co 0118	Subtropical	0.128	2.45
5.	CoLk 94184	Subtropical	0.122	2.33

9.22 Maintenance Breeding

Genetic purity of sugarcane varieties is being maintained through maintenance breeding and micropropagation. Maintenance breeding is being done by following cane-to-row method of planting. Individual canes are cut into single-budded setts to plant in a 6 m length row. Rows are monitored regularly, and the rows, if any, with mixture are excluded from the bulk nucleus seed. In vitro studies were initiated in the late 1970s, and protocols were standardized for tissue and meristem culture of sugarcane for the first time at ICAR-SBI (Sreenivasan and Jalaja 1979). Micropropagation technology has been adopted by many seed-cane production agencies and companies in the country. Micropropagation is also useful in the rejuvenation of old and degenerated varieties, possibly through elimination of endogenous pathogens, and the benefits have been demonstrated in farmers' fields (Neelamathi et al. 2017). About 500,000 micropropagated plants are produced annually by different laboratories in India.

9.23 Coordinated System of Testing

The All India Coordinated Research Project on Sugarcane {AICRP(S)} came into being during 1970. The Project Coordinator's office was housed at the Indian Institute of Sugarcane Research (IISR) to coordinate research efforts and to test the technologies developed by the State Agricultural Universities (SAUs), State Research Stations and Indian Council of Agricultural Research (ICAR) Institute, but with a strong emphasis on the development of improved varieties. Under the AICPR(S) network, presently, there are 24 regular centres and 14 voluntary centres across the country in five agroclimatic zones, viz. Peninsular Zone, North West Zone, East Coast Zone, North Central Zone and North Eastern Zone.

A National Hybridization Garden (NHG) facility was established at ICAR-SBI in 1972 for crossing and for country-wide sugarcane seed (fluff) supply. Out of 38 stations of AICRP(S), 24 stations are full-fledged sugarcane breeding stations and participate in hybridization activities at the NHG. Sugarcane breeders from different research stations in the country visit the NHG during flowering season, chose the parents / crosses according to their local requirements, and the seeds are germinated for selection programmes in the respective stations. On average 200,000 seedlings are raised in these stations. After initial evaluation of 5–6 years at respective research stations, the selected best clones are pooled to conduct common (multi-location) trials in the respective zones under AICRP(S). Once accepted for evaluation under AICRP(S), these clones are multiplied for 2 years to generate sufficient seed material for the subseuent Initial Varietal Trials (IVT-1-year) for 1 year in all centres within each zone. Clones performing better than the check varieties in the IVT are promoted to Advanced Varietal Trials (AVTs), which consist of two plant and one ratoon crop experiments. The test clones performing better than the best check variety are identified by the Varietal Identification Committee of the AICRP(S). Elite selections from AVTs are recommended for commercial release

and official notification of sugarcane varieties for respective zones. This programme (since 2000) has released 63 sugarcane varieties by 16 stations in four zones for cultivation.

The flow chart of varietal development (Table 9.8) followed at ICAR-SBI is generally followed throughout the country with minor variation based on the population available and the seasonal variations which determine planting and harvesting. Taking into consideration the breeding history of the sugarcane stations, the flowchart of sugarcane breeding generally followed in India is included.

Table 9.8 Flow chart of sugarcane breeding followed in India

Year	Activity	Selection criteria and number of clones selected
I	Choice of parents and hybridization (600–700 crosses)	Parents are selected based on pedigree, molecular diversity, combining ability, registered genetic stocks
II	Seedling raising and transplanting in ground nursery (200,000–500,000 seedlings)	Sowing in March to August, transplanting in June to December
III	Ratooning ground nursery	Brix, no. canes/stool, flowering, cane diameter, cane height, cane morphology (~20% selection)
	Selection and planting in I Clonal trial (30,000–40,000 clones)	
IV	Selection in I Clonal trial	Brix, no. canes/stool, flowering, cane yield parameters (~25% selection)
V	II Clonal trials	One row trials in Aug RCBD/RBD (~25% selection)
VI	Pre-Zonal Varietal trials (PZVT)—Multiplication	~250 clones—2 rows trial—Juice quality analysis at 240, 300 and 360 days post-planting
		Screening for red rot, crop stand, flowering
VII	PZVT trials/final clonal trials (RBD[a])	60–100 clones; Yield, quality evaluation, red rot screening by plug and nodal inoculation, smut screening, ethanol and fibre content, Pol % cane
VIII	Selection elite clones for multilocation testing	20–25 Co canes, 30–40 Co allied, 2–5 other centre selections
IX, X	Multiplication and exchange of AICRP (S) entries	60–75 to AICRPS trials (5–25 clones for the five agroclimatic zones); multiplication for 2 years
XI	Initial Varietal Trial	1 year in RBD[a]
XII, XIII	Advanced Varietal Trial	Two plant crops and one ratoon crop to select the best clones (with >10% improvement in cane yield and no reduction in quality or 5% improvement in sucrose content and no reduction in cane yield over the zonal standard)
		Varietal release (within 2 years of AICRP multilocation testing)

[a] RBD randomized block design; RCBD augmented randomized complete block design

In addition to hybridization at NHG at Coimbatore, efforts have also been made in some states to develop varieties utilizing their local hybridization gardens. These include Bihar and Orissa (developing cultivars including BO 10, BO 54, BO 70, BO 72, BO 91, BO 108, BO 110, BO 137, BO 145, BO 147, BO 154), Uttar Pradesh (UP 1, UP 3, UP 5, UP 15, UP 39, UP 0097, UP 9530, UP 05125), Maharashtra (MS 68/47, MS 7110, MS 7455, MS 10001) and Karnataka (HM—Hebbel Mysore 320). Vasantdada Sugar Institute, Pune, has an in-house crossing facility to partially meet the varietal development needs of their local region and has developed varieties including VSI 12121, in cultivation in Maharashtra state. M/S EID Parry (India) Ltd. the only private company in the country to breed sugarcane varieties, with its hybridization facility in Bangalore, produces and releases varieties designated with the prefix PI (Parry India). A few locally adapted PI varieties have been released. PI 1110 is one such clone with excellent performance under irrigated conditions. Table 9.9 summarizes the details of the research stations engaged in sugarcane improvement in India and names of prominent varieties produced from those centres.

9.24 Future Thrust Areas

The sugarcane breeding efforts continues in India since the inception of ICAR-Sugarcane Breeding Institute, Coimbatore (ICAR-SBI), in 1912. The first sugarcane interspecific hybrid variety Co 205 (*S. officinarum* × *S. spontaneum*) was released in 1918, which replaced all existing north Indian canes (*S. barberi*) in the subtropical region. Since then, and till 2021 about 3179 Co canes have been evolved by the ICAR-Sugarcane Breeding Institute. The ICAR-SBI is keeping its National Hybridization Garden dynamic with additions of new releases and phasing out of old and unproductive parents for the larger gains of sugarcane research stations all over the country under AICRP.

Sugarcane is coming up as an energy crop with options like co-generation and second-generation ethanol production. Research on development of high biomass clones with high fibre content might be strengthened, depending on demands, for developing Type 1 and Type 2 energy canes which are characterized and found promising as biofuel crops. Selection of the right clones starting from ground nursery has to be scientific based on parameters that are visibly scorable and indirect selection aided by statistical tools to add prediction.

With availability of 'omics' technologies in 'genomics era' of modern plant breeding, sugarcane research has gone rapid transformation. To harness the benefits of these technologies, linking modern techniques with the conventional breeding is very essential in sugarcane. ICAR-SBI has integrated the biotechnological tools in breeding programmes to improve precision and efficiency. Genomics has been the key in unravelling the crop complexities and in developing and adopting several molecular markers with wide applications including DNA fingerprinting, diversity

Table 9.9 Organizations involved in sugarcane improvement in different crop production zones in India

Organization	Affiliations/funding source	Key activities	Major varieties
Peninsular zone			
ICAR-Sugarcane Breeding Institute, Coimbatore	ICAR	Germplasm collection evaluation, utilization, breeding, selection, varietal trials, tissue culture, genomics, transgenics	Co 205, Co 285, Co 312, Co 313, Co 419, Co 740, Co 6304, Co 62175, Co 86032
Vasantdada Sugar Institute, Manjari Block, Pune, Maharashtra	Co-operative members of the Sugar factories in the state of Maharashtra	Germplasm evaluation, utilization, breeding, selection, varietal trials, tissue culture, genomics, transgenics	Co VSI 9805, VSI 12121
Central Sugarcane Research Station, Padegaon, Maharashtra	Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri	Breeding, selection, varietal trials	CoM 88121, CoM 0265
Zonal Agricultural Research Station, Powarkheda (Madhya Pradesh)	Jawaharlal Nehru Krishi Vishwa Vidayalaya, Jabalpur, MP	Breeding, selection, varietal trials	CoJn 86141
Regional Sugarcane and Rice Research Station, Rudrur, Telengana	Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad	Breeding, selection, varietal trials	98R 278, CoR 9301
Regional Sugarcane and Jaggery Research Station, Kolhapur, Maharashtra	Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar, Maharashtra	Selection, varietal trials	–
Sugarcane Research Centre, Akola, Maharashtra	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Krishinagar	Selection, varietal trials	–
Regional Sugarcane Research Station, Navasari, Gujarat	Gujarat Agrl. University Navasari	Breeding, selection, varietal trials	CoN 05071
Sugarcane Research Station. Kallunkal, Tiruvalla, Kerala	Kerala Agricultural University, Kerala	Breeding, selection, varietal trials	CoTl 88322
Agricuture Research Station, Perumalapalle	Acharya N.G. Ranga Agricultural University, Andhra Pradesh	Breeding, selection, varietal trials	CoT 8201, CoT 10367
Zonal Agricultural Research Station, V. C. Farm Mandya (Karnataka)	University of Agricultural Sciences, Bangalore (Karnataka)	Breeding, selection, tissue culture, varietal trials	CoVc 14061
Sugarcane Research Station, Melalathur, Tamil Nadu	Tamil Nadu Agricultural University, Coimbatore	Breeding, selection, varietal trials	CoG 773, CoG (SC) 5

(continued)

Table 9.9 (continued)

Organization	Affiliations/funding source	Key activities	Major varieties
Sugarcane Research Station, Sirugamani, Tamil Nadu	Tamil Nadu Agricultural University, Coimbatore	Breeding, selection, varietal trials	CoSi 776, CoSi 96071, CoSi (SC) 6
M/s E I D Parry, Pugalur, Tamil Nadu	M/s E I D Parry (India), Chennai	Breeding, Selection, Tissue culture	PI 00-1110
K.J. Somaiya Institute of Applied Agricultural	Godavari Refineries	Varietal trials	–
North west zone			
ICAR Indian Institute of Sugarcane Research, Lucknow	ICAR	Breeding, selection, germplasm utilization, tissue culture, varietal trials, genomics, transgenics	CoLk 8102, CoLk 94184
U.P. Council of Sugarcane Research (UPCSR), Shahjahanpur, Uttar Pradesh	Government of Uttar Pradesh	Breeding, selection, germplasm utilization, tissue culture, varietal trials, genomics	CoS 510, CoS 687, CoS 767, CoS 8432, CoS 8436, CoS 88230, CoS 95255, CoS 96268, CoS 08279
Punjab Agricultural University, Regional Research Station, Faridkot (Punjab)	Punjab Agricultural University-Ludhiana, Punjab	Breeding, selection, varietal trials	–
CCS HAU Regional Agrl. Research Station, Uchani (Haryana)	Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana)	Breeding, selection, varietal trials	CoH 56, CoH 119
PAU, Regional Research Station, Kapurthala (Punjab)	Punjab Agricultural University-Ludhiana, Punjab	Breeding, selection, varietal trials	CoJ 46, CoJ 64, CoJ 83, CoJ 85, CoPb 91
G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand	G.B. Pant University of Agriculture and Technology, Pantnagar	Breeding, selection, varietal trials	CoPant 84212, CoPant 97222
Agricultural Research Station, Kota, Rajastan	Agriculture University, Kota, Rajastan	Selection, varietal trials	CoPK 05191
Sugarcane Research Station, Sriganganagar, Rajasthan	Swami Keshwanand Rajasthan Agricultural University, Bikaner	selection, varietal trials	–

Regional Centre of Sugarcane Breeding Institute, Karnal	ICAR SBI Coimbatore	Breeding, selection, germplasm utilization, varietal trials	Co 285, Co 312, Co 1148, Co 1148, Co 1158, Co 62399, Co 7717, Co 89003, Co 98014, Co 0118, Co 0238, Co 15023
North central zone			
Sugarcane Research Institute, PUSA (Bihar)	Rajendra Agricultural University, Pusa, Bihar	Breeding, selection, varietal trials	BO 17, BO 54, BO 70, BO 91, CoP 9301
Sugarcane Research Station, Gorakhpur (UP)	UPSCR, Shahjahanpur	Selection, varietal trials	
Genda Singh Sugarcane Breeding and Research Institute, Seorahi (UP)	UPSCR, Shahjahanpur	Breeding, selection, varietal trials	CoSe 92423, CoSe 95422, CoSe 98231, CoSe 01434
Sugarcane Research Station, Bethuadahari (West Bengal)	Department of Agriculture, Govt. of West Bengal	Breeding, selection, varietal trials	CoB 94164
Indian Institute of Sugarcane Research Centre, Motipur, Bihar	ICAR IISR, Lucknow	Breeding, selection, varietal trials	Co 89029, Co 0233
North eastern zone			
Sugarcane Research Station, Buralikson (Assam)	Assam Agricultural University, Jorhat, Assam	Breeding, selection, varietal trials	CoBln 9101, CoBln 9105
East coast zone			
Regional Agricultural Research Station, Anakapalle, Andhra Pradesh	Acharya N.G. Ranga Agricultural University, Guntur, Andhra Pradesh	Breeding, selection, varietal trials	CoA 7601, CoA 92081, 2001A 63
Sugarcane Research Station, Vuyyuru, Andhra Pradesh	Acharya N.G. Ranga Agricultural University	Breeding, selection, varietal trials	CoV 94102, CoV 09356
Sugarcane Research Station, Panipoila, Nayagarh	Orissa University of Agriculture and Technology, Bhubaneshwar	Breeding, selection, varietal trials	CoOr 03151
Sugarcane Research Station Cuddalore, Tamil Nadu	Tamil Nadu Agricultural University,	Breeding, selection, tissue culture, varietal trials	CoC 671, CoC (SC) 25
M/s. E I D Parry (India) Ltd. Nellikuppam, Cuddalore, Tamil Nadu	M/s. E I D Parry (India) Ltd, Chennai	Breeding, selection, tissue culture, varietal trials	PI 00-1110

analysis, markers linked to traits, marker-assisted selection, etc. Transcriptomics, metabolomics and proteomics approaches are employed for important traits such as sugar accumulation, yield, resistance to diseases and tolerance to drought, salinity, waterlogging, cold and pests. However, being a complex polyploid, no single technique is found to be the best. Biotechnological approaches combined with bioinformatics analysis will pave way for identification of new genes, regulatory elements and promoters and expressed sequence tags (ESTs), etc. The Brazilian initiative of SUCEST database with over 2 lakhs of sequence tags and the SUCEST-FUN database created to manage sugarcane genome data and provide tools of interest for sugarcane functional genomicists and molecular breeders (sucest-Fun.org) owe a lot of applications for translation to the field level.

Success in genetic transformation in sugarcane has opened up newer avenues of crop improvement and product diversification. In India, ever since the first sugarcane transgenic was developed during 1999 (Subramonian et al. 1999a, b), several transgenics with improved tolerance to drought and sugarcane borers are developed and awaiting field trials (Sruthy et al. 2015) along with isolation of effective promoters (Chakravarthi et al. 2016). Opportunities on viral and fungal resistance, increased sugar content, lignin synthesis and sugar accumulation need to be explored. Sugarcane is also considered as a biofactory to produce high-value molecules and pharmaceuticals, and ICAR-SBI has perfected a vacuolar targeting protocol for localizing the proteins in the cell vacuoles (Harunipriya et al. 2016), which makes it easy to extract from the sugarcane juice.

At present, transportation of sugarcane seed is a difficult task as a truckload of sugarcane material is required as seed for planting 1 ha of area. The settling transplanting technique economizes planting cost with the requirement of just 5000–6000 plants for planting an acre. Guided by this success, the ICAR-SBI has been working on a programme on sugarcane true seed development through developing homozygous parental lines by repeated selfing, creation of haploids through various means and standardizing the seed defuzzing technique. It is expected that the research in this direction based on conventional and biotechnological means would deliver the right product in the form of a pocketfull of homogeneous seed in a packet from the present level of a truckload of seed.

Sugarcane breeding, the continuous process of evolving better varieties, has been evolving with newer and improved technologies and approaches. We can be proud that the target for 2030 AD (32.5 million tonnes) has already been achieved with a record production of over 33.2 million tonnes of sugar during 2018–2019. Now sugarcane can be projected as the crop to cater to multiple needs of food, fuel, fibre and fodder under normal and constraint situations. The research at ICAR-SBI has been reoriented towards product diversification employing the technical know-how from all the disciplines so that future sugarcane varieties would have specialized roles to make the country self-reliant in sugar and energy apart from yielding value-added products.

9.25 Conclusions

A major breakthrough in sugarcane improvement was achieved through the use of wild species, viz. *S. spontaneum*, which led to the development of the first sugarcane interspecific hybrid variety Co 205. The variety became very popular and replaced all existing north Indian canes (*S. barberi*) in the subtropical region. Since then, about 3179 Co canes have been evolved by the ICAR-Sugarcane Breeding Institute. In efforts to increase diversity in the progeny of commercial crosses, tropical parents were crossed with subtropical parents to facilitate genetic recombination and to break the linkage drag. Screening of large seedling populations under conditions of winter and selection has been a success, which resulted in a new series of varieties under Karan series from ICAR-SBI, Regional Centre, Karnal (Ram 2007). Notable among these are Co 98014, Co 0118, Co 0238, Co 05011 and Co 15023. The institute holds the unique distinction that its varieties cover over 78% of the area in the country, and among these Co 86032, a tropical cane (about 0.89 million ha), and Co 0238 (2.79 million ha) together cover over 3.6 millon ha (70% area under sugarcane) in the country during 2020–2021.

AICRP trials provide opportunity to identify stable clones with high sugar yield. As utilization of such clones from the four agroclimatic zones in breeding programmes is expected to earn better genetic gains, the Institute is keeping its National Hybridization Garden dynamic with additions of new releases and phasing out of old and unproductive parents for the larger gains of sugarcane research stations all over the country.

Sugarcane breeding has evolved into a systematic activity with the inputs in the form of new and diverse genetic material, statistical and analytical approaches to improve precision and to bring scientific touch in crop improvement. Achievements made through breeding new varieties have been quantified, and a steady improvement in juice quality has been noticed over decades. The yield plateauing necessitated the use of new genetic resources. Evaluation of selected hybrids under ISH (interspecific hybrids) series in multi-locations and for multiple stresses has yielded valuable parents for sugarcane improvement.

The rate of replacement, variety as well as seed, is very slow in sugarcane due the requirement of huge quantity of seed per unit area. The cost of the seed itself contributes to 16–18% of cultivation cost. At present, transportation of sugarcane seed is a difficult task. The success of single bud/bud chip settling raising and transplanting has been demonstrated resulting in increased productivity and profitability. It is expected that the research on development of true seeds initiated at ICAR-SBI would change the transportation of sugarcane seed from truck to pocket.

Linking modern 'omics' technologies in 'genomics era' of modern plant breeding with the conventional breeding is expected to achieve introgression of targeted trait in the desired varieties of sugarcane. Success in genetic transformation in sugarcane has opened up newer avenues of crop improvement and product diversification. Sugarcane is also considered as a biofactory to produce high-value molecules and pharmaceuticals, and ICAR-SBI has perfected a vacuolar targeting protocol for

localizing the proteins in the cell vacuoles, which makes it easy to extract from the sugarcane juice.

Sugarcane breeding, the continuous process of evolving better varieties, has been evolving with newer and improved technologies, and it can be expected that the pace of development can initially sustain sugarcane production and then accelerate crop production for multiple needs as food, fuel, fibre and fodder under normal and constraint situations.

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Jute Breeding

10

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Abstract

Jute (*Corchorus* spp.; $2n = 14$) is an annual crop and ranks next only to cotton as a source of natural bast fibre. Unlike other field crops, the economic product is fibre from stem which is a vegetative part. Jute breeding is a challenge to plant breeders as selection of better genotype is cumbersome as generation advancement of selections requires two growing seasons compared to one season in other field crops. Moreover, hybridization is also a tedious process. In spite of these difficulties, a number of high-yielding varieties of both *C. olitorius* and *C. capsularis* have been developed in India, Bangladesh and China using different breeding methods. During the last 50 years, the improvement of fibre yield potential has almost doubled. Advanced plant breeding methods along with genomic tools are only way to bring next quantum jump by breaking yield plateau. Draft genome sequencing of jute has been accomplished by India, Bangladesh and China independently in recent years. But due to the narrow genetic base of *Corchorus* species, lack of suitable transformation protocol using tissue culture and lack of high-throughput phenotyping technology, the pace of jute crop improvement is slow. The use of genomic tools and advanced breeding methods like marker aided selection, speed breeding and transgenic research and the use of genome editing tools may open a new avenue in future in crop improvement in jute to establish it as a climate-smart crop by imparting biotic and abiotic stress resistance along with better fibre quality.

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_10

Keywords

Germplasm · Molecular breeding · Breeding objectives · Coordinated system of testing · Varietal development

10.1 Introduction

Jute is the second most important fibre crop next to cotton in terms of global production and use. The term jute is used for fibre obtained from the bark of two cultivated species (*Corchorus olitorius* and *C. capsularis*). Jute is grown in India and in Bangladesh in the pre-monsoon (pre-kharif) season within a small growing window of mid-March to mid-July (110–120 days) in the Eastern Gangetic Plain (EGP). The north-western monsoon during April and the heavy monsoon shower during June–July (80% of the total rainfall) in the EGP are not suitable for cultivation of other crops during pre-monsoon. Competitive alternate crops of jute are *boro* rice, wheat, sesame, maize and summer vegetables. *Boro* rice and wheat require longer duration (November–May), and vegetables require protection from rain. However, during the past 20 years, monsoon rainfall decreased significantly in the river basins of EGP (Yaduvanshi and Ranade 2017). More interestingly, it was observed that short-term fluctuations (<10 years) are the major cause for variability (77.6%) in annual rainfall (Yaduvanshi and Ranade 2017). Such abrupt changes in rainfall distribution and occurrence of frequent drought spells have increased vulnerability of jute cultivation in the recent decades, which is reflected in the reduction of area of jute cultivation during the past few decades (Fig. 10.1). Despite this, production of jute has remained comparatively stable due to an increase in productivity. Progress in breeding and management of jute crop has resulted in an increase of both average yield and potential yield of jute crop. The jute-rice cropping system is prevalent in 90% of the jute growing area.

Learning objectives include (1) importance of jute crop as a source of natural fibre; (2) origin and evolution of *Corchorus* species, distribution of wild relatives and genetic resources; (3) morphological differences, floral biology and hybridization technique of *Corchorus* species; (4) genetics of quantitative and qualitative traits; (5) breeding for fibre yield improvement and associated traits including heterosis, biotic and abiotic stress; (6) maintenance breeding and evaluation system in jute; and (7) knowledge of advanced breeding methods including genomic tools.

10.2 Origin, Evolution and Distribution of Species and Forms—Wild Relatives

According to the Linnaean classification, jute was first classified in the family Tiliaceae under the order Malvales, but was later placed under the family Malvaceae (Table 10.1). The family distribution under the order Malvales has been debated

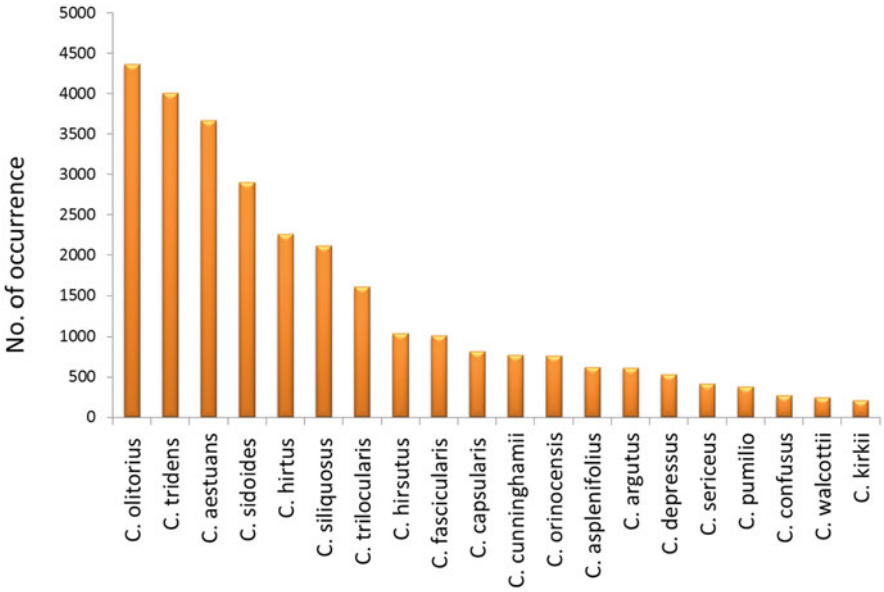

Fig. 10.1 Occurrence distribution of major *Corchorus* species (GBIF data; accessed on 23 June 2021)

Table 10.1 Taxonomic classification of jute

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Angiospermae
Category	Malvids
Order	Malvales
Family	Malvaceae
Subfamily	Grewioideae
Tribe	Sparmannieae
Genus	*Corchorus*
Species	*C. capsularis*, *C. olitorius*

repeatedly in recent decades. According to the Angiosperm Phylogeny Group (APG) classification system, *Corchorus* is classified under the subfamily Grewioideae. However, Grewioideae has been divided into two tribes, Grewieae and Sparmannieae. The tribe includes *Corchorus* (jute) and *Oceano papaver*.

C. olitorius has a pantropical distribution, with a higher concentration in African countries around the equatorial line, such as Ethiopia, Kenya, Tanzania, South Sudan, Nigeria, Ghana, Benin, Togo and Guinea. In addition, it is also distributed in Australia, South China, India and Bangladesh and sporadically found in South East Asia, Europe, North America and South America. Natural distribution of *C. capsularis* is limited mostly in South China, India, South East Asia, Australia and Zambia. Initial assessments on the origin of *C. olitorius* were based on

geographical distribution, morphological features and species richness. Based on these parameters, Kundu (1951) proposed Africa as the primary centre of origin of *C. olitorius* and India or the Indo-Burma region as secondary centres. Molecular studies confirmed an African origin of *C. olitorius* (Benor et al. 2012; Kundu et al. 2013; Satya et al. 2014; Yang et al. 2018). Further, Sarkar et al. (2019) reconfirmed the African origin of *C. olitorius* using SNP markers and proposed peninsular India as a secondary centre of origin. The origin of *C. capsularis*, however, is still debated. Kundu (1951) suggested Indo-Burma as the origin of *C. capsularis*, while Vavilov placed the species under China centre. Archaeological evidences of *C. capsularis* jute fibre in Harappa around 2200–1900 BC (Wright et al. 2012) suggest an Indian origin of *C. capsularis*.

Basu et al. (2016) noticed that while the nuclear genomes of *C. capsularis* and wild *Corchorus* species distributed in India show high genetic distance, the organelle genomes of the two species were quite close. They suggested that *C. capsularis* evolved earlier than *C. olitorius* and a specific haplotype of *C. capsularis* might have originated in the Indo-China region. Contrary to this, Benor et al. (2012) and Benor (2018) suggested an African origin of *C. capsularis* based on the distribution of wild *Corchorus* species and genome size variation. Since a natural distribution of *C. capsularis* was not documented from Africa, it may be possible that *C. capsularis* was originated from the wild *Corchorus* species in India. The closest relative of *C. capsularis* was found to be *C. pseudo-capsularis* (Benor 2018). Most of the studies identified *C. aestuans* as the closest wild relative of *C. olitorius* (Kundu et al. 2013), although *C. pseudo-olitorius* (Kundu et al. 2013) or *C. orinocensis* and *C. pilosus* (Benor 2018) might also be the progenitor of *C. olitorius*.

The two cultivated species *C. capsularis* and *C. olitorius* have similar morphology, growth duration and physiology. They can be distinguished by a number of features, the most prominent of which are fruit shape and size. While pods of *C. capsularis* are round, pods of *C. olitorius* are smooth cylindrical shaped. Besides, they have differences in several other plant and fibre characteristics. The general features of *C. capsularis* and *C. olitorius*, as noted by Kundu (1956) and Maiti (1988), are described in Table 10.2.

10.2.1 Wild Relatives of Jute

There are several contradictory reports on the number, occurrence and distribution of wild *Corchorus* species. Since these species have been reported in different continents with partial information, there is a lot of ambiguity, particularly over the species relationship in *Corchorus*. Possibly the number of distinct species is only 60–100, while the rest might be duplications and sub-types. The Global Biodiversity Information Facility (GBIF, www.gbif.org) enlists 81 *Corchorus* species. The occurrence distribution of these species is in the order of *C.olitorius*>*C.tridens*>*C.aestuans*>*C.sidoides*>*C.hirtus*>*C.siliquosus*>*C.trilocu-*

Table 10.2 Morphological features of the two cultivated species of jute

Characters	*C. capsularis*	*C. olitorius*
Seed	Chocolate brown seed coat colour; 1 g contains about 300 seeds	Seed coat colour varies from bluish green to steel grey or even black; 1 g contains about 500 seeds
Seedling	Seed germinates uniformly by 24 h; seedling growth faster; full-grown cotyledon larger	Seed germinates in instalment by 48 h; seedling growth slower; full-grown cotyledon smaller
Stem	Stem tapers from the base to the apex; the rate of transverse growth is quicker at the base, resulting in higher basal diameter; pigment on the stem varies from full green to dark red with intermediate shades; periderm at the base develops predominantly, the extent of which varies with stage of maturity; after 40–50 days of sowing, stem grew slower; height at flowering is comparatively shorter; more lodging resistant	Stem tapers gradually from the base to the apex forming nearly a cylinder; the rate of transverse growth is slower at the base, resulting in lower base diameter; stem pigments are full green, light red and deep red; periderm formation is almost absent; height at flowering is comparatively taller; less lodging resistant
Branching habit	Auxiliary buds may or may not be present, resulting in branched or non-branched stem, respectively; auxiliary buds if present may or may not develop into full-grown branches	Axillary buds present but branches usually develops less vigorously
Leaf stipule	Leaf base contains two free lateral stipules; green or red pigmented; may be modified into foliaceous or even full-grown leaf like but smaller in size in comparison to normal leaf; in exceptional cases, the number may vary even up to 6 at each node (3 on each side of the leaf base)	Leaf base contains two free lateral stipules; green or red pigmented
Petiole	4–8 cm length; varies in colour from green to dark red	4–9 cm in length; may be green or red pigmented
Lamina	Usually, ovate-lanceolate in shape with coarsely serrated margins; lower most pair of serrations develop into filiform appendages, in dark red types, red pigmented develop uniformly on leaf surface, particularly on veins; taste of leaf is bitter; rate of leaf fall is less	Usually, ellipsoidal lanceolate with smooth serrated margin, lower most pair of serrations develop into filiform appendages more predominantly; in deep red types, red pigment develops in patches on leaf surface; taste of leaves non-bitter; rate of leaf fall is more
Root	Tap root less deep, more lateral roots; more tolerant to waterlogging; produces a greater number of adventitious roots	Tap root deeper, less lateral roots; less tolerant to waterlogging condition; produces a smaller number of adventitious roots

>*C.tridens*>*C.aestuans*>*C.sidoides*>*C.hirtus*>*C.siliquosus*>*C.trilocularis*>*C.hi*-C.tridens>C.aestuans>C.sidoides>C.hirtus>C.siliquosus>C.trilocularis>C.hirsu-*C.tridens*>*C.aestuans*>*C.sidoides*>*C.hirtus*>*C.siliquosus*>*C.trilocularis*>*C.hirs*->*C.aestuans*>*C.sidoides*>*C.hirtus*>*C.siliquosus*>*C.trilocularis*>*C.hirsutus*>*C.f*-C.aestuans>C.sidoides>C.hirtus>C.siliquosus>C.trilocularis>C.hirsutus>C.fascicularis > C. capsularis *(Fig. 10.1). Most of the species, except C.* aestuans*, show very little/nil crossability with* C. olitorius *and no crossability with* C. capsularis*, limiting the primary gene pool within the respective species.* C. aestuans *has a pantropical distribution with high occurrence in China, Australia, India, South East Asia and West Africa. Surprisingly, report of natural distribution of* C. aestuans *in East Africa, particularly in Kenya and Ethiopia, is very low (GBIF, www.gbif.org).*

The Flora of China (2007) enlists four species, *C. olitorius*, *C. capsularis*, *C. aestuans* and *C. trilocularis*, to be present in China. Eight wild *Corchorus* species (*C. aestuans*, *C. depressus*, *C. fascicularis*, *C. pseudo-olitorius*, *C. tridens*, *C. trilocularis*, *C. urticifolius* and *C. velutinus*) and the two cultivated species are found in natural habitats in different parts of India. Among these, *C. aestuans* has the highest distribution, followed by *C. olitorius*, *C. capsularis*, *C. tridens*, *C. trilocularis* and *C. fascicularis*, respectively. The wild jute species harbour several important traits that can be useful in jute breeding. For example, *C. aestuans* exhibits higher resistance to diseases, particularly to stem rot. A wild accession of *C. aestuans* has been registered in India as a resistant genetic stock and is being utilized in jute breeding programme. Another interspecific derivative of *C. olitorius* and *C. aestuans*, RS-6, exhibits high resistance to stem rot disease, has considerable resistance to early flower initiation and produces fibre comparable to the popular cultivars (Mandal et al. 2021).

10.3 Plant Genetic Resources

Based on species richness of *Corchorus*, explorations were made by the International Jute Organization (IJO) in 1987 in Kenya and Tanzania, and a total of 374 seed samples representing 12 *Corchorus* species were collected. Explorations were also made in different countries including China, Indonesia, Nepal, Thailand and Pakistan to collect wild *Corchorus* germplasm from their natural habitats. A total of 2300 accessions were collected by the IJO and distributed to different countries for evaluation, conservation and utilization in *Corchorus* breeding programmes. The Gene Bank of the Germplasm Division, Bangladesh Jute Research Institute (BJRI) was designated as the IJO Centralized Germplasm Repository. Presently, BJRI maintains about 6000 germplasm of jute and mesta, including 2380 accessions of *C. capsularis* and 1450 accessions of *C. olitorius* (Miah et al. 2020). A total of 655 accessions covering landraces and wild relatives of *Corchorus* and allied fibre crop species from different agroclimatic regions were collected during 1999–2004 under NATP (National Agricultural Technology Project) and

characterized. As of 2021, India has a working collection of 3500 *Corchorus* accessions being conserved in the midterm gene bank of Central Research Institute for Jute and Allied Fibres (ICAR-CRIJAF), Barrackpore. A base collection is maintained in the National Gene Bank at the National Bureau of Plant Genetic Resources, New Delhi. The Institute of Bast Fibre Crops, Changsha, and the Fujian Agriculture and Forestry University, Fuzhou, in China hold 10,970 germplasm of bast fibre crops including jute (Zhang et al. 2019).

10.4 Floral Biology, Emasculation and Pollination Techniques

10.4.1 Key to Cultivated Species

- Capsule globose, seed brown, without transverse septa between seeds (*C. capsularis*).
- Capsule elongated, cylindrical, seed dark greyish to bluish with transverse septa between seeds (*C. olitorius*).

10.4.2 Floral Biology

10.4.2.1 *Corchorus olitorius*

Inflorescence is cymose, opposite to leaves, 2–5 flowered, sometimes solitary, peduncle about 1 cm long, pedicle 1–3 mm long, bract up to 3 mm long and all glabrous. Sepals are usually 5–6, free green 5–7 cm long, tips prolonged into flower bud, ciliate at the basal margin. Petals are usually 5 in number, rarely 6 or more, pale yellow, oblanceolate, 4–7 mm long. Stamens are numerous, free, about 30–60 anthers, yellow. Ovary is superior, elongated, cylindrical, up to 3.5 mm long, 5 carpelled usually, rarely 6 or more, placentation axile, ovule linearly placed in each locule, style 3–5 cm long, stigma globular, entire, pubescent. Fruit is cylindrical, 10 ribbed, beaked capsule, 5–10 × 0.5–0.8 cm in size, dehiscing by 5 valves with transverse septa between seeds. Seeds are small, pyramidal, angular, 1–2 mm long, dark greyish, bluish in colour, about 500 in number per gram.

Floral formula: $\otimes \, ♂♀ K_{5-6} C_5 A_\infty \underline{G(5)}$

10.4.2.2 *Corchorus capsularis*

Inflorescence is cymose or solitary, 2–5 flowered, opposite to leaves; sepals are usually 5, free, narrow, 4–5 cm long. Petals are usually 5, yellow, 4–7 mm long, oblanceolate. Stamens are 20–30, free, filament short, anthers small, bi-lobed; ovary is superior, 5 carpelled, syncarpous with numerous ovules, placentation axile; styles short, 2–4 mm, stigma flattened, 2–3 fid, pubescent. Fruit is globose capsule, 1.2–2 cm in diameter, wrinkled, 10 ridged, flattened at the top, dehiscing

loculicidally into 5 valves, without transverse partition between seeds. Seeds are small, 2–3 mm long, oval, pointed, conclave on one surface, copper brown in colour, about 300 in number per gram.

Floral formula: $\otimes \, ♂ ♀ \, K_5C_5A_{\infty}\underline{G}\,(5)$

10.4.3 Emasculation

Emasculation is done 1 day ahead or at the time of opening of the flowers. In *C. capsularis*, emasculation could be done in the very early morning before 5: 30 a.m. on the date of pollination, as anthesis in the species starts one after sunrise. Emasculation at that time is sometimes preferred because of larger bud size. In *C. olitorius*, emasculation in the morning is not advised because anthesis starts about 1 h prior to sunrise. The most advanced bud in the inflorescence can be identified from its size and yellow colour of the petals and anthers. The buds selected are opened and the stamens are removed with fine pointed forceps. The emasculated flowers are covered with small butter paper bags to protect them from the dew and rain.

10.4.4 Pollination Technique

Self-pollination is the rule in this crop in both *Corchorus capsularis* and *Corchorus olitorius*. The natural crossing in *C. capsularis* is higher rate, and it is due to the wind pollination and insect visitation. Anthesis starts 1–2 h after sunrise in *C. capsularis* and about an hour before sunrise in *C. olitorius*. The stamens usually burst before anthesis.

10.4.4.1 Selfing

To ensure self-fertilization, the flowers may be protected by covering them with fine mesh muslin bags or a polyethylene lantern. This is necessary in the *C. capsularis* species where cross-pollination is much higher. The supports by bamboo stakes are given to bags which are covering the flowers, since the jute is a tall plant and inflorescence is at the top.

10.4.4.2 Crossing

Cross-pollination between varieties within a species is readily made, but the inter-specific crosses are rarely successful. This is due to endosperm abortion. The flowers which will be used as pollen source are wrapped with cotton in the afternoon of the day preceding pollination. This process precludes contamination. The stigma of the emasculated flower is lightly touched with a ripe anther, and the pollen is dusted on the stigma. Pollination should be started immediately after opening of the flower. In *C. olitorius*, flowers open around 7:30 a.m., and in *C. capsularis*, that happens

around 8:30 a.m. It is desirable that pollination in both the species be completed within half an hour before blooming. Rain immediately after pollination washes the pollen and poses a hindrance to pod setting. For assured pod setting at least 2 h, rain-free weather after pollination is essential in both species. After pollination, the flowers are bagged for 24 h. Seed capsules matured in about 6 weeks.

10.5 Molecular Cytogenetics and Breeding

Studies on molecular cytogenetic in jute species *C. olitorius* and *C. capsularis* very less as it is a neglected crop but a major cash crop of South East Asian countries. The genomes and chromosomes of tossa jute and white jute (*Corchorus* species) are poorly studied. Chromosome-specific physical localization of genes in jute was investigated by studying association and localization of single copy expressed sequence tag (EST) loci in the *Corchorus olitorius* genome (Joshi et al. 2014) in mitotic interphase nuclei of specific trisomic(s) for fluorescence in situ hybridization and validating using a cDNA fragment of the 26S rRNA gene (600 bp) as a molecular probe. When same probe was hybridized, the pachytene chromosomes of diploids confirmed that 26S rRNA occupies the terminal end of the short arm of chromosome 5 in *C. olitorius*. Similarly, physical localization of 63 single copy EST chromosome-specific association were determined on chromosomes 2, 4, 5 and 7. This will be useful in the construction of genome-wide physical maps of jute.

Begum et al. (2013) used a comparative analysis through FISH karyotyping of a prominent satellite DNA family, identified in this study, to reveal its diversification and emerging subfamily structure in both jute genomes with conserved heterogeneous distribution along chromosomes. A reference karyotype for both jute species also generated using ribosomal genes and retrotransposon sequences for chromosome-specific distribution of the satellite DNA for in situ hybridization. This study will be useful for genetic mapping and analysis of hybridity.

10.6 Genetic Studies of Qualitative and Quantitative Traits

Genetic analysis in jute was initiated in India during the first decades of the twentieth century. Finlow and Burkill (1912) first reported that red pigmentation in stem was dominant over green pigmentation and exhibits monogenic inheritance. Later, more genes were reported to control plant pigmentation (Basak et al. 1993). Although this trait is controlled by few genes, its expression changes with age, exposure to sunlight and stress. In India, jute farmers prefer green stem over red stem believing red pigmentation might interfere with fibre colour. But the pigmentation is formed in the outer epidermal layer, which is eaten away by microbes during retting; thus, there is no valid reason to support this idea. However, most of the elite cultivars of *C. capsularis* and *C. olitorius* bear green pigmentation considering the farmers' preference.

10.6.1 Genetics of Qualitative Characters

Among the various qualitative traits in jute, anthocyanin pigmentation of stem has been studied more extensively, and it was found complex in nature. Other qualitative characters studied are related to stem, leaf colour, leaf texture, leaf surface, shape, serration, flower seed coat colour, leaf taste, pod shape, stipule, pod shattering and fibre colour in both *C. olitorius* and *C. capsularis*. Almost all characters are found to be controlled by monogenic recessive genes with exceptions of digenic leaf characters (leaf glossiness, leaf rolling in *C. olitorius* and narrow leaf in *C. capsularis*) and digenic duplicate for fuzzy seed coat colour (Table 10.3).

The three major plant characters that form the ideotype of present-day elite jute cultivars are non-shattering property of pod, resistance to earliness in flowering and non-branching plant type. Earliness in flowering (also referred to as premature flowering) is a typical problem of jute cultivation. Being a short-day plant, jute flowers bloom during September–October (white jute) and October–November (tossa jute). For fibre cultivation, the recommended sowing period of jute is first week of April. As it is a rainfed crop, sowing depends on the onset of monsoon, and farmers often sow the crop in early or late March. *C. capsularis* is comparatively more tolerant to premature flowering than *C. olitorius*. Resistance to earliness in

Table 10.3 Genetics of some important qualitative characters in jute

Character	Inheritance pattern	Reference
Undulated hypocotyl	Duplicate dominance	Satya and Sarkar (2018)
Dwarf stem	Monogenic recessive	Basak et al. (1993)
Stiff stem	Monogenic recessive	Basak et al. (1993)
Broad/narrow leaf	Three interacting genes each with two alleles	Basak et al. (1971)
Chlorina leaf	Monogenic recessive	Thakare et al. (1973)
Yellow leaf	Monogenic recessive	Thakare et al. (1973)
Waxy leaf	Monogenic recessive	Basak et al. (1993)
Leathery leaf	Monogenic recessive	Basak et al. (1993)
Rolled leaf	Digenic complementary	Basak et al. (1973)
Drooping leaf	Monogenic recessive	Basak et al. (1993)
Crumpled leaf	Monogenic recessive	Basak et al. (1993)
Palmate leaf	Monogenic recessive	Ghosh and Sen (1971)
White flower	Monogenic recessive	Basak et al. (1993)
Round pod	Monogenic recessive	Basak et al. (1993)
Fruit dehiscence	Monogenic recessive	Joseph (1972)
Fuzzy seed coat	Digenic duplicate	Basak et al. (1971)
Green seed coat colour	Monogenic recessive	Basak et al. (1971)
Ribbon leaf	Monogenic recessive	Mitra (1977)
Bitter leaf test	Monogenic recessive	Ghosh et al. (1948)

flowering in *C. olitorius* was first identified in an African accession Sudan Green, which was then transferred to the new cultivars.

Since flowering itself is a complex organized process at the cellular level, change in the external environment such as onset of drought can cause drastic morphological changes. With global warming and change in climatic conditions, the tolerant cultivars are also showing sign of susceptibility during the past few years under harsher climatic conditions. The inheritance of premature flowering is not well understood. However, insensitivity to photoperiod in jute is controlled by a monogenic dominant gene. Branching is an undesirable character in jute, because it reduces fibre quality. The branching habit in jute is controlled by a single gene, branched type being dominant over non-branched type. The same genotype, when sown for seed crop, exhibits branching under long day. Thus, branching is also controlled by environmental conditions and crop management.

Transition to non-shattering pod type from shattering pod type is a common sign of plant domestication of many crop species, such as rice, wheat, grain legumes, etc. Certain genetic stocks of jute are shattering type, but the released cultivars are all non-shattering type. This character exhibits monogenic inheritance, non-shattering type being dominant over shattering type.

10.6.2 Genetics of Quantitative Characters

As jute is a bast fibre crop, the product fibre is obtained from the bark of the plant. Also, commercially useful fibre and seed cannot be obtained from the same crop. A variety of mating designs, including line × tester, diallel and triallel, have been used to partition the genetic variance into additive and dominance components. Most of these reports suggest that fibre yield is controlled predominantly by dominance gene action, although additive gene actions are also important. As the fibre yield in jute is polygenically inherited and is highly influenced by genotype and environment interactions, selection of plant is based indirectly on two most important component characters, i.e. plant height and base diameter of the stem. Other traits like fibre percentage, green weight, top diameter and fibre wood ratio are also considered for indirect selection criteria. Genetic association studies revealed that these two characters along with other characters such as leaf biomass, internode length and node number have good correlation with fibre yield. Both these characters are under control of quantitative gene action. Contradictory reports have been published regarding genetics of these two characters, although dominance gene action seems to be more important than additive gene action. Most of the reproductive characters are also controlled by quantitative gene action (Table 10.4).

Table 10.4 Inheritance of major quantitative characters in jute

Character	Mating design	Inheritance pattern	Reference
Fibre yield	Diallel (12-parent)	$V_A = V_D$	Rahman (1968)
	Diallel (5-parent)	$V_D > V_A$	Jana (1972)
	Diallel (10-parent)	$V_A > V_D$	Jana (1972)
	Diallel (7-parent)	$V_D >> V_A$	Singh (1975)
	Generation mean analysis	$h > d$	Paul et al. (1977)
	Diallel (7-parent)	$V_A > V_D$	Kumar (1987)
	Half-diallel (7-parent)	$V_A = V_D$	Mandal and Choudhury (1988)
	Diallel (11-parent)	$V_D > V_A$	Kumar and Palve (1995)
	Diallel (11-parent)	$V_D > V_A$	Mitra et al. (2005)
	Half-diallel (7-parent)	$V_D > V_A$	Kumar et al. (2016)
Basal diameter	Diallel (7-parent)	V_D only	Singh (1975)
	Diallel (11-parent)	$V_D > V_A$	Kumar and Palve (1995)
	Diallel (10-parent)	$V_A > V_D$	Khatun et al. (2010)
	Half-diallel (7-parent)	$V_D > V_A$	Kumar et al. (2016)
Plant height	Diallel (5-parent)	$V_A > V_D$	Jana (1972)
	Diallel (10-parent)	$V_D > V_A$	Jana (1972)
	Generation mean analysis	Duplicate epistasis	Basak and Dana (1971)
	Diallel (11-parent)	$V_A > V_D$	Kumar and Palve (1995)
	Diallel (11-parent)	$V_D > V_A$	Mitra et al. (2005)
	Diallel (10-parent)	$V_A > V_D$	Khatun et al. (2010)
	Half-diallel (7-parent)	$V_D > V_A$	Kumar et al. (2016)
Number of nodes	Generation mean analysis	Duplicate epistasis	Basak and Dana (1971)
	Diallel (5-parent)	$V_A > V_D$	Jana (1972)
	Diallel (10-parent)	$V_D > V_A$	Jana (1972)
	Diallel (11-parent)	$V_A > V_D$	Mitra et al. (2005)
Fibre percentage	Diallel (11-parent)	$V_A > V_D$	Kumar and Palve (1995)
	Diallel (11-parent)	$V_A > V_D$	Mitra et al. (2005)
Days to flowering	Diallel (5-parent)	$V_A > V_D$	Jana (1972)
	Diallel (10-parent)	$V_A > V_D$	Jana (1972)
	Diallel (11-parent)	$V_A > V_D$	Kumar and Palve (1995)
Internode length	Diallel (8-parent)	$V_D > V_A$	Ghosh and Das (1980)
Root weight	Diallel (8-parent)	V_D only	Basak et al. (1973)

10.7 Breeding Objectives

Till the 1970s, jute was grown in the filed for a period of 150–160 days, and plants were harvested at early pod setting stage. *Tossa* jute flowers at early vegetative stage if sown during March as it is a short-day plant, so the usual sowing practice of *tossa* jute was end of April to mid-May. As most farmers grew one crop in a year, jute and rice were not grown in the same field in a year. However, with the introduction of semi-dwarf photo-insensitive rice varieties, a new jute-rice cropping system emerged in the Gangetic delta. To fit to this cultivation practice, jute was harvested by 120–130 days, and farmers started to be pre-pone sowing of *tossa* jute to mid-March to mid-April. Thus, the ideotype of jute in India and Bangladesh was changed to suit the growth duration. New cultivars developed in the later part of the twentieth century (JRO-524) and the early twenty-first century (JRO-204, CO-58, JRO-128) have been developed considering the growth period of 110–120 days and sowing in mid-March to mid-April.

10.7.1 Fibre Yield and Yield Contributing Characters

The economic product of jute, the fibre strand, is a vegetative tissue, which lies within the secondary phloem of the bark. The fibre strands are cells with thickened lignocellulosic cell wall. The primary breeding objective is to increase the amount of fibre strands, which can be achieved by increasing the number of fibre cells, increasing the length of each fibre cell or increasing the thickness of individual fibre cells. Since the length and thickness of each fibre cell do not vary much, increasing the number of fibre cells through more biomass accumulation is the only practical breeding target. Breeders have traditionally targeted to increase this by selecting for more plant height and increased radius of the stem. Under favourable condition, jute plant reaches a height of 3.5–4.5 ft within a period of 120 days. Since the growth duration is the main limiting factor, selection should be made for faster-growing genotypes. However, jute is not a deep-rooted crop, so increasing above-ground biomass without giving attention to root architecture increases the chance of lodging. This can be observed in the rainy season when jute is grown in highly fertile loose soil. The plants tend to lodge also under wind pressure at higher dose of nitrogenous fertilizer application. Thus, the development of lodging tolerant jute varieties which can also utilize more nitrogen to increase fibre content is also becoming a priority. The present-day *tosha* jute varieties are capable to produce 40–45 q/ha fibre under favourable conditions and 30–32 q/ha under moderately fertile soil with proper crop management practices. There is not much scope for fibre yield improvement for the present plant type, and farmers are more concerned with quality and marketability of jute fibre.

10.7.2 Quality Characters Including Biofortification

Farmers are more concerned with quality and marketability of jute fibre. Therefore, the current focus on genetic improvement is to develop varieties for specific target environments and diverse end uses. In addition to its traditional use as bags and sacks, alternate use of jute fibre is becoming more popular day by day as people are becoming more concerned with the ill fates of using synthetic fibres. Demand for jute plant and jute fibre as geotextiles, fibre composites, upholsteries, value-enhanced carrying case and textile blends is on the rise. In addition, industries are being established for the production of biofuel, biochar, herbal products and vegetable jute, which have high market demand in both Eastern and Western countries. Considering the present and future jute cultivation scenario, jute breeders are trying to develop jute varieties for alternate use. For example, for the production of diversified jute products, fibre fineness is an important parameter. On the other hand, for geotextile, fibre strength and meshiness of the fibre are important so that coal tar can better adhere to the fibre surface.

10.7.3 Biotic Stresses

Over 40 insect pest species infect jute. However, only a few of them are major insect pests of jute, namely yellow mite (*Polyphagotarsonemus latus*), hairy caterpillar (*Spilosoma obliqua*), stem weevil (*Apion corchori*), indigo caterpillar (*Spodoptera exigua*) and jute semilooper (*Anomis sabulifera*). The jute cultivars, in general, exhibit good field resistance to insect pests, except yellow mite. Only a few resistance sources have been identified against these insect pests in jute. Most of the resistance sources have come from the indigenous and exotic germplasm of jute. The cultivar JRO-204 exhibits moderate resistance to yellow mite. The major diseases of jute are stem rot (c.o. *Macrophomina phaseolina*), anthracnose (c.o. *Colletotrichum corchorum* and *C. gloeosporioides*), black band (c.o. *Botryodiplodia theobromae*) and soft rot (c.o. *Sclerotium rolfsii*). Stem rot is the most prevalent disease of jute causing a crop loss of 15–20%. However, in specific zones, 100% crop loss can occur. The causal organism *Macrophomina phaseolina* is a soil-borne/seed-borne/air-borne pathogen and infects over 150 species. Despite many attempts, genetics of resistance to stem rot is not well understood. One of the major reasons is non-availability of suitable artificial screening techniques for screening and definite disease scoring pattern. Of the cultivars grown in India, JRO-204 exhibits moderate resistance to stem rot. Wild species are good sources of resistance to insect pests and diseases. *C. aestuans*, a close relative of cultivated jute, exhibits good resistance against hairy caterpillar. A *C. aestuans* genotype, WCIN-136-1 (INGR21036), exhibits high resistance against stem rot. Interspecific hybridization between *C. olitorius* and *C. aestuans* has resulted in the development of advanced breeding lines showing resistance to stem rot (Mandal et al. 2021). Another wild species, *C. fascicularis*, has resistance against indigo caterpillar. A list of jute genotypes that exhibit resistance to major insect pests and diseases of jute is provided in Table 10.5.

Table 10.5 Important target traits in jute breeding

S. no.	Trait	Genotype	Reference
1.	High fibre yield	*C. olitorius*: Chinsurah Green, JRO-524, JRO-204, JRO-8432, JRO-878, S-19, Tarun, JRO-128, JROMU-1, JROB-2; O-4, O-9897, OM-1, O-72, Yueyuan-5hao, Cuigreen, Guangfengchangguo *C. capsularis*: D 154, JRC-321, JRC-212, JRC-517, JRC-532, JRCJ-11, Huangma-971, Huangma-179, Xinyuan-2, Minma-91, CVL-1, C-83	Pandey et al. (2015)
2.	Resistance to earliness in flowering	*C. capsularis*: All varieties (early march) *C. olitorius*: JRO-204 (mid-March); NJ-7010, JROBA-3 (early March), RS-6 (early March)	
3.	High fibre strength	JRO-204, JRO-128	
4.	Better fibre fineness	*C. capsularis*: JRC-212, JRCM-2 *C. olitorius*: JROG-1, JROM-1, S-19, JROB-2	Pandey et al. (2015)
5.	High plant biomass	*C. olitorius*: JROB-2	AINPJAF Annual Report, 2018–2019
6.	Vegetable jute	*C. olitorius* (India): JRO 204, BJRI Deshi pat Shak-1 and BINA pat Shak-1	Islam (2019)
7.	Climate resilient jute	JROB-2	Sharma et al. (2019)
8.	High fertilizer use efficiency	JROB-2, JRO-204	AINPJAF Annual Report, 2018–2019
9.	Insect pest resistance	Yellow mite: JRO-204, JROG-1 Stem weevil: *C. capsularis*—JRC-5145, Mogra, Maniksari, Capsularis Hard Stem, BJRI Deshi Pat-7 *C. olitorius*—JRO-878, JRO-514	Dikshit et al. (1989) and Roy et al. (2019)
10.	Disease resistance	Stem rot: *C. olitorius*—JRO-204, RS-6, OIN-154 *C. capsularis*—CIM-036	Mandal et al. (2021)
11.	High biomass	JROB-2	Indian Gazette notification
12.	High cellulose content in biomass	JROB-2	Sharma et al. (2019)
13.	High β-carotene	JRO-204, JRO-8432	Choudhary et al. (2013)
14.	High foliage yield	JRO-204, JRO-8432	Choudhary et al. (2013)
15.	High potassium content	JRO-204, JRO-8432	Choudhary et al. (2013)

(continued)

Table 10.5 (continued)

S. no.	Trait	Genotype	Reference
16.	Low lignin	*C. olitorius*: *C. capsularis*: dlpf (7%)	Sengupta and Palit (2004) and Kundu et al. (2013)
17.	Phytoremediation	Cu-phytoremediation—HongTieGuXuan (HT), C-3 As tolerance—CVE-3	Nizam et al. (2016) and Saleem et al. (2020)
18.	High flavonoids	*C. olitorius*—T-8, Kuangyechangguo, Funong-6	Biswas et al. (2020)
19.	Anti-oxidation capacity	*C. olitorius*—T-8, Funong-6	Biswas et al. (2020)
20.	Tolerance to waterlogging stress	D-154, CVL-1; *C. capsularis* more tolerant than *C. olitorius*	Prodhan et al. (2001)
21.	Tolerance to drought stress	JRO-204, O-4; *C. olitorius* more tolerant than *C. capsularis*	Yang et al. (2017)
22.	Tolerance to salinity stress	JRC-517, CIN-536, CIN-538	Sharma et al. (2012)

10.7.4 Abiotic Stress Resistance Including Climate Change

Jute yields are seriously impacted by biotic factors (~30% loss from stem rot diseases *Macrophomina phaseolina*) and by abiotic stresses (such as waterlogging, drought and salinity). Among the two cultivated species, *C. capsularis* L. is a drought-sensitive species, and *C. olitorius* L. is a drought-tolerant species. The jute crop faces drought stress in early growth phase and waterlogging stress during later growth stage. In competition with food crops and other remunerative crops, jute cultivation is declining gradually. Genotype-environment interaction is more prominent in crops like jute where economic produce is obtained from the vegetative part of the plant. Commercially valued fibre should fulfil a certain criterion for proper grade jute fibre, which is largely impacted by abiotic stress. One of the abiotic stress-induced traits is early flowering of jute specifically in *C. olitorius* jute. Low night temperature, cloudy sky and short daylength coupled with drought often induce early flowering in jute, which is detrimental for fibre quality. Early flowering of jute is always followed by stem bifurcation at top and branching which impact fibre grade. India is successful in intro-gressing late flowering gene from African genotypes to adapted Indian varieties of tossa jute. Therefore, it is necessary to develop jute varieties that are tolerant to changing environmental conditions via molecular breeding strategies. Gene and QTL mapping involved in jute drought stress is not reported till date. Drought tolerance in jute plants is carried out mainly on the evaluation of drought-resistance germplasm and morphological, physiological and biochemical changes during drought response and transcriptome sequencing (Kabir et al. 2021; Yang et al. 2017).

10.7.5 Exploitation of Heterosis and Hybrid Development

Several attempts were made to exploit heterosis in jute for the development of new cultivars. Although dominant genetic variation contributes significantly to fibre yield and its component characters like plant height and basal diameter, the extent of heterosis was low in most of the cross combinations tested. Moreover, flowering in jute is indeterminate that continues for a period of 64–72 days (Mukherjee and Kumar 2002) on both primary and secondary branches. Moreover, *C. olitorius* exhibits 10–12% cross-pollination. Under such condition, a mechanism for controlled pollination is essential to develop an economic hybrid seed development method. However, no such controlled pollination (male sterility/self-incompatibility) is available in jute. A 'ribbon' mutant of *C. capsularis* cv. JRC 212 was reported to exhibit male sterility (Rakshit 1967), but was later lost. Moreover, sterility in this mutant had pleiotropic relation with several undesirable characters, such as delayed anthesis, small flower and weak growth, rendering it unsuitable for hybrid breeding (Mitra 1977). A ribbon leaf mutant (*bfs*) of *C. olitorius* (Kundu et al. 2012) exhibits high pollen fertility. A total of 1541 accessions of *C. olitorius* germplasm were screened by Mukherjee and Kumar (2002), but no male sterile line could be identified. Sharma et al. (2017) used various chemical agents to induce male sterility in jute and reported maleic hydrazide as a promising chemical hybridizing agent. Induced male sterility, therefore, may be a promising option for hybrid breeding in jute.

10.8 Breeding Approaches—Conventional and Non-Conventional Including Use of Genomic Tools

10.8.1 Conventional Breeding

Jute breeding was initiated a century ago by R. S. Finlow and I. H. Burkill in India using (1) selection of superior genotypes from the cultivated types and (2) hybridization of promising genotypes (Roy 1968). The initial selection was based on 'single plant culture' using characters like plant height, sparse branching and freedom from chlorosis. Two mega-varieties of jute, 'D-154' of *C. capsularis* and 'Chinsurah Green', were identified based on the principles of pure line selection, for which R. S. Finlow must be given due credit. Later, R L. M. Ghosh and J. S. Patel established high correlation of fibre yield with plant height ($r = +0.76$) and thickness of the plant (basal diameter) ($r = +0.91$) and used replicated progeny row trials, selecting the most promising families, resulting in selection of promising lines like C 39–212 and C 42Kj-321 in *C. capsularis* and 040–632, 040–753 and 039–620 in *C. olitorius* (Roy 1968). Partitioning of India and Pakistan destabilized the breeding programme, resulting in loss of most of the genetic material. Indian breeding programmes were re-initiated at newly established Jute Agricultural Research Institute (JARI), Nilganj, Barrackpore (later renamed as ICAR-CRIJAF). Jute breeding work continued at Pakistan under the Jute Agricultural Research Laboratory (JARL),

Dhaka, which was renamed first as Jute Research Institute in 1951 and then as Bangladesh Jute Research Institute in 1971 after the independence of Bangladesh. Roy (1968) proposed an additional criterion, fibre/wood ratio for selection. Overall, in a segregating progeny, characters like germination, pigmentation, plant height, basal diameter, uniformity of the population and dry weight of fibre are considered. Based on the breeding objectives, the following breeding methods have been adopted in jute.

10.8.1.1 Direct Introduction of Cultivars from Other Countries

Often, successful cultivars developed in other countries are introduced for direct cultivation. The most prominent example of direct introduction of jute is cultivation of *C. olitorius* cultivar JRO-524 in Bangladesh which was developed in India (Mukul and Akter 2021; USAID/EAT 2014). While the cultivar JRO-524 is not notified in Bangladesh (USAID/Enabling Agricultural Trade (EAT) project report 2014), about 2500–4000 ton seed of JRO-524 is exported from India to Bangladesh each year, which is 80–85% of the total jute seed requirement of Bangladesh (IMED 2016). D-154, developed in India, was introduced in China and is cultivated for a long period (Yang et al. 2018). Tanganyika-1, an African landrace, was introduced and domesticated in India as a fibre-type cultivar. Several tossa jute varieties were introduced from India (Cuilv), Pakistan (Bana 72–1, Bana 72–1, Bana 72–1, Bachang 4/O-4) and Mali (Maliyeshengchangguo) to China (Zhang et al. 2019).

10.8.1.2 Pure Line Selection

Pure line selection is performed either to select superior lines from landrace/germplasm through selfing and progeny test or to purify old cultivars. Replicated trials are performed in later generations to minimize environmental effects. A number of jute cultivars have been developed through pure line selection (Table 10.6). It can be observed from the table that cultivars like D-154, JRC-212 and Xinxuan-1 were purified through this method for the development of new cultivars. For re-purification of cultivars, Roy (1968) suggested to take 200 seeds of the initial cultivar and grow single plant progenies to identify the original pure line.

10.8.1.3 Pedigree Breeding

Pedigree breeding involves hybridization between two or more homozygous genotypes (pure line/inbred) and selection of improved genotypes from segregating generation through single plant selection. Pedigree breeding and its modified schemes are widely utilized to develop new improved genotypes in all the sexually propagating crops including jute. For comparative evaluation, Roy (1968) suggested growing of F_3 nursery along with parents and selection of superior F_3 lines using modified mass selection method. For microplot trials of advanced generations, simple lattice design should be followed. Several jute varieties have been developed through this method in India, Bangladesh and China (Table 10.7).

Table 10.6 Cultivars developed through pure line selection in jute

	C. capsularis		*C. olitorius*	
Country	Variety	Source	Variety	Source
India	D-154	Kakya Bombai	Chinsurah Green	Local Landrace
	JRC-206	Liza	Fanduk	Local Landrace
	JRC-212	Local Landrace	JRO-632	Local Landrace
	JRC-321	Hewti	JRO-620	Local Landrace
	KJC-7	Local Landrace	Guangfong	Local Landrace
	KTC-1	IC-30730		
	BCCC-1	CIJ-123		
	BCCC-2	CIN-492		
China	Hongtiegu	Local variety	Zhema-1	Cuilv
	Hepingzhuhaoma	Local variety	Yuanjiang-101	Cuilv
	Hainanqiongshan	Local variety	Heganhuangma	Local Landrace
	Xinfeng	Xinfengqingpi	Guangfengchangguo	Local Landrace
	Yuanguo-564	Meifeng-4	Xianhuang-2	Guangfengchangguo
	Xinyuan-1	D-154	Changguo-134	Yuanjiang-101
	Xinyuan-2	JRC-212		
	Yueyuan-1	Taiwan local		
	Yueyuan-2	Xinxuan-1		
	Yueyuan-1	Xinxuan-1		
Bangladesh	D-154-2	D-154	OM-1 (BJRI Tossa Pat-3)	–
	CVL-1	–	BJRI Tossa Pat-7	OM-1
	CVE-3	–	BJRI Tossa Pat-8	Mutant line

(Derived from: Sinha and Satya 2014; Zhang et al. 2019; Islam 2019; Mukul and Akter 2021)

Table 10.7 Varieties developed through pedigree breeding method in different countries

Country	*C. capsularis*	*C. olitorius*
India	JRC-4444, UPC-94, Padma, JRC-698, JRC-80, C-517, C-532, Monalisa, NDC-2008, JBC-5, JRCM-2, KJC-7, JRC-9057, AAU-CJ-2, JRCJ-11	JRO-878, JRO-7835, JRO-524, JRO-3690, JRO-66, JRO-8432, JRO-128, S-19, JRO-204, AAU-OJ-1, JBO-2003-H, CO-58, JBO-1, JRO-2407
Bangladesh	BJRI Deshi Pat-5, BJRI Deshi Pat-6, BJRI Deshi Pat-7, BJRI Deshi Pat-8, BJRI Deshi Pat Shak-1, BJRI Deshi Pat-9	O-9897, BJRI Tossa Pat-4, BJRI Tossa Pat-5
China	Huangma-971, Yueyuan-4, Yueyuan-5, Yueyuan-6, Meifeng-1, Meifeng-2, Meifeng-4, Minma-5, Huangma-179, Fuma 1, Minma-273, Minma-407, Minma-603, Qiongyueqing, Huangma-71–10, Zhonghuangma-1, Fuhuangma-3	Guangbaai, Kuanyechangguo, Xianghuangma-1, Xianghuangma-2, Y007–10, Funong-4

(Derived from: Sinha and Satya 2014; Zhang et al. 2019; Islam 2019; Mukul and Akter 2021)

Table 10.8 List of mutant cultivars developed in jute

Crop	Country	Mutant variety	Source	Mutagen
C. capsularis	India	JRC-7447	JRC-212	X-ray
		Bidhan Pat-1	D-154	γ-ray
		KC-1 (Jaydev)	JRC-4444	γ-ray
	China	912	Huangma 179	γ-ray
		C2005–43	Zhonghuangma 1	γ-ray
C. olitorius	India	KOM-62	JRO 878	γ-ray
		JROMU-1	JRO 204	γ-ray
		JROB-2	JRO-204	γ-ray
	China	Changguo-751	Guangfengchangguo	–
		Funong-1	Taizi-4	γ-ray
		Xianghuangma-3	Kuanyechangguo	γ-ray

(Derived from: Sinha and Satya 2014; Zhang et al. 2019; Islam 2019; Mukul and Akter 2021)

10.8.1.4 Mutation Breeding

Mutation breeding, the process of induction of mutation through physical/chemical mutagenesis and selection of superior lines from the mutant progenies, has been very successful to develop new cultivars. The application of γ-ray, a physical mutagen, has been most successful for the development of new jute varieties (Table 10.8).

10.8.2 Genomics-Assisted Breeding

The development of molecular markers and genomics technologies during the twenty-first century has triggered augmentation of these technologies in traditional plant breeding. Though a number of molecular markers, particularly SSRs, have

been developed in jute, the application of these technologies is limited due to several bottlenecks, such as delayed developments in marker and genomics technologies, low genetic diversity, incomplete genetic maps, low marker coverage in genetic maps, difficulty in trait-marker linkage establishment, low power of QTL detection and preponderance of dominance variation for the quantitative traits related to fibre yield and component characters. Despite these biological and developmental roadblocks, significant achievement has been made in marker discovery and genetic map construction.

Genome size as determined by various authors for *C. capsularis* and *C. olitorius* are ~280 Mb and ~324 Mb, respectively (Sarkar et al. 2011), *C. capsularis* ~ 274 mb (Akashi et al. 2012); *C. capsularis* and *C. olitorius* ~ 336 Mb and 361 Mb (Zhang et al. 2021), respectively. In general, *C. capsularis* has a smaller genome compared to *C. olitorius.*

A preliminary genetic map of *C. olitorius* was developed by Das et al. (2012) that placed 36 SSR markers on six linkage groups (LGs) covering 784.3 cM. Another genetic map was developed by Topdar et al. (2013) identifying 7 LGs carrying 82 SSR markers over 799.9 cM. The first genetic map of *C. capsularis* was developed by Chen et al. (2014) that contained 18 RAPD, 57 ISSR and 44 SRAP markers. But the map contained 8 LGs and was stretched to 2185.7 cM. The first high-density genetic map of *C. olitorius* was developed by Kundu et al. (2015) that contained 503 RAD markers in 7 LGs over a much smaller distance of 358.5 cM. Following this, a high-density genetic map of *C. capsularis* was developed that contained 913 specific locus amplified fragment (SLAF) markers on 11 LGs covering 1621.4 cM (Tao et al. 2017). Yang et al. (2019) developed a *C. olitorius* map containing 4839 SNP markers over a length of 1375.41 cM. Both the maps, though have many markers, are much longer than the map length reported by Kundu et al. (2015).

A few QTLs for fibre yield and component characters have been mapped on these maps, such as QTLs for plant height, stem diameter, node number, fibre yield, wood yield, green biomass and root weight (Sarkar et al. 2016). Three fibre quality associated traits, namely fibre strength, fibre fineness and histological fibre content, were also mapped by Kundu et al. (2015). A total of 16 QTLs for salt tolerance were identified in *C. olitorius* with LOD values ranging from 2 to 4 (Yang et al. 2019). In addition, a number of mapping populations using multiple parents are being developed to reduce linkage drag. A multi-parent advanced generation intercross (MAGIC) population involving 20 parental lines of diverse geographical origins has been developed (Sarkar et al. 2016), which show significant variability for fibre yield, plant height, base diameter and green biomass. While the genetic markers developed in jute have shown good potential for population structure and diversity analyses, jute breeding is yet to gain benefits of genomic selection approaches.

10.9 Precise and High-Throughput Phenotyping Protocols for Key Traits

Since the economic product of jute is fibre, a precise phenotyping system for the estimation of physical and chemical properties of jute fibre is a priority. An automated fibre quality testing system has been developed in India by ICAR-NINFET, which can estimate fibre strength, fibre fineness, colour and lustre for grading the quality of jute fibre. Till date, no high-throughput phenotyping system for morphological characters has been developed.

10.10 Emerging Challenges at National and International Levels

- Narrow genetic base.
- Lack of a high-throughput phenotyping system.
- Lack of efficient transformation protocol through tissue culture or other method.
- Establishment of jute crop suitable for diversified uses like biomass, biofuel, etc.

10.11 Breeding Progress/Varietal Development

Since the inception of concerted crop improvement efforts in jute during the early twentieth century, several varieties of jute have been developed worldwide. In accordance with the contemporary cultivation practices and demand of farming community, each of these varieties was targeted for certain specific traits. While the primary goal of the jute breeders is to increase fibre yield, the plant type of jute has changed considerably over this long time period, and a number of diversified applications are upcoming.

10.11.1 Modernization of Crop Improvement Programme

As jute is a fibre crop of regional importance, the research thrust is also limited in national and international levels. With the increase in awareness about the detrimental effect of synthetic fibres including plastics, more emphasis has been directed towards research on environment-friendly jute crop. Modern crop improvement programme includes genomic research in jute in different countries like India, China and Bangladesh. The use of genomic tools and advanced breeding methods like marker aided selection, speed breeding and transgenic research and the use of genome editing tools may open a new avenue in future in crop improvement in jute.

10.11.2 Status of Varietal Development and Maintenance Breeding

The breeding history of jute is very short. In the fertile tracts of Eastern India, jute has been fitted to a cropping season starting from March to April which favours vegetative growth and delays initiation of reproductive phase. During earlier years, i.e. up to the 1960s, jute was being cultivated for longer crop duration, starting from March extending up to August.

Under high rainfall conditions, *C. capsularis* (white jute or guti pat) was much preferred over *C. olitorius* (tossa jute or shuti pat). Capsularis jute had three major advantages: tolerance to premature flowering during early growth phase, ability to withstand waterlogging condition and production of fibre having better quality. *C. olitorius* was less cultivated by farmers during that time, and the cultivation was limited to certain pockets. However, the situation has reversed during the last 50 years. Tossa jute is now cultivated over 95% of the jute area, whereas the area under white jute has reduced to 5% of the total area under cultivation.

The system of jute-based agriculture has also changed over the past 50–60 years considerably, challenging breeders to change the ideotype for enhancement of productivity and quality. Thus, the crop ideotype of jute has undergone considerable changes, challenging of directed breeding efforts. Systematic crop improvement for jute was started by R. S. Finlow in 1904 at Burdwan district of West Bengal, followed by establishment of Jute Agricultural Research Laboratory (JARL) at Dhaka in 1939 where some breeding works were carried out. Varietal development in *capsularis* jute was initiated through selection during 1900–1920. The first white jute variety *Kakya Bombai* was developed in 1916. Further improvement of *Kakya Bombai* resulted in the development of D-154 in 1919, which was less susceptible to chlorosis and more resistant to stem rot. The first tossa jute variety D-38, commonly known as *Chinsurah Green* (CG), was developed in 1915 through selection. It was the only *tossa* jute variety for general cultivation for a long period.

10.11.3 Varietal Development: Post-Independence of India

After India gained independence in 1947, the major jute area goes to Bangladesh, whereas the Hooghly River based jute industries remained in India. The major challenge for Indian jute breeders was to develop varieties suitable for new areas having wider adaptation. After 1947, jute researches were carried out initially at Rice Research Station, Chinsurah, Hooghly of West Bengal from 1948 to 1952. The Jute Agricultural Research Institute came into existence at Barrackpore, West Bengal, during 1953 and was renamed as Central Research Institute for Jute and Allied Fibres in 1990. Two very popular varieties, JRC-212 and JRC-321, were developed through selection and released in 1954. JRC-212 has same maturity as of D-154 but with much higher yield than the latter. JRC-321, besides high yield and early maturity, produces finer quality fibre and is suitable for growing in low-lying areas. The development of varieties like JRC-321 enabled farmers to fit jute into the jute-rice cropping system, harvesting two crops from same land in a year.

However, other capsularis varieties developed during the 1970s and 1980s were of longer duration (150–160 days), which gradually were replaced by higher-yielding *olitorius* varieties.

Introduction of resistance to premature flowering was a path-breaking achievement in tossa jute. Although tossa jute had higher productivity, it did not fit well to the cropping system as farmers had to sow the crop almost 1 month later than white jute (Tables 10.9 and 10.10). Moreover, under the high rainfall situation, capsularis jute was more advantageous. However, rainfall pattern gradually changed, particularly in South Bengal where olitorius jute started to replace capsularis jute. Still, during the 1970s, the area under jute cultivation was dominated by *capsularis* varieties (*capsularis*: *olitorius* = 75: *25*). Sudan Green (SG), an exotic germplasm from Sudan, Africa, having premature flowering resistance was identified and hybridized with JRO-632 and JRO-620, followed by pedigree selection, which resulted into varieties like JRO-524, JRO-7835 and JRO-878 during the 1970s at CRIJAF, Barrackpore.

Among these three varieties, JRO-524 (Navin) began to supplant *capsularis* varieties rapidly. Being released in 1977, it became popular among the farmers during the early 1990s and still maintains dominance. It reaches harvestable maturity within 120 days, has premature resistance to flowering derived from Sudan Green, has moderate tolerance to pest and disease attack and can produce up to 34–36 q fibre/ha under high-input agriculture. The major concern of jute farmers was the fluctuating price of produce, rather than its productivity. The new olitorius variety JRO-524 satisfied the farmers' need. Like *Sudan Green*, *Tanganyika-1*, an exotic strain from Tanzania, was identified to possess resistance to premature flowering character. Utilizing this strain in the hybridization programme (IC-15901 × Tanganyika-1), JRO-8432 (*Shakti*) was developed and released in 1999, which exhibits 8–12% higher productivity than JRO-524. Another variety, JRO-204 (Suren), a promising variety, has been released in 2007, which outperformed both JRO-524 and JRO-8432 with a yield potential of 35–40 q fibre/ha. This variety has high strength and is becoming increasingly popular among the farmers.

10.11.4 Need of Varieties for Diversified Applications and Climate Resilience

The resurgence of jute-based products over rising concerns of environmentally hazardous synthetic fibres in the past decade has opened up new avenues for jute demanding new types of jute varieties. Jute cultivation is one of the major solutions for reducing environmental pollution and increasing carbon credit. Besides, the application of jute fibre has been extended over the past years from fibre composites to textile blends and geotextiles, each of which needs tailor-made varieties suitable for each application. Geotextile materials need durability and strength, for which varieties with better fibre strength and low degradability are required. Both of these are influenced heavily by the presence of lignin in fibre. On the other extreme, some

Table 10.9 Characteristic features of improved varieties of *tossa* jute (*C. olitorius* L.) in India

Variety	Developing institute and year	Parentage	Significant attributes
'JRO-632' (Baisakhi *Tossa*)	CRIJAF, Barrackpore, West Bengal	Selection from indigenous type	Suitable for late sowing; pods shattering type; fibre fineness—3.06 tex Yield: 3.0–3.2 ton/ha
'JRO-878' (Chaitali *Tossa*)	CRIJAF, Barrackpore, West Bengal (1974)	'JRO-620' × 'Sudan green'	Premature flowering resistance; suitable for early sowing; pods non-shattering type; very fine fibre (2.60 tex). Yield: 3.0–3.2 ton/ha
'JRO-7835' (Basudev)	CRIJAF, Barrackpore, West Bengal (1974)	'JRO-632' × 'Sudan Green'	Premature flowering resistance; pods non-shattering type; withstand waterlogging to some extent at later stage; coarse fibre (3.50 tex). Yield: 3.2–3.4 ton/ha
'JRO-524' (Navin)	CRIJAF, Barrackpore, West Bengal (1977)	'Sudan Green' × 'JRO-632'	Premature flowering resistance; suitable for early sowing; pods non-shattering type; fairly tolerant to yellow mite and root rot disease and drought; coarse fibre (3.40 tex). Yield: 3.4–3.6 ton/ha
'TJ-40' (Mahadev)	BARC, Trombay, Maharashtra (1981)	Selection from inter-mutant cross	Premature flowering resistance; pods non-shattering type. Yield: 3.0–3.5 ton/ha
'JRO-3690' (Savitri)	CRIJAF, Barrackpore, West Bengal (1985)	'Tobacco leaf' × 'long inter-node'	Premature flowering resistance; pods non-shattering type; coarse fibre. Yield: 3.0–3.3 ton/ha
'KOM-62' (Rebati)	Jute Research Station, Kendrapara, Orissa (1993)	Gamma-ray derivative of 'JRO-878'	Premature flowering resistance; pods non-shattering; stem colour—Purple red; coarse fibre (3.80 tex). Yield: 3.0–3.5 ton/ha
'JRO-66' (Golden Jubilee *Tossa*)	CRIJAF, Barrackpore, West Bengal (1998)	Multiple crosses involving six parents	Premature flowering resistance; suitable for late sowing; pods non-shattering; fibre fineness—3.10 tex; strength—Good (25.60 tex). Yield: 3.5–4.0 ton/ha
'JRO-8432' (Shakti)	CRIJAF, Barrackpore, West Bengal (1999)	'IC-15901' × 'Tanganyika 1'	Pods non-shattering type; resistant to premature flowering; fine fibre (2.80 tex). Yield: 3.5–4.0 ton/ha
'JRO-128' (Surya)	CRIJAF, Barrackpore, West Bengal (2002)	'TJ-6' × 'Tanganyika 1'	Pods non-shattering type; resistant to premature flowering; very fine fibre (2.57 tex). Yield: 3.2–3.8 ton/ha

(continued)

Table 10.9 (continued)

Variety	Developing institute and year	Parentage	Significant attributes
'S-19' (Subala)	CRIJAF, Barrackpore, West Bengal (2005)	('JRO-620' × 'Sudan green') × 'Tanganyika 1'	Pods non-shattering type; resistant to premature flowering; fine fibre (2.70 tex). Yield: 3.5–4.0 ton/ha
'JRO-204' (Suren)	CRIJAF, Barrackpore, West Bengal (2007)	'IDN/SU/053' × 'KEN/DS/060'	Pods non-shattering type; resistant to premature flowering; very fine fibre (2.38 tex). Yield: 3.6–4.0 ton/ha
'AAU-OJ-1' (Tarun)	RARS (AAU), Nagaon, Assam (2007)	'Tanganyika 1' × 'JRO 640'	Pods non-shattering type; resistant to premature flowering; fine fibre (2.60 tex). Yield: 2.8–3.0 ton/ha
'JBO-2003—H' (Ira)	CRIJAF, Barrackpore, West Bengal (2008)	('JRO-632') × ('Sudan green') × 'Tanganyika 1'	Pods non-shattering type; resistant to premature flowering; grade TD2; strength 23.89 g/tex; fine fibre (2.86 tex). Yield: 3.4–3.6 ton/ha
'CO-58' (Sourav)	CRIJAF, Barrackpore, West Bengal (2010)	'TJ-40' × 'Tanganyika 1'	Pods non-shattering type; resistant to premature flowering; tolerant to stem rot, root rot, yellow mite, semilooper; fibre strength 26.61 g/tex; very fine fibre (2.49 tex). Yield: 3.0–3.4 ton/ha
'JBO-1' (Sudhangshu)	CRIJAF, Barrackpore, West Bengal (2010)	'JRO-632' × 'Sudan green' × 'Sudan green'	Pods non-shattering type; resistant to premature flowering; low lignin; very fine fibre (2.38 tex); strength—Fairly good (25.25 g/tex). Yield: 3.0–3.4 ton/ha
'JROM-1' (Pradip)	CRIJAF, Barrackpore, West Bengal (2013)	Selection from 'JRO-524' × 'TAN/NY/018C'	Highly tolerant to stem rot, root rot and anthracnose disease of jute; tolerant to yellow mite, semilooper and stem weevil; very fine fibre (2.57 tex). Yield: 3.0–3.1 ton/ha
'JROG-1' (Rithika)	CRIJAF, Barrackpore, West Bengal (2015)	Selection from 'JBO-1' × 'JRO-524'	Resistant to premature flowering; resistant to root rot, yellow mite, semilooper and moderately resistant to stem rot; fine fibre (2.87 tex). Yield: 2.7–2.8 ton/ha

(continued)

Table 10.9 (continued)

Variety	Developing institute and year	Parentage	Significant attributes
'JRO-2407' (Samapti)	CRIJAF, Barrackpore, West Bengal (2015)	Selection from 'KEN/SM/024' × 'JRO-524'	Resistant to premature flowering; tolerant stem rot, root rot, yellow mite, semilooper and stem weevil; tolerant to drought at early stage; very fine fibre (2.30 tex). Yield: 3.3–3.4 ton/ha
'KRO-4' (Gouranga)	ZARS, Krishnanagar (Dept. of Ag.), West Bengal (2017)	Selection from 'OIM-028' × 'JBO-2003-H'	Resistant to premature flowering; fine fibre (2.70 tex). Yield: 2.9–3.0 ton/ha
'BCCO-6' (Kisan Pat)	BCKV, West Bengal (2017)	Selection from 'OEX-05'	Resistant to premature flowering; fine fibre (2.81 tex). Yield: 2.8–2.85 ton/ha
'NJ-7010' (Rani)	Nuziveedu Seeds Pvt. Ltd. (2018)	Mutant selection (EMS induced) from 'JRO-524'	Resistant to premature flowering; fine fibre (2.66 tex). Yield: 3.0–3.1 ton/ha
'JROMU-1'	CRIJAF, Barrackpore, West Bengal (2020)	Mutant selection (gamma-ray induced) from 'JRO-204'	Resistant to premature flowering; tolerant stem rot, yellow mite, semilooper and apion; fine fibre (2.90 tex). Yield: 3.2–3.3 ton/ha
'JROB-2' (Purnendu)	CRIJAF, Barrackpore, West Bengal (2020)	Mutant of JRO-204	Stem colour green; suitable for both fibre as well as biomass for paper pulp industries; resistant to yellow mite, stem weevil and hairy caterpillar. Yield: 3.2 ton/ha. Biomass: 55–60 ton/ha

varieties need to have finer fibre with low strength and lower meshiness for blending in textile material. During 2002, JRO-128 (Surya) was released for quality fibre (fineness 2.7 tex) with a yield potential of 35–40 q/ha. S-19 (Subala) was released during 2005 with high yield (35–40 q/ha) and better-quality fibre (fineness 2.7 tex, strength 25.95 g/tex with less lignin content). It can be sown during middle of March.

Newer varieties like JBO-1 (Sudhangshu), JRO-2407 (2.30 tex) and JROM-1 (2.57 tex) have better fibre fineness being more suitable for numerous textile and non-textile diversified applications. In capsularis jute, JRCM-2 (1.25 tex), KJC-7 (1.30 tex) and JBC-5 (1.45 tex) are promising white jute varieties for textile blending. The value of these new varieties lies in higher fibre fineness with appreciable yield potential, which is expected to fetch higher income for jute farmers of the country.

Table 10.10 Characteristic features of improved varieties of white jute (*C. capsularis* L.) in India

Variety	Developing institute	Year of release	Parentage	Significant attributes
'JRC-321' (Sonali)	ICAR-CRIJAF, Barrackpore, West Bengal	1954	Selection from indigenous type 'Hewti'	Premature flowering resistant; pods non-shattering type; very fine fibre (1.50 tex); suitable for jute-cotton blended yarn and fabric. Yield: 2.5–2.8 ton/ha
'JRC-212' (Sabuj Sona)	ICAR-CRIJAF, Barrackpore, West Bengal	1954	Selection from indigenous type (Dacca)	Premature flowering resistant; pods non-shattering type; very fine fibre (1.61 tex). Yield: 2.0–2.1 ton/ha
'JRC-7447' (Shyamali)	ICAR-CRIJAF, Barrackpore, West Bengal	1971	X-ray derivative of 'JRC-212'	Premature flowering resistant; pods non-shattering type; responds to higher N dose; very fine fibre (1.71 tex). Yield: 2.2–2.5 ton/ha
'JRC-4444' (Baldev)	ICAR-CRIJAF, Barrackpore, West Bengal	1980	Selection from 'JRC-212' × 'D-154'	Premature flowering resistant; pods non-shattering type; very fine fibre (1.90 tex). Yield: 3.0–3.2 ton/ha
'UPC-94' (Reshma)	JRStation Bahraich, NDUAT, Uttar Pradesh	1983	Selection from 'JRC-321' × 'JRC-212'	Premature flowering resistant; pods non-shattering type; very fine fibre (1.50 tex). Yield: 2.5–2.7 ton/ha
'Hybrid-C' (Padma)	ICAR-CRIJAF, Barrackpore, West Bengal	1983	Selection from 'JRC-6165' × 'JRC-412'	Premature flowering resistant; pods non-shattering type; coarse fibre (2.50 tex). Yield: 2.5–2.8 ton/ha
'KC-1' (Jaydev)	JRS Kendrapara, OUAT, Odisha	1992	Gamma-ray derivative of 'JRC-4444'	Premature flowering resistance; pods non-shattering type; fine fibre (2.10 tex). Yield: 2.6–2.7 ton/ha
'KTC-1' (Rajendra pat 1)	Jute Research Station (BAU), Katihar, Bihar	1994	Selection from 'IC-30730' collected from Tripura	Premature flowering resistance; pods non-shattering type; less infestation of pest and diseases. Yield: 2.7–2.8 ton/ha

(continued)

Table 10.10 (continued)

Variety	Developing institute	Year of release	Parentage	Significant attributes
'JRC-698' (Shrabanti white)	ICAR-CRIJAF, Barrackpore, West Bengal	1999	Directional disruptive selection from multiple crosses of five indigenous and eight exotic parents	Premature flowering resistance; pods non-shattering type; very fine fibre (1.80 tex). Yield: 2.0–2.5 ton/ha
'Bidhan Pat-1'	B.C.K.V., Mohanpur, West Bengal	2001	Gamma-ray derivatives of 'D-154'	Photoperiod insensitive; pods non-shattering type; can be harvested in 60–65 days. Yield: 1.2–1.3 ton/ha
'Bidhan Pat-2'	B.C.K.V., Mohanpur, West Bengal	2001	Selection from 'D-154' × 'D-18' (mutant)	Photoperiod insensitive; pods non-shattering type; can be harvested in 90–110 days. Yield: 2.0–2.2 ton/ha
'Bidhan Pat-3'	B.C.K.V., Mohanpur, West Bengal	2001	Selection from 'D-154' × 'D-18' (mutant)	Photo-insensitive; pods non-shattering type; suitable for paper pulp industry; can be harvested in 110 days; very fine fibre (1.80 tex). Yield: 2.4–2.5 ton/ha
'JRC-80' (Mitali)	ICAR-CRIJAF, Barrackpore, West Bengal	2005	Selection from 'CIN-114' × 'JRC-321'	Premature flowering resistant; pods non shattering type; very fine fibre (1.25 tex). Yield: 2.2–2.3 ton/ha
'JRC-517' (Siddhartha)	ICAR-CRIJAF, Barrackpore, West Bengal	2009	Selection from 'JRC-212' × 'JRC-4444'	Premature flowering resistant; pod non-shattering; very fine fibre (1.49 tex). Yield: 2.2–2.5 ton/ha
'JRC-532' (Sashi)	ICAR-CRIJAF, Barrackpore, West Bengal	2009	Selection from 'CHN'/'FJ'/'044C' × 'JRC-321'	Premature flowering resistant; pod non-shattering; very fine fibre (1.83 tex). Yield: 2.5–2.6 ton/ha
'RRPS -27-C-3' (Monalisa)	ICAR-CRIJAF, Barrackpore, West Bengal	2009	'JRC-321' × 'NPL'/'KUC'/'094C'	Premature flowering resistant; pod non-shattering; red stem; very fine fibre (1.61 tex). Yield: 2.9–3.0 ton/ha

(continued)

Table 10.10 (continued)

Variety	Developing institute	Year of release	Parentage	Significant attributes
'NDC-2008' (Ankit)	Jute Research Station (NDUAT), Faizabad, Uttar Pradesh	2009	'7 I'/'20' × 'JRC-321'	Premature flowering resistant; pod non-shattering; light red stem. Yield: 2.5–2.6 ton/ha
JBC-5 (Arpita)	ICAR-CRIJAF, Barrackpore, West Bengal	2010	'JRC-321' × 'THA/Y/086C'	Premature flowering resistant; pod non-shattering; stem green with light red pigmentation; very fine fibre (1.45 tex). Yield: 2.8–2.9 ton/ha
'JRCM-2' (Partha)	ICAR-CRIJAF, Barrackpore, West Bengal	2013	'JRC-321' × 'THA/Y/086C'	Premature flowering resistant; pod non-shattering; stem green; tolerant to stem rot; hairy caterpillar; very fine fibre (1.25 tex). Yield: 2.7–2.8 ton/ha
'KJC-7' (Shrestha)	JRS Kendrapara, OUAT, Odisha	2016	'KC-1' × 'JRC-212'	Premature flowering resistant; pod non-shattering; stem green; very fine fibre (1.30 tex). Yield: 2.8–2.9 ton/ha
'JRC 9057' (Ishani)	ICAR-CRIJAF, Barrackpore, West Bengal	2016	'JRC-698' × 'CIJ-121'	Premature flowering resistant; tolerant to stem rot and semilooper; very fine fibre (1.31 tex). Yield: 2.5–2.8 ton/ha
'AAU-CJ-2' (Kkhyati)	RARS (AAU), Nagaon, Assam	2017	'CEX-045' × 'CEX-050'	Resistant to lodging and pod shattering; stem rot and root rot; tolerant to semilooper and yellow mite; fine fibre (1.93 tex). Yield: 2.7–2.8 ton/ha
'BCCC-1' (Shweta)	B.C.K.V., Mohanpur, West Bengal	2018	Selection from 'CIJ-123'	Tolerant to stem rot, semilooper and hairy caterpillar; very fine fibre (1.65 tex). Yield: 2.7–2.8 ton/ha

(continued)

Table 10.10 (continued)

Variety	Developing institute	Year of release	Parentage	Significant attributes
'BCCC-2' (Shweta)	B.C.K.V., Mohanpur, West Bengal	2019	Pure line selection from 'CIN-492'	Tolerant to apion, semilooper and hairy caterpillar; very fine fibre (1.68 tex). Yield: 2.7–2.8 ton/ha
'JRCJ-11' (Shweta)	ICAR-CRIJAF, Barrackpore, West Bengal	2021	Pedigree selection from a cross CIN-146 × JRC-321	Premature flowering resistant; high tolerance to Bihar hairy caterpillar and moderate tolerance to stem rot; fine fibre (1.78 tex). Yield: 3.1–3.2 ton/ha

10.12 Maintenance Breeding

The production of nucleus seed is the starting point in maintenance breeding of jute crop. When an entry enters advance varietal trial (AVT-II) stage in All India Network Trial and its performance is promising, the concerned breeder initiates the nucleus seed production and continues the process after the identification and release till the variety enters seed production chain and the indent or demand for breeder seed exists. Jute is a self-pollinated crop, although some percentage of cross-pollination is observed in both species. The nucleus seed is produced by growing plant to progeny rows. The various steps to be followed in the production of nucleus seed are listed below.

Cycle I A given variety is grown (minimum 10,000 plants) under optimum conditions with an isolation of 100 m and free from preceding jute crop. The appearance of volunteer plants is avoided resulting from mechanical mixture. About 500–600 true-to-type single uniform plants are selected to maintain the genetic constitution and to avoid any genetic drift. The selected plants are harvested and threshed separately. The seeds are examined for post-harvest characters like seed colour, size, etc., and the seeds not conforming true to type are rejected.

Cycle II Finally selected plants (400–500) are grown in isolations of 100 m following plant to progeny rows. Each block should contain single row plant progenies along with two rows of parental variety for comparison. As jute seed is small in size, 3–7 g of seed may be collected from a single plant. Five border rows of the same variety are grown around the seed plot to set a barrier. Varieties of the same species are grown at a safe isolation distance of 100 m. Fast-growing barrier crops like *Sesbania* are grown between two varieties of the same species. Interspecific cross

incompatibility is also utilized by sowing varieties of two cultivated species of jute adjacently. Full package of practices recommended for a variety is followed. A wide space of 60 cm is kept after every five rows to facilitate roughing and other field operations. The progeny rows are examined periodically throughout the growing season, especially at early growth stage, flowering stage, capsule formation and at maturity stage. The progeny rows which show off-type(s) or phenotypic variation may be rejected and uprooted as and when the off-types are detected. The diseased and agronomically poor progeny rows should also be rejected. The single plant progenies selected are harvested separately, which are true to type of the original variety. The single plant progenies, which meet all the standards mentioned in the descriptor, are bulked to get the nucleus seed.

Selection of individual plants followed by progeny row evaluation is done in two seasons, and this cycle of nucleus seed production is to be carried out every year compulsorily. Grow-out test is carried out to ascertain the genetic purity of nucleus seed and to test the fibre yield potential of the variety retaining the allelic frequencies of the original population for the character. Prior to harvesting, another batch of true-to-type single plants is selected for the next cycle.

10.13 Coordinated System of Testing

Various trials are conducted for the evaluation of a line for identification and release as variety if it is found superior over the best-existing variety(ies) in fibre yield and other traits like biotic or abiotic stresses and fibre quality parameters under All India Network Project on Jute and Allied fibre crops (AINPJAF). In general, there are six different types of trials/tests: (1) station trial; (2) multilocational trials (IET, AVT-I, AVT-II); (3) disease and insect trials; (4) agronomic-fertilizer responsiveness trial; (5) adaptive trial; (6) quality test.

10.13.1 Station Trial

This trial is conducted by Breeder developing the variety and may be conducted for one or more years with the objective of identify superior to be included in AINPJAF for multilocation trial. In station trials of jute, the plot size is generally 3 m $\times$ 1.8 m with a spacing of 30 cm between rows. Plant-to-plant spacing is maintained at 5–7 cm with a minimum replication of 3–4 and best-existing varieties as checks. Disease and pest reaction of the new line are also evaluated. The data from station trials are required for the inclusion of an entry in the multilocation trials.

10.13.2 Multilocation Trials

These trials are conducted under All India Network Project on Jute and Allied fibre crops at different test locations across different agroclimatic zones. The objective of

these trials is to evaluate the performance of newly developed lines. The number of zones for jute is four: (1) north-eastern plain zone (eastern UP and Bihar); (2) eastern zone (West Bengal, Assam, Tripura, Meghalaya); (3) south-eastern zone (Odisha & AP); and (4) southern or peninsular zone (Maharashtra and Tamil Nadu). The various trials conducted under AINPJAF are IET, AVT-I, AVT-II and adaptive trails. The IET trials are conducted in seven to eight locations across these zones with larger plot size, and the best entries are promoted to AVT-I. The AVT-I trials are conducted in seven to eight locations in larger size plots. The AVT-I is generally repeated in next season and termed as AVT-II. Simultaneously, fibre quality tests of all entries in IET and AVT-I are carried out. The best one to two entries are identified every year based on yield and other characters like fibre quality parameters and disease and pest resistance and recommended for adaptive trials in farmers' field in larger plots. Based on combined data over 4 years, a variety superior in yield and other characters compared to check varieties is recommended for identification and subsequent release by Central Sub-Committee on Crop Standards Notification & Release of Varieties for Agricultural Crops and Horticultural Crops. For State release of a variety in jute, in addition to 3-year state varietal trial's data, 1-year evaluation at AINPJAF trial is mandatory.

10.14 Future Thrust Area

Jute being a regional and orphan crop, there are opportunities for future research on different emerging areas. The main objective of future research is to orient research activities, particularly plant breeding activities, towards establishing it as a climate-smart crop. The present monopolistic use jute as fibre (~3% fibre of total biomass) should be transformed to a crop having diversified uses (~97% waste) with 100 product conversion efficacy. In fibre aspect, the main challenge is to improve the fibre quality aspect, particularly fibre fineness, which makes jute fibre for manufacturing diversified products. The emerging areas are to develop varieties which will be source of non-timber-based source of paper pulp with continuous round the year supply, varieties suitable for biofuel, leafy vegetable (high antioxidant), etc. Thus, environment-friendly jute crop may play an important role in future as an alternative to plastic use which poses threat to the environment.

10.15 Conclusions

Jute is the second most important fibre crop next to cotton globally. Jute denotes fibre obtained from the bark of two cultivated species (*Corchorus olitorius* and *C. capsularis*). This crop is grown in India and in Bangladesh in the pre-monsoon (pre-kharif) season within a small growing window of mid-March to mid-July (110–120 days) in the Eastern Gangetic Plain. Unlike other crops, genotype-environment interaction is comparatively high in jute. Progress in jute breeding and management has resulted in an increase of both average yield and potential yield

of this crop. Being environment friendly, this crop is drawing attention to policymakers to combat the ill effect of synthetic fibre including plastics and to increase the carbon credit related to the environment. After achieving yield improvement through a series of high-yielding varieties, breeding objective has shifted towards improved fibre quality suitable for diversified fibre uses. In addition, diversified use of jute is anticipated through use as biomass, biofuel, paper pulp and leafy vegetables in the near future. Plant breeding techniques, assisted by advanced genomic tools and gene editing tools, have eminent scope for the improvement of jute for yield, quality and resistance to diseases and pests.

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Cotton Breeding

11

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Abstract

Cotton (*Gossypium* spp.) is an economically important cash crop grown in more than 90 countries in tropical, sub-tropical and temperate climate for its fibre, oil and protein. Cotton belongs to the genus *Gossypium* that contains 50 species, of which 43 are diploids ($2n = 26$) and seven tetraploids ($2n = 4x = 52$). The diploid species are grouped in seven genomes designated as A–G and K. The tetraploid species with AADD genome originated from natural crossing involving cultivated diploid *G. herbaceum* (A1) and wild diploid species *G. raimondii* (D5), followed by polyploidization. Cultivated cotton has a narrow genetic base which is becoming a hindrance in sustaining cotton productivity worldwide. Broadening the genetic base of cultivated cotton by mobilizing the useful genetic variations from diverse exotic accessions, races of cultivated species and wild accessions requires to be the top priority. The use of molecular markers and advances in sequencing technology has resulted in the development of huge genomic resources that includes molecular markers, several linkage maps and more than 6497 quantitative trait loci (QTL) representing more than 30 agronomically important traits mapped on specific chromosomes. To facilitate high-throughput genotyping of the breeding populations, SNP arrays have been developed and extensively used for genetic mapping and marker-assisted breeding programmes. The last decade witnessed complete genome sequencing and resequencing of cultivated and more than a dozen wild species of cotton. Whole cotton genome sequence data provides a major source of candidate genes with potential for genetic improvement of cotton quality and productivity. Insect- and herbicide-resistant transgenics are under cultivation across the cotton-growing

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_11

countries. Genotype-dependent genetic transformation is known in cotton. Versatile and robust somatic regeneration protocol suiting to a diverse set of genotypes would facilitate transgenic development for economic traits. Precision genome editing tool CRISPR/Cas9 and further refinement in the technology has demonstrated successful simultaneous multiple gene-targeted mutagenesis in several crops including cotton. This technology holds promise to develop transgene-free edited plants for economic, quality, resistance and adaptation traits in cotton. This chapter dwells upon all broad aspects of conventional breeding and molecular tools for cotton improvement, present status and perspectives for cotton production sustainability.

Keywords

Cotton · Genetic resources · Breeding methods · DNA markers · Marker-assisted selection (MAS) · Genome-wide association studies (GWAS) · Genotyping by sequencing (GBS) · Transgenics, Genome editing

11.1 Introduction

Cotton (*Gossypium* spp.) is an economically important cash crop grown in more than 90 countries in tropical and sub-tropical climate for its fibre. Now, cotton is extensively being cultivated in temperate climates. Globally, cotton is cultivated on an area of 31.36 million ha which accounts for about 2.5% of the world's arable area and an estimated production of 24.612 million tonnes in 2020–2021. Among the leading cotton-producing countries, India, China, the United States, Brazil and Pakistan account for about more than 80% of the world's cotton production. The world's cotton consumption for the year 2020–2021 stands at 25.658 million tonnes. The total cotton trade (import and export) is about 9.9 million tonnes, and the ending stock stands at 20.349 million tonnes by April 2021 (USDA 2021).

Cotton played a significant role in industrial revolution that began in the eighteenth century. It also played an important role in evolution of the textile industry. Cotton and its value-added products are among heavily traded agricultural commodity across 150 countries. Asian countries dominate the global cotton production, but most of the produce is domestically consumed. For several years, China and India have been the core markets for cotton consumption. In India, the highly evolved textile sector consumes most of the country's cotton. In recent years, Bangladesh, Vietnam and Uzbekistan have emerged as major consumers of cotton, next to China and India, and consequently the core importers are China, Bangladesh and Vietnam. Similarly, among considerable cotton exporters are the United States, Brazil and India. The United States has been the largest exporter for many years, accounting to 37.8% of global cotton exports in 2019.

11.2 Cotton Production and Consumption Situation in India

At the time of independence (1947–1948), India produced a meagre 0.39 million tonnes of cotton from 4.4 million ha with a productivity of 88 kg lint/ha. The production steadily increased with adoption of improved varieties, production and protection technologies and reached an all-time high of 6.77 million tonnes in 2013–2014 from 11.96 million ha (Fig. 11.1). Currently, India has the largest area under cotton, is the largest producer and the second largest consumer of cotton (Figs. 11.1 and 11.2). The average productivity during the last decade was 512 kg lint/ha. There was a 5.2-fold increase in domestic consumption in the last six decades. From 2005 to 2006, India became the net exporter of cotton. It imports extra-long-staple and long-staple cotton from the United States, Egypt, Sudan or Australia. India exports its cotton mainly to Bangladesh, China and Pakistan and in small quantity to other countries.

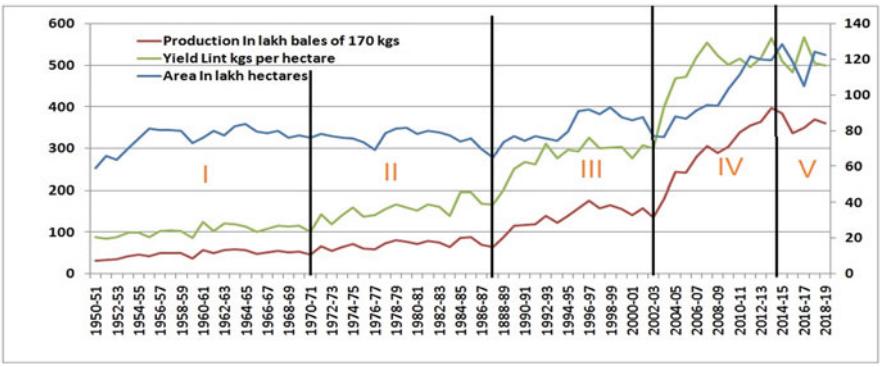

Fig. 11.1 Cotton area, production and productivity in India during 1950–2019

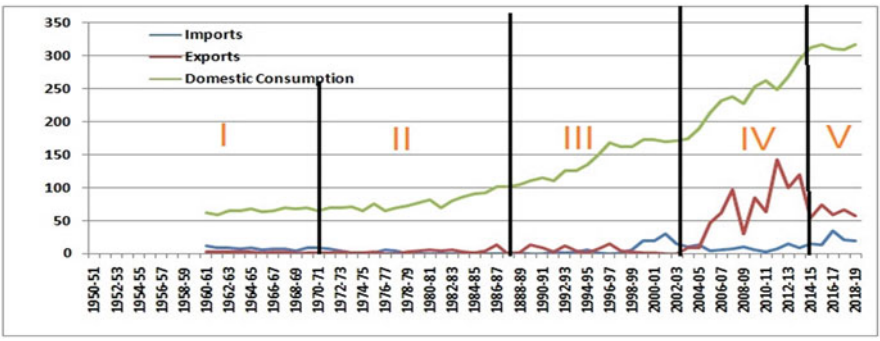

Fig. 11.2 India's cotton consumption, import and export during 1950–2019

11.3 Origin, Evolution and Distribution of Species

India has a history of cotton cultivation of more than 3000 years. The latest archaeological discovery in Mehrgarh (now in Pakistan) puts the dating of early cotton cultivation and its use to 5000 BC (Menon and Uzramma 2017). The ancient Indus Valley Civilization discovered through Mohen-jo-daro relics treats the time of cotton cultivation and manufacture of cotton fabrics to about 5000 years ago.

A close study of these relics at Technological Laboratory of the Indian Central Cotton Committee (now ICAR- Central Institute for Research on Cotton Technology) indicates the coarse cotton from which fabrics were manufactured related to *G. arboreum* types (Sethi 1960). Alexander the Great, during his sojourn in India, described cotton 'as a plant from which the natives plucked the vegetable wool which they spun to admirable clothing'. Herodotus, an ancient Greek historian, described Indian cotton in the fifth century BC as 'tree, bearing as their fruit, fleeces which surpass those of sheep in beauty and excellence'. Sufficient evidence has been recorded by the Arabian travellers describing Indian fabrics and flourishing export trade in cotton and cotton goods as early as 569–525 BC. The available evidence proves that India was the original habitat of cotton and an exporter of fine fabrics since the ancient times. Marco Polo, who travelled to India in the thirteenth century, Chinese travellers to Buddhist pilgrim centres, Vasco Da Gama, who entered Calicut in 1498, and Tavernier in the seventeenth century all have praised the superiority of Indian fabrics.

Historically, cotton is domesticated for its fibre (lint), mainly used for clothing by the textile industry. Besides, cotton provides cellulose from lint and fuzz (short fibres) for several industries (foods, wood, paper, pharmaceutical and cosmetic industries), oil for human consumption and seed as feed for animals. Practically, every part of the cotton plant is used for one or other purpose, i.e. shoot (stem) for manufacturing particle boards and fuel and acids are extracted from leaves.

Cotton is classified as a malvaceous plant in the genus *Gossypium* that belongs to a small taxonomic tribe, the Gossypieae (Fryxell 1968, 1979). *Gossypium* appears to have diverged from its closest relatives during the Miocene, perhaps 10–15 mya, subsequently spreading around the world via trans-oceanic dispersal to acquire its modern geographic range. With the recent addition of new tetraploid species, *Gossypium ekmanianum*, the genus *Gossypium* contains approximately 50 species (Fryxell 1992; Stewart et al. 2008; Wendel and Grover 2015), of which 43 are diploids with chromosome number $2n = 2x = 26$ and 7 tetraploids with chromosome number $2n = 4x = 52$. The diploid species are grouped in seven genomes designated as A–G and K (Table 11.1). The species belonging to the genome A, B, E or F are of African or Asian origin; the species of C, G or K genome are of Australian origin, while species with D genome are of American origin. Although all diploid species share the same chromosome number ($n = 13$), there is two- to threefold variation in DNA content per genome (Hendrix and Stewart 2005). The chromosomes of African and Asian diploids are larger in size and have more DNA content than the American diploid species. The DNA content of the African Asian diploids (2C = 3.47 pg) is 1.92 times higher than the American diploids (2C = 1.81 pg), while the K genome

Table 11.1 *Gossypium* species with their designated genome and geographic origin

Species	Genome	Geographic origin/distribution
Diploid species ($2n = 13$)		
G. herbaceum	A1	Africa
G. arboreum	A2	Asia/India
G. anomalum	B1	Africa
G. triphyllum	B2	Africa
G. capitis-viridis	B3	Cape Verde Islands
G. trifurcatum	B*	Somalia
G. sturtianum	C1	Australia
G. robinsonii	C2	Australia
G. thurberi	D1	Mexico, Arizona
G. armourianum	D2-1	Mexico
G. harknessii	D2-2	Mexico
G. davidsonii	D3-d	Mexico
G. klotzschianum	D3-k	Galapagos Island
G. aridum	D4	Mexico
G. raimondii	D5	Peru
G. gossypioides	D6	Mexico
G. lobatum	D7	Mexico
G. trilobum	D8	Mexico
G. laxum	D9	Mexico
G. turneri	D10	Mexico
G. schwendimanii	D11	Mexico
G. stocksii	E1	East Africa, Indo-Arabia
G. somalense	E2	Africa
G. areysianum	E3	Arabia
G. incanum	E4	Arabia
G. trifurcatum	E5	Arabia
G. benidirense	E*	Somalia, Ethiopia, Kenya
G. bricchettii	E*	Somalia
G. vollensenii	E*	Somalia
G. longicalyx	F1	Africa
G. bickii	G1	Australia
G. australe	G2	North Trans Australia
G. nelsonii	G3	Central Australia
G. costulatum	K1	North Kimberley of W Australia
G. populifolium	K2	Kimberley, Australia
G. cunninghamii	K3	The northern tip of NT, Australia
G. pulchelium	K4	Kimberley, Australia
G. anapoides	K6	N Kimberley, Australia
G. enthyle	K7	Australia
G. exiguum	K8	Australia
G. londonderriense	K9	Australia
G. marchantii	K10	Australia

(continued)

Table 11.1 (continued)

Species	Genome	Geographic origin/distribution
G. nobile	K11	Australia
G. rotundifolium	K12	Australia
G. pilosum	K*	Australia
Tetraploid ($2n = 52$)		
G. hirsutum	(AD)1	America
G. barbadense	(AD)2	South America
G. tomentosum	(AD)3	Hawaii Island
G. mustelinum	(AD)4	Brazil
G. darwinii	(AD)5	Galapagos Island
G. ekmanianum	(AD)6	Dominican Republic
G. stephensii	(AD)7	Wake Atoll

*Not yet recognized

($2n = 5.26$ pg) is 2.9 times higher than the D genome. The tetraploid species with AADD genome ($2n = 4x = 4.91$ pg) originated from natural crossing involving cultivated diploid species *G. herbaceum* (A1 genome) and wild diploid species *G. raimondii* (D5 genome), followed by polyploidization (Phillips 1963).

The available evidence establishes that the New World tetraploid cottons are allopolyploids containing an 'A' genome similar to the Old World cultivated diploids and a 'D' genome similar to the New World diploid species (Endrizzi et al. 1985; Wendel 1989). Molecular data and cytological analysis indicate that all allopolyploids in *Gossypium* share a common ancestry, monophyletic origin, i.e. polyploid formation occurred only once. In addition, all allopolyploids contain an Old World (A genome) chloroplast genome, indicating that the seed parent involving the initial hybridization event was an African or Asian A genome (Wendel 1989). Contrary to the views of varied polyploid formation, the molecular data suggest a geologically recent (Pleistocene) origin of the allopolyploids, perhaps in the last 1–2 million years, is consistent with cytogenetic analysis (Phillips 1963) and ecological considerations (Fryxell 1979). Subsequent to polyploidization, the morphological diversification and spread of allopolyploid must have occurred relatively rapidly; however, the spread of wild forms remained restricted to small regions. At present, there are seven allopolyploid species of *Gossypium*, of which two are cultivated and five are wild. *G. darwinii* is native to the Galapagos Islands, *G. tomentosum* to the Hawaiian Islands (DeJoode and Wendel 1992) and *G. mustelinum* is restricted to a relatively small region of north-east Brazil (Wendel et al. 1994). *G. ekmanianum* Wittm. was recently included in the cotton genus and validated (Grover et al. 2015) by molecular sequence data. *G. stephensii* relatively closely related to *G. hirsutum* from two islands (Wake, Peale) in the Wake Atoll in the Pacific Ocean has also been included in the *Gossypium* genus (Gallagher et al. 2017).

Among the total *Gossypium* species, four species producing spinnable fibre (lint) were extensively domesticated and are under cultivation. These include *Gossypium arboreum* L. and *Gossypium herbaceum* L. commonly referred to as Asiatic or Old

Table 11.2 Races of cultivated species

Cultivated species	Races
Gossypium herbaceum	Acerifolium, Kuljianum, Persicum, Wightianum
Gossypium arboreum	Bengalense, Burmanicum, Cernuum, Indicum, Sinense, Soudanense
Gossypium hirsutum	Punctatum, Palmeri, Marie-Galante, Mirilli, Latifolium, Richmondii, Yukatenance
Gossypium barbadense	Brasiliense

World cottons, native of India and Africa, respectively; *Gossypium hirsutum* L., an American cotton, native of Central America; and *Gossypium barbadense* L., an Egyptian or Sea Island cotton originated in Peru (South America). *G. herbaceum* is considered to be earliest cultivated cotton, originated in the Middle East. It may have been carried to India by travellers, which later differentiated to give rise to *G. arboreum*. *G. arboreum* has wide adaptability and widely cultivated in India covering all the states. *G. hirsutum* is the major cultivated cotton which accounts for about more than 90% of the area and also the world's cotton production. *G. barbadense* is known for its superior fibre quality and extra-long staple, also known as Sea Island, Egyptian or Pima cotton. It is now grown around the world, including China, Egypt, Sudan, India, Australia, Peru, Israel, South Western United States, Tajikistan, Turkmenistan and Uzbekistan, and accounts for about 5% of the world's cotton production. In the course of domestication, cultivated species in different geographical areas over the extended period of time differentiated to give rise to sub-species or races (Table 11.2). Races of cultivated species are important sources of resistance to biotic and abiotic stresses besides traits of agronomic importance.

11.4 Plant Genetic Resources

Plant genetic resources are the foundation and key drivers of any crop improvement programme. Most of the major cotton-producing countries maintain its cotton germplasm resources for utilization in cotton improvement programmes (Table 11.3). Uzbekistan has, to its credit, the highest germplasm holding of 32,580 accessions, followed by India (12,335), the United States (10,311), China (8868), Russia (4296) and France (3069). The germplasm maintained by different countries include working collection, wild resources, interspecific derivatives, aneuploid lines, mutants, varieties, mapping populations, RILs and trait-specific resources.

In India, ICAR-Central Institute for Cotton Research (ICAR-CICR) has the responsibility of maintaining a National Cotton Gene Bank at Nagpur since its establishment in 1976. ICAR-CICR has the mandate of exploration, collection, conservation, maintenance, characterization, documentation, utilization and distribution of cotton germplasm among cotton researchers. National Cotton Gene Bank was initially started by obtaining representative seed samples from the genetic

Table 11.3 Germplasm resources maintained in major cotton-growing countries

Country	*Gossypium hirsutum*	*Gossypium barbadense*	*Gossypium herbaceum*	*Gossypium arboreum*	Other species	Total	Source
Uzbekistan	24,571	4190	1292	1623	937	32,580	Abdurakhmonov et al. (2014a)
India	8851	536	565	2053	330	12,335	CICR Annual Report (2019)
United States	6302	1584	194	1729	502	10,311	Percy et al. (2014)
China	7752	633	18	433	32	8868	Jia et al. (2014)
Russia	4503	1057	336	365	15	6261	Campbell et al. (2010)
Brazil	1660	1509	19	219	889	4296	Campbell et al. (2010)
France	2173	483	50	69	294	3069	Campbell et al. (2010)

resources scattered within the country. ICAR-CICR, in collaboration with ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, and State Agricultural Universities, has collected cotton germplasm by launching germplasm collection missions in areas of origin and diversity of the mandate crops. Further, the cotton gene bank was enriched through donations from various domestic as well as institutions around the world dealing with cotton. India's National Cotton Gene Bank is now maintaining over 12,335 accessions, consisting of geographically diverse germplasm accessions of *Gossypium hirsutum* (8851), *Gossypium barbadense* (523), *Gossypium arboreum* (2094) and *Gossypium herbaceum* (565), wild species of *Gossypium* (24), perennials (254) and races, viz. *G. hirsutum* (4), *G. barbadense* (1), *G. arboreum* (6), *G. herbaceum* (1), and synthetic derivatives (40). A complete set of cotton germplasm is stored at ICAR-NBPGR, New Delhi, under long-term storage (LTS), while the other set is conserved as an active collection at ICAR-CICR, Nagpur, under medium-term storage (MTS, at 5 °C and below 30% relative humidity). Wild species are being maintained in situ in the species garden.

11.4.1 Unique Germplasm

Genotypes unique for specific traits, viz. morphological, economic or quality traits, are being regularly added to the existing germplasm. Fifty (50) genetic stocks [*Gossypium hirsutum* (28), introgressed (6) and *Gossypium arboreum* (16)] have been registered for their unique, novel and distinct characteristics. These would serve as an important source for specific traits and unique morphological markers.

11.5 Floral Biology: Emasculation and Pollination Technique

Cotton flower is extra-axillary, terminal and solitary. It emerges as tiny triangular bud surrounded by three triangular bracts generally referred to as squares. As the square grows in size, the twitted corolla becomes prominent, and it is termed as bud (unopened flower). The twisted corolla emerges from the bud taking shape of fully open flower before anthesis. When the flower first opens, the corolla is either cream, yellow or white in colour. In *G. hirsutum*, generally, corolla colour is cream at the flower opening but turns pink or red after anthesis and later remains attached or falls from the developing boll. Pistil consists of three to five carpels united to form a cylinder. The number of carpels fairly corresponds to the number of locks or locules in the boll. The stamens are united at the base forming a tube that encloses the styles and is united at the base of the corolla. Anthesis occurs after the flower opens and the pollen sheds directly on the stigma or carried to the stigma by insects.

11.5.1 Mode of Pollination

Cotton is normally considered to be a self-pollinated crop. Cotton pollen is heavy and sticky, normally not transferred by wind. The extent of cross-pollination depends on the abundance of vector population that primarily constitutes various wild bees, bumble bees (*Bombus* spp.), honey bees (*Apis mellifera*) and other insects. In normal circumstances, natural cross-pollination ranging from 0% to 10% is considered as a self-pollinated crop. When natural cross-pollination exceeds 50%, it may be treated as a cross-pollinated crop. Usually, in the presence of vector population, cross-pollination varies from 5% to 30%; hence, cotton is grouped in the category of often cross-pollinated crops. Cross-pollination in excess of 50% has been reported in Tennessee, Georgia and North Carolina. However, the intensive use of insecticides to control dozens of major harmful insect pests of cotton has reduced the pollinating insects' populations in the cotton ecosystem, thus reducing the relative extent of cross-pollination in cotton. Cross-pollination also varies with different varieties and their morphological attributes including flower opening, pigmentation, position of stigma and anthers and presence and prominence of flower nectaries.

11.5.2 Self-Pollination

Cotton flower is relatively bigger in size than the leguminous crops and thus easy for selfing and hybridization. Cross-pollination in cotton is controlled by preventing the flower bud to open until anthesis, pollination and early boll development. The flower bud which would normally open the next day is selected and sealed using malleable wire, thread string, paper clips, sticky mud and other similar materials to prevent opening of the flower and entry of insects visiting flowers for nectar or responsible for cross-pollination. Alternately, the flower bud may be covered with a small paper bag to prevent cross-pollination. This simple technique ensures self-pollination and to get pure selfed seeds of the genotypes in the breeding programme or for the maintenance of genotypes/varieties.

11.5.3 Crossing Technique

Hybridization is relatively easy to perform in cotton. Emasculation is performed by removing the corolla and the staminal column by hand, curved scalpel or small scissors on the preceding day the flower would normally open. The emasculated bud is covered with a paper bag to avoid cross-pollination and desiccation. On the following day, the pollen from the male flower is collected and placed on the stigma of the emasculated flower either by brush, directly rubbing the male flower dehisced anthers on the stigma or by soda straw partially filled with anthers slipped over the stigma. The pollinated flower bud is covered with paper bag and appropriately labelled recording name of male and female parent and date of pollination. This

method of hybridization is popularly known as Doak method (Doak 1934). Normally, emasculation is done in the afternoon and pollination on the following day morning. However, pollination may be effected late on the same day of emasculation if male flower buds of appropriate size are available. With good emasculation and pollination technique by a skilled/trained person, success of artificial crossing in terms of boll and seed setting would be as high as 75% of natural pollination.

Intensive research has been done on male sterility in cotton, and the genetic male sterility is being widely used in hybrid seed production in India. With the availability of male sterility, crossing in cotton has become easier. The female flower buds, before opening, are covered with paper bags, and pollination is effected on the following day. Use of male sterility eliminates the tedious process of emasculation and saves about 50% labour in commercial hybrid seed production.

11.6 Molecular Cytogenetics and Breeding

Accurate chromosome count of diploid and tetraploid cottons was reported by Nikolajeva (1923) and Denham (1924). Following these studies, cytological relationship between the species in the genus, their size and pairing behaviour were established. Skovsted (1934) showed that the New World tetraploid cotton is allotetraploid, containing 13 large and 13 small chromosomes; large chromosomes were homologues to large chromosomes of Asiatic cotton, while 13 small chromosomes were homologues to New World diploid species. It was concluded that the tetraploid species were amphidiploids combining diploid Asiatic 'A' genome and American wild diploid 'D' genome species (Skovsted 1934). Beasley (1940) and Harland (1940) independently synthesized amphidiploids between *G. arboreum* (A genome) and *G. thurberi* (D genome) and confirmed Skovsted hypothesis. The genomes of *G. herbaceum* and *G. arboreum* were shown to differ from one reciprocal interchange, indicating that *G. arboreum* was derived from *G. herbaceum* like genome. A sub-genome of allotetraploids in chromosome pairing in triploid F_1 revealed two reciprocal translocations with 'A' genome of *G. herbaceum* and three reciprocal translocations with 'A' genome of *G. arboreum*. The data clearly showed that 'A' genome of *G. herbaceum* was structurally more close (*G. herbaceum* also available in wild form i.e. *G. herbaceum* var. *africanum* while *G. arboreum* has no wild forms) to 'A' sub-genome of allotetraploid and considered as progenitor of present-day allotetraploids (Gerstel 1953; Menzel and Brown 1954). Similarly, morphological characteristics of F_1 hybrids involving allotetraploid and American diploids and chromosome pairing of (AD)D and (AD)A hexaploids clearly revealed that *G. raimondii* is the probable progenitor of D sub-genome of allotetraploid cotton (Stephens 1944a, b; Hutchinson et al. 1945, 1947). Further, the chromosomes of the 'A' and 'D' genomes of two diploid progenitors and the allohexaploids exhibited little or no evidence of divergence during evolutionary history (Phillips 1963). All the recognized allotetraploid species were having the same chromosome end arrangements including multivalent formation and segregation ratios in synthetic hexaploids (Gerstel and Sarvella 1956; Endrizi 1966; Hasenkampf and Menzel

1980) favoured hypothesis of monophyletic origin of amphidiploids and subsequent radiation and divergence.

In recent years, molecular cytogenetics is greatly adding to the understanding of genome organization and evolutionary studies. Besides an increase in the speed, sensitivity and specificity of conventional cytogenetic techniques, molecular cytogenetics offers opportunities to perform a variety of tasks which include analysis and distribution of repeated sequences, assignment of repetitive and single copy DNA sequences to positions on chromosomes, relationship between specific chromosomes and linkage groups, relationships between physical and genetic distances, differentiation of the genomes, detection of alien DNA in introgressed lines and others (Konan et al. 2009). Over the decades, various kinds of tools have been used (Wendel and Cronn 2003) collectively demonstrating that the best extant models of the ancestral genome donors are *G. arboreum* or *G. herbaceum* (A genome) and *G. raimondii* (D genome).

The repetitive DNA fraction accounts for most nuclear DNA in higher plants and animals which can be remarkably different, even in closely related taxa. Zhao et al. (1998) reported some A genome dispersed repeats spread to D genome chromosomes through FISH analysis in tetraploid cotton. The spread of dispersed repeats in the early stages of polyploid formation may provide an indication to identify diploid progenitors of a polyploid. Analysis of ($2n$) nuclear DNA content of all diploid species indicated two- to threefold variation in DNA content per genome, although all diploid species ($n = 13$) share the same chromosome number (Hendrix and Stewart 2005). Phylogenetic and genomic in situ hybridization (GISH) analysis of nine diploids (two A genome and seven D genome) and four tetraploids (AD genome) supported the hypothesis of 'A' sub-genome donor as *G. arboreum* or *G. herbaceum* and 'D' sub-genome donor as *G. raimondii* and provided direct evidence for the monophyletic origin of the polyploidy *Gossypium* species (Wu et al. 2013).

The 2,4-D and kinetin induced relatively high somaclonal variation in tissue culture regenerated plantlets via somatic embryogenesis which could be effectively detected using RAPD and SSR markers. Chromosome number counting and flow cytometry analysis revealed stability for the number of chromosomes and ploidy levels in all regenerated plants except two regenerated plantlets (lost 4 and 5 chromosomes, respectively). The study also implicates that these cytological changes were not correlated with the marker polymorphisms (Jin et al. 2008). Price et al. (1990) using A biotin-labelled cloned fragment of 18S–28S ribosomal DNA from soybean following in situ hybridization of metaphase I meiocytes from two translocation heterozygotes and monosomics involving chromosome 9 mapped cloned DNA sequences to chromosomes 9 arms of cotton. Cui et al. (2015) compared the cytogenetic map with genetic linkage maps and showed that most of the identified marker-tagged BAC clones appeared in same orders in different maps except three markers showing different positions indicating chromosomal segmental rearrangements.

Tafvizei et al. (2010) in cytogenetic and molecular studies of ten tetraploid cotton cultivars (*G. hirsutum*) including four parental genotypes and their F_1 progenies

showed that the parental genotypes differed significantly in their chiasma frequency and distribution as well as chromosome pairing indicating their genetic differences. Adjacent and alternate quadrivalents were detected in the cultivars which might facilitate new genetic recombination in the progenies. Variety-specific markers were identified which will be useful for varietal identification. Morphological, molecular and cytological analyses confirmed the hybridity of the hexaploid (involving *G. hirsutum* and *G. anomalum* and with 0.15% colchicine obtained a putative fertile hexaploid) with 78 chromosomes. Genome-wide molecular analysis with 683 EST-SSR markers revealed that a high polymorphism between species and A genome-derived markers were found to be helpful in distinguishing the genomic differences than the D genome-derived markers (Zhang et al. 2014).

11.7 Genetic Studies of Qualitative and Quantitative Traits

Cotton is one of the ideal crops for genetic studies of various morphological and economic traits. Availability of distinct morphological variation, easy for hybridization and differences in ploidy level in cultivated and wild species make this crop ideal to study genetics and to unravel events of its evolution. Cotton was one of the first crops to which rediscovered Mendelian principles were applied. Qualitative inherited traits controlled by major genes consisting of distinct morphological variation and mutants provided the base for genetic studies in cotton. Balls (1906) reported the inheritance of lint colour in *G. barbadense*. Subsequently, inheritance of okra leaf (Shoemaker 1908), inheritance of red leaf (Mc Lendon 1912) and several other traits were reported in Asiatic diploid and tetraploid cottons. Early genetic studies involving crosses between the cultivated species led to the understanding of ploidy level differences as diploids (*G. arboreum* and *G. herbaceum*) and allotetraploids (*G. hirsutum* and *G. barbadense*), the relationship between the diploids and allotetraploids and probable progenitors of the allotetraploid cotton.

Distinct mutant stocks in cotton helped to study the mechanism of inheritance, metabolism, biochemical and developmental pathways of plant traits. Examples of mutants that helped to get insight into the developmental pathways include the virescent mutant for the study of chlorophyll development and photosynthesis (Benedict and Kohel 1968) and fibre mutants for the development of lint fibre (Kohel et al. 1974). Morphological mutants also play an important role in cotton varietal improvement. Okra leaf, a leaf variant, has potential to reduce the incidence of boll rot and enhance early maturity (Jones and Andries 1967). Frego bract was found to affect boll weevil incidence (Clower et al. 1970). Leaf and stem hairiness or hirsute types effectively control jassids (Knight 1952), while the absence of leaf hairs, i.e. glabrousness, helps to reduce the incidence of whitefly. Nectariless trait has a significant effect on bollworms and on insects those feed on nectarines. The pigmented gossypol glands confer resistance to several pests while glandless mutant produce cotton seeds free from gossypol (Mc Michael 1960). Endrizzi et al. (1984) reviewed the inheritance of different morphological trait mutants and compiled

available mutants in Asiatic and tetraploid cotton with assigned gene symbols, names and source. Recently, Percy et al. (2015) reviewed morphological markers, comprehensively updated the mutants list and summarized integration of morphological markers with molecular marker maps and application of qualitative traits in breeding.

Most economic traits are quantitative in nature controlled by several genes with variable effect on the trait and highly influenced by the environment. Finding genetic association among various traits contributing to an increase in yield and appropriate procedure to be applied for combining several positively associated characteristics had been the focus of conventional breeders. Various mathematical models to analyse the genetic variability in the breeding material, genotype and environment interaction with certain basic assumptions were developed to improve breeding strategies for crop improvement. For quantitative inheritance, understanding of gene action and its partitioning in additive, dominance, epistatic effects and their interaction with the environment is most important. The additive effects refer to average effects of genes, dominance as interaction of allelic genes and epistasis as the interaction of non-allelic genes influencing specific traits. The diallel cross has been the widely used method for the analysis of gene action in cotton (Hayman 1954). Generation mean analysis is often used to estimate additive, dominant and epistatic effects. Different generations of material are used, and the standard errors are relatively smaller than estimated by variance component analysis. When several lines are used in crossing programme, reciprocal differences can also arise. The use of full diallel design in cotton genetic studies effectively compares reciprocal differences. In general, reciprocal differences are not observed in the segregation of nuclear genes, except in cases where cytoplasmic differences exist and maternal parent contribute to such differences. Contribution of maternal parent in cytoplasmic male sterility and fertility restorer from *G. harkensii* (Meyer 1975) and oil content in seeds of F_1s has been reported (Kohel 1980).

The combining ability as proposed by Sprague and Tatum (1942) has great significance for breeding programmes designed to explore heterosis through the development of F_1 hybrids. Combining ability studies also provide information on gene action thereby help in selection of parents for generating segregating populations. Matzinger (1963) indicated that the general combining ability (average performance of the line in hybrid combination) consists of additive and additive × additive epistatic variances, while specific combining ability (refers to cases where the performance of certain combinations is relatively better or least on the basis of average performance of the line involved) consists of dominance and all types of epistatic variances. Heterosis breeding makes use of dominance, dominance × dominance and epistasis gene action. High specific combining ability for yield and higher heterosis is generally preferred for hybrid development. In most of the self-pollinated crops, the near homozygous lines developed that equal or surpass the F_1 (Matzinger 1963). El-Adl and Miller (1971) conducted cotton breeding experiments and reinforced the same conclusion.

The phenotypic performance of a genotype is influenced by its environment, and phenotypic responses may not be the same for all genotypes.

Genotype × environment interactions and their statistical significance are important in estimating various genetic and environmental parameters that help to decide the most likely area of adaption of a cultivar. Large interaction components than the genetic component limit adaptions of cultivar to a specific environment where as if interaction component is small, the cultivar is considered to have general adaptability and may be recommended for general cultivation in all environments, also referred to as universally accepted cultivars. Miller and Rawlings (1967b) demonstrated a method of computing phenotypic and genetic correlation from covariance analysis in cotton. If genetic correlations among the traits are high, selection for one trait facilitates simultaneous improvement of both the traits. But if negatively correlated, selection for one may affect the performance of the other. The correlated responses were caused normally by pleiotropy or linkage. It is assumed that recombination and arrangements of linked genes can occur in heterozygous condition and there would be little chance of new recombination to occur after F_2 generation. To overcome this situation, Hanson (1959) recommended several generations of random inter-crossing to break undesirable linkages. Similar results were obtained in independent studies of Miller and Rawlings (1967a), Meredith and Bridge (1971), Singh et al. (1989) and Waghmare (unpublished).

Heritability of the trait under selection is one of the important parameters to predict success of selection. Heritability in a broad sense includes the total genetic variance but in a narrow sense includes only additive genetic variance. Narrow-sense genetic heritability is of much significance for making steady progress during selection for improving the desired traits. Heritability of yield is generally lower than its yield components and fibre quality traits. That is the reason for slow gains in yields, and it is imperative to base selection for major yield components and quality traits concurrently to make steady gains for yield and quality during selection.

With recent advancements in molecular marker technology and adoption of transgenic Bt cottons, there is a shift in application of techniques and methodology, though the principles used in conventional cotton breeding remains the same. Improvements in host plant resistance, selection for yield and fibre quality are being facilitated by DNA markers. Improvement of lint yield still remains the major selection criterion in the changed scenario; however, the pace and way of collecting phenotypic data of plant attributes contributing to yield component traits and host plant resistance is slowly changing. Availability of improved version of machines for fibre quality measurements, new digital tools and platforms for collecting phenological data in high throughput is making a difference slowly but steadily on future cotton breeding.

11.8 Breeding Objectives

In general, the major breeding objectives in cotton are high lint yield, early maturity, fibre quality, plant type to adapt higher population density and mechanical harvesting, wide adaptability and resistance to biotic (insect pests and diseases) and abiotic (drought, salinity and heat) stresses.

11.8.1 Breeding for Higher Yield

Cotton is cultivated mainly for its natural fibre which is the most economic produce. Fibre yield can be enhanced through improvement of positively associated yield components. Various studies indicate that yield is majorly influenced by boll number per plant, size/weight of boll and proportion of lint (lint percent). These major components of yield are highly interrelated affecting the final lint yield. Plants with prolific boll bearing potential give higher yield. Higher the boll number, the boll size (expressed as weight of the seed cotton per boll) may reduce; on the contrary, plants with high boll weight may have less number of bolls. Lint is produced on the surface of the seed, and the density of lint on the seed affects lint production which is a varietal character. Thus, higher seed set per boll is desirable to increase lint yield. Seed size is associated with size of boll. Bigger seed size normally has low lint proportion or lint percent, while small boll varieties invariably have smaller seeds and high lint percent. Varieties with high proportion of five locule bolls would produce high yield than the variety with four lock bolls. Along with lint yield, fibre quality is also most important. Cotton lint yield and fibre quality are negatively associated. Selection for higher yield often results in reduction in fibre quality. Thus, while improving lint yield, cautiously maintaining requisite fibre quality or simultaneous improvement for lint yield and quality is more desirable.

11.8.2 Early Maturity

Earliness is closely related to determinate growth habit. Earliness in cotton is difficult to define and measure, since receipt of rain or enough moisture in the soil induces the cotton plant to flower and continue to set bolls over longer period. In fact, cotton is a perennial plant induced to annual, with domestication and selection. Early maturity in cotton has several advantages. Early maturity shortens the crop duration and reduces management for late appearing insects, pests and diseases. It also reduces production cost and allows a double-cropping system. Among the other advantages, it helps to reduce losses from late appearing pests such as boll weevil and pink bollworm and also facilitate cotton harvesting in a lesser number of manual pickings and mechanical harvesting.

Earliness in cotton is measured based on the proportion of seed cotton harvested in the first and second picking which is expressed as percentage of total seed cotton and the same may be used as an index (generally above 0.8) for practical measure of earliness. The number of nodes appearing before the first fruiting branch, smaller plant size, small bolls and seeds and boll sets closure to the ground are some of the morphological traits associated with early maturity. Early maturity is a varietal character varying in different varieties and also influenced by environmental factors. It is a common observation that moisture stress induces early maturity. The commencement of flowering, duration of flowering after appearance of the first flower and time required for bolls to mature are few more factors that determine varietal characters and maturity.

11.8.3 Fibre Quality

Enhancement of fibre quality is an important and inclusive objective of any cotton breeding programme. Cotton fibre is an outgrowth of a single epidermal cell, an outer layer of the seed. Cotton seed produces two types of fibres, long and short fibres. The long fibres form an outer layer and get easily separated from the seed; the process is referred to as ginning. The long fibres, generally referred to as 'lint', are spinnable, extensively used for spinning yarn and in the textile industry. The short fibres, inner layer, that remain attached to the seed after ginning are called as 'fuzz' or 'linters'. In cultivated varieties, the proportion of fuzz fibre varies from 1.0% to 10.5%. It is minimal in *G. barbadense*, 4.3–5.9% in Asiatic cottons and maximum (up to 10.5%) in *G. hirsutum*. The fuzz composing of pure form of cellulose are used in making rayon, writing and photographic papers, currency notes, X-ray films, explosives and various cellulosic products.

With a stiff competition from synthetic fibres and to sustain its share in the apparel market, cotton fibre property improvement becomes imperative. Advances in fibre technology has made it possible to measure the characteristics of cotton fibres and to compare and facilitate improvement so as to meet the requirements of the spinners and textile manufacturers. The spinning performance of cotton fibres depends on its properties; the most important are fibre length, strength, fineness and maturity.

11.8.3.1 Fibre Length

This has a direct relation with spinning performance. Uniform staple length of fibre enables to spin yarn with uniform size and strength. Fibre length along with uniformity of fibres is measured with an optical instrument called Digital Fibrograph, which scans cotton fibres at 50% and 2.5% span length (SL). The 50% SL is the length that 50% of fibre in the sample equal or exceed; while in 2.5% SL, 2.5% of the fibres in the sample will equal or exceed. The uniformity ratio for the sample is calculated as the ratio of 50% SL to 2.5% SL expressed as percent.

11.8.3.2 Fibre Strength

This determines yarn strength. Strength is an important fibre attribute essential for high-speed spinning. Weaker fibres frequently break and do not stand rigours of the manufacturing process. The Stelometer and the Pressley strength tester are commonly used to measure cotton fibre strength of small samples. Strength is expressed as pounds of force required to break a bundle of fibres with a cross-sectional area and denoted as tenacity (g/tex). In general, upland and Asiatic cotton varieties have weaker fibre than the Pima and Acala types. Through normal breeding approaches, transferring fibre strength trait from *G. barbadense* varieties to upland cotton is not easy. Several researchers working with upland cotton reported negative association between fibre strength and lint yield. However, years of breeding and selection has helped to improve lint yield and fibre strength simultaneously in upland cotton.

11.8.3.3 Fibre Fineness

This is associated with fibre diameter and thickness of the fibre wall. Fibres of *G. barbadense* varieties have small diameter and smooth or fine texture, while fibres of upland and Asiatic varieties have larger diameter, thicker fibre wall and coarse texture. Fibres with a well-developed inner wall are said to be matured and provide good strength. When the average amount of the inner wall fails to develop, the fibre is said to be an immature fibre. Fibre fineness and maturity is measured by an instrument called 'micronaire'. It measures the rate of airflow at a standard pressure through a known weight of cotton in standard volume chamber. The rate of airflow is slower with fine fibres than with coarse fibres and is expressed as standard micronaire units.

The other parameters affecting quality include colour and extent of trash. Cotton varieties grown across the globe vary in fibre characteristics, and these are inherent to the variety. Generally, the cotton fibres of cultivated upland and Asiatic cottons are white with high reflectance, while the Pima or *G. barbadense* cotton appears yellow in colour. Insect and fungal infestations also result in discolouration affecting fibre colour and quality. Normally, dried leaves, leaf hairs and bracts adhering to the fibres represent a major part of trash affecting fibre quality.

11.8.4 Plant Type

Breeding for plant type has contributed significantly to increase yields in cereals and other crops. Cotton, being a perennial type, induced to annual, recommending a plant type for all situations is not possible. Concerted breeding efforts for developing high-yielding varieties and adoption of mechanization in a cotton production system, especially harvesting, have resulted in the development of varieties with medium stature, compact plant with relatively shorter boll bearing branches and short fruiting period. Such compact varieties have significantly contributed to increasing cotton productivity to more than 2000 kg lint/ha in countries such as Australia. Developing such compact plant through rigorous selection procedure and further maintaining straight varieties become easy than in hybrids. Such compact plant type will help to adapt higher plant density and facilitate mechanized harvesting. After adoption of GM cotton, cultivation of hybrid cotton in India has increased to above 90% of its acreage under cotton. Efforts need to be made to develop varieties or hybrids with compact plant type, big bolls with fluffy opening and early maturing with short fruiting period. Varieties with small or deciduous bracts and smooth leaves (free or less trichome density) would help in the production of cleaner and least trash cotton.

11.8.5 Wide Adaptability

Adaptability refers to the capacity of a variety to produce to its potential in a given environmental condition. In India, cotton is grown in varied agro-climate in North, Central and South from 9°N to 31°N latitude and diverse situations ranged from

irrigated areas characterized by intensive management to assured rainfall and dry rainfed situation characterized by low rainfall (less than 500 mm) accompanied by high temperature. Varieties or genotypes adapted to a particular growing condition may not have good performance under other environmental conditions. This differential response of genotypes is attributed to genotype-environment (GE) interaction (Cruz et al. 2012) and one of the reasons for low yields. To avoid multiplicity of varieties in narrow areas, it is imperative to develop and identify varieties with wide adaptability. Wide adaptability and stability of varieties recommended for cultivation across the agro-eco-regions will help to enhance productivity, minimize variation in fibre quality parameters and better returns to growers.

11.8.6 Biotic Stresses

Cotton crop is attacked by various insects, pests and diseases resulting in considerable economic losses each year. To minimize the losses, growers frequently resort to use of insecticides and pesticides which, in turn, increase cost of production and increase the risk of development of resistance in insects against chemical insecticides and exposure of workers and animals to deadly pesticides and risk of life. Host plant resistance is the best option to reduce economic losses and minimize the use of hazardous chemical pesticides.

11.9 Insect Pests

In a cotton crop, the presence of 252 arthropod pest species (including insects and mites), 173 species of predators and 192 species of parasitoids/parasites have been documented (Nagrare et al. 2022). However, about a dozen species of insects are identified as major pest causing significant losses to cotton crop, while the remaining species are occasional, sporadic, localized or minor in nature. The major pests of cotton in India are bollworms [American bollworm (*Helicoverpa armigera*), spotted bollworms (*Earias insulana*, *E. vittella*) and pink bollworm (*Pectinophora gossypiella*)] and sucking insect pests [leafhopper (*Amrasca biguttula biguttula*), aphid (*Aphis gossypii*), thrips (*Thrips tabaci*), whitefly (*Bemisia tabaci*), mealybug (*Phenacoccus solenopsis*) and papaya mealybug (*Paracoccus marginatus*)]. Other pests such as Indian cotton mirid bug (*Creontiades biseratense*), stem weevil (*Pempherulus affinis*) and tobacco caterpillar (*Spodoptera litura*) are also categorized as major pests especially in South India. Prior to introduction of Bt cotton, the above pests were reported to be attacking cotton crop at different stages of growth causing losses ranging between 50% and 60% (Puri et al. 1999). Currently, losses are estimated ranging from 20% to 30%.

In cotton, innate plant characters that suppress insect pest population were normally explored with considerable success in resistance breeding. These plant characteristics and their significance are as follows:

1. Hairiness—Leaf and stem hairiness is associated with jassid resistance (Jenkins 1989; Watson 1989); however, hairiness attracts more incidence of bollworm (*H. armigera*) and whitefly (Niles 1980) and also results in high trash content in lint.
2. Glabrous or smooth leaves—The absence of leaf hairs is found to have significantly reduced oviposition of bollworm (*H. armigera*) and larval population than hairy leaves (Lukefahr et al. 1971). Eggs laid on glabrous leaves do not remain attached and freely get dislodged by wind. Thin and glabrous leaves were also found to be tolerant to whitefly (Butter and Vir 1989).
3. Absence of nectarines—Nectaries are normally present on the lower midrib of leaves and inside the flower bracts. Nectaries secrete nectar which attracts insects. A significant reduction in oviposition by bollworm has been reported on nectariless plants and no adverse effect on plant growth in the absence of nectarines (Jenkins 1989).
4. High gossypol content—Pigmented gossypol glands are normally present on all parts of cotton plant. Gossypol is a polyphenolic compound with insecticidal properties. High gossypol content confers resistance to American bollworm, tobacco budworm and spider mites. However, gossypol content in seed makes the cotton seed oil unfit for human consumption without gossypol detoxification.
5. Leaf traits—Narrow leaf lobes with hairiness show tolerance to jassids. Okra leaf character reported a significant reduction in damage by pink bollworm (Wilson and George 1982; Wilson 1986).

11.10 Cotton Diseases

Cotton crop is also affected by several diseases. The major diseases of cotton include fungal diseases [root rot, grey mildew (*Ramularia areola*), *Alternaria* leaf spots, *Myrothecium* leaf spot, *Corynespora* leaf spot], bacterial diseases [bacterial blight (*Xanthomonas citri* pv. *malvacearum*), inner boll rot] and viral diseases [cotton leaf curl disease (CLCuD) and tobacco streak virus (TSV)].

11.10.1 Root Rot

Root rot or seedling rot in cotton is caused by soil fungi *Rhizoctonia solani*, *R. bataticola* and *Sclerotium rolfsii*. It results in sudden wilting and drooping of plants that can be easily pulled out. Shredded bark of roots gives yellowish appearance. *R. solani* is one of the most important pathogens of seedling complex of cotton (Rothrock 1996); infected root becomes brown and wet with sunken lesions on stems known as ‘sore shin’ (Atkinson 1892). *R. bataticola* causes black and dry root; *S. rolfsii* infection noticed with white mycelial growth on the collar region leads to the rotting of roots and drying of seedlings. The disease is prevalent in all cotton-growing zones of India. A sick plot has been maintained at ICAR-CICR, Regional Station, Sirsa, to screen segregating and advanced materials to identify resistant

lines. Several resistant lines have been identified and regularly being used in breeding programme in India.

11.10.2 Grey Mildew

Grey mildew is caused by *Ramularia areola*. Pale, irregular and angular spots initially appear on older leaves delimited by veinlets (Chohan et al. 2020). Dirty white powdery growth spreads on the lower and upper surface of the leaves. As the disease advances, leaves turn yellow, necrotic and dark brown and dry leading to premature defoliation and forced boll opening. Grey mildew disease is prominently occurring in diploid cotton. However, recently, it is being observed in *G. hirsutum* cotton in central and south zones of India. In Maharashtra, the grey mildew is commonly referred to as 'dahiya' or 'dahya' disease because symptoms resemble sprinkled curd on foliage (Gokhale and Moghe 1965). A total of 1489 *G. arboreum* germplasm accessions were evaluated for grey mildew reaction at ICAR-CICR, Nagpur, and seven immune accessions (no disease symptoms), namely Bangladesh (EC 174092), G-135-49, 30805, 30814, 30826, 30838 and 30856; and 17 highly resistant accessions were identified (Mohan et al. 2006). These accessions are being used in *G. arboreum* improvement programme.

11.10.3 *Alternaria* Leaf Spot

The disease is caused by *Alternaria macrospora* and *A. alternata*. Initially, the disease appears as brown or tan spots on cotyledons, leaves, bracts and bolls. Concentric rings develop within the spots mostly on the upper surface. Later on, spots coalesce and cause blighting of the leaves. Favourable conditions lead to severe defoliation. The disease is prevalent in all major cotton-growing tracts of the country (Rane and Patel 1956). Recently, two genotypes, i.e. GSHV-159 and GBHV-184, were identified as immune (disease-free) and ten genotypes resistant against *Alternaria* leaf spot disease (Patel et al. 2019). These identified genotypes may be used for breeding host plant resistance.

11.10.4 *Corynespora* Leaf Spot

The disease is caused by *Corynespora cassiicola* and *C. torulosa*. Initially, affected leaves show circular to irregular, dark red spots which turn to brown lesions surrounded by a dark border. Later, alternate light and dark brown rings may develop on the lesions with 'shot hole' appearance. Under severe conditions, defoliation may occur. The disease is prominently emerging in Central India (Salunkhe et al. 2019). It is an emerging foliar disease, spreads very fast if congenial weather conditions prevail and may cause severe economic damage to cotton crop. Available

germplasm may be subjected to intensive screening against this disease to identify sources of resistance.

11.10.5 Bacterial Leaf Blight

Bacterial leaf blight (BLB) is a bacterial disease caused by *Xanthomonas citri* pv. *malvacearum*. BLB is prevalent throughout the world. The disease appears as water-soaked, light to dark green, small spots measuring 1–5 mm on cotyledons and lower surface of leaves. The lesions darken and veins also become black with age. Leaves shed prematurely resulting in extensive defoliation. As symptoms progress and stage advances, it is known as angular leaf spot, black arm and boll rot (Hillocks 1992). Asiatic cotton varieties of *G. arboreum* and *G. herbaceum* are reported to be tolerant, but upland *G. hirsutum* varieties are very susceptible to BLB. At least 18 races of bacterial leaf blight of cotton and corresponding genes conferring resistance have been identified. In India, race 18 is prevalent in all cotton-growing states. Depending on the prevalence of the pathogenic races, it is pertinent to combine two or more genes conferring BLB resistance in new varieties. Several existing varieties are resistant to BLB which may be promoted in hot spot areas for cultivation and also involved in breeding gene pool.

11.10.6 Boll Rot

Boll rot is caused by several saprophytic fungal and bacterial pathogens. Recent detailed studies identified *Pantoea* spp. as the causal organism for inner boll rot (Nagrale et al. 2020). The infected, apparent green healthy bolls, when cross-sectioned, the developing seeds and fibres or lint gets discoloured (yellowish orange to reddish in colour). The developing seeds swell and rot in one or two locules (Hudson 2000) and occasionally the complete bolls. The disease is emerging and currently prevalent in Maharashtra and Central India. Developing varieties with open canopies, okra to super okra leaf types and frego bracts would help to reduce the boll rot to a greater extent. Incorporating nectariless trait in the varieties may also reduce entry of fungal pathogen in the developing boll and subsequent boll rot.

11.10.7 Cotton Leaf Curl Disease (CLCuD)

The cotton leaf curl disease is caused by *Begomoviruses* of the family Geminiviridae. The prominent symptoms of CLCuD include yellowing and small veins thickening (SVT) on the lower surface of young leaves and downward or upward curling of leaves with stunted plant growth. Under severe conditions, a small leaf like outgrowth on the lower side of the infected leaves (enations) may also be visible. This disease is transmitted by insect vector whitefly (*Bemisia tabaci*). Currently, this disease is prevalent only in Pakistan and North Indian states, viz.

Punjab, Haryana and Rajasthan (Rajagopalan et al. 2012). In India, a complete set of *G. hirsutum* germplasm and introgressed derivatives were screened at multi-locations in hot spots of CLCuD in Northern India. However, not a single accession resistant to CLCuD could be identified. Recently, two accessions GSV 8 and GSV 9 obtained from the United States tested for CLCuD incidence showed immune to tolerant reaction. These two accessions have now extensively been used to transfer resistant gene in breeding material for the development of *G. hirsutum* varieties.

11.10.8 Tobacco Streak Virus (TSV) Disease

This disease is caused by *Ilarvirus*. Initial symptoms include chlorotic growing tip in young leaves, subsequent bronzing, curling with necrosis of leaves and plants become stunted (Gawande et al. 2019). It is transmitted by thrips (*Thrips tabaci*) and usually prevalent in southern states of India, but recently reported from Maharashtra and Andhra Pradesh as well. TSV is an emerging disease; studies on the identification of host plant resistance are required to be taken in areas of its prevalence.

Sappenfield et al. (1980) suggested two procedures referred to as 'multiple disease resistant breeding' and 'multiadversity resistance breeding'. It consists of the sequential inoculation of cotton seedlings grown in controlled environments with different disease pathogens or infestations with insect pests, followed by selection of resistant plants. In this procedure, sick plots developed for multiple pathogens can effectively be made use of. The procedure may differ as per the combination of insect pests or disease pathogens for which the resistance is being desired. In this procedure, simultaneously several common disease pathogens and insect pests are evaluated, and plants with multiple disease resistance can be identified. Resistant plants are allowed to be grown to maturity to get seeds and for further use.

11.11 Abiotic (Drought, Salinity and Heat) Stresses

Cotton crop suffers from various abiotic stresses during various stages of crop growth. Abiotic stresses mainly include drought, high temperature (heat) and salinity.

11.11.1 Drought

In India, of the total acreage under cotton, about 60% cotton is grown under rain-dependent situation. In such areas, cotton crop often suffers from moisture stress. It affects the crop growth development and production. Cotton germplasm and cultivated varieties exhibit genetic variability for various traits that contributes to resistance for drought. The major plant characteristics that impart drought tolerance include leaf traits, i.e. small and thick leaves, leaf hairiness, thick cuticle and

waxiness of surface; stomatal characteristics, i.e. small size, sunken type and less in number per unit area; and a deep root system with increased lateral root growth.

Small and thick leaves, thick cuticle, leaf surface waxiness and hairiness reduce water loss through transpiration (Eslick and Hackett 1975; Bhatt and Andal 1979) and contribute towards stress tolerance, but reduced transpiration results in the reduction in photosynthesis. Stomata, small in size, sunken type and less in number per unit area are associated with drought tolerance. Control of stomatal aperture and rapid closing features helps in reducing the evapotranspiration and maintaining high turgor potential of leaf tissues under drought stress. The ability of a plant to maintain high turgor potential is considered to be a manifestation of drought tolerance. The deep root system penetrates deeper in soil extracting more moisture, while increased lateral root growth extracts moisture from lateral root zone and maintains water requirement of the plant under drought stress situation. Availability of sufficient moisture to the plant under stress does not make it drought tolerant, but how efficiently used by the plant makes it drought tolerant. In other words, water use efficiency, reduced transpiration and high photosynthetic rate are also considered to be the reliable parameters of drought tolerance. High proline content in leaves under moisture stress has been recorded (Singh and Sahay 1989) and can be used as an indicator of water stress, but it cannot be used as a measure of drought tolerance.

Drought tolerance is governed by polygenes and influenced to a great extent by several environmental factors. Combining several traits contributing to drought tolerance is a difficult task. Conventional pedigree and backcross methods may be used if the desired traits in biparental crosses are to be combined or introgressed. It is difficult to find a combination of traits in single genotype. Recurrent selection involving several trait contributing genotypes to develop a source population and then intensive selection under drought-induced conditions would help to develop drought-tolerant genotypes.

11.11.2 High Temperature

Temperature and heat stress has become a major problem in cotton production; it affects the normal plant development, growth and productivity. The degree of occurrence of heat stress varies greatly across agro-climatic zones and also depends on the period of high diurnal temperature. Plant response to high temperatures also varies within and across species, as well as developmental stages. A high night temperature has been reported to cause excessive square and boll shedding. Development of more heat-tolerant cotton needs to be prioritized; however, identification of sources of heat tolerance in cotton is a challenge due to reduced genetic diversity for thermo-tolerance in wild-type cotton.

11.11.3 Soil Salinity

Cotton is considered as a moderately salt-tolerant crop and may be grown with a salinity threshold level of 7.7 dS/m (Zhang et al. 2013). Most of the varieties are found sensitive during germination and early growth stages but observed to be tolerant during late growth stages. Salinity is a serious threat for cotton in irrigated areas. Salt stress results in delayed flowering, reduced fruiting, fruit shedding and reduced boll weight that affect seed cotton yield. Excessive sodium exclusion or its compartmentalization is found to be the main adaptive mechanism in cotton under salt stress. Seed priming is suggested for improving cotton germination in saline soils (Bradford 1986). Differential varietal response to varying salt concentration may be used to identify tolerant sources and to develop salt-tolerant varieties with the aid of marker-assisted selection.

11.12 Coloured Cotton

Normally, cotton produced worldwide has white lint. Coloured cotton is being grown and used by mankind since 2500 BC. Coloured cotton varieties known in diploid cottons were under cultivation in Asia, particularly Indian subcontinent, China and Central Asian Republics of the former Soviet Union. In India, brown linted varieties of tree cotton (*G. arboreum*), namely Cocanada 1, Cocanada 2 and Red Northerns, were under commercial cultivation mainly on black soils under rainfed condition in parts of Andhra Pradesh. Red linted types were predominant and high in demand for their better dying qualities and colour fastness. Coloured linted varieties lost their glory, mainly because of low productivity, poor fibre characteristics and non-uniformity of colours (Waghmare and Koranne 1998). In the cultivated species, brown and green colours are most common. Some of the genotypes in germplasm collection of the United States and Russian Republics are reported to have coloured lint with shades of pink, red, blue, green and also black. Ms. Sally Fox of Vreseis Ltd. claimed to have developed multi-coloured lint, i.e. development of more than one colour on the same lint strand (Fox 1987). However, genotypes with multi-coloured lint have not yet been made available to the researchers nor produced on a large scale.

About 40 coloured genotypes of upland cotton (*G. hirsutum*), mostly in various shades of brown and green colour, are available in the National Gene Bank of Cotton maintained at ICAR-CICR, Nagpur. These genetic stocks are indigenous collections as well as exotic accessions from the United States, erstwhile USSR, Israel, Peru, Mexico, Egypt, etc. In Asiatic diploid cottons (*G. arboreum* and *G. herbaceum*), about 10 germplasm lines possessing mostly brown lint colour are also available. Wild species are important source of coloured lint. As many as 22 wild species of the genus *Gossypium*, including putative donors of the present-day tetraploid cotton, i.e. *G. herbaceum* race *africanum* and *G. raimondii*, and all 4 cultivated species of *Gossypium* possess coloured lint.

Development of lint colour starts with accumulation of pigments in the lumen of lint before boll bursting. In upland cotton, pigmentation starts appearing in the developing lint 32 days (46–47 days in Asiatic cotton) after fertilization, and it takes nearly 6 days to develop colour. However, complete expression of lint colour takes place only when the boll bursts open and lint is exposed to sunlight. It takes about a week for the lint to develop a complete natural colour. The intensity and the time taken for complete development of colour vary with the genetic background of the genotypes. Lint colour is a genetically controlled character and has mostly monogenic inheritance. Six loci governing lint colour in upland cotton have been identified and designated with gene symbols Lc_1, Lc_2, Lc_3, Lc_4, Lc_5 and Lc_6 for brown lint, Dw for dirty white and Lg for green lint colour (Endrizzi and Kohel 1966; Kohel 1985). Lc_1 locus governing brown coloured lint was mapped to chromosome 7 and Lc_2 on chromosome 6 (Wang et al. 2014). The breeding methods for improvement of coloured cotton are the same as applicable to white linted cotton.

11.13 Conventional Breeding Approaches

Cotton, being an often cross-pollinated crop, the breeding methods employed differ from methods used for self- and cross-pollinated crops. Though cotton is predominantly self-pollinated, generally, cross-pollination ranges from 3% to 5% owing to sparse insect populations. With the intensive use of inputs, particularly insecticides to control invasive insect pests, the natural population of useful insects that serve as pollinators has drastically been reduced in the cotton ecosystem. However, in some instances, cross-pollination has been reported as high as 30–50% where insect population is abundant in high rainfall areas of the South Eastern United States.

Mode of pollination generally affects the genetic makeup of breeding population. Owing to partial (often) mode of cross-pollination, cotton breeding makes use of breeding methods relevant to both self- and cross-pollinated crops. In practice, cotton breeders exercise considerable flexibility in employing breeding approaches depending upon the extent of available genetic variability and breeding objectives. The principal objective in cotton breeding is to improve the productivity and fibre quality with sufficient degree of adaptability and resistance to insect pests and diseases. Unlike the self-pollinated crops such as wheat, rice and soybean and cross-pollinated crops such as maize, partial heterozygosity is desired in the cotton varieties to be vigorous and productive. It is, in this context, cotton breeding method differs from the self- and cross-pollinated crops.

Several researchers reviewed cotton breeding procedures and methods (Richmond 1951; Sikka and Joshi 1960; Singh and Raut 1983). For success of any plant breeding programme, the prerequisite is availability of sufficient genetic variability in the desired species. To augment the genetic variability, one may need to assemble it from various sources, i.e. germplasm collection, and, if required, create the genetic variability. Variation in the plant species can be enhanced through introduction, hybridization, mutation and combination of these methods. Genetic variability thus generated is channelized following certain selection procedures to

obtain improved varieties. Based on the procedure followed for generation of genetic variability and selection scheme employed, cotton breeding methods may be classified distinctly that have been described with suitable examples wherever necessary.

11.13.1 Introduction

Introduction of crop species and varieties in new habitat has played an important role in the development of early cotton varieties. Cultivated tetraploid cotton (*G. hirsutum*) originated in America was introduced and now commercially cultivated in more than 90 countries in the world. It is one of the striking examples of successful introduction and acclimatization in India (Sethi 1960). *G. hirsutum* was first introduced in India in the latter half of the eighteenth century. The upland cotton from the United States was introduced by East India Company in the middle of the nineteenth century (Gammie 1908). The Cambodian cotton whose origin could be traced from Mexico to the Philippines by way of Cambodia and other parts of Southeast Asia was introduced in Madras state in 1906 (Anonymous 1954). Despite several attempts since 1931, for introduction of *G. barbadense*, the variety 'Andrews' introduced from West Indies was adapted and directly released for commercial cultivation in Kerala and Mysore (Kalyanaraman et al. 1955). Several introduced cotton genotypes, though directly could not be released as varieties, but used for the development of hybrids. Some of the notable examples are American nectariless was crossed with the Indian variety G 67 to produce the world's first intra-*hirsutum* hybrid 'H4' (Patel 1971). Similarly, Russian introduction of *G. barbadense* SB 289E was crossed with the upland variety 'Laxmi' to obtain the first interspecific hybrid 'Varalaxmi' (Katarki 1972). Another Russian *barbadense* introduction SB 1085-6 was crossed with Acala Glandless for the development of the interspecific hybrid 'CBS 156' released in Tamil Nadu.

11.13.2 Selection

Selection has emerged as the most important breeding method from time immemorial for improvement of crop plants. It is the process of picking up individuals in the population with naturally occurring variation or the variation created through artificial hybridization or induced mutations which show marked improvement for one or combination of characters over the existing population. The various selection techniques followed in cotton improvement include empirical mass selection, pure line selection and progeny selection.

11.13.2.1 Mass Selection

This is the simplest type of selection often practised traditionally on an introduced material during initial stages of crop improvement. It is known that many introductions, especially landraces and open pollinated varieties, harbour a considerable extent of genetic variability/heterogeneity; on such genetic variability, mass

selection is usually preferred. In mass selection, desirable high-yielding plants with phenotypic similarity are selected and ginned and the seed is bulked for planting in the next season. This process is repeated for 2–3 years till an improved bulk with higher yield and quality than the existing population is obtained. Release of new variety by this method takes 7–8 years. Few of the released varieties in India include Bikaneri Narma, F 414, H 777, SRT 1, Narmada, L 147 and PRS 72 in *G. hirsutum* and G 27, HD 11, LD 133, Gaorani, Cocanada white and Saraswati in *G. arboreum*. But this method is now seldom used because of its less precision than the progeny selection.

11.13.2.2 Pure Line Selection

This refers to the identification of homozygous plant progeny. Pure lines are developed by inbreeding, controlled pollination to avoid outcrossing or from double haploids. Pure line selection leads to complete homozygosity, little or no heritable variation, reduced vigour and lower yields; hence generally not practised in cotton breeding. However, modified pure line selection is practised especially for maintaining lines with specific traits or for the development of pure lines to be used as parents in hybrid development programme. The varieties developed through pure line selection include MCU 5 VT, CO2 and LSS in upland cotton; Cocanada 2, Gaorani 22, Gaorani 46 and Lohit in *G. arboreum*; Western and Selection 69 in *G. herbaceum*; and Sujata in *G. barbadense*.

11.13.2.3 Progeny Selection

This refers to the selection of single plants in a segregating population for pedigree selection. The progeny of each selected plant is grown separately, and single plants in the superior progenies are selected again. The process of reselection of superior plants is repeated until uniform progenies are obtained. Alternately, progeny selection can be used for maintaining the genetic purity of the variety or progressively improving the variety, if enough heterogeneity persists. Punjab American cv 320F is a good example of intra-varietal selection from LSS that led to the development of superior variety.

11.13.3 Hybridization

Hybridization is the most common method of producing new genetic variability, exercising selection and developing new improved cotton varieties. Hybridization (crossing) between two distinct genotypes or varieties of the same (intraspecific) or different (interspecific) species has widely been used to generate variability for economic and fibre quality traits and to combine the desirable traits of the parents involved in crossing. Besides artificial crossing of known parents, in cotton, natural crossing (open pollination) occurs to a considerable extent either at intraspecific or interspecific level creating variability in the open population. This is one of the reasons that open pollinated varieties rapidly get deteriorated depending on insect vector population in the cotton ecosystem. An excellent example of natural crossing

in cotton is available, i.e. Acala 1517 lines of upland cotton in the United States got tolerance to wilt and excellent fibre properties through natural outcrossing with *G. barbadense* (Harrison 1950). In India, hybrid between *G. hirsutum* (tetraploid) and *G. arboreum* (diploid) is reported to have been resulted from natural outcrossing at Sirsa.

Hybridization breeding procedures in cotton slightly differ from the self-pollinated crops. Artificial hybridization to combine the parental traits in cotton is explored in two ways, i.e. first, development of varieties through pedigree and backcross breeding and second through developing F_1 hybrids to exploit heterosis.

11.13.3.1 Pedigree Procedure

This is generally followed to select superior genotypes from the segregating generations. Segregating populations of single cross, double cross, three way and multiple crosses can be handled following pedigree scheme to develop new varieties. In cotton, the selection may be terminated at early stages (before attaining complete homozygosity) so as to retain residual heterozygosity which is considered desirable in cotton. Using the pedigree method, the majority of cotton varieties have been developed for commercial cultivation in India.

11.13.3.2 Backcross

This refers to crossing of F_1 hybrid to either of its parents. Backcross breeding, a system of repeated backcrossing, is used to transfer genes for disease or insect pests' resistance or other simply inherited characters to the well-adapted varieties. The variety developed through this method resembles more to the recurrent parent variety except for the character transferred. This method is very effective even to transfer male sterility or male fertility restorer genes or chromosome transfer from one compatible background to another. In cotton, several desirable traits have been transferred through backcrossing from wild and cultivated species in elite varieties.

11.13.4 Recurrent Selection

Recurrent selection refers to the reselection of superior plants generation after generation with intermating of selected plants to produce population for the next cycle of selection. The essence of this method is to accumulate desirable alleles in the breeding population through cyclic selection and intermating of superior individuals from the population subjected to recurrent selection. The source population may be created by crossing among a group of genotypes, varieties, breeding lines or exotic germplasm with distinct desirable trait. If the number of genotypes involved to develop source population is large, crossing each genotype in all possible combinations becomes more tedious, time and resource consuming. Moreover, natural crossing may occur only on a limited scale depending on natural insect pollinators. An extent of natural cross-pollination may be augmented by introduction of male sterility genes, especially genetic male sterility, into the population. Once the source population is created, to operate recurrent selection involves three steps:

1. Identification of superior single plant with improved desired trait(s)
2. Evaluation of selected plant progenies
3. Recombination of superior progenies to form the next cycle

In cotton, recurrent selection has been used to a very limited extent. However, it is a very useful and effective method to achieve improvement in cotton yield and fibre quality traits simultaneously. For instance, three cycles of recurrent selection increased seed cotton yield by 29.7% in upland cotton (Miller and Rawlings 1967a, b), while the same number of recurrent selection cycles increased lint percentage from 33.8% to 38% in upland cotton (Meredith and Bridge 1973).

11.13.5 Biparental Mating

The concept of biparental mating was originally developed by Comstock and Robinson (1948, 1952). It refers to crossing among randomly selected plants in F_2 or subsequent generations of a cross in a definite fashion. Biparental mating serves the purpose of releasing additional variability due to opportunity of more recombination to occur. It also helps in breaking undesirable linkages and concentrating favourable genes in the population. Singh et al. (1989) reported breakage of negative association between boll number and boll weight in cotton. This approach has rarely been used in cotton though it is very effective in reducing linkage drag and realizing promising transgressants in interspecific crosses between *G. hirsutum* and *G. barbadense* (Palve et al. 2020).

11.13.6 Mutation Breeding

Mutation breeding has successfully been used in cotton and several other crops to induce genetic variability for qualitative and quantitative characters. As early as in 1911, perhaps for the first time, nectariless mutant was reported by Leake (1911) in Asiatic cotton. In India, Ramaih and Bholanath (1946) were the first to report induced mutation for ginning percent and fibre length in American cotton; and consequently, the first induced mutant variety Indore-2 was released in the same year. Through the use of X-ray irradiation, a drought-resistant mutant, MA 9, was identified from variety Co.2 of American cotton by Doraisamy and Iyenger (1948). A jassid-resistant mutant was obtained through 48 kr X-ray irradiation of dry seeds by Jagathesan et al. (1963). An early maturing mutant, MCU 7, with higher yield and increased spinning performance was obtained through X-ray irradiation and released for cultivation in Tamil Nadu in 1971 (Selvaraj 1976). Among the noted useful mutations, a photo-insensitive mutant from MCU 5, a photosensitive variety, was obtained through gamma-ray irradiation and released as variety 'Rashmi' in 1976. Of the 16 varieties developed through induced mutations in cotton, 8 were developed in India and released for commercial cultivation. Mutation breeding, although not a preferred method over the other methods of breeding, however, it has distinct

advantages of creating enormous variability for the numerous traits and even for the traits not existing in the germplasm.

11.13.7 Heterosis Breeding

Heterosis is the increase in size or vigour of first-generation hybrids over its parents. The term 'heterosis' was first proposed by Shull to denote the simulation in size and vigour in a hybrid as an expression of heterozygosis. The terms 'heterosis' and 'hybrid vigour' are synonymous and most often used interchangeably. Hybrid vigour of first-generation hybrids in cotton was first observed by Mell (1894) for fibre length and agronomic traits. Cook (1909) suggested the possibility of commercial exploitation of heterosis in cotton. Several researchers observed high heterosis in F_1 hybrids obtained between the distantly related parents than the closely related ones. In cotton, high heterosis has been reported in both inter- and intraspecific crosses in diploids and tetraploids.

The first successful attempt to exploit heterosis was made at Cotton Research Station, Surat, India, and the first commercial hybrid 'H4' was released in 1970 (Patel 1971). The hybrid 'H4' was obtained from a cross between two *hirsutum* parental lines/varieties (intraspecific hybrid) Gujarat 67 (G 67) and American nectariless (an exotic line from the United States) which recorded 137% heterosis over the better parent. Subsequently, in 1972, an interspecific hybrid, Varalaxmi, involving *G. hirsutum* cv. Laxmi and *G. barbadense* cv. SB 289E was released from Cotton Research Station, Dharwad, India (Katarki 1972). Both the hybrids H4 and Varalaxmi became very popular among the farmers in Central and South India. The success of both the hybrids provided significant momentum for hybrid research, and later several intraspecific (intra-*hirsutum* and intra-*arboreum*) and interspecific (*G. hirsutum* × *G. barbadense* and *G. arboreum* × *G. herbaceum*) hybrids have been released for commercial cultivation in India.

Production of hybrid seed by hand emasculation and pollination (Doak method) is successfully practised on a large scale in India. However, huge requirement of labour for manual emasculation and pollination makes this procedure economically unfeasible in countries where labour cost is high. The alternative to Doak method of seed production is the use of genetic or cytoplasmic genetic male sterility.

11.13.7.1 Genetic Male Sterility (GMS)

There are several known sources of genetic male sterility in cotton. Sixteen different genes controlling genetic male sterility in tetraploid cottons (13 in *G. hirsutum* and three in *G. barbadense*) and two in *G. arboreum* have been identified (Meshram et al. 1994; Singh and Kumar 1993). Sterility is conditioned by dominant alleles at five loci, viz. MS_4, MS_7, MS_{10}, MS_{11} and MS_{12}, and by recessive allele at other loci, viz. ms_l, ms_2, ms_3, ms_{l3}, ms_{l4} (Dong A), ms_{l5} (Lang A) and ms_{l6} (81A). Genetic male sterility conditioned by duplicate recessive genes includes ms_5ms_6 and ms_8ms_9. The expression of male sterility among male sterility genes greatly varies in extent based on genotypic background. In India, Gregg male sterility source is extensively used.

Among them, the male sterility controlled by two recessive genes (ms_5ms_6) is preferred for transfer to the desired female parents by repeated backcrossing. The male sterile lines so produced can be maintained by sib-mating with the heterozygous male fertile sib segregating at only one locus (ms_5ms_5/Ms_6ms_6 or Ms_5ms_5/ms_6ms_6). The progeny of such male sterile and fertile sibs will segregate in a 1:1 ratio of sterile and fertile plants. From the progeny of male sterile line, the fertile plants may be identified at flowering and removed. The male sterile plants in a hybridization block can be manually pollinated with the pollens of desired male parent to produce hybrid seed. The use of genetic male sterility eliminates hand emasculation and thus reduces cost of hybrid seed production than by the Doak method.

11.13.7.2 Cytoplasmic Genetic Male Sterility (CGMS)

Interspecific hybrids are one of the most common sources of male sterility in crop plants. Meyer (1971, 1975) obtained a cytoplasmically controlled male sterility in *G. hirsutum* by transferring its genome to the cytoplasm of *G. harknessii* Brandegee. From the segregating progenies, fertility restoration lines (fertility restorer gene, *Rf*) were also identified from *G. harknessii* (Meyer 1975) and a gene that enhances fertility restoration (*E*) from Pima cotton (Weaver and Weaver 1977). The fertility restoration was attributed to two gene pairs (Meyer 1975), while a single gene shows partial dominance (Weaver and Weaver 1977). In India, Shroff (1980) transferred cytoplasmic male sterility of *G. harknessii* to *G. hirsutum* cultivars such as Khandwa 2, Bikaneri Nerma and GS23 and produced hybrids using Egyptian (*G. barbadense*) restorer lines. *G. anomalum* as source of CMS has been reported by Rhyne (1971); when *G. arboreum* genome is transferred to the cytoplasm of *G. anomalum*, stamina column becomes petaloid. Similar observations were made by Tayiab (1983) in a first backcross population of a cross cv. AK 235 (*G. arboreum*) × (*G. anomalum* × cv. AK 235) at Akola, Maharashtra. He has also identified male sterility restorer in *G. herbaceum* cv. V797, Nageri and Russian *herbaceum*. Identification of CMS and fertility restoration sources was considered as a significant development towards exploitation of heterosis and commercial scale cultivation of hybrid cotton. However, practical difficulties limit the utilization of male sterility in hybrid seed production: (1) lack of simply inherited restorer gene with stable expression over the environments, (2) lack of good combiners with CGMS and fertility restorer genes and (3) lack of controlled and effective pollination system for hybrid seed production.

11.14 Genomic Tools

11.14.1 Molecular Markers in Cotton

Molecular DNA-based markers can be classified in three categories based on their working mechanism, i.e. hybridization-based, PCR-based and sequence-based markers. The hybridization-based markers mainly include restriction fragment

length polymorphism (RFLP). RFLP markers are the first type of markers used in cotton genetic studies. It reveals the differences among individuals by variation in size of DNA fragments produced by restriction enzymes. PCR-based markers include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and inter-simple sequence repeats (ISSRs). The sequence-based markers include single nucleotide polymorphism (SNP) and genotyping by sequencing (GBS). Molecular markers serve as landmarks in the genome of an organism effectively used to differentiate one from another. The above DNA-based markers have effectively been used in cotton for characterization of genetic resources, genetic diversity and DNA fingerprinting (Rana et al. 2007; Sapkal et al. 2011), linkage and QTL mapping for important economic traits (Reinisch et al. 1994; Rong et al. 2004; Waghmare et al. 2005), genome-wide association studies (GWAS) (Abdurakhmonov et al. 2009; Edwards and Batley 2010) and marker-assisted selection (MAS) (Guo et al. 2003; Fang et al. 2010).

The recent advances in sequencing technologies have resulted in the development of huge genomic resources for cotton over the last two decades. The publicly available resources include SSR (109837), SNP (459825), RFLP (4576), RAD (3984), AFLP (1962) and other scores of markers for genetic studies in cotton (Yu et al. 2014). To facilitate high-throughput genotyping of the breeding populations, few SNP arrays such as TAMU CottonSNP63K array (Hulse-Kemp et al. 2015), NAU 80K SNP array (Cai et al. 2017) and 40K SNP array (Sawant et al. CSIR-NBRI, Lucknow, India, unpublished) have been developed and extensively used for genetic mapping and marker-assisted breeding programmes. Using these genetic resources, about 119 genetic linkage maps involving intraspecific and interspecific populations have been developed, and more than 6497 quantitative trait loci (QTL) representing more than 30 agronomically important traits were mapped on specific chromosomes.

11.14.2 Genetic Diversity

Understanding genetic relationship and extent of genetic variation among genotypes is important to use them in the improvement programmes. Genetic diversity based on the molecular markers gives insights into the genetic relationship and structure of a population. A narrow genetic base is reported to be the reason for stagnation and decline in global cotton yield. The genetic diversity in cotton using different molecular markers by several workers has been reported (Tatineni et al. 1996; Saha et al. 2003; Rana et al. 2007; Rakshit et al. 2010; Sapkal et al. 2011). SSRs have also been used to assess the genetic purity of the cotton hybrids (Selvakumar et al. 2010). The SNPs as markers have also been used to detect genetic variations within and between the species of cotton (Van Deynze et al. 2009).

11.14.3 Linkage and QTL Mapping for Economic Trait

All types of DNA markers can be used for constructing a linkage map. However, the codominant markers (SSR, SNPs) are more informative and detect heterozygous condition that may be preferred over the dominant markers (RAPD). Different types of populations such as F_2, backcross populations Bc_1 and Bc_2, backcross inbred lines (BILs), recombinant inbred lines (RILs) and multi-parent advanced generation intercross (MAGIC) populations are commonly used in genetic mapping. The first genetic linkage map of cotton was constructed by Reinisch et al. (1994) using RFLP markers which included 705 polymorphic loci in 41 linkage groups and a total map length of 4675 cM. Afterwards, several genetic linkage maps were constructed (Shappley et al. 1998; Jiang et al. 1998; Zuo et al. 2000; Ulloa et al. 2002; Zhang et al. 2002; Rong et al. 2004; Waghmare et al. 2005; Yu et al. 2012) with different marker densities.

RFLPs have been widely used to map genes of economic interest in cotton. An RFLP map of *G. hirsutum* and *G. barbadense* was used to map 14 QTLs for fibre-related traits (Jiang et al. 1998). Genes influencing density of stem and leaf trichomes (Wright et al. 1998), low seed gossypol contents in seeds and high gossypol in plant (Vroh Bi et al. 1999), plant adaptation traits including leaf chlorophyll contents (Saranga et al. 2001) and leaf nectaries (Waghmare et al. 2005) were mapped on an RFLP map developed using F_2 interspecific populations. Using $F_{2:3}$ population, 26 QTLs were identified for agronomic and fibre quality traits (Ulloa et al. 2002). In a backcross population of *G. hirsutum* and *G. barbadense*, 28 QTLs for fibre length, nine for length uniformity and eight for short fibre contents were mapped (Chee et al. 2005) using F_2-based RFLP map.

Shen et al. (2005) conducted an extensive SSR genotyping of F_2 populations from three diverse upland cotton genotypes using 1378 markers and identified 39 fibre-related QTLs. Using recombinant inbred lines (RILs), Wang et al. (2006) reported several QTLs related to plant architecture, yield and fibre quality in upland cotton. Qin et al. (2008) used four-way cross populations developed from the 4 inbred lines of *G. hirsutum* to map 31 QTLs linked to the yield and fibre quality traits. The genetic map developed using two F_2 populations with 2072 loci covering 3380 cm was used for QTL analysis, which detects 54 QTLs linked to early maturity (Li et al. 2013). QTL mapping using different genotypes and mapping populations phenotyped in varying environments yield heterogeneous results. Rong et al. (2007) compiled 432 QTLs mapped in one diploid and ten tetraploid interspecific cotton populations, aligned using a reference map and depicted in a cMap resource. The meta-analysis resulted in more complete picture of the genetic control of a trait and trait variation. It supported the hypothesis of non-fibre-producing diploid ancestor contributed to tetraploid lint fibre gain, and both sub-genomes contribute QTL at largely non-homeologous locations, suggesting divergent selection acting on many corresponding genes before and/or after polyploid formation. Several studies dealing with QTL mapping in cotton are available, and more than 6497 QTLs available in CottonGen database (https://www.cottongen.org/) promise the future strategy for marker-assisted breeding (Yu et al. 2014).

11.14.4 Genome-Wide Association Studies (GWAS)

Genome-wide association study involves rapidly scanning markers across the complete sets of DNA, or genomes, of several individuals to find genetic variations associated with a particular trait. Association mapping, also referred to as linkage disequilibrium (LD) mapping, is effectively being used to determine the variation in complex traits in cotton. With the availability of multiple cotton genome sequence data (Wang et al. 2012a, b; Paterson et al. 2012; Zhang et al. 2015), a large number of SNP markers have been identified at the whole-genome level in cotton. In association studies, natural (nonstructured) populations are phenotyped and genotyped to identify the trait associated with marker (Barnaud et al. 2006) so as to capture natural allelic variation (Huang and Han 2014). In cotton, huge genetic diversity conserved is available in the worldwide collections of cotton germplasm. Using association studies, many QTLs and candidate genes associated with fibre quality have been identified (Yu et al. 2013). Though tetraploid genome size is large, considering the total map length of 5200 cm, Abdurakhmonov et al. (2009) worked out about 1000 polymorphic markers for successful and reliable association mapping in cotton.

Sun et al. (2017a) performed a GWAS for fibre quality traits using 719 diverse accessions of upland cotton and 10,511 polymorphic SNPs using the CottonSNP63K array and identified 46 significant SNPs associated with five fibre quality traits. In a combined GWAS and transcriptome analysis, they could identify 19 promising genes related to FL and FS (Sun et al. 2017b). Using a natural population containing 503 *G. hirsutum* accessions through CottonSNP63K genotyping, Huang et al. (2017) detected 79 significant SNPs associated with fibre quality traits. Ma et al. (2018) resequenced a core collection comprising of 419 accessions. Phenotyping was done across 12 environments and three genes associated with fibre quality traits were identified using the association mapping, two GhFL1 and GhFL2 for FL, and Gh_A07G1769 for FS through transgenic experiments in *Arabidopsis*. Liu et al. (2020) identified 42 single nucleotide polymorphisms (SNPs) and 31 QTLs associated with five fibre quality traits in a genome-wide association study. They also identified two pleiotropic SNPs, SNP locus i52359Gb and SNP locus i11316Gh, for fibre traits.

11.14.5 Marker-Assisted Selection (MAS)

Plant breeders select plants which appear phenotypically more promising for the desired traits. Most of the economic traits are controlled by polygenes with complex non-allelic and environmental interactions. In some instances, the same genotype or specific locus associated with QTL may not be detected (Edwards et al. 1987). In such instances, tightly linked loci associated with desired trait, if linked with molecular marker, can effectively support identification of genotypes for specific phenotype. Thus, in marker-assisted selection (MAS), a phenotype is selected on the basis of genotype of a marker (Collard et al. 2005). In the segregating population,

selection of plants with desired gene combinations is important in plant breeding to realize the desired performance. On the identification of tightly linked markers to the genes of interest, breeders may use the known identified DNA marker to select the plants carrying the gene(s)/QTL (Young 1996). The effectiveness of MAS is majorly dependent on marker types and size of the population being handled.

Application of MAS for the traits with high heritability such as disease resistance is more effective. Yield-related components are polygenic in nature with low heritability, which is a major challenge for the utilization of MAS (Elshire et al. 2011). Several QTLs have been identified for seed cotton yield, fibre quality, plant architecture, resistance to diseases such as bacterial blight and *Verticillium* wilt (Bolek et al. 2005), resistance to pests like root knot nematode and flowering date (Voss-Fels and Snowdon 2015) as well as for abiotic stresses (drought, salt tolerance) (Elshire et al. 2011; Wang et al. 2015) in different populations. This provides an evidence of putative loci related to specific traits. But these identified QTLs, through either linkage mapping or association mapping-based approaches, have low resolution and do not serve the purpose to be used for MAS unless QTL regions identified by flanking markers are subjected to high-density genetic mapping which is a prerequisite for MAS. A composite fine-mapping approach will help to dissect the markers close to the target genes and enhance the reliability of MAS. The genomic sequences of cotton provide precious resources to develop high-density SSR- or SNP-based genetic maps. Establishing linkage between phenotypic and genotypic interactions, the identification of stable QTLs lays a basis for fine mapping.

Zhang et al. (2003) identified a major QTL for fibre strength, QTL(FS1), found to be associated with eight markers that explained more than 30% of the phenotypic variation. QTL(FS1) was mapped to chromosome 10, and the markers linked to this QTL could be used in MAS. RAPD markers were converted into sequence characterized amplified region (SCAR) markers to screen the BC_1F_4 population and successfully used, i.e. SCAR 1920 marker, for improvement of fibre strength and marker-assisted selection (Guo et al. 2003). The cotton blue disease (CBD) controlled by single dominant gene was mapped in the telomere region of chromosome 10. Screening of the SNP markers laid to identification of three SNP markers associated with CBD which were employed to efficiently tag a trait enabling MAS for high levels of blue disease resistance in cotton (Fang et al. 2010).

Recently, two genomic loci for lint yield (ghlyi-A02 and ghlyi-D08) were identified with the mix of GWAS and gene-based association that provided a quick method to identify the candidate genes associated with fibre quality (Fang et al. 2017a, b). Ma et al. (2018) characterized genes related to fibre length (*ghfl1* and *ghfl2*) and fibre strength (Gh_A07G1769), which may be further used for MAS. Zhang et al. (2018a) analysed linked SSR marker BNL3232 in the F_2 segregating population of upland cotton following bulked segregant analysis (BSA) and found that one SNP locus was closely associated with the fruiting branches trait. They verified that this SNP marker could be used for molecular-assisted selection of cotton architecture. Kushanov et al. (2017) claimed to have developed two varieties possessing higher fibre strength and improved length, namely Ravnaq-1 and

Ravnaq-2. Ravnaq-1 has superior fibre quality with improved fibre strength (37 g/tex) and staple length (38 mm) compared to its recurrent parent Andijan-35 (32 g/tex and 35 mm staple length). Similarly in Ravnaq-2, molecular markers effectively mobilized superior fibre quality loci that improved fibre strength by 17% and staple length by 9% compared to its recurrent parent Mekhnat. They concluded that the markers and donors have been proved to be useful in MAS to obtain superior cotton cultivars.

11.14.6 Genome Sequencing

In view of the economic importance of cotton, intensive efforts were made by the cotton community to uncover the genome mysteries of cotton species. For sequencing the cotton genome, the smallest D genome of *G. raimondii* (D5) was selected, and a first draft genome assembly was published simultaneously in 2012 (Paterson et al. 2012; Wang et al. 2012a). Subsequently, in the last one decade, all four cultivated species of cotton and about 11 wild diploid species have been sequenced; and sequence data and several thousands of annotated protein-coding genes have been made available (Paterson et al. 2012; Wang et al. 2012a, 2019; Li et al. 2014a, b, 2015; Zhang et al. 2015; Yuan et al. 2016; Du et al. 2018; Udall et al. 2019; Cai et al. 2019; Hu et al. 2019; Grover et al. 2019, 2020; Chen et al. 2020; Huang et al. 2020). The availability of the number of whole cotton genome sequence data provides a major source of candidate genes with potential for genetic improvement of cotton quality and productivity. The integrated whole-genome sequence data and marker data have been made available on CottonGen database (Yu et al. 2014) for easy accessibility and retrieval including search and online analysis tools.

The ever-increasing information of DNA sequencing of different *Gossypium* genomes enables the discovery of genes, their variants and new markers associated with different traits, opening new avenues for crop improvement (Edwards and Batley 2010). Sequencing of new *Gossypium* genomes allows comparing the extent of structural and sequence variation among the genomes and displaying the spectrum of diversity. The genomes of cultivated diploid and tetraploid cottons have been repeatedly sequenced so as to obtain complete and fine sequences (Pan et al. 2020). The comparative genome sequence data sets are likely to reveal the evolutionary history and insights into the formation of present-day cultivated cotton. Repeated sequencing of genomes is expected to address issues of genome instability involving structural changes, gene loss, DNA inversion, translocation, illegitimate recombination, accumulation of repetitive sequences and changes related to the functional evolution of genes. The whole-genome sequences have paved the way to identify and clone functional genes (Pan et al. 2020) and to enhance breeding efforts to develop cotton to produce high yield, superior quality fibres and resisting effects of climate change.

11.14.7 Genotyping by Sequencing (GBS)

Advances in DNA sequencing technologies enabled researchers to rapidly develop large numbers of SNP markers at a relatively low cost. The next-generation sequencing (NGS) technologies have successfully been used for whole-genome sequencing (Paterson et al. 2012; Wang et al. 2012a, b; Li et al. 2014a), gene expression analysis (Naoumkina et al. 2014) and SNP discovery in cotton (Byers et al. 2012; Gore et al. 2014). Among the methods used to discover SNPs, a robust and simple approach is GBS, which facilitates the detection of a wide range of SNPs using several individuals simultaneously. The GBS protocols usually use methylation-sensitive restriction enzymes (RE) to get reduced representation of the genome by targeting the genomic sequence flanking RE sites (Elshire et al. 2011; Poland et al. 2012). The approach of reduced representation and restriction site-associated DNA construction of GBS library has been simplified, made extremely specific, highly reproducible, needing less DNA, with targeted lower copy regions and two- to threefold higher efficiency. The procedure is completed in only two steps on plates, followed by polymerase chain reaction (PCR) amplification of the pooled library (Elshire et al. 2011). With the above approach, GBS can be utilized in any polymorphic species or any segregating population with any number of individuals (Schnable et al. 2013). Genotyping by sequencing (GBS) is a rapid way to identify single nucleotide polymorphism (SNP) markers; however, these SNPs may be specific to the sequenced cotton lines (Islam et al. 2015). GBS technique has been modified and improved to a large extent (Baird et al. 2008). The restriction enzymes that cut upstream and downstream of target site (Wang et al. 2012a, b) allows marker intensity adjustment by producing same length tags and analysis of about all the restriction sites.

Genotyping by sequencing has several potential applications that include gene pool maintenance, diversity analysis, genomic selection, gene mapping and other plant improvement methodologies (Elshire et al. 2011). GBS is cost-effective for studying populations in association mapping and further to employ genomic selection on a large scale (Poland and Trevor 2012). In cotton, Gore et al. (2014) detected SNPs in *G. hirsutum* RIL population by GBS and constructed SSR-SNP linkage map for mapping ten agronomic and fibre traits. Fan et al. (2018) constructed one of the first genetic maps using 3557 GBS SNPs spanning a total genetic distance of 3076.23 cM in a RIL population of *G. barbadense* and identified 42 QTLs for the fibre quality and lint yield traits. A BC_2F_2 population, involving *G. tomentosum* and *G. hirsutum* as the recurrent parent, was genotyped through genotyping by sequencing (GBS) wherein 10,888 SNPs were generated and used to construct a genetic map (Magwanga et al. 2018).

Analysis of serine/threonine protein kinases through miRNA targets in the segregating subfamilies revealed that most of the genes were involved in enhancing abiotic stress tolerance. Further analysis and qRT-PCR validation revealed 16 putative genes, which were highly upregulated under drought stress condition, and were found to be associated with NAC (NAM, ATAF1/2 and CUC2) and myeloblastosis (MYB), the known stress tolerance genes. Ahmed et al. (2020) reported significant

QTLs associated with leaf and stem pubescence in F_2 intra-*hirsutum* population and the response of plant under pest (aphid) infestation. The putative genes were co-localized on chromosome A06 governing mechanism for trichome development and host-pest interaction. The identified GBS SNP markers may be explored for marker-assisted breeding to develop sucking pests' resistant cotton cultivars.

In a study to determine genetic structure and relationship of *G. hirsutum* races, following GBS and phylogenetic analysis revealed that the Latifolium, Richmondii and Marie-Galante race accessions were more genetically related to the *G. hirsutum* cultivars (Zhang et al. 2019). Further, three SNPs were identified located in genes related to the processes of plant responding to stress conditions which were confirmed through genome-wide signals of marker-phenotype association analysis.

11.15 Transgenic Cotton

'Transgenic' refers to the introduction of one or more genes or DNA sequences from another species by artificial means. The recombinant DNA technology allows the transfer of genetic material across a wide range of species and has removed the traditional limits of cross-breeding. It involves transfer of desired genes into the plant genome through genetic transformation and then regeneration of a whole plant from the transformed tissue/cell. For successful development of transgenic plants, the essential requirements include an efficient transformation protocol for the specific crop species, transformation vector and gene to be transferred and suitable target tissues. Various transformation methods such as *Agrobacterium*-mediated gene transfer, particle bombardment (biolistics) method (Klein et al. 1987) and in planta pollen tube transformation (Kalbande and Patil 2016) are being followed to transfer genes into cotton genome. *Agrobacterium*-mediated transformation is a widely used and reliable method of transformation that involves co-cultivation of explants with *Agrobacterium* culture. Cotton is a recalcitrant crop to regenerate from in vitro tissue cultures. Genotype-dependent genetic transformation is known in cotton. Coker genotypes, namely Coker 312 and Coker 201, or its introgressed lines (United States), YZ-1, Simian-3, CRI 24 (in China) and Siokral 1–3 (Australia), are amenable for regeneration in vitro by somatic embryogenesis and are widely used in cotton genetic transformation (Leelavathi et al. 2004). However, the genetic transformation frequency is very low.

The first transgenic cotton was produced by transferring *Cry1Ac* gene encoding crystal toxin protein from the soil bacterium *Bacillus thuringiensis* which is harmful to lepidopteran insects. The genetically engineered cotton with gene from *B. thuringiensis* was popularly referred to as Bt cotton. Bt cotton was first approved for field trials in the United States in 1993 and then for commercial use in 1995. Subsequently, it spread to several cotton-growing countries. Bollgard II, with two genes *Cry1Ac* and *Cry2Ab*, was introduced in 2003 representing the next generation of Bt cotton. So far, some 67 transgenic cotton events carrying insect and herbicide resistance with two to three genes have been released for general cultivation in various parts of the world (Yu et al. 2014).

Development of GM cotton to control bollworms in cotton was more relied on toxin producing *cry* genes from *B. thuringiensis*. Several variants of Bt genes have been introduced to cotton and reported their efficacy on target pests, viz. *H. armigera*, *P. gossypiella* and *Spodoptera litura*. Silencing of CYP6AE14 gossypol detoxifying enzyme in insect midgut using GM cotton RNAi lines for *cyp6ae14* gene is observed to reduce the survivability chance of bollworms by the action of gossypol in cotton plants (Mao et al. 2011). *Amaranthus caudatus* agglutinin (ACA) (Wu et al. 2006), *Allium sativum* agglutinin (ASAL) (Vajhala et al. 2013), *Galanthus nivalis* agglutinin lectin (GNA) (Liu et al. 2013) and insecticidal Tma12 gene from *Tectaria macrodonta* (Shukla et al. 2016) reported their efficacy against target sucking pests of cotton.

The transgenic technology has also been explored for improvement of economic yield and fibre quality traits of cotton. Spatio-temporal regulated expression of the auxin biosynthesis gene *iaaM* (Zhang et al. 2011), sucrose synthase gene from potato (Xu et al. 2012) using FPB7 and S7 promoter, respectively realized 15–30% higher yield compared to control. An increased fibre yield and quality is reported due to overexpression of sucrose synthase *GhsusA1* from superior quality germplasm line (Jiang et al. 2012). Knockdown lines of phytochrome *PHYA1* (Abdurakhmonov et al. 2014b) and overexpression of *PHYB* (Rao et al. 2011) were reported to improve fibre yield (10–35%), quality and agronomic traits of cotton. Significant improvement in fibre strength and micronaire in all transgenic lines having higher expression of the expansin gene was reported due to the action of *CpEXPA3*, an expansin gene from *Calotropis procera* in cotton (Bajwa et al. 2013). A number of actin-binding proteins, viz. *GhADF1* (Wang et al. 2009) and *WLIM1a* (Han et al. 2013), participate in the regulation of actin cytoskeleton dynamics and are reported to be associated with the regulation of fibre quality traits in cotton. Overexpression of *WLIM1a*, a LIM domain protein of elongation and secondary wall synthesis stages, improved fibre strength and fineness traits through modulation of actin cytoskeleton dynamics and transcription factor for lignin biosynthesis (Han et al. 2013). Modification in fibre properties was also reported in transgenic cotton lines expressing *GhXLIM* 6 in fibre development and mainly attributed to dual role of the target protein on F-actin cytoskeleton and transcriptional regulation of cellulose biosynthesis (Li et al. 2018).

The transgenic approach for improvement of abiotic stress tolerance traits utilizing gene sourced from different species is reviewed in different crop species including cotton (Ullah et al. 2017; Mahmood et al. 2020). The heterologous expression of genes coding for transcription factors, viz. NAC transcription factor (*SNAC1*) (Liu et al. 2014), *StDREB2* (El-Esawi and Alayafi 2019), *AtEDT1/HDG11* (Yu et al. 2016), membrane transporters *AtNHX1* (He et al. 2005), *AtAVP1* (Pasapula et al. 2011), *TsVP1* (Lv et al. 2008, 2009), ROS scavenging system and osmotic regulation *ScALDH21* (Yang et al. 2016), *NtOsmotin* gene (Parkhi et al. 2009) enhanced drought and salt tolerance and agronomic performance in transgenic cotton compared to control. The ultralow gossypol in cotton seed is found to be associated with reduction of seed-specific *δ cadinene synthase* expression by utilizing the seed-specific alpha globulin promoter. The low gossypol transgenic

event TAM66274 is approved for commercialization in the United States in 2018 (Sunilkumar et al. 2006; Rathore et al. 2020).

Bt cotton is currently occupying the majority of the area under cotton. It occupies about 85% of the total cotton area in the United States, more than 90% in India and Pakistan and above 65% in China (Anderson and Rajasekar 2016). Success of Bt cotton varies across the countries. Bt cotton has greatly impacted on the cotton cultivation in India. The cotton area increased from 9 million ha to about 13 million ha, and the country's production has almost doubled. The technology has reduced the pesticide usages, reduced yield losses and increased the profitability of the farmers. However, field-evolved resistance of pink bollworm against Bollgard and Bollgard II cotton has been reported in the United States (Dennehy et al. 2002), China (Tabashnik et al. 2012) and India (Dhurua and Gujar 2011; Naik et al. 2018). Sucking pests (whitefly and jassids) have emerged as major pests demanding more attention and resources for management after PBW. For effective working of the transgenic technology, newer gene sources conferring resistance to PBW and sucking pest need to be involved and stacked to provide durable resistance.

11.15.1 Bt Cotton in India

Genetically modified (GM) cotton was developed as an alternate strategy to the previously used hazardous concoction of insecticide mixtures to circumvent bollworm problem in cotton. Bt cotton was the first of GM technologies introduced in India in 2002 as Bollgard (*cry1Ac* gene—Mon531 event) and in 2006 as Bollgard II (*cry1Ac* + *cry2Ab* genes—Mon15985 event). Indian farmers preferred Bt cotton instead of the hazardous insecticide cocktails for bollworm control. With continued higher adaption, Bt cotton is being grown on over 90% of cotton area. Bt cotton effectively controlled bollworms, especially the American bollworm, *Helicoverpa armigera*. Yields are estimated to have increased at least by 30% due to effective protection from bollworm damage. The biggest gain from Bt cotton was in the form of reduced insecticide usage from 46% to less than 20%. Insecticide usage for bollworm control decreased from 9410 metric tonnes (worth INR 747.6 crores) in 2001–2002 to 222 metric tonnes (worth INR 96.3 crores) in 2011–2012. The intensity of bollworms reduced significantly on cotton and also on other host crops. The number of pesticide sprays in cotton declined rapidly from 15 to 20 applications to very few. Protection of early fruiting parts resulted in earliness and determinate habit eventually leading to fewer pickings, reduced labour, improved quality, better price and possibility of second crop after cotton.

In contrast to other countries, Bt technology was introduced in India exclusively in the form of Bt hybrids. Area under cotton (from 76 to >129 lakh ha) and yields (from 300 kg to >500 kg lint/ha) increased after adoption of Bt cotton hybrids in India. This productivity enhancement achieved was said to be a combined result of exploitation of heterosis (hybrids) and protection from Bt technology. Despite more than 90% area under Bt hybrids, the cotton productivity in India is lowest than world average (>750 kg/ha) and remains stagnated at around 500 kg lint/ha. The reasons

for low productivity and stagnation in yield include deployment of long duration Bt hybrids, less genetic diversity in cultivated hybrids and cultivation of cotton in over 60% area under rain-dependent situation. The majority of Bt hybrids are long duration that suffer moisture stress at boll formation stage due to poor water retention of shallow soils in rainfed regions. Productivity enhancement in India can come from yield improvement in rainfed ecosystems through deployment of climate-resilient Bt cotton varieties.

11.16 Genome Editing in Cotton: CRISPR/Cas

Precision in transfer and editing of the target gene is of more significance in the genetic manipulation of crop plants. Engineered nucleases, viz. zinc finger nucleases (ZFNs), meganucleases, transcription activator-like effector nucleases (TALENs) and recently introduced clustered regularly interspaced short palindromic repeats-CRISPR-associated system (CRISPR-Cas)-mediated genome editing, have been successfully deployed in different crop species. The intrinsic versatility, simplicity and high efficiency of CRISPR/Cas9 have resulted in an explosion of research using genome editing as the preferred method to generate precise alterations in the genome of numerous plant species. CRISPR/Cas9 derives from a microbial adaptive immune system, and its major components are the Cas9 nuclease capable of producing double-strand breaks and a small guide RNA (sgRNA) which directs the Cas9 protein to the target site. A number of factors influence the efficiency of the CRISPR/Cas9 system with strong expression of Cas9 and sgRNA being essential to obtain high mutation rates (Jiang et al. 2013).

The first report of genome editing in cotton was based on the re-engineered meganuclease for herbicide and insect resistance in cotton (D'Halluin et al. 2013). Janga et al. (2017) reported successful targeted knockdown of single copy reporter green fluorescent protein (GFP) gene in cotton genome using CRISPR-Cas. Gao et al. (2017) developed a fast and efficient method and validated the functionality of *sgRNAs* in cotton using three different genes, *GhPDS*, *GhCLA1* and *GhEF1*, and a transient expression system. They demonstrated that multiple gene targeting can be achieved in cotton with simultaneous expression of several sgRNAs. Targeted mutagenesis of Cloroplastos alterados 1 (*GhCLA1*) and vacuolar H^+-pyrophosphatase (*GhVP*) genes (Chen et al. 2017), *GhMYB25-like* (Li et al. 2017) and *GhALARP*, a gene encoding alanine-rich protein (Zhu et al. 2018) in both A and D sub-genomes of tetraploid *Gossypium hirsutum* cotton with no traceable off targets, demonstrated the possible utilization of tool for precision genome editing in cotton.

The CRISPR/Cas9 system was successfully utilized to generate two sgRNAs in a single vector for multiple sites genome editing using Discosoma red fluorescent protein2 (DsRed2) and an endogenous gene GhCLA1 in allotetraploid cotton (Wang et al. 2018). Long et al. (2018) optimized the cotton CRISPR/Cas9 system to achieve vastly improved mutagenesis efficiency by incorporating an endogenous GhU6 promoter that increases sgRNA expression level six to seven times higher and

mutation efficiency four to six times over the *Arabidopsis* AtU6-29 promoter. Recently, a variant of CRISPR-Cas (Cas9 nickase) with base editing ability known as '*G. hirsutum*-Base Editor 3 (GhBE3) base-editing system' has been successfully demonstrated for their ability to create single base mutations in cotton (Qin et al. 2020). Ramadan et al. (2021) used pooled sgRNAs assembly and successfully targeted multiple genes; 112 plant development-related genes were knocked out via an optimized CRISPR/Cas9 system. All targeted genes were successfully edited with high specificity.

The *Gh14-3-3d*-edited plants free of T-DNA called transgene-clean editing plants through CRISPR/Cas9 significantly enhanced resistance to *Verticillium dahlia*, a serious pathogen in cotton (Zhang et al. 2018b). CRISPR/Cas9 knockout of the arginase gene significantly enhanced the number of lateral roots and the root surface area in both normal and nitrogen deficiency conditions (Wang et al. 2017) which may help in water and nutrient uptake and improve resistance to other abiotic stresses in cotton.

11.17 High-Throughput Phenotyping

Expression of plant phenotype is the function of genotype and environment interaction. Traditionally, plant phenotype has been used for selection and improvement of crop plants. Selection of stable phenotype, through experience, was the fundamental of breeding during early domestications of crops and until rediscovery of Mendel's laws of inheritance. Phenotyping on a large population of plants has traditionally been challenging, both time and resource consuming and sometimes destructive. Pfeffer (1887) was the first to use an apparatus for plant growth measurement. In the last two decades, several sensors, automation, quantitative data analysis and vision tools have been developed that have become pivotal for quantifying the plant traits with increasing throughput and accuracy (Fiorani and Schurr 2013). Application of non-destructive and non-invasive technology for plant phenotyping in high throughput started with the model plant *Arabidopsis* (Granier et al. 2006). Now, increasingly mobile and higher-throughput field phenotyping systems using ground- and aerial-based [satellite imagery and unmanned aerial vehicles (UAVs)] imaging with a variety of sensors become available that allowed breeders to monitor genotype performance in breeding plots and crop management.

In cotton, recent studies indicating the application of high-throughput phenotyping (HTP) using imaging techniques have successfully explored possibility to improve the efficiency of phenotyping. Li et al. (2016) introduced a region-based semantic segmentation method for infield cotton boll detection based on colour and texture features using two-dimensional (2D) colour images. The method was found superior and could also detect the boll opening stage automatically. However, the 2D image-based method has a limitation of plant occlusion, and image quality is significantly affected by variable illumination conditions in the field which limits automation in data processing (Li et al. 2014b). The use of 3D model-based method

over 2D digital image method for plant phenotyping is getting more prominence as they permit multiple morphological trait data recording simultaneously.

Jiang et al. (2016) developed and evaluated HTP using depth images for measurement of plant height that correlated (R^2 = 0.922–0.987) with the plant height measured manually and with accuracies of over 92%. Sharma and Ritchie (2015) tested automated measurements of plant height, ground cover fraction (GCF), normalized difference vegetation index (NDVI), and canopy temperature (Tc) using a ground-based platform under ten different irrigation levels and found high correlations with lint yield.

Revathi and Hemalatha (2012) proposed the use of image processing edge detection techniques and homogeneous pixel counting technique, neural network, for cotton foliar diseases. Using the pattern recognition algorithm called convolutional neural networks, Xu et al. (2018a) confirmed that the system developed for identifying and automatic counting of cotton flower was comparable with manual counting. Xu et al. (2019) demonstrated the application of aerial multispectral images captured by a multispectral camera on an unmanned aerial system for phenotyping of plant height, canopy cover, vegetation index and flower. McCarthy et al. (2010) succeeded in measurement of internode lengths using an infield machine vision system in upland cotton and observed that visual occlusion of the main stem nodes by foliage and variations in natural lighting conditions as principal reasons for internode lengths not being detected for every plant. An autonomous ground robot system equipped with real-time kinematics (RTK)-GPS system, inertial measurement unit and waypoint to count the number of cotton bolls was developed (Xu et al. 2018b). This study demonstrated that opened cotton bolls can be counted from 3D point cloud with less human intervention. Ritchie and Bednarz (2005) used a photosynthetically active near-infrared spectrometer to investigate the relationship of red edge-based NDVI and leaf area index and to quantify cotton defoliation. Results showed that spectral data based on red edge measurements can provide accurate defoliation estimates which could possibly improve defoliation efficiency.

Thermal images by an infrared thermal camera were used for an infield estimation of the water status of cotton under a range of irrigation regimes as a potential tool for irrigation scheduling (Cohen et al. 2005). Andrade-Sanchez et al. (2014) developed a field-based HTP platform with a set of sensors to measure canopy height, reflectance and temperature simultaneously collecting phenotypic data at a rate of 0.84 ha/h. Hansen et al. (2014) proposed the use of a time series to monitor the changes in growth characteristics of cotton over time. Wu et al. (2018) monitored the progression of cotton root rot based on the extracted NDVI time series profiles.

Light detection and ranging (LiDAR) technology provides an alternative approach for 3D plant model reconstruction. LiDAR is a remote sensing technology to measure the distance between the sensor and the object of interest by illuminating the object with a laser and analysing the time of flight (ToF). LiDAR is also a potential alternative to image-based methods for phenotyping morphological traits at plot or plant level under field conditions. French et al. (2016) and Sun et al. (2017a, 2018) prominently used LiDAR to scan cotton plants. Both the studies used a global

positioning system mounted on a tractor platform. French et al. (2016) succeeded in achieving high-resolution and low-distortion mapping of cotton heights, width, leaf area and boll counts. While multiple traits, viz. plant height, projected canopy area and plant volume, were simultaneously extracted from repeated measurements over the growing season (Sun et al. 2018).

With the extensive worldwide production of cotton and its importance as a natural fibre crop, the HTP offers greater potential in improving the accuracy, efficiency, speed and quality of data acquisition. However, cotton is grown on vast area, due to the heterogeneity of field plots and variations in environmental conditions in cotton production, area wide implementation of HTP has the limitation. It is expected that the future HTP systems with improved robustness, accuracy, effectivity and affordability will pave the way for its application in cotton production. Hence, HTP platforms that facilitate to capture the variability across spatial and temporal scales in cotton fields will be increasingly important.

11.18 Emerging Challenges at National and International Levels

Cotton is a sensitive and challenging crop for management. Cotton production across the world is constrained by high incidence of insect pests and diseases, weeds, soil salinity, soil degradation, drought stresses, heat and frequent climatic aberrations. These constraints may vary with cotton production regions, but all regions experience one or the other from mild to severe form. Extreme weather conditions during early and boll development stage present a major challenge for cotton production in most of the cotton-growing regions. Drought stress in rain-dependent areas affects the cotton yields to a larger extent. During the summer of 2019 in Alabama, at least one-third of the state was impacted by drought according to the US Drought Monitor. This unpredictable, extreme weather is changing the reproductive and feeding patterns of pests, i.e. tarnished plant bugs, which are moving from their wild host plants to the cotton crop earlier than expected. In the regions where planting is undertaken in summer months, germination of seeds and growth of seedlings get affected due to high temperature and heat. Soil salinity coupled with high temperature in irrigated areas of Northern India affects germination and seedling stand due to soil crust formation.

Among the major pests of cotton, in recent years, pink bollworm (*Pectinophora gossypiella*) has emerged as a major problem on Bt cotton in Southeast Asia. Field-evolved resistance of pink bollworm against Bollgard and Bollgard II cotton has been reported in the United States, China and India. Pink bollworm was effectively controlled in the United States following diverse strategies. However, it is causing menace and severe crop losses to the extent of 10–30% in India. Sucking pests (whitefly and jassids) have emerged as major pests on Bt cotton demanding more attention and resources for management. Whitefly is a significant pest in Northern Indian states of Punjab, Haryana and Rajasthan and also in Pakistan.

Among the major diseases, cotton leaf curl disease (CLCuD) caused by *Begomoviruses* is a major threat to cotton production in North India and Pakistan.

The disease is transmitted by insect vector whitefly (*Bemisia tabaci*); hence, its effective control is possible through management of whitefly. Tobacco streak virus (TSV), caused by *Ilarvirus*, is normally prevalent in southern states but emerging as a new challenge in central and northern states of India. Grey mildew (*Ramularia areola*), *Myrothecium* leaf spot and *Corynespora* leaf spot are the fungal diseases demanding timely interventions to contain spread and minimize losses. Recently, inner boll rot is an emerging problem in central and southern states during boll development stage. Fall armyworm (FAW) *Spodoptera frugiperda*, a pest of maize, has become an important pest of cotton in Brazil due to changes in cotton cropping systems. Since 2017, increasing migratory incidences of FAW on cotton were observed in India and have become a potential threat to cotton (Arya and Ahmed 2019).

Natural (mainly cotton) fibres face a stiff competition from synthetic or man-made fibres. Synthetic fibres are generally made from petrochemicals by a process known as polymerization, which involves combining monomers to make a long chain or polymer. These fibres are generally longer, stronger and durable and provide smooth and excellent finishing to the fabrics. At present, the worldwide consumption of different fibres includes 63% synthetic fibres, 25% cotton, 7% wood-based fibres and 5% other natural fibres (Garside 2021). Contrary to the world fibre consumption pattern, the annual consumption of total fibre is to the tune of 5 million tonnes with a synthetic fibre contribution of 40%, while natural fibres together contribute 60%. China is the largest producer of synthetic fibres contributing 66% of the world synthetic fibre production (Ruiz 2019). To increase the contribution of cotton fibre in textile fabrics, emphasis needs to be given on increasing strength and length of cotton fibres.

11.19 Breeding Progress/Varietal Development

11.19.1 Conventional Breeding

Conventional breeding has been the base for improvement of lint yield, fibre quality, adaptation and disease and pest resistance. Initial varieties grown in many of the countries were mixtures of several types due to cross-pollination that provided much needed genetic variability to operate selection. Later, diverse germplasm with adaptability to a wide range of environmental conditions facilitated the development of numerous varieties worldwide. The development of cotton that matures early and possesses enhanced host plant resistance received much attention from about 1970 until the mid-1990s (Bourland and Myers 2015). However, with the advent of transgenic Bt cotton and advancement of molecular tools, emphasis on traits has changed to high productivity inclusive of quality and resistance.

11.19.2 Status of Varietal Development in India

The history of cotton cultivation and its use in India puts the dating of early cotton to 5000 BC (Menon and Uzramma 2017). Until the middle of the nineteenth century, only the *G. arboreum* and *G. herbaceum* varieties of cotton were grown in different regions of the country. The first attempt of introduction of American cotton in India was made in 1790 when the seeds of Bourbon cotton from Malta and Mauritius were distributed in Bombay state, but failed. It was only in Hubli-Dharwar areas in the 1840s seeds from New Orleans were grown successfully. Dharwar American cotton soon became popular, and the acclimatized American upland Georgian cotton variety 'Buri' was released for the first time from Nagpur Farm in 1903–1904.

Scientific studies of cotton cultivation started only after the establishment of the agricultural departments in various provinces and princely states in 1904 and the Indian Cotton Committee (ICC) in 1917 at Bombay facilitated cultivation of long-staple cotton in India. This committee established the Indian Central Cotton Committee (ICCC) in 1921 which assisted the agricultural departments to develop improved cotton varieties. Until 1947 when India became independent, predominantly, the diploid Asiatic cottons, *G. arboreum* and *G. herbaceum*, were grown covering 97% of the acreage under cotton. The cotton improvement efforts got further fillips with the abolition of the ICCC in 1966 and establishment of the All India Coordinated Cotton Improvement Project (AICCIP) at Coimbatore in 1967 and the ICAR-Central Institute for Cotton Research (ICAR-CICR) at Nagpur. Since then, 268 high-yielding improved varieties and 109 hybrids (non-GM) were released for commercial cultivation in different cotton-growing states.

Some of the prominent landmark varieties and hybrids in India include release of the world's first intra-*hirsutum* cotton hybrid 'H4' in 1970 (Patel 1971), the first interspecific hybrid between *G. hirsutum* and *G. barbadense* 'Varalaxmi' in 1972 (Katarki 1972), *G. barbadense* Sea Island cotton variety 'Suvin' in 1978 and 'LRA5166', a *G. hirsutum* variety with wide adaptability in all three cotton-growing zones which occupied >30% area under cotton for about a decade. Successful development of hybrids led to exploitation of heterosis in cotton for higher yields. Interspecific hybridization and introgression from wild species resulted in the development of several varieties and hybrids, viz. Badnawar 1, Khandwa 1, Khandwa 2 (from *G. hirsutum* × *G. tomentosum*); PKV 081, Rajat, Arogya, AKA 8401 (*G. hirsutum* × *G. anomalum*); Deviraj, G 67 (*G. hirsutum* × *G. arboreum*); Devitej (*G. hirsutum* × *G. herbaceum*); MCU 2 and MCU 5, Varalaxmi, DCH 32, DHB 105, NHB 12, TCHB 213, HB 24 (*G. hirsutum* × *G. barbadense*); and DDH 2, DH 7, DH 9 (*G. herbaceum* × *G. arboreum*). Some of the popular varieties and hybrids released for cultivation in different cotton-growing states are given (Tables 11.4 and 11.5).

Table 11.4 Cotton varieties released for different states of India

Name of state	Tetraploid cotton	Diploid cotton
Punjab	F 1378, LH 1556, LH 900, F 846, F 1054, LH 1134, F 505, F 1861	LD 327, LD 491, LD694
Haryana	H 1098, H 777, HS 6, H 974, HS 182, H 1117	HD 107, HD 123
Rajasthan	Bikaneri Narma, RST 9, RST 875, G. Ageti, RS 810	RG 8, RD 18
Uttar Pradesh	Vikas	Lohit, CAD 4
MP	Khandwa 2, Khandwa 3, Vikram, JK 4	Maljari, Jawahar Tapti, Sarvottam
Gujarat	G.Cot 10, G.Cot 12, G.Cot 16, G. Cot 18, LRA 5166, LRK 516	G.Cot 15, G.Cot 19, G.Cot 13, G.Cot 17, G.Cot 21, G.Cot 23
Maharashtra	DHY 286, PKV 081, Rajat, LRA 5166, LRK 516	AKH 4, AKA 5, AKA 8401, PA 183, PA 255, AKA 7, Y1, PA 402, CNA 1028, CNA 1032, CNA 1054
Andhra Pradesh	MCU 5, LRA 5166, L 389, L 603, Kanchana, LK 861	Srisailam, Mahanandi, Raghvendra, Arvinda
Telangana	MCU 5, LRA 5166, LRK 516, L 389, L 603, Kanchana, LK 861	Srisailam, Mahanandi, Raghvendra, Arvinda
Karnataka	Sharda, Abadhita, Sahana	DB 3-12*, Raichur-51, DLSA 17
Tamil Nadu	MCU 7, MCU 5 VT, LRA 5166, LRK 516, Surabhi, Sumangala, MCU 12, SVPR 2, Suvin	K 10, K 11, CNA 1003 (Roja)

**G. herbaceum*

Table 11.5 Popular non-GM hybrids in India

Name of state	Tetraploid cotton	Diploid cotton
Punjab	FHH 209, F 2276, FATEH, LHH 144	DDH 11, Moti (LMDH 8), PAU 626 H (FMDH-3), FMDH-8, FMDH-9
Haryana	DhanLaxmi, OM Shankar	AAH1, CICR-2, AAH 32
Rajasthan	Maru Vikas	RAJH-9
Uttar Pradesh	–	–
Madhya	LAHH 4 and JKHy-1 and JKHy-2, JKHY 11	–
Gujarat	H4, H6, H8, H10	DH 7, DH9
Maharashtra	PKV Hy 2 and NHH 44, NHH 250, Savitri, RHH 195, NHH 302, CICR HH 1	AKDH-7, AKDH-5, PhA 46
Andhra Pradesh	LAHH 1, LAHH 4, NHB 80	–
Telangana	LAHH 1, LAHH 4, NHB 80	–
Karnataka	Varalaxmi, DCH 32, DHB 105 and DHH 11, RAHH 455	DDH 2
Tamil Nadu	Savita, TCHB 213, Surya and Sruthi, TSHH 0629, CBS 156, Suguna	K9, K10

11.19.3 Genomics-Assisted Breeding

Advances in molecular marker technologies and genome sequencing have facilitated dissection of determinants of various economic traits in crop plants. Transgenic technology and genomics have made significant contributions in enhancing the efficiency of cotton breeding. To date, several QTLs associated with economic and fibre quality traits have been mapped and few of them subjected to fine mapping. However, the development of products using marker-assisted breeding in cotton are very few. Two varieties, namely Ravnaq-1 and Ravnaq-2, possessing higher fibre strength and improved length have been developed through marker-assisted breeding (Abdurakhmonov 2016; Kushanov et al. 2017). Ravnaq MAS cultivars were tested by State Variety Testing Committee of Uzbekistan across different cotton-growing soil-climatic zones of the country during 2013 and 2014 and found superior in agronomic performance over conventional upland cultivars. Using PHYA1 RNAi GE cotton, a series of varieties viz., Porloq-1, Porloq-2, Porloq-3 and Porloq-4 were developed (Abdurakhmonov 2016). These RNAi cultivars were successfully tested for 3 years (2012–2014) in different soil-climatic regions of Uzbekistan. The RNAi cultivars demonstrated superiority to traditional varieties both in terms of fibre quality and adaptation to harsh environmental conditions across Uzbekistan. ICAR-CICR, Nagpur, deployed MON 531 event (*cry1Ac* gene, used as a gene-based marker) in elite varieties and developed ten varieties through marker-assisted breeding, tested under the AICRP system of evaluation and released for commercial cultivation.

11.20 Modernization of Cotton Improvement Programmes

The conventional breeding is based on the concept of selecting single and best high-yielding progeny from the segregating populations to develop a cultivar. Conventional breeding helped in the release of high-yielding varieties with superior fibre quality. With the adoption of improved varieties and hybrids, refined agronomy and integrated plant protection measures, cotton production has increased manifold until the beginning of the last decade. However, lately, most of the cotton-growing countries are facing yield stagnation and uncertainties due to changing climatic conditions during the past few years. Depending on the availability of resources, the research programmes may be modulated to meet the pressing needs. At the current status of cotton research, research gaps and future areas can be identified; accordingly, the National Research Programmes may be modulated to bridge the gaps.

11.20.1 Creating Additional Genetic Variability

Conventional breeding has limitation in transferring desired traits from unadapted exotic accessions to cultivated varieties lacking the trait because of negative linkages

and linkage drag. Continuing use of well-adapted base germplasm in the breeding programme and selection and evolving varieties with similar genetic base has resulted in narrow genetic base of the cultivated varieties. In India, about 95% of the cotton area is under Bt cotton hybrid cultivation. Most of the hybrids have at least one or both the parental lines possessing Bt gene(s). The parental lines are derived using Bt base line in Coker background through limited backcrossing. Thus, most of the hybrids under cultivation have one or other parental line in common contributing to narrow genetic base of the varieties/hybrids under cultivation.

The major cotton-growing countries maintain huge genetic resources of cultivated species and wild accessions; however, their utilization is minimal. The exotic and wild accessions possess numerous potential genes that contribute to disease and pest resistance, economic traits, fibre quality attributes and resistance to abiotic stress. Therefore, broadening the genetic base of cultivated cotton by mobilizing the useful genetic variations from diverse exotic accessions, races of cultivated species and wild accessions requires to be the top priority. Countries with cotton as priority crop need to have such exclusive research programme(s) on the utilization of wild resources.

11.20.2 Use of Speed Breeding

Cotton generally takes 150–180 days to complete a crop cycle that makes difficult to grow more than one normal crop in the same year. Growing more than one crop a year of a breeding material will save on time and resources and also speed up the development of varieties or product with desired introgressed traits.

11.20.3 Greater Access to Genomic Breeding Tools

Continuing improvement of molecular tools and advances in sequencing technology has resulted in the development of huge genomic resources. However, the gap between information on available genomic resources and its conversion into a tool for use in cotton breeding is huge that needs to be bridged through capacity building and collaboration across the national and international laboratories.

11.20.4 Developing High-Yielding, Stress-Tolerant, Climate-Resilient Varieties

Prioritization is needed to develop verities or lines that will sustain climate change and emerging stresses. The use of marker-assisted breeding will help to pyramid genes for different traits in one background.

11.21 Maintenance Breeding

Cotton, being an often cross-pollinated crop, genetic makeup of its varieties gradually changes year after year. Cook, as early as in 1932, reported the problems of preservation of varieties of cotton. He emphasized 'selection' as the approved method of keeping a variety uniform and of maintaining its productiveness; an account must be taken of many more features of diversity to preserve the essential characters of varieties by continued selection (Cook 1932).

The genetic purity of varietal seeds is related to the genetic potential of a variety/hybrid for realizing yield, quality and resistance to biotic and abiotic stresses. The genetic purity of a variety may deteriorate during seed production due to various factors. These include natural outcrossing, spontaneous mutation, residual variability, adaptive or developmental variation and mechanical mixture. Varietal deterioration is the most common and serious problem that makes it necessary to maintain the genetic purity of varieties under cultivation. For varietal maintenance in cotton, the probable approaches which can be used include:

1. Rouging off-type plants
2. Mass selection
3. Progeny selection
4. Maintaining seed stocks of the original and seed increase

Rouging out off-type plants, also referred to as negative mass selection, is a widely used procedure for the maintenance of genetic purity. Removal of off-types is based on plant phenotype which can be identified visually. It reduces the mixture, provided the off-type plants are removed before flowering, else cross-pollination involving pollen from off-type plants may build up mixtures. Mass selection involves identifying true-to-type superior plants of a variety based on phenotype and mixing the seed of selected plants to grow next generation. Selfing of selected plants is desirable to eliminate possible cross-pollination with off-type plants. A large number of plants are selected and bulked to raise the next generation so as to reduce variability. Inadvertent inclusion of off-type plants in a pool of a small number of plants may increase variability than in the original population.

The progeny selection method is widely used in the maintenance of varietal purity. It involves selection of a large number of single plants (500–1000), plant progenies are evaluated, and only progeny rows confirming to the varietal type are selected and pooled to produce nucleus seed and further pure seed increase. Maintaining seed stocks is commonly and increasingly being used for the maintenance of varietal purity. In this method, a huge quantity of original variety seed is produced and stored in environmentally controlled conditions to maintain germination and viability for years. If the varietal genetic purity deteriorates, the required quantity of seed is removed from the reserve to produce nucleus and further seed increase. In this approach, the genetic changes in the variety are expected to be minimal over the longer life span of the variety. It also saves breeder's time for the

production of varietal nucleus seeds every year and also resources invested for crop cultivation, isolation and regular monitoring of seed plots and seed processing.

11.22 Coordinated System of Testing

India has established a strong system of cotton genotype evaluation developed by its Research Institutes and Cotton Research Station working under State Agricultural Universities across cotton-growing states. The establishment of the Indian Central Cotton Committee (ICCC) in Bombay as a technical advisory body to the government in 1921 is considered as a major landmark in the history of cotton research in India. The ICCC became a statutory body for promoting research in cotton. The ICCC established the Cotton Technological Research Laboratory (CTRL) [now, ICAR-Central Institute for Research on Cotton Technology (ICAR-CIRCOT)] at Bombay in 1924 for conducting tests on fibre properties of cotton samples to relate fibre properties with the spinning value of cotton. The ICCC was abolished in 1966 and the CTRL was placed under the administrative control of the ICAR. The ICAR reorganized its research set up and established the All India Coordinated Cotton Improvement Project (AICCIP) (now, All India Coordinated Research Project on Cotton (AICRP on Cotton)) in 1967 with its headquarters in Coimbatore (Tamil Nadu). Prior to the AICCIP, different centres conducting research on cotton were under the control of PIRRCOM (Project on Intensification of Regional Research Cotton, Oilseeds and Millets). The AICRP on Cotton has a network of 22 cotton research centres located in 11 cotton-growing states. The AICRP on Cotton conducts multi-location and multidisciplinary research on applied aspects of cotton including varietal development, evaluation/site-specific validation and fine-tuning of agro technologies and pest/disease management strategies. Since its inception in 1967, the AICRP on Cotton has played a stellar role in shaping the cotton sector in India through the development of several varieties/hybrids and fine-tuning agro-eco-region-specific cotton production and protection technologies. The AICRP on Cotton also acts as a nodal centre for transfer of technologies through Front Line Demonstrations (FLDs). The entire cotton-growing area has been divided into three zones, i.e. north, central and south zones, based on agro-climatic conditions and growing season.

The zone-wise promising top-ranking entries after detailed agronomy studies are considered for identification by Varietal Identification Committee. If a variety is intended to be released in a state, it is done by the State Varietal Release Committee. The proposals of identified varieties for one or more cotton-growing zones are then submitted to the Sub-Committee on Crop Standards Notification and Release of Variety of Central Seed Committee where it is released and notified. Further, if a variety is released by the State Committee, it has to be notified by the Central Committee (Basu 1999). The denotification of old/obsolete varieties is also done by the same committee. Through the AICRP on Cotton, 268 high-yielding non-Bt varieties and 109 non-Bt hybrids of cotton have been developed by the network partners and released. Cultivation of Bt transgenic cotton was approved for

commercial cultivation by the Government of India in 2002. The Bt hybrids developed mostly by private seed companies under licence from Monsanto were approved by event-based approval mechanism (EBAM) committee under Review Committee on Genetic Manipulation (RCGM) based on criteria formalized by Genetic Engineering Approval Committee (GEAC). From 2016 onwards, RCGM has entrusted the responsibility of evaluation and release of Bt cotton varieties/hybrids to ICAR and AICRP on Cotton. Following the similar system of evaluation, eight Bt varieties and 57 Bt hybrids have been released through ICAR-AICRP on Cotton.

11.23 Future Prospects

Genetic variability is the base for improvement of any crop plants. Success of selection and consequently development of varieties is directly associated with the extent of genetic variability for several component traits contributing to yield. Cultivated cotton has a narrow genetic base resulting in stagnation of yields the world over. To continue gains in yield and quality, it is imperative to explore primary and secondary sources of gene pool to widen heritable genetic variability in the breeding populations. Countries such as India where yield levels are far below than world average emphasize on research programmes for enhancing genetic variability needed.

Wide scale cultivation of Bt transgenics has provided benefits of protection of cotton crop against bollworms, increased the seed cotton yield and reduced insecticide use during early years of Bt adoption. However, susceptibility of most of the Bt hybrids to sucking pest and subsequent breakdown of resistance/susceptibility of Bt hybrids to pink bollworm have resulted in an increased use of insecticides in cotton. Failure of BGII cotton to pink bollworm is a warning signal for researchers/policymakers and users alike. For success of such technology, adequate precautions are a must while implementation. Alternately, newer sources of genes must be identified, validated and deployed to broaden the horizon of technology.

Advances in next-generation sequencing made the genome sequencing affordable, faster and precise. The sequenced and resequenced genomes of diploid and allotetraploid and also sequencing of about a dozen wild species of cotton provide valuable information on genomic structure, variation, markers, diversity and numerous genes and biological processes associated with important traits such as fibre development and stress responses. Several linkage maps were developed; genes and thousands of QTLs linked to traits of interest were identified. However, large knowledge gaps still persist concerning with the molecular regulation of the biological processes. Characterization of essential genes controlling complex traits is a major challenge for cotton functional genomics studies. Well-characterized functional components of complex traits will facilitate effective application of molecular-assisted breeding in cotton.

Emerging genome engineering technologies such as the CRISPR/Cas9 system have great potential. This technology may be exploited to edit the disease-susceptible genes, negative regulators of yield and fibre quality-related genes and

genes enhancing adaption and resistant to stress situation in cotton. However, success of this technology is dependent on the availability of an efficient regeneration system. In cotton, genotype-dependent regeneration is becoming a major bottleneck for use of CRISPR/Cas9. Development of versatile and robust somatic regeneration protocol suiting to a diverse set of genotypes will provide additional avenues for transgenic development and efficient application of genome editing tools across the genotypes for improvement of economic, fibre quality and adaptation traits.

In recent years, the spinning industry is adopting large, almost fully automated spinning mills to lower yarn manufacturing cost. With this trend, the mills exercise less flexibility with respect to fibre types (blend composition) and yarn composition (count and twist factors). The mills requirement is mostly for fibre length >29–30 mm, matching fibre strength of 29–30 g/tex and micronaire of about 4.0. The mills offer premium price for better fibre quality cotton. The diploid cottons suffer on fibre quality parameters and are less in demand. This trend has also resulted in skewed species composition, *G. hirsutum* occupying about 97% of the area under cotton cultivation in India. Considering variation in market prices for cotton and mills requirement, it is imperative for the cotton breeders to develop varieties with improved fibre quality, viz. fibre length about 30 mm plus, matching fibre strength and micronaire of 3.8–4.2. Asiatic diploids are inherently tolerant to insect pests, diseases and drought stresses than upland cotton. Improved fibre quality parameters matching with mills requirement can help to retain past glory of diploids in future.

11.24 Conclusions

Cotton is the most important source of natural fibre, meeting the most important human needs for clothing. Cotton significantly contributes to the cotton growing economies in terms of export earnings and providing employment to millions in farms and processing industries. In cotton research, the prime objective is to improve lint yield and quality through utilization of diverse germplasm resources employing conventional and recent high-throughput technologies. The phenotypic selection efficiency of the conventional breeding can be maximized using DNA-based markers and recent genomic tools for the future breeding strategies. Stagnation of yields and narrow genetic base of the cultivated cotton is a major issue across the cotton-growing countries implicating need to use available genetic variability in unadapted exotic accessions and to develop gene pools. Currently, insect- and herbicide-resistant transgenic cotton occupy over 80% of world acreage under cotton. Field-evolved resistance of pink bollworm against Bollgard and Bollgard II cotton has been reported in some of the countries including India. Sucking pests (whitefly and jassids) have emerged as major pests demanding more attention and resources for management after PBW. For effective working of the transgenic technology, newer gene sources conferring resistance to PBW and sucking pest need to be involved and stacked to provide durable resistance.

Application of next-generation sequencing (NGS) permitted whole-genome sequencing and building draft assembly at a faster rate. The sequence information across the genomes has become a major source for identifying new SNP markers and candidate genes with potential for genetic improvement of cotton quality and productivity. The refinement and fine-tuning of genotyping by sequencing (GBS) permits scanning of a large number of individuals simultaneously. Cotton genomics research now entered in the phase of functional characterization of genes related to economic traits. These advances have paved the way to identify and clone functional genes (Pan et al. 2020) and enhance breeding efforts to develop cotton so as to produce high yield, superior quality fibres and resisting effects of climate change. Precision genome editing technology such as CRISPR/Cas9 has demonstrated success in gene-targeted mutagenesis in several crops including cotton. This technology holds promise to develop transgene-free edited plants for economic, quality, resistance and adaptation traits in cotton and deserves higher investment by researchers.

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Maintenance Breeding of Pusa Basmati Varieties

12

Rakesh Seth, A. K. Singh, and S. Gopala Krishnan

Abstract

Genetic gain, achieved by any breeding and varietal development programme, can be realized only when there is a robust seed production system, underpinned by a systematic maintenance breeding programme. Breed (variety) and seed are two facets of the same coin—one without another is irrelevant. The full potential impact of a new improved variety, however excellent it may be, cannot be realized, unless it is supported by a strong seed multiplication, distribution and marketing system. The popularity and complete dominance of the ICAR-IARI bred Basmati varieties is evident from the increase in percent share of breeder seed indents of Pusa Basmati varieties in the total breeder seed indents of all Basmati varieties. The details of maintenance breeding and the success of Pusa Basmati varieties are described in this chapter.

Keywords

Basmati · Genetic gain · Mechanical mixtures · Outcrossing · Breeder seed production

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_12

12.1 Introduction

India has achieved significant productivity gains in major cereals post-green revolution (post-GR) era of 1984–2017. The annual gain in rice productivity in different phases after green revolution has been computed as 68% and 117% in 1984–2000 and 2001–2017, respectively (Fig. 12.1) (Yadav et al. 2019). Similarly, impressive genetic gains have also been made in quality and productivity of Basmati varieties, a speciality group of rice varieties, having great aroma and premium culinary attributes (Singh et al. 2018a). This progress has largely been attributed to development and adoption of improved cultivars and crop management technologies. Significant efforts go into breeding programmes and development of trait-specific high-yielding varieties with inbuilt resistance to pests and diseases, besides tolerance to various biotic and abiotic stresses and quality attributes. Seeds are the carriers of these genetic gains to farmers and ultimately to the consumers. Quality seed alone contributes a 15–20% increase in productivity. ICAR-Indian Agricultural Research Institute (ICAR-IARI) bred Basmati varieties (commonly known as 'Pusa Basmati' varieties) have made a significant impact in converting these genetic gains into economic gains for different stakeholders associated with Basmati rice production, processing and export. Cumulative foreign exchange earnings of a single landmark Basmati variety, Pusa Basmati 1121, in one decade (2008–2017) have been to the tune of US $20.8 billion (Singh et al. 2018a). Pusa Basmati varieties (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1, etc.) are dominating the seed chain (Figs. 12.2 and 12.3).

Basmati rices possess unique organoleptic properties (aroma and taste) due to which these are very popular in the international markets. However, cultivation of

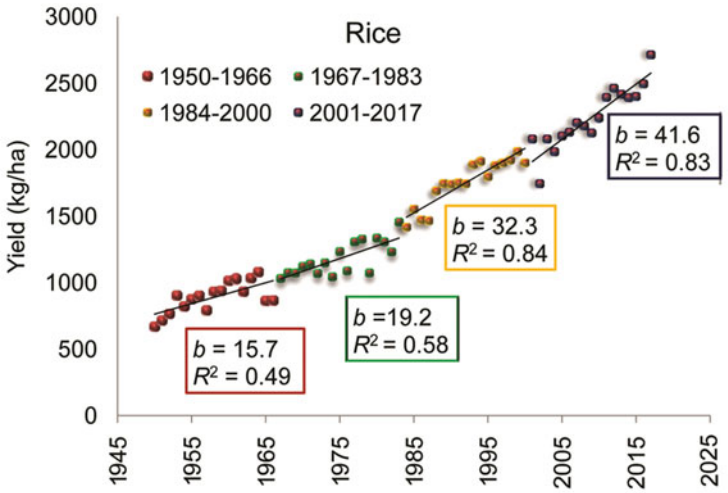

Fig. 12.1 Rice productivity (kg ha^{-1}) trend since 1950 (Yadav et al. 2019)

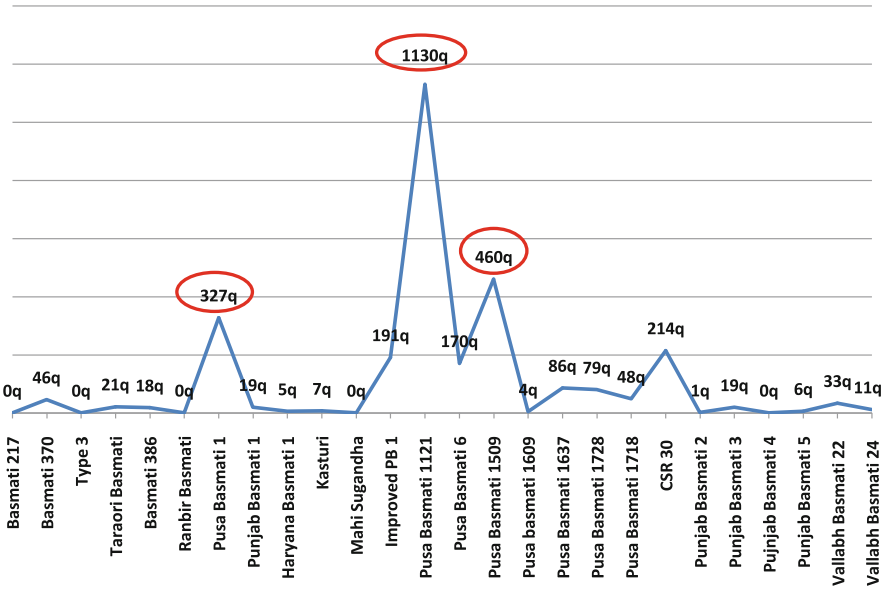

Fig. 12.2 Cumulative breeder seed indent of Basmati varieties during the last 10 years, 2010–2019 (Breeder Seed Allocation Plans 2020)

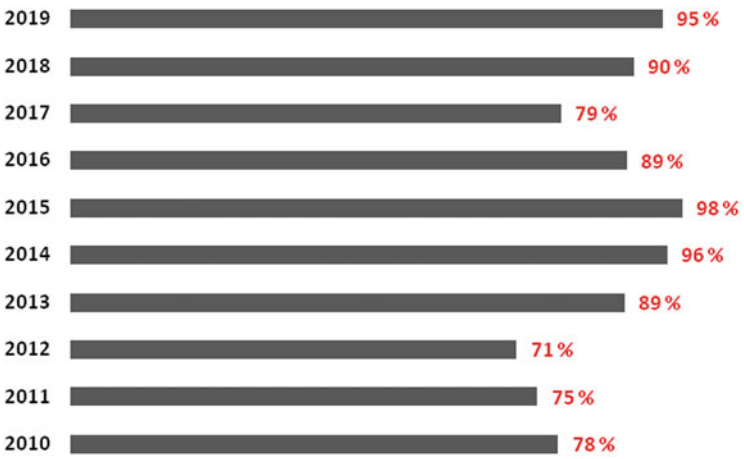

Fig. 12.3 Percentage share of breeder seed indents of Pusa Basmati varieties in total breeder seed indents of all Basmati varieties (2010–2019)

Basmati is geographically limited to North-Western India and Punjab in Pakistan. In India, Basmati production is confined only to seven states (Punjab, Haryana, Himachal Pradesh, Delhi, Uttarakhand, Jammu and Kathua districts of Jammu and Kashmir and 27 districts of Western Uttar Pradesh). This specific region has been earmarked as Geographical Indication (GI) for Basmati rices in 2016 by

Geographical Indication Registry, Government of India (GI No. 145, Certificate No. 238 dt. 15.02.2016). Typically, GI conveys an assurance of quality and distinctiveness which is essentially attributable to the fact of its origin in that defined geographical locality, region or country (Department of Promotion of Industry and Internal Trade 2021).

Basmati—being one of the most popular speciality rices in the EU and the Middle East—attracts premium prices and is subject to stringent tests to differentiate between authentic Basmati from non-Basmati rices (Nader et al. 2019). The authenticity definitions by the UK Code of Practice on Basmati Rice (CoP) of 2017 clearly stipulate that when the description of the product is 'Basmati rice', the non-Basmati rice content must not exceed 7%. This tolerance is in place to take account of **seed impurity and other segregation issues** at origin (http://www.riceassociation.org.uk). This clearly underscores the critical need for maintaining the supply of high-quality genetically pure seed for the production of Basmati rice.

Traditional Basmati varieties are tall, lodging and disease prone, photoperiod sensitive and poor yielders. Pusa Basmati 1, the world's first evolved, semi-dwarf, photoperiod-insensitive, high-yielding Basmati variety developed by ICAR-IARI, New Delhi, in 1989, brought a paradigm shift in Basmati breeding (combining Basmati quality with higher yield and semi-dwarf stature). ICAR-IARI continues to play the flagship role in Basmati breeding with the development of several popular Basmati varieties like Pusa Basmati 1509, Pusa Basmati 6, Pusa Basmati 1637, Pusa Basmati 1718 and the recently released Basmati variety Pusa Basmati 1692 (notified in 2020).

12.2 Maintenance Breeding

Maintenance breeding (used synonymously with varietal maintenance) is a basic technique which primarily deals with the purification and maintenance of varieties. Notwithstanding its simplicity, this process has a profound impact on the spread and enhanced productive life of a variety. 'Variety maintenance' is the perpetuation of a small stock of nucleus seed, as the basis of all future multiplication and production of a variety, either by repeated multiplication of a small stock by a precise procedure, controlling the relationships of the plants in the stock, or by storage (Laverack 1994).

12.2.1 Why Maintenance Breeding?

'Thou shall not sow thy fields with mingled seed', Leviticus 19:19. Thus were the Hebrews enjoined to sow their fields with *unmixed* seed. The early farmers were aware of the fact that varieties of crops deteriorate with time unless directed efforts are made towards maintaining the integrity of the variety (Kadam 1942). Plant breeding is often described as 'plant evolution' directed by man. One of the principal constraints of the conventional breeding methods is that selection decisions about the merits of diverse lines are largely based on their phenotypes. Genotype describes the

allelic constitution of an individual at one or more loci, while phenotype is the observable expression of one or more traits (Singh and Singh 2017). The various traits of an organism can be grouped into two categories: (1) qualitative traits, governed by one or few major genes or oligogenes, each of which produces a large effect on the trait phenotype, and (2) quantitative traits, governed by several genes, each having a small individual effect on trait phenotype, which are usually cumulative. Most of the traits of biological and economic importance are quantitative or metric traits. The phenotypic expression of quantitative traits is significantly influenced by the environment and, often, an interaction between genotype and environment.

The phenotype can be expressed by the following equation:

$$P = \mu + G + E + (G \times E)$$

where P is the phenotype of a quantitative trait (governed by multiple genes), μ is the population mean, G is the effect of genotype of the concerned individual, E is the effect of the environment on the expression of the trait and (G × E) is the interaction component. A precise estimation of G, E and G × E components of phenotypic variation for different quantitative traits is one of the continuing quests for plant breeding (Singh and Singh 2017; Singh 2012). This is also a challenge in maintenance breeding as the production of different classes of seeds including nucleus and breeder seed is essentially done based on the phenotype.

A comprehensive study on the importance of maintenance breeding in the first miracle rice variety IR 8 provided strong justification for continuous maintenance breeding to counter rapidly evolving biotic and abiotic stresses. Maintenance breeding plays a decisive role in the adaptation of newly developed varieties to changing environmental conditions, which have a deleterious impact on older varieties (Peng et al. 2010).

12.2.2 Varietal Deterioration and Maintenance Breeding

Two essential characteristics of a cultivar are (1) identity and (2) reproducibility. In self-fertilizing crops, a cultivar generated from a single, homozygous genotype will be uniform in appearance, whereas a cultivar increased from a mixture of genotypes will exhibit a range of genetic variability according to that present in the mixture. This is assuming that the plant originally selected is homozygous at all loci—an assumption plant breeders often make, but this assumption is seldom met (Sleper and Poehlman 2006). There may be several minor loci still segregating even in the F_{10} generation leading to the production of off-types in a large population. Kadam (1942) in his classical paper on varietal deterioration enumerated seven critical factors responsible for the degradation of varieties over time.

12.2.2.1 Developmental Variations

Seed crops which are grown under different environments over consecutive generations may exhibit differential growth responses leading to the production of such variation. In order to minimize these variations, it is advisable to restrict the seed production of the varieties in their areas of adaptation.

12.2.2.2 Mechanical Mixtures

Mechanical mixtures are one of the most important reasons for varietal deterioration and are mainly attributed to human negligence (Fig. 12.4). These are the leading causes for litigation between seed producers and farmers. Shattering of grains in rice (an important source of mixture) results in volunteer plants (self-sown plants). Thus, care should be taken that the land used for seed production is free from volunteer plants. This stipulation is a mandatory protocol for seed production as per Indian Minimum Seed Certification Standards (IMSCS 2013). Proximity of threshing floors, unclean tarpaulins or the use of the same contaminated seed drills, seed bins and gunny bags and mistakes in handling seed during seed processing are the main reasons for mechanical mixtures. These can be avoided by taking utmost care during every step of seed production and processing.

Fig. 12.4 Mechanical mixtures: major factor for varietal deterioration (result of human negligence)

12.2.2.3 Mutations

Mutation is a sudden heritable change in the genotype of an organism. The organisms with such heritable changes are known as mutants. Mutations are of two types (spontaneous and induced) depending upon their origin. A spontaneous mutation is one that occurs in nature, while an induced mutation results from a mutagenic agent. What appears to be a spontaneous mutation may have been induced, because all plants in nature are subjected to low dosages of natural radiation (Sleper and Poehlman 2006). In nature, plant mutation rates occur between 10^{-5} and 10^{-8} during adaptation and evolutionary processes. These frequencies of natural or spontaneous mutations are extremely low (Zhon-hua et al. 2014). Mutations per se do not pose a serious threat in seed production and varietal maintenance of Basmati rices as well.

12.2.2.4 Natural Outcrossing

The extent of natural outcrossing in rice varies from 0% to 3%, depending on the cultivar and the environment, with an average of about 0.5% (Sleper and Poehlman 2006). Sometimes there is lag between spikelet opening and bursting of the anther resulting in outcrossing. Though rare, but outcrossing in rice is not an exception (Fig. 12.5a, b). In a study on outcrossing (OC) in winter wheat, Martin (1990) observed that there is no question that OC occurs during the multiplication stages of cultivar development. It is likely that OC is most serious when experimental lines are growing side by side in early-generation plant or head rows and, subsequently, in initial small increase plots. If the incidence of outcrossed seed could be reduced in seed replanted from such nurseries, the production cost of all classes of certified seed could be reduced significantly by decreasing the amount of roguing required to meet purity standards for the cultivar.

An interesting observation has been reported by Kadam (1942) that farmers in Konkan generally believe that mixtures suddenly appear in the third year of growing an improved variety. This is due to the fact that in that year natural F_1 plants have segregated for various characters. Perpetuation of such plants, in addition to mechanical mixtures, increases the proportion of dominant types, until in the end a variety resembles a conglomeration of various types. The natural outcrossing can be with off-types, diseased or undesirable plants.

Bateman (1947) in an exhaustive work on contamination of seed crops reported that there are two types of contamination of seed crops: (1) mechanical mixtures and (2) cross-pollination between varieties (admixture of foreign seed at harvesting or admixture of foreign pollen at flowering). The degree of genetic contamination in seed fields due to natural crossing depends upon four variables: (a) the breeding system of species, (b) isolation distance, (c) varietal mass and (d) pollinating agent. Contamination generally decreases, as the isolation distance between the varieties is increased; however, there still may be miniscule traces of contamination over wide distances. Appropriate isolation of seed crops per se is, therefore, a primary prerequisite for the seed production of crop plants cross-pollinated by winds or insects.

Fig. 12.5 (**a**) Outcrossed plants in rice seed field of Pusa Sugandh 5. (**b**) Outcrossed plant with pigmented apiculi in rice seed field of Pusa Sugandh 5

12.2.2.5 Minor Genetic Variations

Varieties appearing phenotypically uniform and homogeneous at the time of their release may still have minor genetic variations. This could be due to the fact that in a typical breeding programme, the seed multiplication is done after its identification/release. Till the identification/release of a variety, the genotypes are grown in smaller

area, and when grown in larger area for seed multiplication, it makes it possible to identify the off-types in a large population of plants. Some of these minor genetic variations may be lost during subsequent production cycles due to selective elimination by the environment (Kadam 1942; Agrawal 1991). Remnant of these genetic variants can be eliminated to a large extent by careful nucleus and breeder seed production.

12.2.2.6 Selective Influence of Diseases

The selective influence of diseases is also an important factor in varietal deterioration. New varieties often become vulnerable to new races of diseases caused by obligate parasites and are often out of seed production programmes (Kadam 1942; Agrawal 1991). With the current focus on incorporating disease resistance in Basmati varieties, a number of disease resistance varieties have been bred by ICAR-IARI through molecular marker-assisted backcross breeding (MABB) (Singh et al. 2019)—for example, Pusa Basmati 1718 (a MAS-derived bacterial blight (BB) resistant Basmati rice variety possessing two genes, *xa13* and *Xa21*) (Singh et al. 2018b), Pusa Basmati 1637 (a MAS-derived near isogenic line of Pusa Basmati 1 possessing *Pi9* gene for blast resistance) (Singh et al. 2017a) and Pusa Basmati 1728 (a MAS-derived near isogenic line of Pusa Basmati 6 carrying two genes for BB resistance, viz. *xa13* and *Xa21*) (Singh et al. 2017b). Taking up systematic seed production programme of these varieties having inherent resistance to specific diseases, the selective influence of these specific diseases can be reduced or eliminated.

12.2.2.7 Segregation Due to Residual Heterozygosity in the Cultivars

Premature release of varieties, still segregating for resistance and susceptibility to diseases or other factors, is also an important factor of varietal deterioration. In addition, heritable variations on account of recombination and polyploidization may occur in varieties during seed production (Kadam 1942; Agrawal 1991). Avoiding hasty release of varieties and putting in seed chain only the stabilized varieties can reduce residual segregation or stability issues to a large extent.

It is apparent from the aforesaid discussion that genetic variation may appear within a seed lot due to multiple reasons, viz. mechanical contamination, undesirable pollination, residual segregation, recombination and mutations. These diverse factors ensure that no variety is likely to retain the precise allele frequencies established by breeder without continuous monitoring (Laverack and Turner 1995). The process of continuous intervention to monitor and maintain the genetic purity of the variety is termed as maintenance breeding. It is usually the fag end of varietal development and the first step in the initiation of seed production. Figure 12.6 depicts an analogy adapted from 0 to 1 (Thiel and Masters 2014). The value 0 to 1 indicates an innovation (variety) and 1 to n is scaling up (seed production). Maintenance breeding is at the cusp of breeding and seed production. In practice, most of the issues pertaining to quality, adaptability and yields are encountered at initial scaling up of the variety and should be sorted out at this stage. Pusa Basmati

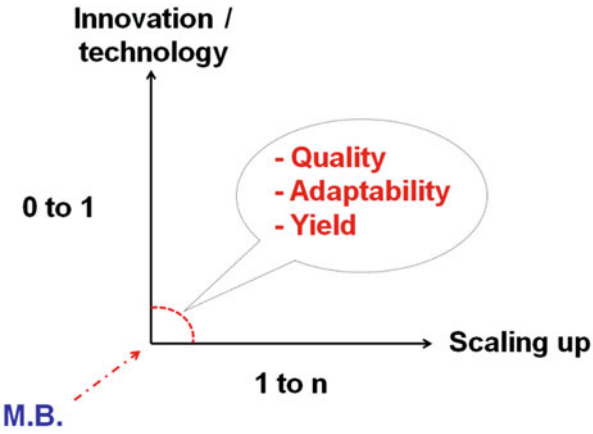

Fig. 12.6 Maintenance breeding (M.B.): challenging cusp of innovation (varietal development) and scaling up (seed production). (Adapted from Thiel and Masters 2014)

1692, a new variety notified in 2020 (Singh et al. 2020), is now at the cusp of maintenance breeding and its large-scale seed production.

12.3 Basmati Varieties Maintenance Breeding and Breeder Seed Production

At present, 34 varieties have been notified as Basmati varieties till 15 July 2021 (Notified Basmati Varieties 2021) (Table 12.1). To understand the varietal dynamics and consumer preferences, a comparison of breeder seed indents of all Basmati varieties in seed chain in the last decade (2010–2019) has been made in Figs. 12.2 and 12.3. A total of 24 Basmati varieties figured in breeder seed indents in the last decade. Two varieties Basmati 217 and Mahi Sugandha have zero breeder seed indent, and three varieties have almost negligible cumulative breeder seed indent of less than 0.50q each ((Type 3 (0.10q), Ranbir Basmati (0.37q), Punjab Basmati 4 (0.32q)) during this decade. Top three peaks were occupied by Pusa Basmati varieties *viz.,* Pusa Basmati 1121, Pusa Basmati 1509 and Pusa Basmati 1 (Fig. 12.2). This complete dominance of Pusa Basmati varieties gives an idea about demand pull of these varieties, implicitly indicating that varieties per se are not only excellent, but also gives an insight about IARI's ability to consistently saturate the seed markets with genetically pure high-quality seeds which is produced from nucleus seed (product of maintenance breeding).

All the IARI (Pusa) bred Basmati varieties undergo systematic maintenance breeding at ICAR-IARI, Regional Station, Karnal (dedicated to maintenance breeding and seed production). A brief description of some important Basmati varieties (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 6, Pusa Basmati 1718) along with specific comments on the maintenance breeding and breeder seed production is discussed in following sections.

Table 12.1 Notified Basmati varieties as per APEDA (The Agricultural and Processed Foods Exports Development Authority)

S. no.	Variety[a]	Notification no. and date	S. no.	Variety	Notification no. and date
1	Basmati 217	4045—24.09.1969 361 (E)—30.06.1973	18	Malviya Basmati Dhan 10–9 (IET 21669)	2817 (E)—19.09.2013
2	Basmati 370	361 (E)—30.06.1973 786—02.02.1976	19	Vallabh Basmati 21 (IET 19493)	2817 (E)—19.09.2013
3	Type 3 (Dehraduni Basmati)	13—19.12.1978	20	Pusa Basmati 1509 (IET 21960)	2817 (E)—19.09.2013
4	Punjab Basmati 1 (Bauni Basmati)	596 (E)—13.08.1984	21	Basmati 564	268 (E)—28.01.2015
5	Pusa Basmati 1	615 (E)—06.11.1989	22	Vallabh Basmati 23	268 (E)—28.01.2015
6	Kasturi	615 (E)—06.11.1989	23	Vallabh Basmati 24	268 (E)—28.01.2015
7	Haryana Basmati 1	793 (E)—22.11.1991	24	Pusa Basmati 1609	2680(E)—01.10.2015
8	Mahi Sugandha	408 (E)—04.05.1995	25	Pant Basmati 1 (IET 21665)	112 (E)—13.01.2016
9	Taraori Basmati (HBC 19/Karnal Local)	1 (E)—01.01.1996	26	Pant Basmati 2 (IET 21953)	112 (E)—13.01.2016
10	Ranbir Basmati	1 (E)—01.01.1996	27	Punjab Basmati 3	3540 (E)—24.11.2016
11	Basmati 386	647 (E)—09.09.1997	28	Pusa Basmati 1637	3540 (E)—24.11.2016
12	Improved Pusa Basmati 1	1178 (E)—20.07.2007	29	Pusa Basmati 1728	3540 (E)—24.11.2016
13	Pusa Basmati 1121 After amendment	1566 (E)—05.11.2005 2547 (E)—29.10.2008	30	Pusa Basmati 1718	2805 (E)—25.08.2017
14	Vallabh Basmati 22	2187 (E)—27.08.2009	31	Punjab Basmati 4	1379 (E)—27.03.2018
15	Pusa Basmati 6 (Pusa 1401)	733 (E)—01.04.2010	32	Punjab Basmati 5	1379 (E)—27.03.2018
16	Punjab Basmati 2	1708 (E)—26.07.2012	33	Haryana Basmati 2	3220 (E)—05.09.2019
17	Basmati CSR 30 After amendment	1134 (E)—25.11.2001 2126 (E)—10.09.2012	34	Pusa Basmati 1692	3482 (E)—07.10.2020

[a]Notified till 15 July 2021

12.3.1 Pusa Basmati 1

Pusa Basmati 1 was released for commercial cultivation in 1989 (Fig. 12.7). It is the first evolved Basmati variety having semi-dwarf stature, high yield potential and photoperiod insensitivity. Development of this variety by ICAR-IARI was a turning point in Basmati breeding in India. Pusa Basmati 1 is a product of cross between Pusa 150 and Karnal Local. It combines unique traits from diverse lineage. Pusa 150 is a breeding line derived through a convergent breeding approach involving many high-yielding non-aromatic rice varieties (Taichung Native 1, IR 8, IR 22, etc.) and traditional Basmati rice variety (Basmati 370) used as quality trait donor. Karnal Local was a selection from the traditional Basmati rice collection, Haryana Basmati Collection 19 (HBC 19) from the Karnal district of Haryana (with better grain and cooking quality), which was later released as Taraori Basmati in 1996 (Singh et al. 2004). Pusa Basmati 1 is still in demand as regular breeder seed indents of this variety are received till date. It is being maintained at ICAR-IARI, Regional Station, Karnal, since 1989 (notification year). Maintenance breeding comment: kind of variants observed in nucleus/breeder seed plots: (1) awn less off-types; (2) flowering variants.

Fig. 12.7 Pusa Basmati 1: outstanding example of maintenance breeding (notification year 1989)

12.3.2 Pusa Basmati 1121

Pusa Basmati 1121 is an exquisite Basmati rice variety with exceptional grain and cooking quality notified for commercial cultivation in 2005 and subsequently (after the amendment) for the states of Delhi, Punjab and Haryana in 2008 (Table 12.1). The superior linear cooked kernel elongation of this unique variety was derived from parents Basmati 370 and Type 3 (used as donors for grain and cooking quality traits). Accumulation of favourable loci for extra-long grain and exceptionally high linear cooked kernel elongation was possible through transgressive segregation, due to selective inter-mating of the sister lines showing better linear kernel elongation in the segregating generations. As many as 13 rice varieties/enhanced germplasm (including Basmati 370 and Type 3) were used to bring together the favourable alleles at multiple loci for agronomic, grain and cooking quality characteristics in the development of Pusa Basmati 1121 (Fig. 12.8) (Singh et al. 2018a).

Modern varieties being the products of complex lineage and multiple crosses, consequently the varietal maintenance of these varieties also becomes quite arduous. Different types of variants do crop up in these varieties during repeated cycles of seed production. Many times it becomes very difficult to keep the exact combination of the favourable alleles brought together by breeder, almost intact in repeated cycles

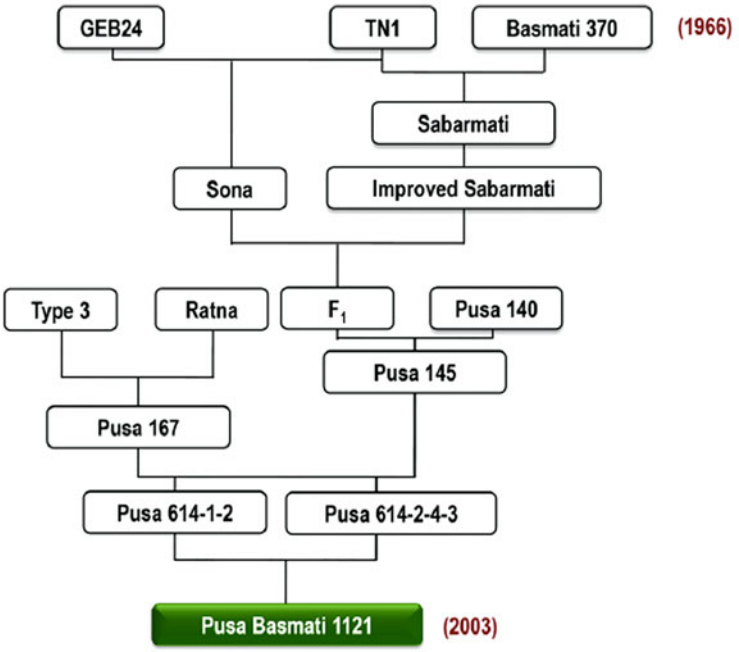

Fig. 12.8 Pusa Basmati 1121 pedigree showing contribution of several varieties. Years in parentheses indicate the year in which crossing was initiated (1966) and the year of release of variety (2003) (Singh et al. 2018a)

of multiplication. Pusa Basmati 1121 is a typical example of a difficult variety to maintain due to its complex ancestry (Fig. 12.8) various types of variants have been observed (Fig. 12.9a, b). Consistent and recurrent cycles of varietal maintenance, nucleus and breeder seed production (Fig. 12.10) have enabled this variety to make a significant contribution in farmer prosperity. Maintenance breeding comment: challenging variety to maintain. Kind of variants observed in nucleus/breeder seed plots: (1) tall off-types; (2) dwarf off-types; (3) grain size off-types; (4) awned off-types.

12.3.3 Pusa Basmati 1509

Pusa Basmati 1509 is a very popular Basmati rice variety notified in 2013 for commercial cultivation. It has semi-dwarf plant stature (a plant height of 95–100 cm), sturdy stem and non-lodging and non-shattering habit as compared to Pusa Basmati 1121. It is suitable for multiple cropping systems with a seed-to-seed maturity of around 115–120. It has an average yield of 4.1 t/ha with potential yield as high as up to 7.0 t/ha under good management conditions. Pusa Basmati 1509 possesses aromatic extra-long slender grains (8.41 mm) and good kernel length after cooking (19.1 mm) (Singh et al. 2014). Its area is fast increasing, and this variety has significant export potential (Fig. 12.11). Maintenance breeding comment: kind of variants observed in nucleus/breeder seed plots: (1) tall off-types; (2) grain size off-types.

12.3.4 Pusa Basmati 6

Pusa Basmati 6 is very popular in niches of southern Punjab and north-western districts of Haryana. The major chunk of breeder seed of this variety is used in these districts (Fig. 12.12). Pusa Basmati 6 is also popularly known as Pusa 1401. It has semi-dwarf plant stature with sturdy stem. This variety has a kernel that retains uniform shape after cooking, as against kernel shape of Pusa Basmati 1121 (tapering end after cooking). Pusa Basmati 6 possesses strong aroma along with minimum chalkiness (<4%) (Singh et al. 2018a). Maintenance breeding comment: kind of variants observed in nucleus/breeder seed plots: (1) grain size off-types; (2) tall off-types; (3) dwarf off-types.

12.3.5 Pusa Basmati 1718

Pusa Basmati 1718 is a product of marker-assisted backcross breeding having two genes (*xa13* and *Xa21*) governing bacterial blight (BB) resistance. It is a MAS-derived near isogenic line of popular variety Pusa Basmati 1121. Pusa Basmati 1718 has been released for Basmati-growing states of Haryana, Punjab and Delhi. With a seed-to-seed maturity of 136–138 days, it has an average productivity of 4.6 t/ha (maximum yield potential 6.0 t/ha) (Singh et al. 2018b). It possesses long slender

Fig. 12.9 (**a**) and (**b**) Pusa Basmati 1121 maintenance and purification. Variants in a paired row, raised from single true-to-type panicle

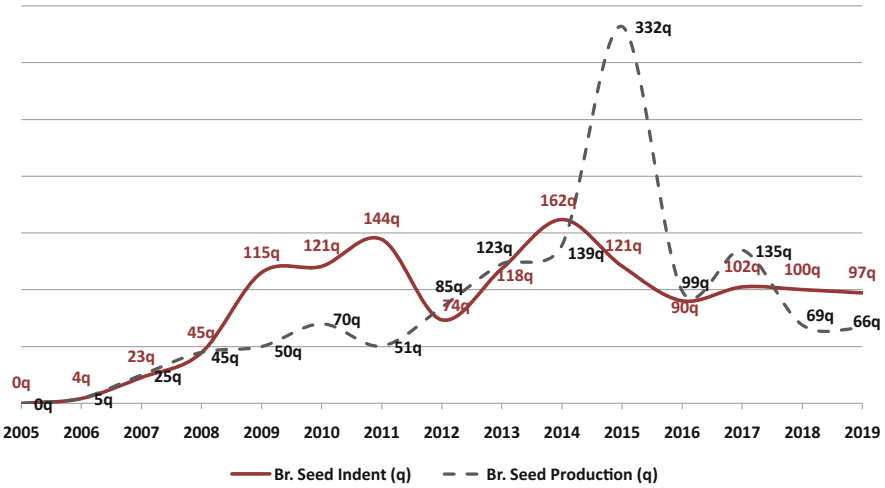

Fig. 12.10 Journey of Pusa Basmati 1121. An established brand. Breeder seed indent and production from 2005 onwards

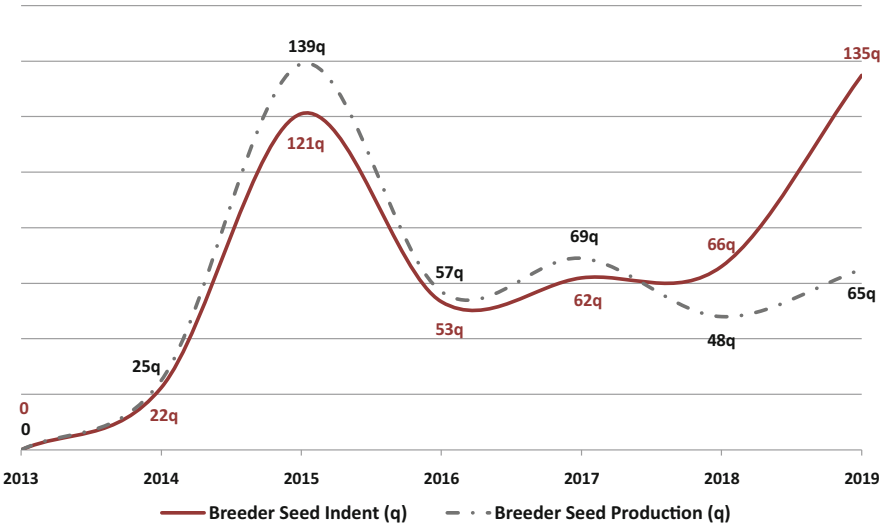

Fig. 12.11 Journey of Pusa Basmati 1509: a brand in making. Breeder seed indents and production from 2013 (notification year) onwards

grains (8.1 mm) and very good kernel length after cooking (17.0 mm). It also has very less grain chalkiness, intermediate amylose content (22.2%) and strong aroma (Singh et al. 2018a). This variety is becoming quite popular and in the near future is likely to occupy significant area under Basmati cultivation. Maintenance breeding comments: (1) tall off-types; (2) grain size variants.

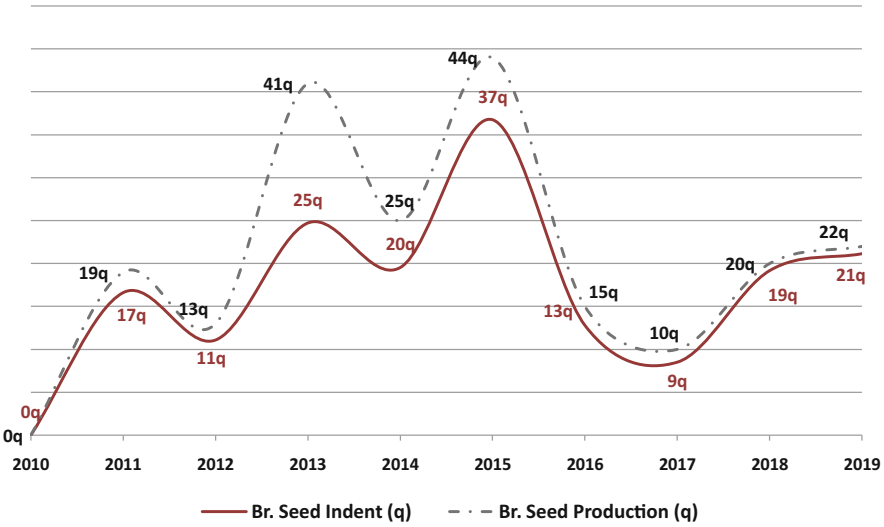

Fig. 12.12 Journey of Pusa Basmati 6: breeder seed indents and production from 2010 (notification year) onwards

12.4 Basmati Varieties Maintenance Breeding Procedure

The procedure adopted is panicle to row method:

- Single 'true-to-type' panicles (350–500 in number) are selected.
- Selected 'true-to-type' panicles are threshed individually. Each threshed panicle is critically screened for seed characteristics.
- For Basmati varieties, a small portion of seed of each panicle is subjected to grain and cooking quality testing.
- Seeds of panicles not conforming to seed characteristics or not performing well in cooking quality are rejected.
- In the case of varieties developed through MABB, an additional step is undertaken. Seedlings are raised from part of the seed from each panicle used for cooking, for screening the presence of target alleles of the genes incorporated using either gene-based or gene-linked markers (Fig. 12.13). Any panicle having any inadvertent plant without possessing the R-allele of the disease resistance genes is summarily rejected.
- Seeds of remaining 200–250 panicles are raised in panicle rows. A slight modification is raising of paired rows from a single panicle. Raising of paired rows from single panicle helps in better comparison amongst the selected panicles (Figs. 12.14a, b).

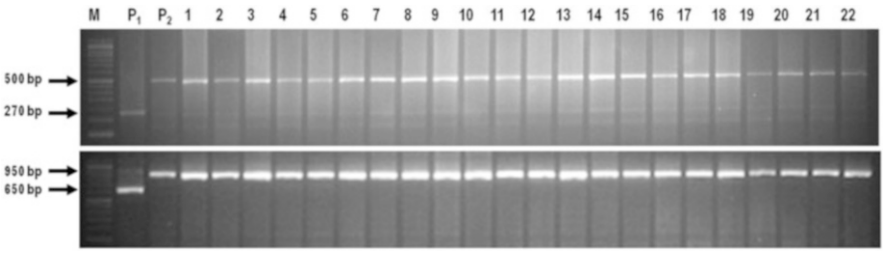

Fig. 12.13 Amplification profile of the molecular analysis of panicles of Pusa Basmati 1718 for the presence of BB resistance genes, *xa13* and *Xa21*, using gene-based marker, *xa13* prom and pTA248, respectively. All the panicles amplified 500 bp fragment for *xa13* prom and 950 bp with pTA248 corresponding to the resistance alleles of *xa13* and *Xa21*. Hence, all these panicles which were possessing desirable grain and cooking quality are only considered for raising panicle to row progenies for nucleus seed production. M, 100 bp ladder; P_1, Pusa Basmati 1121 (susceptible check); and P_2, Improved Pusa Basmati 1 (resistant check); 1–22, panicles harvested from nucleus seed plot for further maintenance, nucleus and breeder seed production

- Generally, the row length is kept 5 m long. Spacing between the rows (30 cm) and between two paired rows (60 cm). The plant-to-plant spacing is kept 20 cm. Thus, a 5 m long *paired row raised from a single panicle* would be having 50 plants (i.e. 25 plants/row and 50 plants/paired row). The wider spacing of 60 cm between two paired rows helps in the proper expression of individual plants and critical observation and screening of different paired rows. This layout can be modified as per availability of seedlings per panicle for transplanting for each paired row.
- A thorough screening of panicle rows at different crop growth stages is done. Diagnostic characteristics based on DUS guidelines (PPV&FRA 2007) for conduct of test for distinctiveness, uniformity and stability on rice are very useful in screening.
- Panicle rows not conforming to 'true-to-type' plant type or showing off-types are totally discarded as and when observed.
- Remaining selected panicle rows are harvested and threshed individually. And again harvested and threshed seed of each individual panicle row is critically examined.
- Finally, seeds of all retained 'true-to-type' panicle rows are bulked to get genetically pure high-quality nucleus seed.

Integration of cooking and grain quality test and screening for disease resistance genes in the maintenance breeding programme itself has significantly enhanced the consumer preference of these varieties. Maintenance breeding plots of specific varieties are raised in such a way that these are surrounded by the breeder seed plots of the same variety. This simple intervention (nucleus seed plots surrounded by breeder seed plots) not only prevents outcrossing with undesirable pollen as breeder seed plots act as buffer but also helps in visual comparison providing both micro and macro views of the variety (Fig. 12.14a, b).

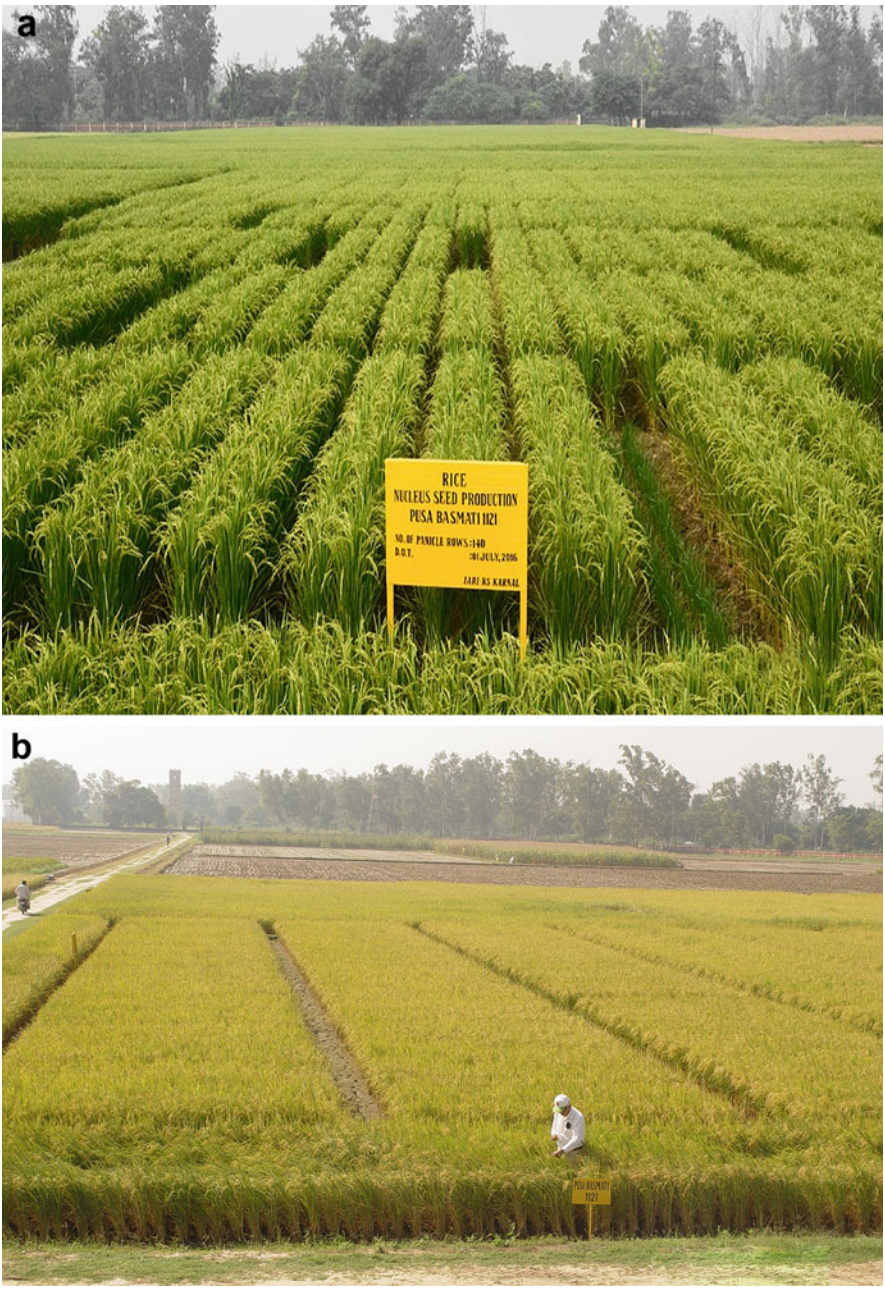

Fig. 12.14 (**a**) Pusa Basmati 1121 nucleus seed production (panicle paired rows). (**b**) Pusa Basmati 1121 maintenance breeding plots (nucleus seed) surrounded by breeder seed plots (to prevent outcrossing)

12.5 Maintenance Breeding, Seed Production, Off-Types and Rogues

Adequate understanding of terminology, namely maintenance breeding, generation system of seed multiplication, seed chain, off-types and rogues, helps in conceiving and executing a proper seed production programme. There are three recognized classes of seed in the Indian generation system of seed multiplication (i.e. breeder, foundation and certified seed), and seed supply chain follows a three- to four-tier system of multiplication (Breeder seed → Foundation seed → Foundation/Certified seed → Certified seed). The seed multiplication cycle starts with 'breeder seed', which is dependent upon availability of high-quality nucleus seed (a product of varietal maintenance). Any issue with the genetic purity of the breeder seed lot (presence of contaminants/off-types/mixtures) gets multiplied and results in an exponential increase in these contaminants in succeeding generations. The presence of these contaminants may lead to loss of identity and requisite traits of the variety for which it has been specifically bred.

12.5.1 Off-Types

Off-types are defined as plants showing a distinct phenotype from the sown variety and are unknown as a variety (Lee et al. 2013). The proportion of off-types in any particular population or seed lot depends on four factors: (a) rate of addition of plants in each generation and the number of generations of multiplication, (b) proportion of progeny of off-types which are also off-types (i.e. the stability of these off-types in subsequent multiplications), (c) relative rate of multiplication of off-type plants and (d) effectiveness of removal of off-types by roguing at each generation (Laverack and Turner 1995). Bateman (1946) described contamination as 'obvious' and 'cryptic' in the deterioration of certain British vegetable seed stocks when isolation distances were inadequate. He classified off-types produced by cross-pollination with other varieties into 'obvious' types, where a distinct phenotype was produced, and 'cryptic' where the resulting variant genotype was not easily seen from phenotypic characters. Obvious off-types would be seen more easily and removed. Cryptic off-types would be more difficult to detect and so could spread in the population with potentially serious consequences for yield and quality. This description of obvious and cryptic contamination is very aptly delineated by the famous optical illusion (Fig. 12.15) titled 'The Young Girl—Old Woman' (Attneave 1971). The cryptic off-types are generally camouflaged in larger seed production plots. These can only be removed effectively in maintenance breeding plots, where limited numbers of panicle rows are being critically observed, as against larger seed production plots (Figs. 12.16 and 12.17).

Fig. 12.15 Obvious or cryptic. The optical illusion 'The Young Girl—Old Woman' (the young woman's chin is also the old woman's nose). (Adapted from Attneave 1971). Cryptic off-type plants are often camouflaged

Fig. 12.16 Obvious (tall off-type)

Fig. 12.17 Cryptic (grain size off-type)

12.5.2 Roguing

Roguing may be defined as the selective removal of undesirable plants from a seed crop on the basis of visual inspection in the field in order to improve one or more quality attributes of the seed lot to be harvested. Roguing for genetic purity is an attempt to maintain the original genetic base of a variety. By defining the limits of phenotypic variation and removing non-conforming plants, it seeks to achieve uniformity expected or required. Roguing represents a continuation of the maintenance process in order to restrict variation within an acceptable level, but it is necessarily imprecise because judgments about genotype (G) are made from the phenotype, which is the result of genotype and environment (G × E) interaction (Laverack and Turner 1995). Proper understanding of rice plant morphology and its descriptive features helps in undertaking effective roguing. DUS guidelines of Rice gives the descriptors of rice plant (62 characteristics; 29 asterisk characteristics) (PPV & FRA 2007). These guidelines are very helpful in identifying off-types from true-to-type plants in maintenance breeding plots as well as for undertaking roguing operations in large-scale seed production plots (Figs. 12.16 and 12.17).

The point to be understood here is that in maintenance breeding plots (nucleus seed plots), we never undertake roguing. It is the summary rejection of panicle rows expressing any sort of variants. Roguing operations are undertaken only in large-scale seed production plots (breeder, foundation, certified or truthfully labelled seed).

12.6 Conclusions

Putting together all the disparate components discussed above (maintenance breeding, seed chain, seed production, true-to-type, off-type and roguing), it can be concluded that varietal development, maintenance breeding and seed production represent a continuum. For any effective crop varietal development programme, maintenance breeding plays a pivotal role in its ability to saturate the area under cultivation with genetically pure seed. Basmati export trade in international markets is going to have a bigger and wider footprint in the coming years. If this sector is to be developed, akin to software industry with a potential to generate more than Rs. 50,000 crores forex in next 3–5 years (Basmati Rice-Life in Science with Pallava Bagla 2020), then the Basmati rice exports have to be tailor-made to meet consumers' preferences. Global Basmati markets are now becoming mature with very discerning buyers in the EU, North America and the Middle East, where authentic Basmati rice gets a premium price. Nader et al. (2019) clearly depict the kind of scrutiny and pedigree checks these markets undertake for authenticity testing of this premium Basmati grain (Fig. 12.18). Another interesting and insightful indicator is UK Code of Practice on Basmati Rice (CoP) of 2017, which clearly

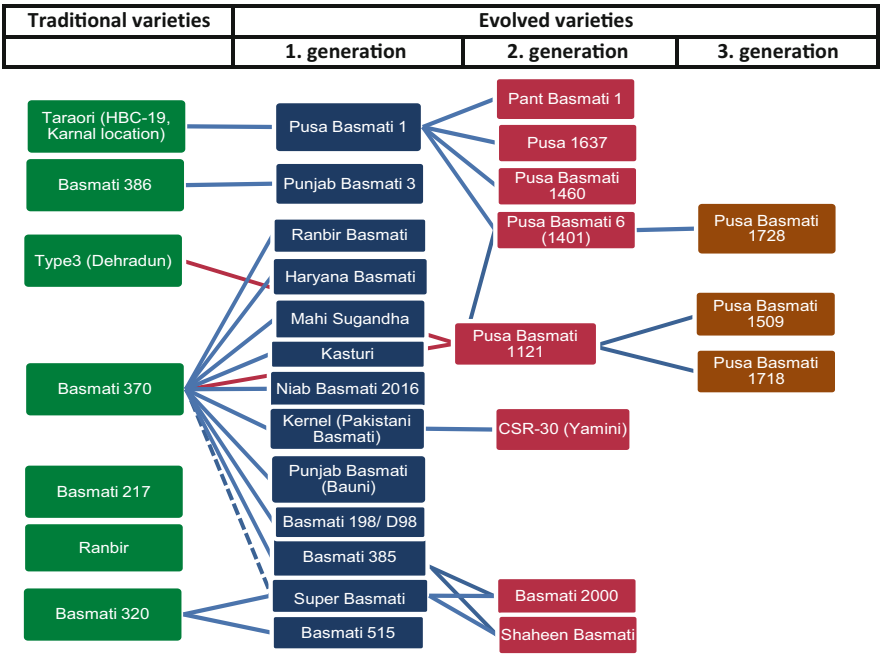

Fig. 12.18 EU strict authenticity checks: pedigree of Basmati rice varieties based on information about their breeding history, which was available in the public domain. (Adapted from Nader et al. 2019)

stipulates that when the description of the product is 'Basmati rice', the non-Basmati rice content must not exceed 7%. This tolerance is in place to take account of seed impurity and other segregation issues at origin (http://www.riceassociation.org.uk/content/1/47/2017-basmati-code-of-practice.html). This type of rigorous scrutiny of Basmati rice is an explicit imperative for a very strong varietal maintenance programme for all the Basmati varieties in seed chain, to keep India's dominance in Basmati markets.

Acknowledgements The authors would like to put on record their gratitude to Dr. S. S. Atwal, Ex-Head, IARI Regional Station, Karnal, and Dr. V. P. Singh, Ex-Principal Scientist, IARI, New Delhi, for initiating the systematic maintenance breeding work of Basmati rice varieties at IARI Regional Station, Karnal. This legacy is being continued, with the hope that systematic maintenance breeding will become a national mandate for all the breeding and seed production units working on various field and vegetable crops.

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Maintenance Breeding

13

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Abstract

The improved varieties play a pivotal role in agricultural development and economy of a country. These developed varieties are evaluated in All India Coordinated Trials prior to identification, release and notification. As per the provisions of Seed Act (1966), only the notified kinds or varieties are eligible for multiplication under certified seed production programme. The Indian seed programme largely adheres to the limited generation system for seed multiplication. The system recognizes three generations, namely breeder, foundation and certified seeds, and provides adequate safeguards for quality assurance in the seed multiplication chain to maintain the purity of the variety as it flows from the breeder to the farmer. However, one of the major constraints in enhancing crop productivity is deterioration of varieties when they are multiplied in subsequent generations. The maintenance of a variety in its original and purest form is referred to as 'maintenance breeding', where a breeder and/or seed technologist maintains the genetic identity and purity of a released variety, as it undergoes production year after year. The terms 'maintenance breeding/varietal maintenance' and 'nucleus seed production' are synonymous. There are different procedures for variety maintenance and nucleus seed production for various

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_13

forms of cultivars in different crops. There is paucity of literature pertaining to the maintenance and multiplication of improved open-pollinated varieties (OPVs) and hybrids in cross- and often cross-pollinated crops. This chapter provides appropriate, user-friendly procedures as well as guidelines for maintenance of inbred lines and varieties or nucleus seed production, which can be helpful for maintaining varietal uniformity and purity applicable to some of the important cross- and often cross-pollinated crops like maize, pearl millet, sunflower, castor, rapeseed and mustard, pigeon pea, sorghum, safflower and cotton.

Keywords

Cross-pollinated crops · Generation system · Inbred lines · Maintenance breeding · Variety maintenance · Reserve seed method · Plant row method · Nucleus seed production · Often cross-pollinated crops · Seed quality

13.1 Introduction

Over the last five decades, India has experienced an impressive growth trajectory, thus transforming from a food scarce country to a food sufficient and to a food surplus one. Having experienced a situation of 'ship to mouth', India has emerged as the largest producer of milk, spices, cotton and pulses and the second largest producer of wheat, rice, fruits and vegetables (DoAHD&F 2019). This became possible with infusion of new technologies, innovative institutional engineering and proper incentives. However, as we look forward, these food systems face many challenges ranging from increasing pressure on natural resources (soils, water, air, forests, etc.) to climate change, fragmented landholdings, increasing urbanization and malnutrition among children (Gulati et al. 2021). Hence, India further needs a right mix of policies from subsidy driven to investment driven, and from price policy to income policy approach, promoting agricultural production and diversification towards more nutritious food to mitigate these problems.

13.2 Development of New Varieties

The improved varieties play a critical role in agricultural development of a country. Hence, the primary objective of crop improvement research is the development of improved/superior varieties. The mode of reproduction (breeding system) of crop plants influences the level of genetic variability present in the crop population as well as the breeding and selection methods suitable for crop improvement. In addition, the particular type of reproduction behaviour can impose practical limits on the efficiency of certain breeding and selection procedures. For this reason, breeders have sought to genetically alter the breeding system of crops. The use of cytoplasmic male sterility and the modification of self-incompatibility are examples of alterations that have been affected in breeding systems to produce new methods for crop

improvement (Ahmar et al. 2020). In addition, the breeding system must be manipulated to produce hybrid cultivars to overcome the stagnation in production potential of varieties. Present-day crop breeding and selection methods are based on the genetic principles propounded in Mendel's laws of inheritance (Allard 2010). The readers are requested to refer to the basic genetics and/or plant breeding and/or plant biotechnology texts to seek explanation of the terminologies and methods, since a complete coverage in the context of chapter is not possible.

13.3 Identification, Release and Notification of New Varieties

In order to reduce the dependence on foreign countries and to ensure the food and nutritional security of burgeoning population, the Indian government established All India Coordinated Research Projects (AICRPs) and other institutes in a systemic manner to produce a large number of varieties with assured seed quality in all major crops. The production of high-quality seeds was one of the pillars to change the position of Indian agriculture into the new world order. The ultimate intention was to introduce the newly evolved high-yielding cultivars to the resource-poor farmers for cultivation in the area of their adoption (Chand et al. 2021b).

In pursuance of these objectives, the Government of India acknowledged seed as an essential commodity under the Essential Commodities Act, 1955. During October 1964, Varietal Release System (VRS) came into existence with the formation of the Central Variety Release Committee (CVRC) at the national level and State Variety Release Committees (SVRCs) at each state level. A Central Seed Committee (CSC) was established under the then Ministry of Agriculture as per the provisions of the Seeds Act, 1966. The functions of the CVRC were taken over by the CSC in 1969 to ensure the notification of the kinds/varieties and regulate the quality of seeds being offered for sale. Further, the CSC constituted a Central Sub-Committee on Crop Standards, Notification & Release of Varieties for Agricultural Crops and Horticultural Crops to perform the functions related to release/notification, provisional notification and de-notification of cultivars at the central level, whereas State Seed Sub-Committee (SSSC) was constituted to perform similar functions at the state level (Mohan and Nigam 2013).

In India, new improved varieties of crops are developed by Crop Research Institutes of Indian Council of Agricultural Research, State Agricultural Universities and few Private Seed Companies. These varieties are tested (evaluated) for a minimum period of 3 years, before identification and consideration of release for cultivation. The official testing of candidate varieties for identification on national or zonal basis is carried out by the All India Coordinated Research Project (AICRP) of a given crop. The AICRP conducts the trials under its supervision at experimental centres of ICAR Research Institutes, State Agricultural Universities and officially recognized Private Seed Companies (Tonapi et al. 2015a).

After due deliberations, the candidate entry is released for general cultivation, if found promising. Once a variety is accepted for release, it can be notified in the gazette. After official release (at state as well as central levels), the cultivars are

notified under the Seeds Act so that the quality of seeds can be regulated. The main purpose of notification is to bring the seeds of a particular crop/variety under the purview of Seed Law Enforcement, mainly to empower the seed inspectors to verify the quality of its seeds by sampling and analysis. The notification is made by the central government on the recommendation of the Central Seed Committee. The proposals for notification of a state variety are forwarded in the prescribed format by the state government after its release in a particular state to the Central Seed Committee for consideration. At this stage, details about All India trials need to be furnished even for state released varieties. Once notified, a variety can be multiplied under certified seed production (Chand et al. 2021b).

13.4 Seed Multiplication Chain

The variety comes into seed multiplication chain soon after its gazette notification. The commercial seed production involves management of complete seed chain which includes three stages of seed, viz. breeder seed, foundation seed and certified seed. Nucleus seed, of course, is not an official class of seed in India, but is the source for production of breeder seed and therefore has highest genetic purity. It is the end product of the maintenance breeding programme. Breeder seed is produced from nucleus seed under the supervision of a qualified plant breeder in a research institute or agricultural university which has developed the variety. This provides for initial and recurring increase of foundation seed. Breeder seed is monitored by a joint inspection team of scientists and officials of certification agency and National Seed Corporation. Breeder seed shall be genetically so pure as to guarantee that in the subsequent generation, i.e. certified foundation class shall conform to the prescribed standards of genetic purity. Foundation seed is the progeny of breeder seed and is produced by National Seed Corporation, State Seed Corporations and SAUs under technical control of qualified plant breeders or technical officers. Its production is supervised and approved by state seed certification agency (SCA). The minimum standards for genetic purity and other quality parameters are available in Indian Minimum Seed Certification Standards for foundation as well as certified seed classes. Foundation seed may also be produced from foundation seed which can be clearly traced to breeder seed. Certified seed is the progeny of foundation seed or certified seed produced from foundation seed, and its production is also supervised and approved by certification agency. The seed of this class is normally produced by the State and National Seeds Corporation and Private Seed Companies on the farms of progressive growers. This is the commercial seed which is made available to the farmers (Kumar et al. 2017a).

In India, seed certification standards have been prescribed for foundation seed and certified seed only. There are two types of standards, i.e. field standards and seed standards. The field standards apply to the seed production plots and standing crops, whereas seed standards are applicable at the seed level. Field standards include land requirements, isolation requirements, maximum permissible level of off-type, inseparable other crop plants, pollen shedders (in male sterile line), plants infected by

seed-borne diseases, etc. Seed standards include genetic purity, physical purity, germination, other crop seeds, moisture content, etc. (Tunwar and Singh 1988).

In the generation system of seed multiplication, the production of a particular class of seed from specific class up to certified seed stage is carried out (Agarwal 2008). The choice of a proper seed multiplication model is the key to further success of a seed programme which basically depends upon:

(a) The rate of genetic deterioration.
(b) Seed multiplication ratio.
(c) Total seed demand.

13.5 Genetic Deterioration

One of the main constraints in the availability of quality seed is deterioration of the variety during multiplication over years primarily due to the lack of knowledge of variety maintenance methodology. There may be several reasons for variety deterioration (Kadam 1942) as listed below:

- Developmental variations.
- Mechanical mixtures.
- Natural crossing.
- Genetic shifts.
- Selected influence of pests and diseases.
- The techniques of the plant breeder.
- Mutations.

The variety deterioration in its physical and physiological traits may also lead to genetic aberrations. The frequency of chromosomal aberrations induced during seed ageing gradually increases with the increase in the time of ageing. The factors like moisture, temperature, relative humidity and activity of insect pests are related to the retention of seed quality and thus the deterioration of variety in storage. High temperature, relative humidity and moisture in the storage environment appear to be the principal factors involved in the deterioration of physiological seed quality (Dahuja and Yadav 2015).

13.6 Seed Production Models

Seed multiplication ratio (SMR) is the number of seeds to be produced from a single seed (broadly ratio of seed yield to seed rate). One of the key elements of a seed production system is estimation of actual demand considering factors like SMR, weather, market, farmers' skill to maintain seed and sources of seed. Fair demand assessment is crucial for actors engaged in the system including government, producers, importers and distributors. Based on actual demand, seed multiplication

models may be derived for each crop, and the seed multiplication agency should decide how quickly the farmers can be supplied with the seed of novel varieties for faster variety replacement, subsequent to the supply of breeder seed to the concerned agency, for faster variety replacement (Kumar et al. 2017a). In view of these basic factors, the chain of seed multiplication models could be:

(a) **Three-generation model**—breeder seed-foundation seed-certified seed.
(b) **Four-generation model**—breeder seed-foundation seed (I)-foundation seed (II)-certified seed.
(c) **Five-generation model**—breeder seed-foundation seed (I)-foundation seed (II)-certified seed (I)-certified seed (II).

13.7 Principles of Maintaining the Genetic Purity During Seed Production

The important safeguards for maintaining genetic purity during seed multiplication (Singhal 2003) are:

(a) Control of seed source.
(b) Preceding crop requirement.
(c) Providing adequate isolation to prevent contamination by natural crossing or mechanical mixtures.
(d) Roguing of seed fields, prior to the stage at which they could contaminate the seed crop.
(e) Periodic testing of varieties for genetic purity through grow-out test.
(f) Avoiding genetic shift by growing crops in areas of their adaptation only.
(g) Certification of seed crops to maintain genetic purity and quality seed.
(h) Strictly adhering to the generation system.

The above mentioned principles will take care of prevention of factors responsible for deterioration of a variety. However understanding of characteristics of specific varieties and their expression in various environments is essential for breeders and or seed technologists involved in seed production and maintenance breeding.

13.8 General Principles of Raising Crops for Maintenance Breeding

Seed production in general and nucleus seed production in particular differ from commercial crop production in several aspects (Pandita et al. 2017). Some important principles in the production of quality (nucleus/breeder) seed that are to be taken into consideration have been discussed below.

13.8.1 Land Requirement

The land selected for seed production should be well fertile, levelled and with proper drainage. It should be completely free from volunteer plants (self-sown plants). Volunteer plants are a big problem, e.g. in *Brassica* sp., legumes, sorghum, pearl millet, etc. The self-sown plants may continue to appear even up to 3–4 years. There is no problem of volunteer plants in maize and wheat. The volunteer plants may contaminate directly by producing seed, if not removed. They may also contaminate through crossing with the main variety, particularly in cross-pollinated crops.

In addition, there should be no weed plants in the field or within isolation distance which are cross compatible to the seed crop, e.g. Johnson grass in sorghum. Selection of proper land for seed production is quite helpful in the prevention of the deterioration of variety through mechanical mixtures and natural outcrossing with volunteer plants (Agrawal 2015).

13.8.2 Isolation Requirements

The seed production plot should be isolated from various sources of contamination by a certain minimum distance, known as isolation distance. Isolation from contamination source is much more important for cross-pollinated crops to prevent genetic contamination through pollen. The contamination source may be volunteer plants in the field, other varieties in nearby fields or same variety not conforming to genetic purity and other cross-compatible species (may be crops or weed plants) that need to be kept at a minimum distance (Tunwar and Singh 1988) as mentioned (Table 13.1).

The figures in the parenthesis are isolation distance from different kernel coloured plants and teosinte in the case of maize and from Johnson grass and high tillering forage sorghum with grassy panicle in the case of sorghum. It may be noted that in

Table 13.1 Isolation requirements for different seed crops for certified seed classes

Crop	Isolation distance (m) for	
	Foundation seed	Certified seed
Cotton	50	30
Maize (inbreds and foundation single cross)	400 (600)	–
Maize (composites, synthetics, OP)	400	200
Maize (hybrids)	–	200 (300)
Pearl millet (comp, syn and OP)	400	200
Pearl millet (hybrids)	1000	200
Pigeon pea	200	100
Rapeseed and mustard (self-compatible types)	200	100
Rapeseed and mustard (self-incompatible types)	50	50
Sorghum (OP varieties)	200 (400)	100 (400)
Sorghum (hybrids)	300 (400)	200 (400)
Sunflower	400	200

any case the isolation for nucleus seed production/maintenance breeding plot should not be kept less than the recommended distance for foundation seed production.

13.8.3 Harvesting, Threshing and Processing

Harvesting and threshing of seed crop needs more care as any damage to seed may lead to loss in germinability. The threshers/combine harvesters should be thoroughly cleaned to avoid mechanical mixing. The crop should be harvested at proper stage and moisture content to minimize mechanical damage to seed. In hybrid/MS line seed production, where two parents are used, harvesting requires special attention. All the male parent rows should be harvested first and moved away from the field. The plot is then inspected for the presence of any male parent plants or ears/panicles. The female parent rows are then harvested as hybrid seed/MS line seed (Benaseer et al. 2018). After harvesting, proper threshing would be of vital importance to avoid any physical impurity. Subsequently the seed has to be conditioned that may involve pre-cleaning, drying and treatment etc. and it should be packed and labeled in the manner that no mixing happens and the seed maintains highest of its quality in storage.

13.8.4 Storage

Storage losses of seeds/grains due to insect pests have been recorded throughout the world. Seed security is the key to attain food security. Before its utilization for sowing purpose, seeds may require storage for some period depending on a particular purpose of seed production. Like most biological materials, nucleus seed is also vulnerable to various factors that can cause the deterioration of seed quality during storage. Apart from temperature and relative humidity of the storage environment, infestation of insects or rodents can contribute to loss of seed quality (FAO 2018).

13.9 Maintenance Breeding/Variety Maintenance

At the time of release of a variety, a small quantity of seed is available with the plant breeder. Commercial quantity of seed is produced after a series of multiplication steps, and it starts with the maintenance breeding programme in which nucleus seed is produced. The nucleus seed is used for the initiation of seed multiplication chain where breeder, foundation and finally certified seed are produced. Maintenance of a variety is required for continuous supply of quality seed to the farmers in sufficient quantity. The maintenance breeding programme helps in:

(a) Purification and maintenance of variety and consequent nucleus seed production.

(b) Reduction in the amount of roguing required in large breeder seed production plots.
(c) Removal of certain specific off-types which can be detected only at nucleus seed production stage.
(d) Extension of useful life of varieties.

Maintenance breeding is the branch/area of plant breeding which deals with principles and methods of nucleus seed production and variety maintenance. The terms maintenance breeding/varietal maintenance and nucleus seed production are synonymous. Laverack (1994) defined variety maintenance as 'the perpetuation of a small stock of parental material through repeated multiplication following a precise procedure'. The precise procedure refers to **plant row method** 'Evaluation of selected (true to the type) plants on the basis of performance of their progenies'. Here, performance does not mean yield per se, but trueness to the variety. Plant row method is suitably modified depending upon growth habit and reproduction behaviour of the crop.

(a) **Self-Pollinated Crops**
In most of the self-pollinated crops like chickpea, garden pea, green gram, soybean, field pea, etc., **plant row method** as such is used for variety maintenance, where it is easy to take out entire single plants. In the case of tillering and closely planted crops like wheat, barley rice, etc., single ear/panicle is selected, and the method is termed **ear row** or **panicle row method**. Cowpea plant has a twining growth habit, so the method is modified to **cluster row method** (Yadav et al. 2003), where single cluster is selected instead of single plant. The part of the plant selected for planting in single rows should have a sufficient number of seeds to be used for evaluation. The plant row method is described below:
- Single plants (true to the variety) are selected, harvested and threshed individually.
- Individual plant seed is screened for seed characteristics. Seed lots having variant seed or seeds are rejected.
- Individual plant seed is planted in single rows (one plant seed-one row).
- Plant rows are critically screened at different growth stages.
- Variant rows or rows with variant plant(s) are discarded as and when detected.
- Single plants typical of the variety are again selected from the remaining rows (for the next cycle of maintenance breeding).
- The rows are harvested and threshed individually. The seeds are again screened for varietal traits.
- Seed packets typical of the variety may be bulked to constitute NSS-I or may be used for sowing in NSS-II plots, if nucleus seed requirement is high.

(b) **Cross-/Often Cross-Pollinated Crops**
In often cross-pollinated crops, there is a certain amount of outcrossing depending upon flower structure and pollination agents. Therefore, the produce of single plant rows cannot be taken as nucleus seed, as there might be some

cross-contamination from adjoining variant rows detected after pollination. So the plant row method is further modified to **reserve/rest seed method** where single plant seed, after screening, is divided into two parts maintaining its identity. One part is used for evaluation in plant rows, while the other one is kept in laboratory as reserve seed. The reserve seed packets of the plants which have true-to-type progeny (as evaluated in plant rows) are bulked to constitute nucleus seed. This method has been found very effective in purification and maintenance of pigeon pea and mustard varieties. In highly cross-pollinated crops, the cultivars are mainly open-pollinated varieties or synthetics or composites; a certain level of gene frequency for desirable traits is maintained following the Hardy-Weinberg principle. So, the cultivars are maintained in isolation with random mating (no selection of single plants). In such crops, some amount of variation is tolerated as it is a part of the variety. Nowadays, the majority of cultivars in cross-pollinated crops are hybrids, where maintenance procedure is applied on parental inbred lines as purity of hybrids is governed by purity of its constituting inbreds. The inbred lines are maintained by selfing. Inbred lines showing extreme inbreeding depression may be maintained by sib-mating or a combination of selfing and sib-mating.

13.9.1 Reserve Seed Method of Variety Maintenance

(a) A large number (300–500) of single plants typical of the variety are selected from an initial seed crop plot. The number of single plants depends upon the age of the variety. However, higher numbers of plants are selected in a newly released variety as compared to an established variety.
(b) These selected single plants are harvested and threshed individually. The threshed seed is examined critically for colour, shape and size. The seed packets that are not true representative of the variety are rejected. The selected individual plant seed is divided into two parts maintaining their identity (the serial number of the selected plant is written on both of the packets).
(c) In the next season, one part of the seed from selected plants is sown in plant rows (3–5 m long depending upon the number of seeds) for evaluation. The remaining seed (rest part) of each plant is stored. It is very important to maintain the identity of the plant row and the reserve seed (e.g. for a particular plant seed, the row planted from one part of the seed and the reserve seed stored should be given the same number).
(d) The individual plant progeny rows are examined critically throughout the growing season. Any row with one or more off-types is rejected as and when detected. The rejection is recorded in the notebook also.
(e) At the time of flowering, it should be examined daily or at alternate days to minimize genetic contamination from off-type plants. All the rejected rows should be removed as and when detected.
(f) Plant rows true to the variety for morphological characters are retained. These rows are harvested separately.

(g) Seed from individual plant progeny rows is again examined for colour, shape and size. The seed of plant rows not conforming to the variety is rejected.
(h) Reserved seed (kept in stores) of single plants representing true-to-type progenies is bulked to constitute the nucleus seed. For the next cycle of variety maintenance, single plants are selected from crop grown using this reserve seed.

In areas with very low pollinator frequency or covered plots, the single plant progeny row (used in evaluation) produce may also be used as nucleus seed, but priority should always be given to reserve seed.

This bulked seed is planted in isolation for further multiplication or breeder seed production.

The reserve seed method is cumbersome and requires more efforts from the maintenance breeder. So after two to three cycles of reserve seed method when the variety gets purified, one may opt for two to three cycles of **mass selection**. In mass selection method, the large number of true-to-type plants is selected from the central part of the field (as border areas of plot have more cross-contamination). After a thorough screening of the single plants' seed, the true-to-type plant seed is bulked to constitute nucleus seed. After two to three cycles of mass selection, there may be a need for purification, and we may go for variety maintenance through reserve seed procedure.

13.9.2 Maintenance of Varieties Derived Through Marker-Assisted Selection (MAS)

The successful adoption of marker-assisted selection (MAS)-derived varieties depends on the appropriate after-release follow-up, particularly in maintenance breeding. Unlike other varieties, the maintenance breeding of MAS-derived varieties involves testing genetic purity of the seeds through gene-based/gene-linked markers for the homozygosity of the target allele(s) for tolerance or stress resistance. This follow-up step is very important not only at the nucleus and breeder seed production levels but also during the certified seed production, for the maintenance of seed quality (Singh et al. 2019).

13.9.3 Reserve Stocks

It is always desirable to retain a reserve stock to safeguard against loss from crop failure. These stocks must be stored under cold storage for at least 2 years. Seed stores should be dry and cool with proper sanitation. The seeds should be dried to 8–12% moisture content for storage in moisture impermeable; polythene or poly lined/tin containers and cloth/jute bags, respectively. The recommended temperatures and relative humidity (Smith 1992) are as follows:

1. Short term (Up to one year): 20 °C/60% RH.
2. Medium term (One year to 2–3 years): 15 °C/40% RH.
3. Long term (<5 years): 5 °C/30% RH.

13.9.4 Grow-Out Test

The quality of nucleus seed can be assured by conducting grow-out test. GOT is also conducted to monitor the genetic purity of the breeder seed lots. A sample of nucleus/breeder seed lot is drawn and subjected to grow-out test as per the procedure. The GOT for nucleus seed acts as pre-control while that of breeder seed as post-control plots for genetic purity of breeder seed (Prasad et al. 2017).

Maintenance breeding/nucleus seed production of major cross- and often cross-pollinated crops has been described in this chapter.

13.10 Maintenance Breeding of Crops

13.10.1 Maize

Maize is a diploid ($2n = 20$) annual plant belonging to the family Gramineae and the tribe Maydeae. Unlike other grass species, maize plants are fairly tall with height ranging from 1.5 to 3 m. Based on endosperm characteristics, maize can be classified (Kumar et al. 2012) as:

Pop type: small smooth kennels with hard endosperm.
Flint type: large smooth kernels, mainly hard endosperm but often with a small floury centre.
Floury type: large smooth kernels with floury endosperm.
Dent type: large kernels with a central core of floury endosperm, which on drying shrinks more than the surrounding hard tissue denting the kernel.
Sweet type: carbohydrates stored largely as sugars, kernels wrinkle and turn translucent when dry.

13.10.1.1 Floral Biology and Pollination

The knowledge of the reproductive biology of maize enables scientists to carry out selfing and sibbing—the two main ways of effecting inbreeding in maize. It also helps in carrying out hybridization by way of crossing desirable parents. The plants are monoecious where the tassels (male flowers) emerge at the top and the female flowers (the cobs) are borne in the axils of the lower leaves. The stigmatic surfaces of the female flowers (called silks) emerge from the leaf axils shortly (after 2–5 days) after tassel emergence, making the crop protandrous in nature. The seeds are formed on the cobs, which are compact stalks of female inflorescences. The male inflorescence is a loose terminal panicle, which has a central rachis bearing lateral branches spirally. The spikelets are arranged in rows, and each spikelet has two glumes which

are equal in size. Lemma is oval and contains palea. Spikelets are lodiculate and staminate (bear three stamens). Palea is membranous and flat with intruded margin. Lodicules are two in number, fleshly and truncated. Pistillate (female) inflorescence is borne at the end of a short axillary branch called shank. Leaves are reduced to leaf sheath, which constitutes husk covering the ear. Ear is modified spike and has a thick central axis called cob, on which numerous paired spikelets are arranged in a series of longitudinal rows. The ovary is superior; superior style is terminal, long and filiform. Stigma is bifid; entire style is hairy and receptive. The ovary has one ovule with basal placentation, fruit is a caryopsis and seed is monocotyledonous with endosperm.

Within the grass family, maize produces the largest pollen grains (90–125 μ × 85 μ). The pollen volume and weight are approximately 700×10^{-9} cc and 250×10^{-9} g, respectively. Maize pollen principally travels through air, but due to its larger size and gravity, the pollen cannot travel long distances as compared to other members of the Maydeae tribe of Poaceae family. Pollen remains viable from a few hours to several days. Higher temperature and low relative humidity adversely affect pollen viability.

Maize is a typically cross-pollinated species because of its monoecious and protandrous nature. It is an anemophilous crop, where pollination is facilitated by wind. The degree of male and female floral synchrony is specific and sensitive to plant population, soil fertility and environmental stress. A tassel sheds pollen for 2–14 days (depending on genotype and environment with major shedding between 5 and 8 days, where pollen is released for approximately 4–5 h, commencing 1 h after sunrise. This period may be delayed by 1–2 h, if the weather is cool and cloudy. Each plant, depending upon the genotype, is capable of producing 9000–50,000 pollens per kernel set. Antlers with a minimum overlap result in approximately 5% self-fertilization. Silks emerge on the ear over a period of time, which grow continuously and are receptive throughout its length. The silks are receptive at emergence and remain receptive up to 10 days. After fertilization, the silks stop elongation and desiccate rapidly. If not fertilized, the silk continues to elongate until it is fertilized or cell elongation is complete (Kumar et al. 2012).

13.10.1.2 Important Diagnostic Characteristics

The characteristics of maize (UPOV 2009) are detailed in Table 13.2 below to help the seed technologists and breeders to identify true-to-type plants/progeny rows and eliminate the off-type/undesirable plants/progenies.

13.10.1.3 Maintenance and Nucleus Seed Production of Inbred Lines

Maize is a cross-pollinated crop; however, it is self-compatible; as a result, up to 5% natural selfing occurs in maize. Practically, it is possible to encourage inbreeding in maize by pollinating with pollen from same (selfing) or related plants (sibbing). Inbreeding leads to homozygosity. This is attainted much faster in the case of selfing, while to a lesser extent by sib-mating. Maize being a cross-pollinated species, it exhibits inbreeding deficiencies. However, over the past few decades, population

Table 13.2 Important diagnostic characteristics of maize

Characteristics	States	Stages of observations
Anthocyanin coloration of sheath	Absent/weak/strong	Seedling
Anthocyanin coloration of brace roots	Absent/present	Reproductive
Width of leaf blade	Narrow/medium/broad	Vegetative
Angle between main axis and lateral branches	Small/medium/large	Reproductive
Number of primacy and lateral branches	Absent/few/many	Vegetative/ reproductive
Plant height	Short/medium/long	Vegetative/ reproductive
Time of silk emergence	Early/medium/late	Silk emergence
Anthocyanin coloration at the base of glume	Absent/present	Reproductive
Time of anthesis	Very early/early/medium/late/very late	Reproductive
Anthocyanin coloration of anthers	Absent/present	Reproductive
Density of spikelets	Lax/medium/dense	Reproductive
Anthocyanin coloration of silk	Absent/present	Silk emergence
Length of peduncle	Short/medium/long	Reproductive
Anthocyanin coloration of glumes of cob	White/light purple/dark purple	Reproductive
Ear diameter	Small/medium/large	Reproductive
Ear shape	Conical/cylindrical	Reproductive
Number of rows of grains	Few/medium/many	After harvest
Type of grains	Flint/semi-flint/semi-dent/pop/waxy/ opaque/opaque tinge/sweet	After harvest
Row arrangement of grains	Straight/spiral/irregular	After harvest
Grain shape	Shrunken/round/indented/pointed	After harvest
Grain size	Small/medium/bold	After harvest

improvement programme, especially recurrent selection, has increased the vigour of inbred lines to a considerable extent.

Inbred lines are derived through rigorous selfing and/or sib-mating (seven to eight cycles). They are considerably homozygous. The goal in inbred line maintenance is to maintain the performance and appearance (physical and genetic purity) of original lines, which includes proper isolation, rigorous elimination of off-types (roguing), care in pollination procedures (selfing or sibbing) and using accurate pedigree records and labels. Inbred line maintenance can be accomplished through self-pollination, sib-pollination or a combination of these. Selfing helps in maintaining inbreds in homozygous condition, while sib-mating tends to prevent excessive loss of vigour. In selfing, representative plants of the inbred lines are self-pollinated, and those with uniform characteristics with the inbred description are harvested

individually. Ears consistent with inbred characteristics are shelled separately. In the next year, parts of the seeds of individually shelled ears are sown as ear to progeny rows. Off-type rows are eliminated and rows with characteristics consistent are selected and self-pollinated. Self-pollinated cobs are harvested individually, and off-types are rejected. Ears are shelled separately. A portion of seeds is retained separately, to be used for future progeny testing, and the rest is bulked as nucleus seed.

Alternatively, out of bulked seeds, inbred line is planted. Off-type plants are rogued out before flowering. This is followed by sib-mating, which is pollination between plants within a row. Moreover, both plant-to-plant as well as bulk sib-pollination are practised. Plant-to-plant sib-pollination is safer.

Inbred lines can also be maintained in isolation, allowing for open pollination after thorough roguing of off-types. True-to-type plants are retained and involved in sib-mating. Off-type ears are rejected after harvest. True-to-type cobs are shelled in bulk. Seeds from best cobs are retained and used as nucleus seed, whereas the rest is used as breeder seed.

The most convenient way of maintaining inbred lines is to grow them in a big seed plot in isolation and execute rigorous roguing at four stages of crop growth, i.e. at knee-high stage, flowering, post-flowering and at harvest. The cobs of all plants are covered with silk bag before silk emergence. Once the breeder is sure that all off-type plants are rogued out of the seed plot, the silk bags are removed and open pollination is allowed to take place. After harvest, selection is made on the basis of ear and grain characters. One hundred best representative ears constitute breeder's seed after bulking the seeds. Out of the selected 100 ears, 50 to 75 seeds are bulked to make up nucleus seed. The rest of the ears harvested from the seed plot are bulked to constitute breeder seed. In the whole process, extreme care is taken to rogue out off-type plants to encourage homogeneity in the material (Singh et al. 2003).

13.10.1.4 Maintenance of Composites/OPVs

Open-pollinated varieties (OPVs) refer to a collection of individuals which share a common gene pool. Synthetics have been derived through interbreeding of lines with good general combining ability, while composite varieties are interbred populations in advanced generation of promising genotypes without any knowledge of their combining ability. Open-pollinated varieties (OPVs) are easier to develop than hybrids; their seed production is simpler, relatively inexpensive and is adapted to the local environment. The subsistence farmers who grow them can save and exchange own seed for planting in the following season, reducing their dependence on external sources. OPVs are particularly suitable for tribal and hilly regions, where seed replacement rate is very low.

In the case of OPVs, care must be taken for actual representation of the variety. Only off-type plants should be removed to minimize inbreeding depression. The number of plants to be used to advance generation is dependent on two factors: the number of plants required to adequately represent the variety and the quantity of the seed required to meet the future seed requirements. Mild selection during seed production and multiplication is inevitable; however, it should be minimized.

Varietal maintenance is normally done in isolation following half-sib method. Fifty to 100 seeds are to be bulked from each cob from representative plants of the variety to form nucleus seed. About 5000–10,000 seeds are normally sufficient to represent OPVs and provide adequate quantity of nucleus seed (Singh et al. 2003).

13.10.2 Pearl Millet

Pearl millet, commonly known as bulrush millet (*Pennisetum glaucum* (L.) R. Br.), also classified as *P. typhoides*, *P. americanum* or *P. spicatum*, is a cultivated, small-grain, tropical cereal grass. Vernacular names include 'bajra' (India), 'gero' (Nigeria, Hausa language), 'hegni' (Niger, Djerma language), 'sanyo' (Mali), 'dukhon' (Sudan, Arabic) and 'mahangu' (Namibia). It is a diploid ($2n = 2x = 14$) with a large genome (2450 Mbp) (Taylor 2004). Pearl millet is quantitatively the most important millet, with a world annual production of about 15–25 million tonnes (Mt). It is cultivated mainly in the semi-arid tropics, almost exclusively by subsistence and small-scale commercial farmers.

India is a major pearl millet-producing country with 43% of world acreage. It is an indispensable food crop and important source of fodder for drier areas of India where no other cereals can be cultivated economically. It is the fourth most important cereal after rice, wheat and sorghum. Five states (Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana) account nearly 90% of the total of around 10 m ha cultivated pearl millet areas in India. Increasing area under cultivation of improved varieties and hybrids necessitates the production of large quantities of hybrid seed, which should meet high quality standards. Improved pearl millet varieties and hybrids are being released by the ICAR institutes and the State Agricultural Universities. The genetic purity of nucleus and breeder seed is a prerequisite for maintenance of high standards of seed quality of a variety/hybrid, which can be maintained only if sound scientific methods of seed production are meticulously practised for nucleus and breeder seed production (Satyavathi et al. 2021).

13.10.2.1 Floral Biology and Pollination

Pearl millet inflorescence is a compound terminal spike or panicle. It consists of a central rachis. It bears fascicles on rachillae, arranged in a spiral form. Each fascicle contains one to two spikelets surrounded by a whorl of bristles (i.e. involucre). A spikelet consists of two glumes and two florets. The lower is staminate, whereas the upper is hermaphrodite. The ovary is free and exposed and monocarpellary. The styles are free or adnate at base and bifid, terminating in brush-like stigmas. There are three stamens with penicillate and versatile anthers.

Pearl millet is a protogynous species. The styles start protruding 2–3 days after the emergence of the panicle. The stylar branches protrude first from the florets in the upper middle region of the panicle and then proceed both upwards and downwards. In the hermaphrodite flowers, the stigmas emerge faster than the anthers, and hence stigmas receive pollen from inflorescence of other plants. The time required for complete stigma emergence varies from 2 to 3 days, depending on the environment

conditions. The two stigmas separate and diverge only after complete exertion on the styles. They remain fresh and receptive for 2–3 days, depending upon the environment. The sequence of flowering practically excludes self-pollination in the same inflorescence, but it may occur within the inflorescence of the same plant (Mangat et al. 1999).

By the time anther emergence commences, all stigmas will have emerged, been already pollinated, which avoids selfing under open-pollination conditions. The emergence of the first anther usually begins about 3–4 days after the first stigma has emerged. The protogyny in pearl millet is exploited for controlled cross-pollination without resorting to emasculation. The inflorescence to be used as a female or male is covered with the glassine paper bag before any stigma is visible. Generally, the safest stage is when about one-third of the inflorescence is out of the flag leaf sheath. When all stigmas have emerged, the panicle can be considered ready for cross-pollination. If selfed seed of the male parent is not required, pollen from it can be collected by bagging even that inflorescence in which stigmas have completely emerged. Fresh pollen from dehiscing anthers, visible as yellow powder in the transparent selfing bags, is collected by tapping the bagged inflorescence. The pollination is carried out by quickly removing the bag from the female inflorescence, dusting the pollen collected from the male inflorescence and then rebagging the pollinated inflorescence again.

13.10.2.2 Important Diagnostic Characteristics

To facilitate easy roguing of the off-type plants from a seed plot, knowledge of the diagnostic characters of a variety is very important. These diagnostic characters should have high heritability so that their expression does not change under the varying environments. In pearl millet, characteristics (UPOV 2010) given in Table 13.3 below are important which should be observed during the crop season or at the seed level.

13.10.2.3 Nucleus Seed Production

The salient features of the procedure for the maintenance breeding/production of nucleus seed of OPVs, male sterile line, maintainer line and restorer line in pearl millet (Bhatnagar et al. 2003) is described below:

Open-Pollinated Variety

Season I

- Basic seed of open-pollinated variety is grown in an area of 0.1–0.2 ha maintaining a strict isolation of at least 1000 m from any other plot of pearl millet or wild millet.
- One-third of the commercial plant population should be maintained keeping at least 3000–5000 plants.
- Carefully observe at critical stages (tillering, pre-flowering, flowering, dough stage and maturity) and select 500–1000 plants with characters identical/typical to the released variety.

Table 13.3 Important diagnostic characteristics of pearl millet

Characteristics	States	Stages of observations
Plant: anthocyanin coloration of the first leaf sheath	Absent/present	Seedling emergence
Plant: growth habit	Erect/intermediate	Vegetative/ spike emergence
Plant: time of spike emergence	Very early/early/medium/late/very late	Spike emergence
Leaf: sheath pubescence	Absent/present	Spike emergence
Plant: node pubescence	Absent/present	Spike emergence
Spike: anther colour	Yellow/brown/purple	Anthesis
Plant: node pigmentation	Whitish/green/brown/red/purple	Reproductive
Spike: exertion	Partial/complete	Reproductive
Spike: length	Very small/small/medium/long/very long	Reproductive
Spike: anthocyanin pigmentation of glume	Absent/present	Reproductive
Spike: bristle	Absent/present	Reproductive
Spike: bristle colour	Green/brown/red/purple	Reproductive
Spike: shape	Cylindrical/conical/spindle/candle/lanceolate/ dumb-bell/club/oblanceolate/globose	Reproductive
Plant: height	Very short/short/medium/tall/very tall	Reproductive
Spike: density	Very loose/loose/semi-compact/compact/very compact	Harvest maturity/lab
Seed: colour	Whitish/cream/yellow/grey/deep grey/grey brown/yellow brown/purple/purplish black	Harvest maturity/lab
Seed: shape	Obovate/elliptical/hexagonal/globular	Harvest maturity/lab
Seed: size	Very low/small/medium/bold/very bold	Harvest maturity/lab

- Harvest and keep seed of each selected plant separately. Evaluate each plant for seed characters.
- Keep half of the seed of each selected plant progeny as remnant seed.

Season II: Progeny Evaluation

- Plant unreplicated progeny rows along with checkrows (grown from the basic bulk seed of OPV) after every 15–20 rows.
- Compare progeny rows at critical stages and select 30–50% progenies confirming to the varietal characters.
- Bulk the remnant seed of selected progenies.

Season III: Nucleus Seed Plot
- From the bulked remnant seed, grow nucleus seed nursery in isolation (1000 m).
- The harvested seed is bulked, can be divided into five to six lots and kept under cold storage.
- One of these lots can be used as base seed for nucleus seed production when required, and the rest may be used for breeder production in the subsequent years.

13.10.2.4 Nucleus Seed Production of Parental Lines of Hybrids

The nucleus seed production of hybrids essentially involves the seed production of their parental lines. In the case of pearl millet, especially single cross hybrids are in vogue; thus, an account of production of nucleus seed of single cross hybrids, i.e. A line (male sterile), B line (maintainer) and R line (restorer), is given below.

Maintainer Line ('B' Line)

Season I
- Grow a large number of plants of B line (0.05 ha).
- Select and self about 1000 plants at the time of flowering.
- Finally, select about 200 selfed plants confirming to the characters of maintainer line.

Season II
- Grow plant to row progeny of selfed plants in two replications, retaining the remnant seed.
- The progeny rows are studied for the diagnostic characters, and rows not confirming to the characteristics of the line are rejected and uprooted.
- Identify the best progeny rows (25–30%).
- Bulk the remnant seed of selected best lines.

MS Line ('A' Line)

Season IV
- Grow A and B lines in alternate rows. Seed of B line will be obtained from rejuvenation as given in the maintenance of B lines.
- Make 200–250 paired crosses between A and B plants.
- Care is taken to cross A and B plants confirming to the line standards only.
- Paired crosses among A and B lines should be labelled, viz. A1 × B1, A2 × B2, etc., and harvested seed of each pair should be kept separately.

Season V
- Grow the pairs, respective A line (crossed seed) and B line (selfed seed) in alternate rows.
- Retain a portion of seed as remnant seed.

- Observe critically pairs of A and B lines for all the characters including height, flowering and typical morphological characters. Off-type and undesirable types should be rejected.
- Observe for pollen shedders in A line. The A line progenies showing pollen shedders and corresponding B lines should also be rejected.
- Identify uniform pairs of A and B lines which confirm to the standards of parental lines.
- Remnant seed of the A lines of the selected pure pairs is bulked. This forms nucleus seed bulk of A line.

Restorer Line ('R' Line)

Season I
- Grow a large number of plants of R line (0.05 ha).
- Self a number of plants (about 1000) confirming to the standards of line at the time of flowering.
- Finally, select about 200 selfed plants based on field studies as well as observations in laboratory for seed character like colour, shape, etc.

Season II
- Grow plant to row progeny of selfed plants in two replications.
- Retain a portion of selfed seed of each plant as remnant seed.
- The progeny rows are studied for the diagnostic characteristics of the line.
- Evaluate the rows for yield and agronomic score for other economic characters.
- Identify the best progeny rows (30–50%) based on all characteristics mentioned above. In the progeny row testing if an adequate number of progeny rows confirming to the line standards are not obtained, selfing for one or more generations will be required. These selfed plants of R line should also be tested for their restoration ability.
- Bulk the remnant seed of best lines.

Season III
- Grow the bulk seed of remnant seed in isolation.
- Bulk the seed of all the plants after the harvest.
- This forms the nucleus seed bulk of R line.

13.10.3 Sunflower

The genus *Helianthus* (Compositae family) contains 60 annual and perennial species originating from America. Among them two have been improved for nutritional use, *H. tuberosus* L., for its succulent tubers, and *H. annuus* L., the cultivated sunflower, the oil from whose seed, and protein-rich oil cakes are utilized. The basic number of chromosomes is $X = 17$. *Helianthus* contains diploid species, the cultivated sunflower (*H. annuus*) with $2n = 2x = 34$, tetraploid species (*H. hirsutus*, *H. laevigatus*)

with $2n = 4x = 68$ and hexaploid species (*H. resinosus*, *H. tuberosus*) with $2n = 6x = 102$. Sunflower is a global oilseed crop of economic importance, introduced in India in 1970 for commercial cultivation. Poor quality of seed is one of the important factors responsible for low productivity in India, which necessitates maintenance of genetic purity of varieties and parental lines of hybrid cultivars (Jonard and Mezzarobba 1990).

13.10.3.1 Floral Biology and Pollination

The flowering process begins with unfolding of outer ray florets. The outer whorl of disc flowers opens first proceeding gradually towards the centre of the head. In general, two to four whorls open daily and complete flowering within a head in 5–8 days. Anthesis takes place in the morning between 06 and 08 AM on warm sunny days. Anthesis is delayed if weather is cool, cloudy or wet. The sunflower is protandrous. Pollen is dehisced within the anther tube. As the style elongates and pushes up through the anther tube, the pollen is mechanically forced out. The style continues to elongate until the stigmas emerge from the anther tube and the lobes separate, exposing their pollen receptive surfaces. Pollination and fertilization occur when the spiny viable pollen is transferred to stigmatic surface. Cross-pollination is favoured by insects, in particular honeybees (Giriraj and Reddy 2003).

13.10.3.2 Diagnostic Characteristics

Following major characteristics of sunflower (UPOV 2000) as listed in Table 13.4 below should be taken into consideration for identification of true-to-type plants/rows and elimination of off-types in nucleus/breeder seed plots/fields.

Table 13.4 Important diagnostic characteristics of sunflower

Characteristics	States	Stages of observations
Leaf size	Very small/small/medium/large/very large	Vegetative
Leaf colour	Light green/medium green/dark green	Vegetative
Leaf blistering	Absent/medium/strong	Vegetative
Fineness of serration	Fine/medium/coarse	Vegetative
Flowering	Early/medium/late	Start of flowering
Ray flower colour	Ivory/pale yellow/yellow/orange/purple/red brown/multi-coloured	Flowering
Head diameter	Small/medium/large	Harvest maturity
Head shape	Concave/flat/convex/mis-shape	Harvest maturity
Plant height	Very short/short/medium/tall/very tall	Vegetative/reproductive
Seed length	Short/medium/long	After harvest
Seed base colour	White/grey/brown/black	After harvest
Stripes on seed	Absent/present	After harvest

13.10.3.3 Nucleus Seed Production of Open-Pollinated Varieties

Pustovoit method of breeding has been recommended for the maintenance of commercially grown open-pollinated varieties (Giriraj and Reddy 2003) as detailed below:

- A total of 5000–8000 individual plants are selected in the base population raised under isolation. (Selection is based on uniform plant and shape of head.)
- Selected plants are evaluated for seed yield, oil content, test weight and hull content, and 20–25% of the best plants are advanced for progeny testing.
- A single row of selected progenies is raised, and for every 10–20 progenies, a check is included which is the best cultivar most similar to the progenies being evaluated.
- Retain a portion of selfed seed of each plant as remnant seed.
- After harvest of progeny trial, laboratory evaluation is undertaken again for seed yield, oil content and other agronomic attributes.
- Finally, select 150–200 superior progenies.
- The remnant (reserve) seeds of selected progenies from the original plants are identified and bulked to form nucleus seed.
- Part of this seed is grown for the next cycle as detailed above, and the other part is channelled into breeder seed production chain.

13.10.3.4 Nucleus Seed Production of Parental Lines of Hybrids

- Performance of released hybrid cultivar over the years is mainly dependent on the initial maintenance of genetic purity of parental lines. Maintenance of parental lines ensures genetic purity in subsequent seed production chain for increasing seed quality. Parental lines have to be purified under the direct supervision of the breeder who has developed the hybrid.

A single cross hybrid involves production and maintenance of:

- 'A' line or CMS line (male sterile)
- 'B' line or maintainer line (male fertile)
- 'R' line or restorer line (male fertile).

Maintenance of 'A' and 'B' Lines

Season I

- Grow 'A' and 'B' lines obtained from original population or nucleus seed source in alternate row of 4–5 m length. Raise at least 300 rows in each 'A' and 'B' line.
- Tag the plants in both 'A' and 'B' lines considering uniformity for plant height, flowering and morphological characteristics prescribed for the line.
- Cover (before flowering) the plants tagged in A and B lines and transfer manually pollen from B line to A line. Label all the capitula of A × B crosses as A1 × B1, A2 × B2 . . . A500 × B500.
- About 400–500 A × B crosses should be ensured.

- Individual selected 'B' lines are harvested first and threshed for seed characters in lab. Undesirable types should be rejected. Seeds of true types should be stored in separate packets after drying.
- After the harvest of 'B' lines, 'A' lines are harvested which received pollen from corresponding 'B' line.
- After threshing and drying, the seeds are stored in packets with proper labelling.

Season II
- Grow plant to progeny line of A × B crosses—A line alternated with corresponding B line.
- Retain a portion of seed as remnant seed for all the A × B crosses and B lines.
- Progeny rows are studied for diagnostic characters. The lines (A and B) which do not conform to prescribed norms are rejected.
- After flowering, observe individual plants carefully for pollen shedders (male fertile) in A line. The lines in which pollen shedders are observed should be rejected. The corresponding B line should also be rejected.
- Identify the best progenies (about 100).
- The remnant (reserve) seeds of selected best progenies from the original plants are identified (A and B) and bulked separately.
- The bulk seeds of 'A' and corresponding bulk seed of 'B' line form the nucleus seed.

13.10.4 Rapeseed and Mustard

Rapeseed-mustard is a unique group of crops having different kinds of breeding behaviour. Some of them are self-compatible (self-pollinated) crops like yellow sarson (*Brassica rapa* var. yellow sarson), gobhi sarson (*Brassica napus*) and Ethiopian mustard (*Brassica carinata*), while others are self-incompatible (cross-pollinated) crops including toria (*Brassica rapa* var. *toria*), brown sarson (*Brassica rapa* var. brown sarson) and taramira (*Eruca sativa*). In Indian mustard (*B. juncea*), the major crop of the group, cross-pollination ranges from 5% to 15%. *Brassica* spp., commonly known as rapeseed-mustard, plays a significant role in the Indian economy by providing edible oils, vegetables, condiments and animal feed. Globally, India holds the second and third position in rapeseed-mustard area under cultivation and production, respectively. However, anthropogenically accelerated climate change thwarts yield potential of rapeseed-mustard by employing abiotic (drought, flood, temperature variation and salinity) and biotic (disease and insects) stresses (Chauhan et al. 2011).

Historically, the cultivation of *Brassica* spp. has been quoted in numerous ancient scriptures and believed to be cultivated on or prior to 5000 BC. It has also been reported that mustard crop was in cultivation around Channhu-daro of Harrapan ancient civilization during 2300–1750 BC. There is ambiguity in the history as the origin of *B. juncea* is concerned. It had been believed that the centre of origin for *B. juncea* is the Middle East, where putative parents, i.e. *B. nigra* and *B. rapa*, would

Table 13.5 The common names, types of pollination, chromosome number, genome and size of different *Brassica* spp.

Species	Common name	Type of pollination	Chromosome no. (2*n*)	Genome	Genome size (Mb)
B. juncea (L.) Czern.	Indian mustard	Often-self	36	AABB	~922
B. carinata A. Braun	Karan rai or Ethiopian mustard	Often-self	34	BBCC	–
B. napus L.	Gobhi sarson	Self and cross	38	AACC	~1130
B. nigra (L.) Koch	Black mustard	Cross	16	BB	~558
B. oleracea L.	Cabbage, cauliflower, etc.	Cross	18	CC	~630
B. rapa L.	var. brown sarson	Lotni type: crossTora type: self	20	AA	~485
	var. toria	Cross			
	var. yellow sarson	Self			
Eruca sativa	Taramira	Self	22	EE	–
B. alba Rab. (syn. *Sinapis alba*)	White mustard	Self	24	SS	–

have crossed with each other. Later on, it had been disseminated to other parts of the world such as Europe, Asia and Africa. Today, there are two centres of diversity, i.e. China and Eastern India, based on the prevalence of their wild progenitors and relatives. At present, it has been proved that there are two geographical races, i.e. Chinese and Indian, of *B. juncea* based on molecular and biochemical studies. In 1935, Nagaharu U proposed a theory known as U's triangle to show genetic relationships based on artificial interspecific hybridization experiments among six species, namely *B. rapa*, *B. nigra*, *B. oleracea*, *B. carinata*, *B. napus* and *B. juncea*. As per theory, three allotetrapolyploid species (*B. napus*, *B. juncea* and *B. carinata*) were derived by natural hybridization of three basic diploid species (*B. rapa*, *B. nigra* and *B. oleracea*), followed by genome doubling (Chand et al. 2021a). Nowadays, with the accomplishments of genome sequencing of *Brassica* taxa, this hypothesis has been increasingly accepted. Furthermore, it has been scientifically proved that allotetraploid *B. napus* and *B. juncea* had been derived from their diploid parents based on comparative genomic analysis, and the results were in accordance with 'U' triangle (Nagaharu 1935) (Table 13.5).

13.10.4.1 Floral Biology and Pollination

The mustard inflorescence is an aggregate of yellow florets at the apex of the raceme that give a field a deep golden appearance when fully open. The structure of the flower, as given under 'Cole Crops', applies equally to the mustard flower. The two outer nectarines may be somewhat functional or inactive. Mustard is an excellent source of nectar and pollen for honeybees. The floret opens between 9 a.m. and noon and remains open for 3 days. Usually, the stigma projects about 2 mm beyond the petals the afternoon preceding opening of the flower and is immediately receptive. Soon afterwards, however, the corolla begins to grow and re-engulfs the stigma. Thereafter, the stamens lengthen so that the anthers are in level with the stigma, but when the corolla opens, they turn half around. At this period, nectar secretion by the inner nectaries begins. Just before the flower closes, the anthers turn to their former position, and if any degree of self-fertility exists, selfing can result (Yadava and Singh 2003).

The position of the anthers in relation to the nectaries and stigma makes cross-fertilization likely but by no means inevitable on the visit of pollinating insects. The flower is so constructed that pollen from another flower is likely to be transported to it before its own pollen comes in contact with the stigma. Mustard is basically an insect-pollinated type of crop, with ample pollen and nectar to attract pollinating insects. Some of the self-pollen may contact the stigma without the aid of insects, but this contact can be abetted by the bees' visit to the flower. The compatibility varies with species, cultivar and even the age of the plant. Yellow mustard (*B. hirta*) is a cross-pollinated crop, while *B. juncea* is self-fertile but can be abetted by wind and/or bees. Bees can help achieve more than double seed production, e.g. in *B. alba*. The flowers are highly attractive to bees for both nectar and pollen, so there is no problem in getting visitation if sufficient bees are in the area and the weather permits floral visitation. Rapeseed-mustard is adversely affected by heat stress (35/15 °C) at the early stage of flowering. Drought can adversely affect plant growth at various stages from seed germination to reproduction and flowering to harvesting and ultimately results into oil and yield penalty.

13.10.4.2 Important Diagnostic Characteristics

Oilseed brassicas have a lengthy taproot system. Leaves are dark green, serrated, pinnatifid and either sessile (rapeseed) or petiolate (mustard). In *B. rapa*, syn. *campestris*, the upper leaves are auriculate and clasp the stem closely. In *B. napus*, only the lower leaves are partially clasping, whereas in *B. juncea* they are petiolate. The inflorescence is elongate raceme, borne terminally on the main stem as well as on the branches carrying bright yellow flowers (colour may vary from dark to cream yellow). The fruit is a long narrow pod or siliqua, usually consisting of two carpels separated by a false septum, which shatters after maturity and is a varietal character detrimental to an oilseed strain. They produce dark brown (black), light brown or yellow coloured seeds. The important characteristics of rapeseed and mustard (UPOV 2013) which may help the seed technologists and breeders to identify true-to-type plants/rows and to eliminate off-type plants during maintenance breeding/seed production are given in Table 13.6 below.

Table 13.6 Important diagnostic characteristics of rapeseed and mustard

Characteristics	States	Stages of observations
Leaf shape	Serrated/non-serrated	Vegetative
Leaf type	Sessile/petiolate	Vegetative
Leaf colour	Light green/medium green/dark green/purple green/purple	Vegetative
Leaf hairiness	Present/absent	Vegetative
Calyx colour	Green/light green	Vegetative
Corolla colour	Dark yellow/yellow/cream yellow/white	Flowering
Petal shape	Narrow/broad	Flowering
Plant type	Erect/semi-erect/spreading	Vegetative/ reproductive
Plant height	Dwarf/medium/tall	Vegetative/ reproductive
Main shoot length	Short/medium/long	Vegetative/ reproductive
Siliqua arrangement	Appressed/semi-appressed/spread	Maturity
Siliqua surface	Smooth/intermediate/constricted	Maturity
Siliqua beak	Short stout/long slender	Maturity
Seeds/siliqua	Less/average/more	Maturity
Pod locule	Unilocular/bilocular/trilocular/tetralocular	Maturity
Seed colour	Yellow/dull grey/reddish brown/brown/black	Maturity/post-harvest
Seed size	Small/medium/bold	Post-harvest
Oil content	Low/medium/high/very high	Post-harvest

13.10.4.3 Nucleus Seed Production of Open-Pollinated Varieties

The procedure of nucleus/breeder seed production varies from crop to crop according to their breeding behaviour. The field should be selected where no *Brassica* species had been grown for the last 3 years, unless the crop was raised for nucleus seed production of the same variety. Field should be properly isolated from the other field of any *Brassica* species. An isolation distance of 200 m is recommended for production of nucleus and breeder seed of self-incompatible (cross-pollinated) crops, including *B. rapa* var. *toria*, *B. rapa* var. brown sarson and *E. sativa* (taramira), and self-compatible (self-pollinated) crops, including *B. juncea* (Indian mustard), *B. rapa* var. yellow sarson and *B. carinata* (Karan rai). For maintenance of varieties in mustard, typical reserve seed method (as outlined above) is followed. As the procedure is somewhat cumbersome, we may also go for plant row method in self-compatible group, if the frequency of pollinators is negligible (Yadava and Singh 2003).

13.10.4.4 Nucleus and Breeder Seed Production of Parental Lines

Hybrids developed in rapeseed-mustard are based upon the cytoplasmic-genetic male sterility (CMS) system. Parental lines are multiplied in different plots. The seed parent (A line) is maintained by growing the rows of A and B lines in a specific ratio. Normally, a 3:1 ratio of seed parent (A line) and maintainer (B line) is followed. The maintainer rows (B line) are harvested first. Later on, the remaining rows of seed (A line) parent are harvested and bulked. Strict roguing is advised during flowering to rogue out the fertile plants from seed parent. The seed production of B and R lines is similar to any other varietal seed production. The commercial F_1 hybrid seed is produced by growing seed parent (A line) and restorer (R line) in a 3:1 ratio as followed in the case of maintenance of seed parent. The rows of restorer parent (R lines) are harvested first and bulked, followed by harvesting of seed parent. The seed from the seed parent is processed and packed as hybrid seed. Honeybees play an important role in enhancing the transfer of pollen; hence, 3–4 honeybee boxes/ha may be kept to ensure proper pollination and good seed set (Yadava and Singh 2003).

13.10.5 Pigeon Pea

The name pigeon pea was first used in Barbados during 1692 where pigeons were fed with the seeds of *Cajanus cajan*. Wide genetic variability exists in India, therefore it is considered as the centre of origin for cultivated pigeon pea. This crop is also been widely cultivated in many African countries, Egypt, and a bunch of Asian countries since prehistoric times. Eastern Africa was considered as centre of origin of pigeon pea by several workers owing to its occurrence in wild form. Based on the occurrence of wild relatives and diversity, it was inferred that India is the primary centre of origin and Africa is the secondary centre of origin for pigeon pea (Kumar et al. 2017b).

Pigeon pea (*Cajanus cajan* (L.) Millspaugh) is the second most important pulse crop in India, next to chickpea, covering an area of 4.42 m hectares. Bestowed with several unique characters, it finds an important place in the cropping systems being adopted by the farmers. The highest production of pigeon pea is from Maharashtra which is around 30% of national production. More than 90% of production contribution of Tur is from 8 states, namely Maharashtra, Karnataka, Madhya Pradesh, Uttar Pradesh, Gujarat, Jharkhand, Telangana and Andhra Pradesh. The existing productivity of pigeon pea (806 kg/ha) can be doubled with the adoption of improved production technology. Seed is a basic input, and unless pure seed is used, the application of other inputs such as fertilizers, irrigation and plant protection measures becomes less effective. However, non-availability of quality seed remains a major constraint in meeting the targeted increase. The genetic identity of a pigeon pea variety/hybrid can be maintained in foundation and certified seed stages if sound scientific methods are meticulously practised at nucleus and breeder seed stage.

13.10.5.1 Floral Biology and Pollination

Pigeon pea is an important legume crop of the Papilionaceae family. It is an often cross-pollinated crop, and breeding principles of both self- and cross-pollinated crops are highly effective in its genetic enhancement. Pigeon pea is a hard woody shrub, extensively adaptable to a range of soil types, temperature and rainfall. It has a deep taproot system extending up to 2 m and can grow to a height of 4 m. Pigeon pea roots form a symbiotic association with *Bradyrhizobium* spp. and perform biological nitrogen fixation. The branching pattern of stem may vary from bush type to compact upright type and is of determinate, semi-determinate and non-determinate types based on the flowering pattern. The primary leaves are simple, opposite and caduceus, while the latter ones are pinnately trifoliate with lanceolate to elliptical leaflets.

Pigeon pea flower is bisexual and zygomorphic, borne on terminal or auxiliary racemes and is normally yellow in colour with some variations. Stamens are 10 in number and diadelphous (9 + 1) with light or dark yellow anthers. The ovary is superior with a long style attached to a thickened, incurved and swollen stigma. The male and female parts of the flower are covered by corolla which consists of standard petal, wing petal and keel petal. Anthers are dorsifixed and light or dark yellow in colour. The ovary is superior, subsessile densely pubescent with two to nine ovules. The stigma is capitate and style is long filiform and glabrous. The floral structure of pigeon pea was initially adapted to self-pollination, which changed over time to partial outbreeding. In 90% flowers, pollination takes place before the opening of the flower (Singh et al. 2016).

Pigeon pea is an often cross-pollinated crop with a range of 3–40% cross-pollination. In a fully developed bud, anthers surround the stigma and dehisce a day before the flower opens. The duration of flower opening varies according to climate and the environment. Anthesis in pigeon pea starts from 06.00 AM and continues till 04.00 PM. Maximum anthesis takes place between 10:30 a.m. and 12: 30 p.m. Flowers that open in the evening usually remain open throughout the night and are closed before noon on the following day. The receptivity of stigmas starts 68 h before anthesis and continues for 20 h after anthesis. The duration of flower opening also depends on the weather and the environment. This varies from 6 to 36 h. Fertilization occurs on the day of pollination. The fruit of pigeon pea is called pod, which is of various colours, with and without deep constrictions. Seeds (with 20–22% proteins and amino acids) can be round or lens shaped, in shades of white and brown colour with yellow colour cotyledon. Pigeon pea is a widely consumed multi-utility pulse crop; thus, the knowledge about the crop botany is vital for modifying it according to future challenges and goals.

13.10.5.2 Important Diagnostic Characteristics

For identification and production of true-to-type seed, some of the important morphological characters of pigeon pea (PPV&FRA 2007) to help the seed technologists and breeders have been listed below (Table 13.7).

Table 13.7 Important diagnostic characteristics of pigeon pea

Characteristics	States	Stages of observations
Plant type	Compact/semi-spreading	Vegetative
Growth habit	Determinate/indeterminate/semi-determinate	Flowering
Pigmentation on stem	Green/purple	Vegetative
Leaf shape	Oblong/obovate/sesamum	Flowering
Days to flowering	Early/medium/late	Start of flowering
Colour of the base of petal	Light yellow/yellow/orange yellow/purple/red	Flowering
Pattern of streaks	Absent/sparse/medium/dense	Flowering
Pod colour	Green/green with brown streaks/green with purple streak/purple/dark purple	Premature pods
Pod pubescence	Absent/present	Full podding
Seed colour	Cream/brown/dark brown/grey/purple	Maturity
Seed shape	Oval (egg shape)/globular (pea shape)/(angular)/ elongate	Maturity
Seed size	Small/medium/large/extra large	Ripe seeds

13.10.5.3 Nucleus Seed Production of Varieties and Male Parents

Variety maintenance and nucleus seed production in varieties and fertile inbred lines (B and R lines) are done following reserve seed method (as described above). We may use produce of individual plant progeny rows (as grown for evaluation purpose) as nucleus seed where insect/pollinator's activity is very low. During nucleus seed production, it is very important to provide sufficient isolation distance and avoid volunteer plants by selection of seed plot (Dhar et al. 2003).

13.10.5.4 Nucleus Seed Production of Female Parent

Nucleus seed production of genetic male sterile (GMS) line constitutes an important part of seed production and determines the purity and quality of seed in the subsequent years of seed production. The important precautions are:

- Use the original seed of the genetic male sterile or female parent available with the breeder to produce nucleus seed.
- For nucleus seed production of genetic male sterile lines, pair-wise selection of male sterile and male fertile plants with characteristics similar to each other and to the original genetic male sterile line is carried out for crossing of male sterile plant with the fertile one for maintenance.
- The crossing is carried out manually under caged conditions to avoid any chance of pollination with alien pollen. At least 50 such pair-wise crosses of male sterile and fertile plants having similar characteristics are made under caged conditions in the original genetic male sterile line.

- Harvest the seeds from male sterile plant of each pair separately and raise plant progeny rows along with the original genetic male sterile line after every ten progenies as a check.
- This enables the evaluation of progenies for different morphological characteristics and determination of segregation ratio of male sterile and fertile plants.
- Cover the uniform individual progenies exhibiting similarity for different traits with the check and segregating for 1 MS: 1 MF plants with the nylon net cages separately at flower initiation stage and maintain them by crossing of male sterile plants with fertile sib counterparts by hand pollination.
- Harvest and thresh the male sterile plants of all the selected progeny rows separately for screening with respect to seed yield and seed characteristics.
- Bulk the seeds of the progenies exhibiting similarity with the check and giving higher seed yield than the check to form the nucleus seed. The progenies with off-type plants are discarded at flowering stage itself.
- The selection of progenies of genetic male sterile lines for nucleus seed production should be very strict and hence bulk the seed uniform from only genetically pure lines to maintain originality in the subsequent generations.

The present-day pigeon pea hybrids are developed using a CMS system. Seed production of parental lines in the CMS system is much easier than the GMS system (Dhar et al. 2003).

13.10.6 Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is grown worldwide for food, feed, fodder, fuel and industrial products. Cytogenetically, sorghum is a diploid of $2n = 2x = 20$, where $2n$ is the somatic chromosome number having two complete sets ($2x$) of chromosomes and a chromosome number of 20. It has a haploid chromosome number of 10, containing approximately 800 Mb with 34,000 protein-coding genes. The genome contains a high level of repeats (61%). In India, sorghum is the most important cereal crop for poor people in the semi-arid zones. The sorghum hybrids were developed and released in India, which resulted in quantum jump in productivity from 570 kg/ha in 1970 to >1000 kg/ha in recent times. However, it is still the lowest among the major sorghum-producing countries in the world, though the world average is 1435 kg per hectare. The purity of commercial seed depends on the genetic purity of parental lines in nucleus, breeder and foundation seeds. Hence, the quality of maintenance breeding is the prerequisite to ensure high genetic purity in multiplication chain during seed production programme (Reddy et al. 2006).

13.10.6.1 Floral Biology and Pollination

In sorghum, the panicle may be short and compact or loose and open. It may be 4–25 cm or more in length and 2–20 cm or more in width. The inflorescence is a raceme, which always consists of one or several spikelets. Racemes vary in length

depending upon the number of nodes and length of internodes. The sessile spikelet varies in shape from lanceolate to almost round and ovate and is sometimes depressed in the middle. The lower glume is usually somewhat flattened and conforms more or less to the shape of the spikelet, while the upper one is more convex or boat shaped. The seed may be enclosed by the glume or may protrude from the glume. The seed may be just visible or almost completely exposed. In sessile spikelets, there are two lemmas, two lodicules and a palea. The pedicelled spikelets are much narrower than the sessile spikelets, usually lanceolate in shape. They are male or neutral in sex, but may rarely have a rudimentary ovary. The lemmas are much reduced in size and only rarely does the upper lemma have an awn. Sorghum has two pistils and three stamens. Each fluffy stigma is attached to a short stout style extending to the ovary. The anthers are attached to long thread-like filaments.

The floral initial forms 30–40 days after germination (but may range from 19 to 70 days or more). Floral initiation marks the end of the vegetative growth due to meristematic activity. Sorghum usually flowers in 55–70 days in warm climates, but it could be as early as 30 days or as late as 100 days or more. The flowering (anthesis) in a panicle starts from the top, and it travels to successively lower whorls. Flowering is completed over a period of 4–5 days (6–8 days under cooler conditions). Pollen is usually available for a period of 10–15 days because the heads in a field do not flower at the same time. Sorghum is predominantly a self-fertilized crop, but the cross-pollination may occur to the range of 2–10%. At flowering, the glumes open and the three anthers fall free, while the two stigmas protrude, each on a stiff style. Flowering occurs just before or just after sunrise, but may be delayed on cloudy damp mornings. The anthers dehisce when they are dry. For selfing, the panicle is merely covered with a paper bag. Pollen is shed freely and can always be collected in bags enclosing the spikes. When crossing is made before 10 AM, it generally set the maximum amount of seed per inch of head (Tonapi et al. 2015b).

13.10.6.2 Important Diagnostic Characteristics

The characteristics of sorghum (UPOV 2015) are given in Table 13.8 below to help the seed technologists and breeders to identify the true-to-type plants and to rogue out the off-types or undesirable plants.

13.10.6.3 Maintenance Breeding

The maintenance breeding of sorghum (Babu and Seetharama 2003) involves the purification of:

- Cytoplasmic male sterile line (A line).
- Maintainer line (B line).
- Restorer line (R line).
- Open-pollinated variety.

Table 13.8 Important diagnostic characteristics of sorghum

Characteristics	States	Stages of observations
Plant height	Tall/medium/dwarf	Vegetative
Plant colour	Pigmented (grey brown groups)/tan (yellowish)	Vegetative
Juiciness	Juicy/corky	Vegetative
Leaf	Erect/semi-erect/droopy	Vegetative
Leaf midrib colour	White/dull green/yellow/brown/purple	Vegetative
Leaf colour	Dark green/green/light green	Vegetative
Stem thickness	Thin/medium/thick	Vegetative
Days to flowering	Early/medium/late	Reproductive
Panicle	Very lax/very loose with erect primary branches/very compactness and shape loose drooping primary branches/ loose erect primary branches/loose drooping primary branches/semi-loose erect primary branches/semi-loose drooping primary branches/semi-compact elliptical/compact elliptical/compact oval/half broom corn/broom corn	Reproductive
Glume colour	White/straw/brown/light red/red/yellow/purple/black	Maturity
Seed covering	25% grain covered/50% grain covered/75% grain covered/ grain fully covered/glumes longer than grain	Maturity
Awns	Awned/awnless	Maturity
Days to maturity	Early/medium/late	Maturity
Shattering	Low/medium/high	Maturity
Seed colour	White/pale yellow/yellow/orange	Maturity
Seed lustre	Lustrous/medium/non-lustrous	Maturity
Seed sub-coat	Present/absent	Maturity
Seed plumpness	Dimple/plump	Maturity
Seed form	Single/twin	Maturity
Endosperm texture	Completely corneous/mostly corneous/intermediate/starchy	Maturity
Endosperm colour	White/yellow	Maturity
Endosperm type	Normal/waxy/sugary	Maturity

13.10.6.4 Nucleus Seed Production of 'A' and 'B' Lines

Seed quality with all its ramifications must be cardinal virtue in the seed chain. The purity of commercial seed depends on the genetic purity of parental lines in nucleus, breeder and foundation seeds. The success of hybrid depends on the maintenance of genetic purity of parental lines, which is essential to obtain high yields in farmer's fields.

Season I

Select the individual plants of A and B lines from good quality breeder seed or plots grown with the breeder in large areas (0.5–1.0 acre) based on distinguishing morphological traits for characterizing the genotypes that are not influenced by the environment.

- Raise 200–300 rows (each 5 m length) from each A and B line obtained from above seed source.
- Examine the panicle morphological traits especially those which are not influenced by genotype × environment interactions, such as compactness from top to bottom and secondary branch distribution, number of whorls, shape of apex, panicle branch distribution at the bottom, middle and apex, glume characteristics (shape, size and colour) and awn characteristics, etc.
- Tag the individual A and B lines which are true-to-type plants. Self the individual tagged plants in B lines and mark the paired crosses between A and B plants during flowering. The ear head of B lines and A × B crosses should be labelled properly such as $A_1 \times B_1$, $A_2 \times B_2$,. . . $A_{200} \times B_{200}$. Harvest the individual plants as per labelling. The A lines should be harvested after B lines. After proper threshing and drying, store the properly labelled packets.
- Carefully examine the seed of each plant on the table for uniformity in colour, shape and size of seed. Discard the seed of ear head appearing deviant from original true-to-type seed characters.

Season II

- Raise plant to progeny rows such as B line and A × B crosses form the selected 200–300 plants. Retain some portion of seed as remnant seed for all the B line plants and A × B crosses.
- Observe the individual plants in progeny rows for diagnostic characters. Uproot the rows not confirming to the characteristics of the line. The A lines showing pollen shedders and the corresponding B lines should also be rejected.
- Examine the seed characters in laboratory as per the descriptors of parental line. Discard the seed of progeny row in the case of doubtful deviants, if any.
- Identify the best progenies (about 50) of A and B lines which confirm to the standards of the parental lines. The remnant seeds of selected best progenies from the original A and B plants are identified and bulked separately, which forms the nucleus seed.

13.10.6.5 Nucleus Seed Production of 'R' Line

Season I

- Grow a large number of plants of R line.
- Self about 1000 plants conforming to the standards of the line at the time of flowering.
- After harvest, rejection may be done based on seed colour, shape, size, etc.

- Finally, select about 200 plants based on field observations as well as observations in the laboratory.

Season II
- Grow plant to row progeny of the selected plants in the two replications. Retain a portion of selfed seed as remnant seeds.
- The progeny rows are studied for the diagnostic characters. The lines not confirming to the characters of the parental line should be rejected. If an adequate number of progeny rows confirming to the lines are not found, selfing for one more generation will be required. It is desirable to test selfed plants of R lines for their restoration ability.
- Evaluate lines for economic traits and disease resistance characters.
- Identify the best progeny rows (about 50) based on all the diagnostic descriptors of R line.

Season III
- Grow the bulk seed of remnant seeds in isolation.
- Ensure adequate pollination during flowering.
- Bulk the seed of all the plants after the harvest.
- This forms the nucleus seed of R line.

13.10.6.6 Nucleus Seed Production of Varieties

Season I
- Grow a large number of plants of a variety.
- Self about 500 confirming to the standards of the cultivar at the time of flowering. Finally, select about 200 plants based on field observations.
- After the harvest, rejection may be done based on laboratory examination for seed colour, shape, size, etc.

Season II
- Grow head to row (5 m length) progeny of the selected plants in two replications. Retain a portion of selfed seed as remnant seeds.
- The progeny rows are studied for the diagnostic characters. The lines not confirming to the characters of the variety are rejected. Variation of plants may be with regard to plant height, leaf traits, flowering and maturity period, insect pests and disease reactions and panicle types. If an adequate number of progeny rows confirming to the lines are not found, selfing for one more generation will be required.
- Evaluate lines for economic and disease resistance traits.
- Identify the best progeny rows (about 50) based on all the diagnostic descriptors of the variety.
- These rows should be harvested separately with labels, and the seed of individual ear to rows should be cleaned and table examined. The deviants should be discarded, if any.

Season III

- Grow the bulk seed of remnant seeds in isolation.
- Ensure adequate pollination during flowering.
- Bulk the seed of all the plants after the harvest.
- This forms the nucleus seed of a variety.

13.10.7 Cotton

The genus *Gossypium* includes ~45 diploid ($2n = 2x = 26$) and five tetraploid ($2n = 4x = 52$) species. Diploid species fall into eight genomic groups (A–G and K) (Percival et al. 1999). The African clade (A, B, E and F genomes) occurs naturally in Africa and Asia, while the D-genome clade is indigenous to the Americas. A third diploid clade (C, G and K genomes) is found in Australia. Allotetraploids arose in the New World from interspecific hybridization between an A-genome-like African species and a D-genome-like American species, which occurred 1–2 million years ago. The closest relatives of the original tetraploid progenitors are the A-genome species *Gossypium herbaceum* L. (A1) and *Gossypium arboreum* L. (A2) and the D-genome species *Gossypium raimondii* (D5) Ulbrich. Two of five allotetraploid species, *Gossypium hirsutum* L. and *Gossypium barbadense* L., are domesticated and cultivated. Interestingly, the A-genome species produce spinnable fibre and are cultivated on a limited scale, whereas the D-genome fibre is rudimentary and not useful (Applequist et al. 2001). The fibre in allotetraploids is much longer and stronger, suggesting activation or silencing of homoeologous fibre-related genes by genetic and epigenetic mechanisms, leading to hybrid vigour. Most production (>97%) in the USA is upland cotton (*G. hirsutum* L., AD1), and the remainder is Pima cotton (*G. barbadense* L, AD2) with extra-long fibre that is sought after for high-quality textiles. Cotton is one of the most important commercial crops of India. Even though India ranks first in the world in respect of total cotton area, it ranks only third in respect of production, next to China and the USA.

India is unique where all the four cultivated species of cotton, viz. *Gossypium hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum*, are grown commercially. During 2020–2021, the production of cotton was 371.0 lakh bales cultivated under an area of 129.57 lakh hectares with a productivity of 487 kg per hectare as per the estimates of Cotton Corporation of India. Nearly 65% of the cotton crop is cultivated under rainfed conditions in the country. Seed plays a major role as a basic input to cotton productivity and sustained production. Studies indicated that the use of good quality seeds alone could increase production by up to 20%. Good seeds enable the farmers to maintain uniform and vigorous stand of crop, which serve as a catalyst to make other inputs productive and cost-effective.

13.10.7.1 Floral Biology and Pollination

Cotton flowers are extra-axillary, terminal and solitary and are borne on the sympodial branches. The flower is subtended by an involucre of usually three unequal leaf-like bracts. Bracteoles, alternating with the bracts on the inside of the involucre or

standing on either side of the small bract, may be present. The calyx, consisting of five undiverged sepals, is persistent and shaped as a shallow cup. The calyx adheres tightly to the base of the boll as it develops (Manickam and Prakash 2016).

The corolla is tubular, consisting of five obcordate petals alternating with calyx lobes and overlapping the next one in the series in a convolute manner. On the first day after anthesis, the corolla changes into pinkish hue and then into red during succeeding days. It withers and falls off on the third day, together with the staminal column and stigma leaving the ovary, calyx and involucre intact.

The stamens are numerous and united to form a tubular sheath which surrounds the pistils, except for the exposed portion of style and stigma at the tip. Under normal conditions, the pollen grains are viable up to 24 h and thereafter lose potency and fail to effect fertilization. The time taken by the pollen tube to traverse the style varies according to the variety and the environment. In general, 10–13 h duration is required to traverse the entire length of style. Generally, the interval between pollination and fertilization varies from 36 to 40 h. The pollen grains of cotton are heavy, sticky and warty, leaving little chance for wind pollination. The insects are the natural agents for the pollen transfer.

The pistil consists of three to five undiverged carpels corresponding to the locular composition of a fully mature dehisced boll. The ovules are attached to the parietal placenta of each locule. The style varies in length and splits near the apex into three, four or five parts depending on the number of carpels. The dehiscence of the boll is along the dorsal sutures.

13.10.7.2 Important Diagnostic Characteristics

The characteristics of cotton (UPOV 2018) are given in Table 13.9 below to help the seed technologists and breeders to identify the true-to-type plants and to rogue out the off-types or undesirable plants.

13.10.7.3 Nucleus Seed Production of Varieties

To start a nucleus seed programme, a base source is a prerequisite. For released varieties, it may be breeder seed or nucleus seed, whereas it may be AVT II seed material for new varieties. Sufficient single plants (minimum 200 plants) may be selected from the base source. The selected plants should conform to the basic morphological (phenotypic) characters given at the time of release of variety. The selected plants are individually observed for various distinguishing morphological characters as mentioned in the table above and are selfed. Mean and standard deviation for various agronomic characters are to be worked out, and the plants that lie within the mean $\pm$ SD for all the characters are selected. Individual plant selection is made on morphological characters identical or typical to released variety in the field. The selected plants are subjected to fibre quality evaluation to determine 2.5% span length, micronaire, uniformity ratio and bundle strength. Plants which conform to the basic fibre characters given in the release proposal or which lie within mean $\pm$ SD are selected (Manickam et al. 2003).

The selfed seeds from the selected plants are grown in a randomized replicated design. The row length of plants may be determined based on the availability of

Table 13.9 Important diagnostic characteristics of cotton

Characteristics	States	Stages of observations
Hypocotyl pigmentation	Absent/present	Seedling
Stem pigmentation	Absent/present	Vegetative/ reproductive
Stem hairiness	Absent/medium/strong	Vegetative/ reproductive
Plant height	Dwarf/medium/tall	Vegetative/ reproductive
Leaf shape	Palmate (normal)/digitate (okra)/semi-digitate (semi-okra)/lanceolate (super okra)	Vegetative/ flowering
Leaf size	Small/medium/large	Vegetative/ flowering
Leaf colour	Light green/green/light red/dark red	Vegetative/ flowering
Leaf pubescence	Absent/medium/strong	Vegetative/ flowering
Leaf nectaries	Absent/present	Vegetative/ flowering
Leaf petiole pigmentation	Absent/present	Vegetative/ flowering
Bract type	Normal/frego	Vegetative/ flowering
Sepal pigmentation	Absent/present	Flowering
Petal colour	White/cream/yellow/pink/red/bicolour	Flowering
Petal spotting	Absent/present	Flowering
Position of stigma	Embedded/exserted	Flowering
Anther colour	White/cream/yellow/purple	Flowering
Boll size	Small/medium/large	First boll bursting
Boll shape (longitudinal section)	Rounded/elliptical/ovate	First boll bursting
Boll tip prominence	Blunt/pointed	First boll bursting
Boll surface	Smooth/pitted	First boll bursting
Boll opening	Close/semi-open/open	First picking
Fibre length	Very short/short/medium/long/extra-long	First picking
Fuzz colour	White/grey/brown/green	Harvest maturity
Fibre colour	White/off-white/brown/green	Harvest maturity
Fibre strength	Weak/medium/strong	Harvest maturity
Ginning percent	Low (<31)/medium (31–35)/high (36–40)/very high (>40)	After ginning
Density of fuzz	Naked/semi-fuzzy/fuzzy	After ginning

seeds. Normally, two rows of two replications per progeny are grown. Appropriate spacing should be adopted to enable full expression of characters. It will be better to take up nucleus seed production in areas where the variety is most adopted and during the best growing season.

Normally, the progeny rows should be grown in compact fields with proper isolation. The nucleus seed production plot should be critically observed by the breeder for all the morphological characters during different growth periods. If any progeny or a plant is found to be deviant at any stage for any character, the whole progeny should be rejected. The lines unusually susceptible to insect pests and diseases may also be rejected. Selfing is to be done to maintain the purity. The progenies are studied for various morphological characters, and the data are analysed statistically. The progenies that fall within the mean ± CD at 5% are selected. The fibre test and micro-spinning test are conducted, and only progenies that conform to the original fibre characters are selected. Equal quantity of selfed seeds of selected progenies is bulked to constitute the nucleus seed. If a large quantity of breeder seed is required, the next stage of progeny bulk seed may be taken as nucleus seed stage II. If required, the cycle of selection could be repeated again to further ensure uniformity.

13.10.7.4 Nucleus/Breeder Seed Production of Parental Lines of Conventional Hybrids

The nucleus and breeder seeds of parental lines of hybrids are produced in a similar way as described above for varieties. However, female and male parents are maintained separately in isolation. At final stage, the selected lines should be tested for heterosis and SCA; the seeds of those lines having highest SCA and heterosis for yield and fibre quality traits are utilized for further multiplication and hybrid seed production. This procedure is repeated in the maintenance of parental lines of hybrids to retain the original productivity and fibre quality of hybrids (Manickam et al. 2003).

13.10.7.5 Nucleus Seed Production of A, B and R Lines in the CMS System

- *Production of 'B' line*: The scheme of production of nucleus and breeder seeds of 'B' line is similar to that of female parental line of conventional hybrids.
- *Production of 'A' line*: The 'A' and 'B' lines are grown in alternate rows, and paired crosses are effected between 'A' and 'B' plants during flowering. Care should be taken to cross A and B plants confirming to the original characters of the female parent. Simultaneously, selfing is done in the B line. In the next year, a portion of the selfed and crossed seeds are sown in pair (A and B) while retaining the portion of remnant seeds. Both A and B lines are evaluated for all the morphological characters. Apart from this, the A lines are also observed for pollen shedders. The A lines showing pollen shedders are rejected, and uniform A and B lines are identified which confirm to the standards of the parental lines. The remnant crossed seeds are bulked to constitute the nucleus seed of A line from which the breeder seeds are produced by sib-matting between A and B lines.

- *Production of 'R' line*: About 200 single plants may be selected based on the morphological characters matching the original R lines. They are simultaneously selfed and test crossed with A lines. In the next year, both test crosses and the progenies from the selfed seeds are raised. A part of selfed seed is retained as remnant seeds. Critical observations are recorded in the progenies for conformity with morphological characters of the original parent. The crosses are observed for the fertility restoration. In those crosses, wherein fertility restoration is incomplete, their corresponding progenies are rejected. Also, the progenies not confirming to the morphological characters of the parents are rejected. The remnant seeds from the selected progenies are bulked to constitute the nucleus seed of R lines. The breeder seed is produced from the nucleus seed as discussed earlier for varieties (Udaya Bhaskar et al. 2016).

13.10.7.6 Nucleus Seed Production of GMS Line

About 200 heterozygous fertile plants are selected in the base population, based on morphological characters matching the original female parental line of the hybrid. Sib-mating is done between the sterile and selected fertile plants. In the next year, progeny rows of each selected single plant are raised from a portion of sib-mated seeds. The progeny rows are evaluated for various morphological and fibre quality characters for confirmation. The segregation for fertile and sterile plants in sib-mated progenies is also observed. Only those progenies which segregate in a 1:1 ratio for sterile and fertile plants and confirm to original morphological characters are sib-mated, while the others are rejected. The remnant sib-mated seeds of the selected progenies are bulked to constitute the nucleus seed. The breeder seed is produced from the nucleus seed by sib-mating sterile plants with their fertile counterparts (Singhal 2003).

Acknowledgements The authors duly acknowledge all the resources consulted and text referred from various books, manuals, papers and resources on the Internet for the preparation of this chapter.

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Efficient Breeding of Crop Plants

14

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Abstract

In crop breeding programs, the rate of genetic gain which is achieved using the traditional breeding is insufficient to meet the increased demand of food for the rapidly expanding global population. The main constraint with the conventional breeding is the time which is required in developing crosses, followed by selection and testing of the experimental cultivars. Although, using this technique, lot of progress has been made in increasing the productivity, the time has come to think beyond this and integrate the recent advances in the area of genomics, phenomics and computational biology into the conventional breeding program for increasing its efficiency. While doing this emphasis on proper characterization and use of plant genetic resources, defining the breeding objectives and use of recent advances in holistic way are also essential. Therefore, in this chapter, we first highlight the importance of plant breeding followed by significance of the plant genetic resources in the breeding program, need of ideotype breeding and the breeding objectives for important traits including resistance against various biotic and abiotic stresses. We then discuss the

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_14

limitations of conventional breeding and advantages of genomics-assisted breeding. While doing this, we also discuss various molecular breeding tools and genomic resources as well as different approaches for efficient breeding including marker-assisted selection, marker-assisted recurrent selection and genomic selection. This is followed by importance of other non-conventional approaches including the recent one on gene editing, speed breeding and role of integrated data management and bioinformatics in the breeding programs. We also discuss the significance of phenomics and phenotyping platforms in the crop breeding as well as role of computational techniques like artificial intelligence and machine learning in analysing the huge data which is being generated in the breeding programs. Finally, we conclude with a brief note on the emerging challenges in breeding which need to be addressed and the thrust areas of research for the future.

Keywords

Plant breeding · Efficient breeding · Ideotype breeding · Genomics · Plant genetic resources · Phenomics · Gene editing · Artificial intelligence

14.1 Importance of Crop Breeding

The history of plant breeding has been very promising, and plant breeding has contributed immensely in consistently increasing the productivity of the crop plants during the last several centuries. This has helped in addressing the issue of hunger and malnutrition to larger extent. The achievements of plant breeding have been manifold and have resulted in tremendous increase in yield either by directly improving the yield component traits or through resistance breeding. The Green Revolution is the best example of this, through which the productivity of cereals has increased remarkably by introducing dwarf genes into wheat varieties responsive to fertilizers (Tester and Langridge 2010). In several countries including India, self-sufficiency has been achieved in almost all the major crops in the last few decades using the practices of conventional breeding coupled with better agronomic practices. However, these methods are not sufficient to produce enough food for the growing population in the coming years, particularly with the growing challenges of environmental changes (Godfray et al. 2010; Tester and Langridge 2010).

In order to achieve the breeding objectives, plant breeders often use various methods and techniques. Although the application of techniques in conventional breeding is often straight forward, they need to be refined from time to time according to the existing needs. It is under such circumstances that modern and efficient breeding approaches involving use of genomics tools should become part of the breeding programs (Kulwal et al. 2012). However, before implementing any such technique, it is essential that the breeding objectives are clearly defined.

The science of plant breeding has evolved over the years. Recently, the timeline of the plant breeding has been partitioned into four different phases (Ramstein et al. 2019). Breeding 1.0 focused on selection with unknown loci during the first 10,000 years before 1900 (early domestication phase), Breeding 2.0 relating to the Mendelian genetics and selection by controlled crosses, Breeding 3.0 involving use of genomics techniques like marker-assisted breeding and Breeding 4.0 focusing on ideotype-based selection and use of techniques like genetic transformation and gene editing (Wallace et al. 2018; Ramstein et al. 2019). Broadly speaking, we can categorize Breeding 1 and 2 as conventional approaches while Breeding 3 and 4 as efficient approaches. While we are already in the phase 4.0, the real potential of genomics tools (phase 3.0) has not yet been fully exploited in all the crops. Nevertheless, the success these efficient breeding techniques have shown (see later) has provided new dimensions to the crop improvement programs.

14.2 Importance of Plant Genetic Resources in Crop Breeding

Efficiency of any breeding program is directly dependent on the availability of the plant genetic resources (PGRs) for that crop. These PGRs include cultivated and wild species and valuable germplasm including landraces (Bošković et al. 2012). In order to deal with the complex traits and to respond to the challenges of climate change, particularly biotic and abiotic stresses, breeders need to exploit as much genetic diversity as they can (Galluzzi et al. 2020). This diversity can be exploited through use of the PGRs (landraces, crop wild relatives and other available germplasm) which are generally conserved in the gene banks. Often, the breeders use superior performing lines, albeit with limited diversity in their breeding programs. Although substantial progress has been made for yield improvement with this approach, the time has come when breeders need to exploit the true potential of the PGRs to address the challenges of the future. There are different ways through which PGRs are used in crop breeding programs. However, this depends on the breeding objective, the trait being studied and the genetic resources available (Bidinger 1992).

The three most common ways through which PGRs are being used in the conventional breeding are the (1) introgression (transfer of one or few genes from PGRs), (2) incorporation (widening of the genetic base) and (3) pre-breeding (use of exotic materials or wild relatives) (Haussmann et al. 2004). Besides this, PGRs can also be effectively used (1) as a parental genotype in developing a mapping population to be used in QTL mapping and (2) simultaneous identification and transfer of desirable QTLs from wild or unadapted germplasm into the cultivated one through advanced backcross QTL mapping and (3) for identification of genes/QTLs through the approach of association mapping/genome-wide association studies (GWAS) and allele mining (Kulwal 2016; Kulwal and Singh 2021). Large numbers of studies have been carried out in different crops, and hundreds of marker-trait associations (MTAs) have been identified for a variety of traits through GWAS (Gupta et al. 2019). In situations when all the PGRs for a crop cannot be utilized for GWAS, then

Table 14.1 Plant genetic resources available in different gene banks of CGIAR institutes

S. no.	Crop	Institute	Number of accessions
1	Wheat	CIMMYT and ICARDA	199,248
2	Rice	IRRI and Africa Rice	151,765
3	Barley	ICARDA	32,790
4	Maize	CIMMYT and IITA	30,055
5	Sorghum	ICRISAT	41,582
6	Bajra	ICRISAT	23,841
7	Small millets	ICRISAT	11,797
8	Chickpea	ICRISAT and ICARDA	36,513
9	Pigeon pea	ICRISAT	13,783
10	Soybean	IITA	4841
11	Groundnut	ICRISAT	15,622
12	Forages	CIAT, ICARDA and ILRI	70,514

core and mini-core collections are developed by identifying most diverse but representative set of germplasm from the total collection for further analysis.

There are quite a few examples where PGRs from the gene banks have been used to identify and introgress important genes/QTLs for abiotic stresses in different crops. For instance, in rice, a landrace FR13A was identified for its resilience to complete submergence. The submergence QTL (*Sub1*) from its derivatives has successfully been introduced into several lines leading to the development of many rice varieties with improved tolerance to submergence (Bailey-Serres et al. 2010). Similarly, in chickpea genotype ICC 4958 available at the ICRISAT gene bank was identified as having a profuse root system and was used in many QTL mapping studies which had led to the identification of *QTL-hotspot* for drought tolerance-related traits (Varshney et al. 2014). This QTL region was subsequently transferred in the genetic background of chickpea variety Pusa-372 leading to the development and release of drought-tolerant variety Pusa Chickpea-10,216 following the approach of marker-assisted selection (MAS). In addition, the *QTL-hotspot* has been introgressed in several genetic background, and many introgression lines have been developed (Roorkiwal et al. 2020; Bharadwaj et al. 2021). These examples clearly demonstrate the vital role PGRs play in the plant breeding programs. There are many more such examples in different crops where PGRs have been used for the improvement of the varieties.

The gene banks are invaluable treasures which contain large number of PGRs and provide an opportunity to utilize these resources to breed for the uncertainties posed by climate change (Bohra et al. 2020; Khan et al. 2020). Large numbers of accessions have been conserved at different gene banks across the world. These include major gene banks of CGIAR (https://www.genebanks.org) which house thousands of accessions collected from world over for the important crops (Table 14.1). The number of accessions per crop which are conserved in different gene banks in the CGIAR institutes is also available at https://www.genebanks.org/resources/crops/ (verified March 04, 2021). In addition, there are several national

gene banks in different countries which also conserve different accessions. In India, National Bureau of Plant Genetic Resources (NBPGR) at New Delhi is the main gene bank entrusted with this responsibility. In addition, there are several crop-specific gene banks at different institutes of ICAR. While conserving the germplasm is an important step, it is equally important to characterize and utilize these PGRs effectively for use in the crop improvement programs for addressing the issue of the world's future food requirements (McCouch et al. 2020). In order to utilize this treasure of PGRs available with the national gene bank in India, recently the Department of Biotechnology, Government of India, has funded several projects for the utilization of these germplasm for their characterization and use in the breeding programs. There is no doubt that utilization of these PGRs in the breeding programs can help in achieving the Sustainable Development Goals as outlined by the United Nations (Halewood et al. 2018).

14.3 Ideotype Breeding

The concept of ideotype breeding was proposed for the first time by Donald (1968) in wheat. The concept given by Donald presumed that most of the plant breeding programs are based on two main objectives including (1) defect elimination and (2) selection for yield. The term defect elimination is appropriately used when the disease resistance trait is introduced into a susceptible variety or when early maturity trait is introduced into a variety prone to water stress in late season (Donald 1968). The defect elimination involves correction of physical imperfections like fragile skin in tomatoes, weak malting performance in barley, poor flavour in potatoes and weak straw in cereals. The elimination of these defects ultimately led to the increase in yield and quality. On the other hand, selection and improving yield is considered ultimate objective of any breeding program in the world. The improvement of yield can be done through hybridization that involves crossing of superior genotypes having broad genetic base. Therefore, in summary, ideotype-based approach in plant breeding improves the efficiency of the selection (Gauffreteau 2018). The process of ideotype breeding involves (1) defining the varietal specifications; (2) designing and building an ideotype; and (3) selecting varieties according to the ideotype and assessing their ability to meet the specifications.

In ideotype breeding, the theoretical models are being developed based on our understanding, knowledge, experience and imagination. The models thus developed are known as "the breeding of model plants or ideotypes". Through this type of breeding, it is possible to design a plant that is capable of greater production in a target environment than the genotype it is to replace. For efficient ideotype breeding, we need to choose model characters. These model characters are presumably very important. For instance, in cereals stout stem is a "model character" and provides lodging resistance. Similarly, presence of awns is another model character and has a role in photosynthesis and yield. Another model character is erect foliage (upright leaves) which have shown advantage in photosynthesis. The concept of crop ideotype initially formulated for wheat crop by Donald has been used for several

other crop species including barley (Rasmusson 1987), chickpea (Siddique and Sedgley 1987), forest trees (Dickmann 1985) such as spruce and pine (Kärki and Tigerstedt 1985) and fruit trees like mango and apple (Dickmann et al. 1994). The attributes of ideotypes are morphological characters based on physiological consideration. The concept of ideotype first relied primarily on the morphological traits. However, later the ideotype definition was extended, and traits like physiological, biochemical, anatomical and phenological traits have been included in defining a particular crop ideotype. However, before including any new parameter or a trait for a plant type, it is important to check its contribution towards crop yield. Thus, study and understanding the concept of plant ideotype for a given crop can prove to be an efficient in the breeding of that crop.

14.4 Breeding Objectives for Important Traits

14.4.1 Breeding Objectives for Yield

Grain yield is one of the most complex quantitative traits known in crop plants, and this trait is significantly influenced by the environment. The genetics of grain yield over the years suggested that grain yield is controlled by large number of genes/QTLs with minor effect. The gene controlling grain yield is often influenced by the environment. Therefore, it is important to study genotype × environment interaction while studying genetics of this complex quantitative trait. In addition, extensive studies of grain yield have uncovered that genes responsible for grain yield are often interacting with other genes. This phenomenon is popularly described as "epistasis". Different kinds of gene × gene interactions and gene × gene × environment interactions have also been discovered for grain yield. The complexity of grain yield as a trait can be realized by the fact that grain yield can be dissected into several component traits with higher heritability. In addition, it has been reported in earlier studies that individual traits showing correlation with grain yield are most often controlled by the same set of QTLs/genes.

The most important component traits of grain yield included number of spikelet's/spike, number of grains per spike and 1000 grain/kernel weight (Mir et al. 2021b). For example, in bread wheat, the important reproductive organ harbouring grains is the spike, and therefore traits related to wheat spike are considered very important for manipulating grain yield. The published literature showed strong and positive correlation between different spike traits like spike length with grain yield and yield-related traits including 1000 grain/kernel weight (Mir et al. 2012a). Therefore, from a breeding point of view, genes/QTLs already identified or to be identified in the future for traits related to wheat spike are of importance for wheat molecular breeding programs aimed at enhancing grain yield. The domestication genes/loci like *Q*, *C* and *S* are also considered important and influence several yield component traits including rachis fragility, spike length, spike compactness, grain morphology traits, grain number, seed shape and glumes of a wheat spike.

The genetic dissection of grain yield has been attempted using a variety of approaches including QTL interval mapping and association mapping and have led to the discovery of genes/QTLs for grain yield component traits in several crops. The QTL/genes that have been identified over years need to be deployed into breeding programs through modern and efficient breeding methods including MAS (see later).

14.4.2 Grain Quality Characters

Grain quality traits are very important in any crop because the market price of the end produce is directly related to the quality parameters. A lot of emphasis is thus being given on the improvement of the quality parameters. While there are some quality parameters which are common to majority of the grain crops (e.g. grain size, shape, protein content, etc.), some are unique (e.g. pre-harvest sprouting tolerance in wheat, sorghum, mungbean; milling quality in rice, etc.). List of some important quality traits in different crops is given in Table 14.2. While a lot of breeding efforts have been made for the improvement yield of crop plant, it is now necessary that focus should be shifted towards quality parameters. Breeding for quality parameters is difficult because their nature of inheritance is complex and they are controlled by polygenes. Moreover, measuring the trait precisely is most of the time technically demanding and expensive. However, with the development of novel, rapid and cost-effective screening techniques, breeders have taken renewed interest in the breeding for quality parameters (Munck 2009).

Any breeding program aimed at improving the quality traits relies most importantly on the availability of the known sources containing genes for these traits (Varshney et al. 2021). The PGRs (germplasm, landraces, wild relatives) can prove very useful in this regard. Genes/QTLs for these traits can be identified in a mapping population developed for this purpose by crossing two genotypes differing for the trait or using germplasm collection and natural population. Several studies have been carried out in different crops where QTLs have been identified for important quality

Table 14.2 Key important quality traits in different crops

S. no.	Crop	Quality traits
1	Wheat	Grain protein content; gluten strength; pre-harvest sprouting tolerance; grain size; grain weight; kernel colour; dough making properties
2	Rice	Grain size; grain shape; aroma; whiteness; long and thin uncooked grains; amylose content; milling, physical appearance, cooking, sensory, palatability, and nutritional value
3	Maize	Protein content; amino acid content; fatty acid content; starch quality
4	Sorghum	Amylose content; starch content; crude protein; gross energy; tannin content; polyphenol content
5	Chickpea	Protein content; grain size; fibre content; carotenoid composition
6	Soybean	Seed weight; seed protein; sucrose concentration; oil content; oil composition

traits (Kulwal et al. 2005, 2010). Some of these QTLs have also been transferred into the desirable genotypes using efficient breeding programs like MAS (Varshney et al. 2021). However, success of any such program also depends on the variation explained by the identified QTL. One of the concerns in breeding for quality traits is that it is often considered that improvement in some trait (e.g. protein content) comes with yield penalty, although not always (Kulwal and Mhase 2017). For this purpose, understanding the nature of gene action and genotype × environment interaction is also essential. In recent years, the techniques like genetic engineering and gene editing offer tremendous scope for improving the quality.

14.4.3 Resistance to Biotic Stresses

Biotic stresses (pests and diseases) are known to cause significant losses to the crop plants resulting in reduction in the yield as well as quality of the produce. Depending on the existing circumstances, the degree of losses caused can vary from minor to severe. It has been reported that almost half of the total yield losses in the world are due to biotic stresses (Balconi et al. 2012). Therefore, breeding for biotic stresses is the major objective in any crop improvement program. While use of chemical pesticides is effective in controlling these stresses, they can add to the cost of production and cause significant damage not only to human and animal being but to the environment as well (Bainsla and Meena 2016). With the issues concerning climate change, the threat of new pests and diseases has also increased in recent years. In order to address this issue in a sustainable way, use of resistance sources in the breeding program coupled with deployment of efficient and precise techniques like MAS, marker-assisted gene pyramiding, genetic engineering and gene editing are very promising. MAS not only has simplified the breeding programs but has also accelerated the process of gene transfer in the desired genetic background (see later). The sources of resistance can be identified by screening large number of germplasm accessions against particular disease/pest. Exploitation of PGR can prove useful under such circumstances.

Although the conventional breeding relying on the principle of back crossing using resistant sources has proved successful in incorporating resistance, it is time-consuming and not very efficient technique. Other limitation is that it is effective only when the *R* genes are used one at a time, thus limiting gene pyramiding. There are many successful examples where the technique of MAS has successfully been used to transfer resistance genes/QTLs against various pests and diseases in the crop plants as well as in gene pyramiding (Dormatey et al. 2020). It has also been reported that the resistance achieved through pyramiding of the resistant QTL is at par or better than that conferred by the *R* genes (Richardson et al. 2006). However, MAS is useful only when the resistance source which is being used in the crossing program is compatible with the recipient genotype. In cases when the compatible resistant sources are not available, the technique like genetic engineering and gene editing (see later) can prove very effective (Dong and Ronald 2019). Several successful examples are available where these techniques have been used for incorporating

resistance against particular pest (Varshney et al. 2021). One thing which is very important in resistance breeding is that with the constant pressure of emerging pests and diseases, the breeders need to keep themselves well equipped with the recent tools and one step ahead of the pest and disease in question so that new varieties with durable resistance can be developed.

14.4.4 Resistance to Abiotic Stresses

The yield of crop plants is mostly influenced by a variety of biotic and abiotic stresses, climatic and agronomic factors. The major abiotic stresses limiting crop yields include drought, heat, cold, freezing, salinity and metal stresses. All these abiotic stresses create adverse effects on morphology, physiology and biochemistry which ultimately lead to the adverse effects on growth and yield of plants. Both vegetative and reproductive phases of plant growth are influenced by the abiotic stresses like heat, drought, cold and freezing. It is estimated that an average of 50% yield losses in agricultural crops is caused by abiotic stresses, and among these abiotic stresses high temperature stress causes 40% loss followed by salinity (20%), drought (17%) and cold/freezing (15%) (Meena et al. 2017). For example, in chickpea, one of the most important grain legume crops in the world the significant economic losses due to drought/heat (1.3 billion US dollars), cold (186 million US dollars) and salinity (354 million US dollars) have raised major concerns among the chickpea-growing countries (Jha et al. 2014; Mir et al. 2021a). This situation is further exacerbated by climate change which may cause higher intensities and frequencies of abiotic stresses, thereby necessitating the identification and development of climate-resilient cultivars having region-specific traits, which can perform well under stress.

Several physiological changes are induced in plants in response to different abiotic stresses. For instance, the physiological changes induced in plants in response to abiotic stress included wilting and abscission of the leaf, transpiration, etc. The most important abiotic stress "drought" results in decrease in turgor pressure and thus affects cell growth. It also creates disruption to water flow from xylem to neighbouring elongating cells and thus stops cell elongation. The visible changes include reduction in leaf area as well as plant height. The different mechanisms adapted by plants during abiotic stress include escape, avoidance and tolerance mechanisms. Like drought and heat stress, cold and freezing stress also adversely affects plant growth and development, membrane structure and photosynthetic activity (Mir et al. 2021a). Low temperature stress is an important issue for winter-sown crops like chickpea, lentil, pea, wheat, barley, etc. in the countries surrounding the Mediterranean Sea, the tropical highlands and temperate growing areas. The most affected regions are northern South Asia and parts of the Australia, where crop faces low-temperature stress (<15 °C) which limits growth and vigour at all phenological stages but particularly during vegetative and reproductive stages leading to chlorosis and necrosis of leaf tips, substantial loss of flowers and pod abortion,

reduced pollen viability and pollen tube growth and, thus, reduced seed quality and yield potential by 30–40% (Rani et al. 2020; Mir et al. 2021a).

Keeping in view the losses due to abiotic stresses, the breeding for climatic resilient varieties having tolerance against abiotic stresses has emerged as one of the important subject areas of crop research and a major goal in plant breeding programs worldwide (Meena et al. 2017). Different breeding methods have been used to breed for different abiotic stresses in different crop plants. The methods of breeding adopted in different crop plants depend on their mode of reproduction (self-pollination, cross-pollination or asexual) and the genetic control of trait of interest. The breeding methods for drought are usually the same as that of yield. In general, the conventional breeding methods like pedigree and bulk method could be used for self-pollinated crop species, and recurrent selection could be used for cross-pollinated crop species.

In some cases if one wishes to transfer more than one trait related to drought into a high yielding variety, then back cross breeding method is considered the most appropriate. The varieties for salinity tolerance were developed through pedigree, modified bulk pedigree and anther culture approach. The different genetic resources including landraces, wild relatives, released varieties, pre-breeding lines, advanced breeding lines and mutants also serve as important sources of resistance against abiotic stresses. Wild species of crop plants serve as an important source and reservoir of genes for abiotic stress tolerance. For instance, in wheat crop, *Aegilops kotschyi*, *Ae. Squarrosa* and *Triticum urartu* serve as an excellent source of drought tolerance and *Aegilops tauschii* as source of salinity tolerance. In chickpea, *Cicer microphyllum*, *Cicer reticulatum* and *Cicer echinospermum* serve as excellent sources of cold tolerance. The genetics of abiotic stresses is considered very complex and controlled by large number of small effect genes/QTLs.

Although conventional breeding methods have been used to address the issues of abiotic stresses, to breed more efficiently, scientists all over the world are now adopting integrated genomics-based tools in their breeding pipelines. A variety of genomics, physiology and breeding approaches have been extensively deployed for the genetic dissection of abiotic stress adaptation (Mir et al. 2012b). Several genomics approaches including QTL interval mapping, GWAS, transcriptomics, etc. have been deployed for the discovery of genes/QTLs/transcripts/markers for their use in genomics-assisted breeding for development of abiotic stress-tolerant crop varieties.

14.5 Crop Breeding in the Era of Genomics

14.5.1 Limitations of Conventional Breeding

While the conventional breeding is being practiced since centuries and has immensely helped in development of large number of varieties in different crops, it has its own limitations. Since the conventional breeding programs are dependent on phenotypic selection, which basically is more of an art than science, the

effectiveness of such program is less. The major limitations which hamper the success of conventional breeding are (a) the time required for improvement of a trait is very long; (b) the accuracy with which the desirable genotypes could be selected is very less (this happens mainly due to linkage drag when many traits are transferred along with the trait(s) of interest—including those traits that have undesirable effects); and (c) the efficiency with which the selection for the trait can be done is also very less. These three limitations not only increase the time and cost required for development of a variety, but many times, the new genotype becomes susceptible to new pest or disease by the time it is ready for release as a new variety.

14.5.2 Advantages of Molecular Breeding/Genomics-Assisted Breeding

The three key limitations associated with the conventional breeding discussed above can effectively be overcome by the use of molecular breeding/genomics-assisted breeding (GAB). This can also be called as precision breeding (Kulwal et al. 2012). The three key advantages of molecular breeding over the conventional breeding are the following.

14.5.2.1 Time Saving

Using molecular breeding, the time and labour savings may arise from the substitution of difficult or time-consuming field trials (that need to be conducted at particular times of year or at specific locations or are technically complicated) with DNA marker tests. This is an important advantage of the molecular breeding, since lesser time will also curtail the cost of the breeding program and selections can be done any time during the year.

14.5.2.2 Increased Efficiency

Molecular breeding can greatly increase the efficiency and effectiveness of breeding compared to conventional breeding because it is simpler compared to phenotypic screening, selection may be carried out at seedling stage and single plants may be selected with high reliability. These key advantages may translate into greater efficiency or accelerated line development in the plant breeding programs.

14.5.2.3 Increased Accuracy

It is well-known that effect/influence of environmental factors on field trials is more, thereby limiting the accuracy of selection. However, selection based on DNA markers is more reliable as effect of environment is not there. Moreover, the total number of lines that need to be tested may be reduced, and specific genotypes can easily be identified and selected.

With the advances in the area of genomics coupled with the availability of large number of genomic resources in different crops (see next section), molecular

breeding or GAB has now become an integral part of the crop improvement program (Varshney et al. 2021).

14.5.3 Molecular Breeding Tools for Efficient Breeding

As already discussed in the above section that there are several advantages of molecular breeding over conventional plant breeding, the traditional/conventional crop improvement techniques are now being replaced by GAB approaches. Recent advances in genomics tools and techniques have facilitated the development of large number of different types of molecular markers, genetic and physical maps, high-throughput and precise genotyping platforms and rapid discovery of genes using different approaches in almost all important crop plants (Bohra et al. 2020). Therefore, efficiency and precision of crop improvement could increase using these genomic tools and techniques. This has become primarily possible due to the advances in the next-generation DNA sequencing technologies and development of large numbers of molecular markers. The efficient breeding program today thus relies on several tools which are described in the following sections.

14.5.3.1 Molecular Markers

The recent years have witnessed the development of large-scale genomics and genetic resources including variety of molecular markers, expressed sequence tags (ESTs) or transcript reads, bacterial artificial chromosome (BAC) libraries, genetic and physical maps and genetic stocks with rich genetic diversity, such as core reference sets and introgression lines in majority of the crop plants (Varshney et al. 2010). The DNA-based markers also known as molecular markers are being used for the detection of genome sequence level differences between two or more than two individuals. The discovery of molecular markers has revolutionized crop improvement programs by providing quick and sophisticated/reliable crop improvement tools and techniques. The marker technology although discovered in the 1980s with the discovery of RFLPs has witnessed continuous evolution from hybrization-based markers to GBS-based markers (Mir et al. 2013; Mir and Varshney 2013; Gupta et al. 2013a, b; Kumar et al. 2021; Tyagi et al. 2019, 2021). A number of classifications have been proposed to classify molecular markers. Some of the classifications of markers include hybridization-based vs. non-hybridization-based, first-generation vs. second-generation vs. third-generation markers, past vs. present vs. future, low-throughput vs. medium-throughput vs. high-throughput markers, sequence based vs. non-sequence based and array-based vs. non-array-based markers (Gupta et al. 2008; Mir et al. 2013; Mir and Varshney 2013).

The advances in genomics has not only resulted in development of large numbers of markers in important crops but also in in the once considered orphan and resource poor crops like chickpea, pigeon pea and groundnut. Now in these crops, thousands of all important types of molecular markers including SSR, diversity arrays technology (DArT), single nucleotide polymorphism (SNP), different SNP platforms, micro-array-based markers, GBS, InDel markers, etc. are available. For instance,

over the years, >2000 SSRs in chickpea, >3000 in pigeon pea and >2500 in groundnut have become available using different approaches of marker development. Similarly, thousands of DArT and SNP markers are available in these crops.

Several genotypic platforms including Kompetitive Allele Specific PCR (KASP) assays, GoldenGate assays, Vera Code assays and 60 K SNP chips using Affymetrix SNP platform and Axiom SNP array with thousands of SNPs uniformly distributed across the genome are available now (Thudi et al. 2021). These marker resources have been used in the study of genetic diversity, population structure, development of genetic maps and QTL mapping/GWAS for key traits in all major food crops. The genes/QTLs once identified are being deployed into molecular breeding programs aimed at enhancing targeted traits in different crop plants through MAS, marker-assisted recurrent selection (MARS) and genomic selection (GS). It is expected that the improved versions of next-generation crop varieties could be developed with enhanced quality traits, better yield and disease resistance.

14.5.3.2 Genetic/Linkage Maps

The molecular genetic map or linkage map refers to linear arrangement of genetic markers (loci) on the genome obtained on the basis of estimates of recombination fractions among the markers. The genetic map may be thought of as a "road map" of the chromosomes developed for a mapping population derived from two different parents. It indicates the position and relative genetic distances between markers along chromosomes, which is analogous to signs or landmarks along a highway. The concept of genetic linkage is known since the studies of Morgan 1911 and Sturtevant published genetic map of chromosome X of Drosophila in 1913. The first partial genetic map of maize was published by Emerson and colleagues in 1935. These linkage maps were prepared by analysing segregating populations derived from crosses of genetically diverse parents and estimating the recombination frequency (RF) among genetic loci. The distance between the markers on a genetic map is related to the RF between the markers. Lower the frequency of recombination between two markers, the closer they are situated on a chromosome, and hence greater the frequency of recombination, more is the genetic distance. Markers that have a recombination frequency of 50% are described as "unlinked" and assumed to be located far apart on the same chromosome or on different chromosomes. Different chromosomal regions vary in their recombination frequency. Because of this, genetic maps cannot be used to measure physical distance between markers on the genome and only provide an approximation of physical distance, as well as a representation of marker order along the chromosome. Genetic maps have in general several functions including (1) providing an insight into genome organization, (2) the evolution of species, (3) synteny between related species, (4) rearrangement across taxa and more importantly (5) identification of genes/QTL for a trait of interest.

The molecular genetic maps based on DNA markers are now available in almost all plants of significant academic and economic interest, and the list of plants is growing regularly. Different types of molecular markers have been used for development of genetic maps, and sometimes genetic maps of only individual chromosomes have been constructed on the basis of needs of researchers. However,

development of whole genome maps covering all the chromosomes in genome is always desirable. Initially RFLP-based genetic maps were developed, but with the discovery of markers of choice like SSRs and SNPs, now almost all maps are based on these markers (Thudi et al. 2021). More recently, several high-throughput genotyping platforms have been developed based on SNP markers, and the use of these genotyping platforms has facilitated the development of high-resolution and high-density genetic maps. These high-density genetic maps prove useful in fine mapping of genes. The different maps developed over the years have been used for mapping, tagging, cloning and characterization of large number of genes/QTLs in all important crop plants, and the information so generated can facilitate efficient breeding.

14.5.3.3 Use of Genome Sequence Information for Crop Breeding

The science of plant genomics research had its beginning with the publication of genome sequence of *Arabidopsis thaliana* in the year 2000. Nowadays, the genome sequence of almost all the major crop plants including cereals and legumes have become available (Thudi et al. 2021). After sequencing of Arabidopsis genome, rice genome was sequenced in year 2005. After sequencing of rice genomes, draft genomes of several cultivars among ssp. *japonica* and ssp. *indica* have also become available. Draft genomes of Australian wild A genome taxa including *O. rufipogon* and *O. meridionalis*, other wild species, core collections and mini-core core collections have also become available in recent years (for a review, see Thudi et al. 2021).

The other most important cereal crop "maize" genome was sequenced using a most widely used female parent "B73" for developing maize hybrids and study of maize genetics (Schnable et al. 2009). Followed by the sequencing of B73 genome, draft genome sequence of other maize inbred including Mo17, W22, HZS, SK and K0326Y were also generated. Bread wheat, one of the most important cereal crops with complex and huge genome size, has also been sequenced by the International Wheat Genome Sequencing Consortium (IWGSC) more than a decade after the initial drafts of the rice genome (International Wheat Genome Sequencing Consortium, IWGSC, http://www.wheatgenome.org/). Similarly, barley genome was also sequenced using six-row malting cultivar Morex (Mascher et al. 2017) followed by sequencing of Tibetan hulless barley (Zeng et al. 2015, 2018) and wild barley species AWCS276.

Like cereals, major legume genomes have also been sequenced. For instance, draft genome of cultivated soybean Williams 82 (Schmutz et al. 2010) and undomesticated ancestor of *G. max*, the *G. soja* (Kim et al. 2010), have become available. The genome sequence of two diploid progenitor species of groundnut (*A. duranensis* V14167 and *A. ipaensis* K30076) was reported by the International Peanut Genome Initiative (IPGI) through the Peanut Genome Consortium (PGC) (Bertioli et al. 2016). This was followed by sequencing of several other groundnut genomes (Chen et al. 2016; Lu et al. 2018; Yin et al. 2018; Bertioli et al. 2019; Zhuang et al. 2019). The common bean genome sequence has also become available for Andean inbred landrace "G19833" (Schmutz et al. 2014). For grain legume crop

chickpea, a draft genome sequence for Kabuli genotype, CDC Frontier was generated by Varshney et al. (2013b) and for desi chickpea genotype ICC 4958 by Jain et al. (2013). For pigeon pea, draft genome assembly was developed for variety Asha (ICPL 87119) (Singh et al. 2012; Varshney et al. 2012).

The sequencing of these plant genomes has played very crucial role in discovering important genes and understanding their biological functions. Due to advances in genome sequencing technologies, the speed of sequencing has increased, and cost of sequencing has drastically decreased. This has resulted in the sequencing of draft genome of >800 plant species, and the number is continuously increasing (Thudi et al. 2021). The sequencing of crop genomes and the information derived have been utilized in both basic and applied research. For instance, this information has been utilized in working out evolutionary relationships, developing better phylogenetic classification and discovery of genes, alleles, markers, etc. The sequencing of large number of genomes has also resulted in the introduction of the concept of plant pan genome, each composed of "core genome" and "dispensable genome". The recent advances in genomics tools and techniques have helped in the development of genomics resources in all major crop plants in the world. Several databases like Legume Information System (LIS https://legumeinfo.org/; LegumeIP, https://plan.tgrn.noble.org/ LegumeIP; and Know Pulse, https://knowpulse.usask.ca) have been developed for providing genomic information.

14.6 Approaches for Efficient Breeding

14.6.1 Genomics-Assisted Breeding

With the advances in the area of genomics in last few years coupled with the availability of large numbers of genomic resources in terms of mapping populations, different types of molecular markers, genome sequence, linkage maps and identified QTLs/MTAs have changed the way plant breeding is being practiced. The important advantage the science of genomics has brought is the increased efficiency of the breeding programs leading to the proper understanding of the genetic architecture of the traits and incorporating this information in the varietal development programs. There are different ways through which this can be accomplished and are discussed below.

14.6.1.1 Marker-Assisted Selection (MAS)

The simple meaning of MAS is the use of molecular marker linked with the QTL/gene of interest as the substitute for making selection for a desirable genotype under laboratory conditions rather than cumbersome field-based phenotypic screening. However, before using the markers in the breeding program, there are few key things which need to be satisfied. These are (1) identification of the marker (s) associated with the trait of interest, (2) validation or testing suitability of these markers in the desirable genetic background and (3) use of these markers in the breeding program though marker-assisted backcrossing (MABC) for transfer

of the required QTL/gene in the desirable genetic background (Kulwal et al. 2012). The general pre-requites for undertaking MAS have also been discussed elsewhere (Jiang 2015). The success of MAS entirely depends on the accuracy with which MTAs are identified. This can be accomplished by the development of linkage maps (preferably high-density maps) using the genotypic data of large numbers of markers generated for a biparental mapping population developed by crossing two genotypes differing for the trait of interest. The biparental mapping populations which can be used for this purpose are F2, doubled haploids (DH) and the recombinant inbred lines (RILs). The advantage of having linkage maps is that, one can place the markers on different chromosomes in linear order and assign distances between these markers. When development of biparental mapping population is not possible/feasible (particularly in case of tree species), one can use the germplasm or natural population and genotype them using molecular markers for identification of the QTLs/MTAs following the approach of association mapping (AM) or GWAS.

It is equally important that the population for which genotypic data has been generated is phenotyped precisely for the trait of interest so that the data can be used in conjunction with the genotypic data for the identification of QTLs/MTAs. Ideally, phenotypic data recorded over seasons and locations is desirable so that QTL × environment interactions can also be worked out. Several approaches of QTL analysis have been proposed for the analysis of the data (reviewed by Kulwal 2018). Large numbers of studies have been carried out following the approach of biparental QTL mapping and GWAS in different crops for variety of traits (Gupta et al. 2011, 2014, 2019). However, not all the markers linked with these identified QTLs can be used in the MAS program. The underlying criterion for this therefore is that the QTL which is to be transferred through MAS must be a major effect QTL. However, in recent years with the availability of wealth of data generated through large numbers of QTL and GWA studies as well as meta-analysis, it has become easy to identify major QTL and choose the type of marker for the MAS program. The advantage which MAS offers is that it can effectively be utilized for traits having low heritability. Large numbers of studies are now available where MAS has been utilized for the transfer of useful QTL in the desirable genetic backgrounds in different crops leading to the development of superior breeding lines and varieties (Varshney et al. 2013a). Given the fact that there has been tremendous reduction in the costs of marker genotyping and the advantages which MAS offers, it is anticipated that MAS will be used on large scale by the breeders for crop improvement in the future.

14.6.2 Marker-Assisted Recurrent Selection (MARS)

It is well established that majority of the traits of interest are polygenic in nature and are controlled by many genes/QTLs, each having minor effect. The problem with deployment of these minor QTLs in the breeding program through MABC is that they are not expressed consistently over different seasons. It therefore become difficult to introgress multiple QTLs in a common background. Although in conventional breeding recurrent selection has been suggested as an effective strategy for

improving the polygenic traits by accumulating the favourable alleles in the population, the strategy is not very effective due to effect of environment on the phenotype and the long time required for genotypic selection (typically 2–3 crop seasons per cycle) (Godiki et al. 2016). In order to address this issue and to utilize these minor effect QTLs in the breeding program, MARS has been proposed and allows genotypic selection and intercrossing in the same crop season (Bernardo and Charcosset 2006).

MARS utilizes markers initially for the identification and then selection of several genomic regions associated with the complex trait(s). This then can be used to assemble the best-performing genotype within a single or across related populations (Ribaut et al. 2010; Jiang 2015). The advantage of MARS over MABC is that the genetic gain achieved through the former method are more as compared to the later since MARS deals with transfer of several QTLs as against that of only selected QTL in the MABC. There are several successful examples in crop plants where MARS has shown increased efficiency of selection in the breeding programs. Some of these examples include in maize for yield improvement (Johnson 2004), for improving grain yield under drought stress (Beyene et al. 2016), in wheat for bread making-related traits (Charmet et al. 2001) and crown rot resistance (Rahman et al. 2020). There are many other examples where MARS has been successfully utilized in different crops.

14.6.3 Genomic Selection (GS)

Although conventional breeding coupled with modern breeding techniques have helped in increasing the genetic gain to a considerable extent, it is necessary that the rate of gain should be increased further to address the challenges of food security (Xu et al. 2020). Genomic selection (GS) also referred to as genome-wide selection (GWS) or genomic prediction is one of the forms of MAS, in which large numbers of markers covering the entire genome are used to predict the genetic value of a trait or individual. Selection for the desirable individuals is based on computing the genomic estimated breeding values (GEBVs) using the markers across the genome (Meuwissen et al. 2001; Crossa et al. 2017). This in contrast to MAS in which only the markers tightly linked with the trait are used in the breeding program

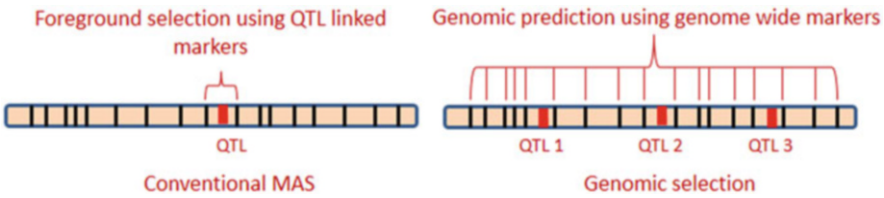

Fig. 14.1 Figure depicting the difference between marker-assisted selection and genomic selection; the horizontal bar represent the chromosome on which mapped markers are shown in black vertical lines, while position of QTL is shown with red rectangle

(Fig. 14.1). The underlying criteria in GS is that rather than focusing on only important or major QTLs in the breeding programs, it utilizes all the QTLs (minor and major) by using the genome-wide markers while making the prediction. In order to increase the accuracy of GEBV and GS, large number of markers across the genome is thus essential so that all the QTLs are in LD with at least one marker (Meuwissen 2007; Jiang 2015).

For undertaking the GS, a training population (TP) for which genotypic and phenotypic data is generated is essential so that a prediction model for understanding the relationship between the two can be developed. The genotypic data of the breeding population is then fed into this model to calculate GEBVs for these lines (Heffner et al. 2010). There are different ways through which these GEBVs can be calculated. The GEBVs calculated represents the sum of the effects of all QTLs across the genome. GS thus outperforms MAS in terms of its effectiveness (Kulwal et al. 2012). Since there are different statistical models to estimate the breeding values, each having its own superiority and limitations under the given scenario, one model cannot fit all the situations (Rahim et al. 2020). It is therefore difficult to suggest which model will work under the given scenario.

Although the technique of GS was initially proposed for use in animal breeding (Meuwissen et al. 2001), in recent years, emphasis is given on the use of GS in the breeding of many crops. It has also been reported that the cost per unit gain was lower up to 55% using GS than the phenotypic selection (PS) in case of oil palm and that GS was superior to MARS and PS (Wong and Bernardo 2008). Similarly, superiority of GS over PS has also been reported in many crops (reviewed by Jiang 2015; Pandey et al. 2020; Rahim et al. 2020). Although the technique of GS appears to be promising, its success primarily depends on the size of the TP which is used for identifying the associations. This TP should be updated frequently by incorporation of new genotypes in the analysis so as to maintain the prediction accuracy (Rahim et al. 2020). However, given the fact that not many breeders are trained in the use of molecular markers on large scale and computer programs dealing with estimation of breeding values, it is necessary that breeder friendly software packages should be developed for such purpose. In addition, developing the efficient models which can involve genotype × environment interaction and achieve greater prediction accuracy are therefore required (Xu et al. 2020). It is expected that with the reduction in the cost of marker genotyping due to advances in the next-generation sequencing techniques, accompanied with advances in the computational analysis, GS will become an integral part of the crop breeding programs (Desta and Ortiz 2014).

14.6.4 Gene Editing in Plant Breeding

Crop improvement is a continuous process for ensuring food and nutritional security for burgeoning world population. Great success has been achieved through conventional plant breeding and through use of transgenics. The advantages, success stories and limitations of transgenic technology have been reviewed in detail in several

earlier publications (see Datta et al. 2004; Husaini et al. 2011; Ahmar et al. 2020). Due to several concerns associated with transgenic technology, new plant breeding techniques including RNA interference (RNAi), gene silencing and gene editing are gaining worldwide attention in different crop improvement programs. Among the different new breeding technologies, gene/genome editing (GE) making use of site-directed nucleases (SDNs) is considered the most important and one of the promising technologies that can overcome the inherent limitations associated with classical/conventional plant breeding and transgenic technology. The GE tools and techniques are considered effective for modifying the target genome and creating desired and novel new traits/phenotypes in crop plants. The breakthrough technology started with sequence-specific nucleases including zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and now the most important and emerging clustered regularly interspaced short palindromic repeats, CRISPR/Cas technology (Lloyd et al. 2005; Cermak et al. 2011; Mahfouz et al. 2011; Li et al. 2013; Nekrasov et al. 2013; Shan et al. 2013; Tan et al. 2020).

Discovered by Emmanuelle Charpentier and Jennifer A. Doudna (Nobel Laureates in chemistry for the year 2020), CRISPR/Cas9 genetic scissor is considered one of gene technology's sharpest tools that can help to change the DNA of crop plants with extremely high precision. The noble prize winning technology "CRISPR/Cas9" can help to change the code of life of crop plants over the course of a few weeks. CRISPR technology has been used extensively in plant genome editing over the past few years and has a great potential for precision breeding (Zhang et al. 2020). However, this system is also subject to some technical limitations and some other main obstacles including consumer preference of gene-edited food product that may hinder its application in food crops (Ahmar et al. 2020). The progress and perspectives of the use of genomic editing tools and technologies for their use in plant breeding have been extensively reviewed (see Varshney et al. 2019; Zimny et al. 2019; Zhang et al. 2019, 2020).

The technology has been mostly used for improvement of crop yields, quality and stress resistance by simply knocking out one or more than one genes that control a particular trait (Zhang et al. 2018). For instance, knocking out of genes (*Gn1a*, *DEP1* and *GS3)* in cereal crop rice led to the increase in grain number, dense erect panicles and increased grain size (Zhang et al. 2020). Similarly, in maize disruption of waxy gene "*Wx1*" resulted in increase in concentration of amylopectin with enhanced digestibility of grain. In wheat and tomato, knocking out of gene/allele "*MLO*" resulted in the development of powdery mildew-resistant wheat and tomato plants (for a review, see Zhang et al. 2020). The technology has recently revolutionized agriculture by helping in fixing heterosis in rice hybrids (Khanday et al. 2019; Wang et al. 2019), which is otherwise lost in subsequent generations due to segregation of alleles in F_1 hybrids.

In these studies involving fixing heterosis in rice, a genotype known as *MiMe* (Mitosis instead of Meiosis) was produced by targeting important genes responsible for meiosis, and thereby haploid plants were developed using CRISPR technology. When *MiMe* genotype was combined with haploidy in hybrid rice, the clonal progeny maintained genome-wide parental heterozygosity that demonstrates the

possibility of asexual reproduction through seed propagation in crops (Khanday et al. 2019; Wang et al. 2019). The technology of fixing heterosis in rice hybrids will allow the maintenance of rice hybrids during propagation to subsequent generations. This revolutionary process can reduce the cost of hybrid seed production as well will allow farmers to produce their own hybrid seeds, reducing dependence of subsistence farmers on commercial seed producers. In a study in wheat, *A3A-PBE*-mediated cytidine base editing was used for editing of all the six *TaALS* alleles that resulted in the development of nicosulfuron-resistant wheat lines (Zong et al. 2018). In an another study considered one of the best examples of the use of CRISPR/Cas technology for crop improvement is CRISPR-mediated gene regulation in tomato, where CRISPR technology was used to mutate the promoters of genes responsible for controlling the most important quantitative traits including fruit size, inflorescence branching and plant architecture (Rodriguez-Leal et al. 2017). In summary, the technique has already been used in ~20 crop species improvement programs for the development of crops that possess high yield and withstand biotic and abiotic stresses. The list will keep on increasing in more crops involving improvement of important traits in the near future.

14.6.5 Role of Bioinformatics in Breeding

Bioinformatics is multi-disciplinary science, integrating biology, statistics, mathematics, computer science, etc. that helps in solving the biological problems. The need for the science of bioinformatics was strongly felt with the evolution of next-generation sequencing technologies that helped in sequencing of genomes of almost all important crop species. The sequencing of genomes through genome sequencing projects led to the explosion of sequencing data produced and hence demands conception/creation of novel discipline called "bioinformatics". The aim of bioinformatics is primarily storage, acquisition, analysis, distribution and modelling of various types of sequence data (Aslam et al. 2004). Therefore, computational biology and bioinformatics have their roots in life science and helps to find out the function of macromolecules, biological sequence data and genome content/genes.

In the last two to three decades, the science of bioinformatics has emerged as a significant tool for the use of large volumes of data that have been generated using different omic-technologies. The analysis of data through different software programs provided vital role in extracting useful information, interpretation of data and future decision-making process (Batley and Edwards 2008, 2009; Aslam et al. 2004). The analysis of genome sequencing data and other genotyping data through bioinformatics tools have helped in discovery of thousands of genes in important crop species. The identified genes will prove useful in plant breeding programs aimed at enhancing trait performance of varieties leading to the development of next-generation crop varieties.

The huge volume of genome sequencing data generated required development of databases for its storage, and therefore in 1986 largest sequence databases were developed in association of GenBank with European molecular biology laboratory

(EMBL). A number of bioinformatics online databases including crop-specific databases are now available including BGI Rice Information System, Gateway of Brassica Genome, ChloroplastDB, EMBL, GRAINGENES, GRAMENE, GRIN, NCBI, LIS, KOME database, KEGG PLANT, OryGenesDb, TAIR, TREP, etc. The complete list has been tabulated elsewhere (Aslam et al. 2004). The details of classification of databases, databases for transcription factor in plants, small RNA databases, genomic databases, crop-specific databases and list of different bioinformatics tools for analysis of NGS data, dbEST available in different crops, etc. are also available in different review articles (see Vassilev et al. 2005; Agarwal and Narayan 2015; Aslam et al. 2004; Kushwaha et al. 2017). In general, there are three primary sequence databases including GenBank (NCBI), the Nucleotide Sequence Database (EMBL) and the DNA Databank of Japan (DDBJ). These databases are actually repositories used for storing of raw sequence data. However, each data entry is also extensively annotated, and important properties and features of each sequence are also highlighted. These three important databases exchange data routinely. Similarly, databases are also available for storing protein sequence like SWISS-PROT and TrEMBL. The constant serge in the omics data and the emergence of molecular breeding technologies coupled with advances in genomics and computational biology provide ample opportunities for bioinformatics to develop efficient approaches for plant breeding.

14.6.6 Integrated System of Data Management and Delivery

With the challenges posed by the varying environments, the activities of plant breeding have expanded in the last few decades. This has resulted in multi-disciplinary and multi-institutional collaborations and generation of large-scale phenotypic data, typically collected over different environments having increased dimensionality due to use of sensors and techniques like phenomics. This is in addition to the large-scale genomics information which is being generated routinely due to the availability of high-throughput techniques resulting in big data. This big wealth of data on one hand has increased the capabilities of the breeders in achieving their goals and on the other hand has made it necessary to think about proper management of the information. While the scientific community spends most part of their time on generation of valuable data, only limited time is spent on proper documentation, analysis and interpretation process.

Although there is no doubt that the key to success for any breeding program is careful collection of the data, it is equally important that there should be proper integration of the other parameters in the analysis process leading to the meaningful interpretation of the results. Any wrong decision or improper interpretation of the data can cause huge loss to any breeding program. It is therefore very important that there should be an integrated system of data management and delivery in a plant breeding program so that one can access, analyse and recombine the vast wealth of data (Kuriakose et al. 2020). The advantage with this system is that, the information generated in the experiment is not only stored carefully, but can be retrieved at any

given time as per the need. This generally is not possible in the traditional way of collecting the data. Therefore the success of any future breeding program will depend on how strong the data management and delivery system is in place. An excellent overview of this aspect is discussed by Kuriakose et al. (2020).

This type of setup is generally seen in many private seed companies, but lacked in the public sector breeding programs in developing countries. It is therefore envisioned that the primary challenge for the plant breeders in coming years will be to design the efficient system to handle and analyse the massive amounts of multifarious data that is generated in the breeding programs rather than access to the modern technology (Cobb et al. 2019).

14.6.7 Speed Breeding

Ideally, any conventional breeding program involve crossing and/or selection for successive generations (typically 4–6) followed by yield evaluation trial before a variety is released for cultivation. Majority of the varieties so far in the world have been released using the same approach during the last several decades. Generally 1–2 generations/cycles are possible per year in majority of the crop plants. With this slow speed, the time required in a breeding process is too long and also slows down the process of variety development. Although the alternate techniques like shuttle breeding and doubled haploid can be used to shorten the time required in a breeding program, they have their own limitations. Therefore, in order to accelerate the process of breeding and generation advancement, a technique called speed breeding was proposed recently (Hickey et al. 2019). As the name suggests, speed breeding relies on use of environment-controlled growth chambers equipped with artificial lights which can accelerate the plant growth and development so that multiple generations of crop plants can be advanced per year (Ahmar et al. 2020). This is very much required in today's context because in order to produce more to feed the growing world population, there is an urgent need to accelerate the rate of genetic gain. While the molecular breeding techniques are efficient in introgression of the desired gene/QTL, their utility will be enhanced only if more number of generations are advanced per year. Speed breeding enhances the growth of the plant by regulating light and temperature, thereby promoting early flowering and rapid generation advancement (Bhatta et al. 2019).

Since each crop plant has differential requirement of photoperiod for normal growth and development, the protocol established for one crop plant may not be suitable for the other. The proper understanding of the physiology of the plant is thus necessary. Ideally, in speed breeding, vegetative growth is enhanced by increasing the temperature in the chamber, while it is lowered during the reproductive growth (Hickey et al. 2019). However any such improvement comes with a cost. For instance, flowering can be hastened in the environment-controlled chambers; however, the total biomass and yield will be impacted due to this (Bhatta et al. 2019). However, the success achieved in achieving four to six generations per year in crops like wheat, barley and canola (Hickey et al. 2017; Ghosh et al. 2018; Watson et al.

2018) using speed breeding shows the great promise this technique offers in the crop improvement programs and in accelerating the speed of variety development. However, one need to weigh the cost involved in developing the facility and the associated gains through it before investment is made on this technique.

14.7 Advances in High-Throughput Phenotyping

One of the major factors limiting progress in GAB is lack of precise phenotypic data. Therefore, plant phenomics is considered one of the most important factors for translating the progress made in the area of plant genomics. The area of plant phenotyping has made huge progress in the last decade by replacing invasive or destructive methods of phenotyping by the high-throughput precise non-destructive methods of phenotyping (Mir et al. 2019). The advancement made has revolutionized crop phenomics and allowed screening of large germplasm (mapping populations, core collections and breeding material) with high precision/accuracy with less efforts, time and labour. These advances not only have generated huge amount of information but have also necessitated use of novel techniques for the analysis of the big data. These issues have been discussed in the following sections.

14.7.1 Plant Phenotyping Platforms

The non-destructive high-throughput phenotyping (HTP) platforms developed and used routinely include infrared cameras, fluorescent microscopy/spectroscopy, three-dimensional camera, lidars (light detection and ranging), magnetic resonance imaging (MRI) and positron emission tomography (PET), canopy spectral reflectance (SR) and infrared thermography (IRT), nuclear magnetic resonance (NMR) and digital imaging (see Mir et al. 2019 for review). The use of these HTP platforms helps in recording trait data on thousands of plants in a single day similar to next-generation sequencing technology in the field of plant genomics (Finkel 2009). A number of state-of-the-art international phenomics centres/facilities have been developed for precisely recording high-throughput phenotyping data in cost-effective manner. Some of the important phenomics facilities include the Plant Accelerator in Adelaide, Australia (http://www.plantaccelerator.org.au/); High Resolution Plant Phenomics Centre (http://www.plantphenomics.org/HRPPC) in South Australia; the Jülich Plant Phenotyping Centre (http://www.fz-juelich.de/ibg/ibg-2/EN/methods_jppc/methods_node.html) in Jülich, Germany; Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany; and the National Plant Phenomics Centre (http://www.phenomics.org.uk/temp-site/about.html) in the UK to name a few (Gupta et al. 2012; Mir et al. 2015, 2019). The relevant information about plant phenotyping is being provided by the world's major plant phenotyping centres "International Plant Phenotyping Network (IPPN)" (https://www.plant-phenotyping.org/). In addition, different private companies like LemnaTec, Phenokey, PhenoSpex, Photon System Instruments, Wiwam and We

Provide Solutions are offering large-scale, custom high-throughput phenotyping platforms for the field and laboratory (Mir et al. 2019).

The different HTP phenotyping platforms that have been developed for recording data on variety of traits in almost all crop species include "LEAF-E" developed and used for the analyses of leaf growth parameters, "Zeppelin NT aircraft" used aerial phenotyping "Phenovator" and "GROWSCREEN FLUORO" used for phenotyping for photosynthesis and growth "TRiP (Tracking Rhythms in Plants)" used for determination of circadian period. Similarly, image-based phenotyping methods have been developed and used for measuring plant stresses including cold tolerance and spikelet anthesis. The other phenomics platforms like" PHENOPSIS" was used to dissect plant responses to soil water deficit, and "Unmanned Aerial Platforms (UAP)" was used for measuring low-nitrogen (low-N) stress tolerance. The "Hyperspectral Imaging (HIS)" was used to determine spectral changes on the leaf and cellular level in plants during resistance reactions/host-pathogen interactions. Like phenotypic platforms, a number of different software programs have been also developed for recording trait data on variety of traits. A list of software programs and phenotyping platforms for high-throughput precise phenotyping being used in several laboratories across the world are available elsewhere (see Mir et al. 2019).

The different precise high-throughput phenomics methods/platforms already developed have been used for trait phenotyping of variety of traits including growth traits, phenological traits, physiological traits, scoring disease incidence, insect damage, drought tolerance and recording data on different plant organs like roots, seeds and shoots (for review see Mir et al. 2019). For instance, in crops like rice, wheat, barley, maize, pea, Arabidopsis, potato, soybean, etc., different phenotyping platforms have been used, and data has been recorded for spikelet anthesis, circadian period, plant height, leaf growth parameters including leaf area, area phenotyping of canopies, photosynthesis, photosynthesis efficiency, chlorophyll content, leaf nitrogen content and canopy height (see Mir et al. 2019 for more details). Phenomics has also been used for the study of plant responses to various abiotic stresses including drought, heat, cold tolerance, salinity and nutrient-starving. For drought tolerance, trait phenotyping either in glasshouse or in field have been conducted and approaches like osmotic balance in hydroponics to conveyer systems in glass house to rainout shelters in the field have been used very extensively.

Several important methods and platforms that are now routinely being used for precise high-throughput phenotyping of drought tolerance have been discussed in detail elsewhere (Mir et al. 2012b). These methods are based on imaging, robotics and computers that allow recording of trait data of thousands of plants in a day in non-destructive manner. Like abiotic stresses, phenomics platforms have been also used for recording trait data on biotic stresses like insect pests. For instance, automated video tracking "a phenomics platform" has been developed and used to record the aphid feeding behaviour on leaf discs that is helpful to measure plant resistance. This platform of automated video tracking can be also used to measure data on aphids and other piercing-sucking insects in plants in high-throughput manner. Like insects, the platforms can be used for recording data on disease reactions and for characterization/selection of resistant plants against fungal

pathogens. In summary, the phenomics platforms/methods/software have been used to record data in high-throughput fashion for variety of traits in almost all important crops, and the trait evaluation has also led to the genetic dissection leading to discovery of genes/QTLs for several traits including root system architecture traits, seed shape, osmotic tolerance and biomass traits in crops like rice, wheat, barley and mustard.

14.7.2 Applications of Artificial Intelligence (AI) and Machine Learning (ML) in Crop Breeding

Classical plant breeding techniques mainly focus on estimation of genetic diversity, analysis of stability for different traits over different seasons and environments, hybrid prediction using different parental combinations and related things and rely on routine statistical methods for analysis of the data (Niazian and Niedbała 2020). Besides this, in order to identify the desirable plant, breeder often needs to take repeated observations in the field and make careful selection. This not only requires lot of time, but skill and experience of a breeder. It has now been realized that in any plant breeding program, rapid and precise phenotyping for the desired trait is very essential. Since this involves recoding thousands of data points in shorter time, in recent years a shift from traditional way of phenotyping to use of sensor based phenotyping has been seen. This has been facilitated by the advances in the area of phenomics and phenotyping platforms as discussed above. The important advantage with these techniques is that it can enable collection of enormous and high-dimensional data in a very short span of time. Similarly, with the advances in the omics techniques (genomics, proteomics, metabolomics, epigenomics), the volume of data which is being generated in any such experiment is huge. In order to handle this huge amount of data efficiently in a breeding program and to make meaningful interpretations, the methods which involve minimal human efforts are required for analysing the data with increased precision (Harfouche et al. 2019).

Modern technology has been of great help to the breeders in this endeavour. For instance, digital images of standing crop in the fields are taken from the surface or through air with the help of drones or unmanned aerial vehicles (UAVs). This not only saves time in recording the data but also reduces the error associated with the manual way of recording observations. The large numbers of images or data points so captured can be analysed using computer tools for understanding the traits and interpreting the results. Artificial intelligence (AI) and machine learning (ML) and variants thereof (neural networks, deep learning, etc.) are considered as the important breakthrough in dealing with this big-data. The ML tools can collectively analyse the phenotypic, genetic and environmental data to help breeders better understand the relationships between genetics, environment and plant performance. While doing this, ML method uses approximations to find out the patterns which are embedded in the data so that it can be used to predict the future data (Murphy 2012). Thus, AI and ML can be used practically in all aspects of breeding (including prediction of phenotype, image identification, disease identification and genomics

experiment including GWAS and GS studies) (Harfouche et al. 2019). This not only will accelerate the process of breeding but will allow screening of large number of accessions in a breeding experiment with increased precision in shorter span of time. This can enable breeders in identifying the desirable plant suited for a particular climate and soil type and identifying desirable cross combination of genes for increased yield (Beans 2020). In recent years, different approaches of ML have been proposed and used in plant breeding programs (Parmley et al. 2019; Kuriakose et al. 2020; Niazian and Niedbała 2020).

Although sensors and machines can increase the volume of data, they cannot replace experience of a breeder. Moreover, since breeders generally are not experienced in the algorithms which are used in ML, therefore, any such experiment requires close cooperation between statisticians, IT specialists and experienced breeders. It is expected that with the growing awareness about AI and ML and quest for better algorithms involved in analysis and interpretation of the data, they will become an integral part of breeding programs in the future.

14.8 Emerging Challenges at National and International Level

Although plant breeding has contributed immensely and resulted in achieving self-sufficiency in many crops in several countries, with the pressure of ever-growing world population and the impact of climate change resulting in uncertain environments, the task of plant breeders has become more challenging. In the future, the main task before the breeders will be to develop varieties with higher productivity having better adaptability to the changing climates. The important challenge which breeders need to address is to develop the varieties which are suited to the specific agroecological regions rather than developing mega-varieties. In addition, emphasis need to be given on varieties which offer better nutrient use efficiency and requiring less resources so that they can offer economic benefits to the farmers. Similarly, another challenge before the breeders will be to develop varieties which can offer food security for the increasing world population and sustainable agriculture.

14.9 Future Thrust Areas and Conclusions

While plant breeding is often considered as an art and science of genetically improving plants and no technique can substitute plant breeding, it needs to adapt to the advances in digital revolution so as to integrate it with molecular techniques for the benefit of humankind. For this purpose, plant breeders need to sync themselves with the advances in the area of genomics and phenomics and embrace modern techniques like AI and ML to increase its effectiveness and to address the challenges of the future. Although the impact of plant breeding in increasing the food productivity is known to everyone, it is still necessary that the subject should be considered as priority by all. Integration of promising techniques like MAS and GS

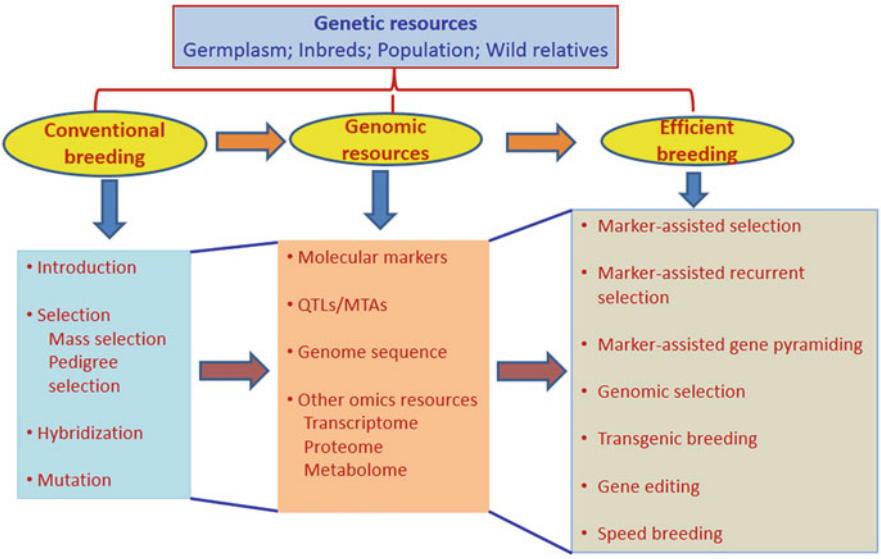

Fig. 14.2 Figure depicting different breeding schemes from conventional to efficient using genomic resources

in the breeding program can offer increased genetic gains. This probably seems to be the only way through which we can ensure/achieve food security in more sustainable way. In addition, emphasis on exploiting the potential of the PGRs is very essential. Moreover, the novel methods like speed breeding and genome editing technique like CRISPR/Cas can allow rapid generation advancements of a breeding cycle and development of genetic diversity for breeding purpose, respectively, and are very promising if used in proper manner. To conclude, rather than using technologies in isolation, integration of modern and efficient techniques in the breeding program can help in achieving food security in a sustainable manner in long run (Fig. 14.2).

Acknowledgements PLK would like to thank Department of Biotechnology, Govt. of India for the research grants during the course of writing this article. RKV thanks Bill and Melinda Gates Foundation, USA, for supporting several projects related to genomics-assisted breeding at ICRISAT and acknowledges Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India, for awarding JC Bose National Fellowship.

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Brassica Breeding

15

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Abstract

Rapeseed-mustard belongs to Brassicaceae (syn. Cruciferae) family, is the third major oilseed crop globally. It is the second most important edible oil crop and ranks first in terms of contribution (>30%) to the indegenous edible oil production in India. Though significant progress has been made in India in terms of area (1.5 times), production (3.4 times), and productivity (2.2 times) of rapeseed-mustard since the launching of the National Mission on Oilseeds during 1985–1986, there is a need to increase the productivity vis-à-vis production to check the increasing import bills on edible oils. The global programmes on *Brassica* are more focused on *Brassica napus* and *B. rapa*, whereas Indian programme is concentrating more on *Brassica juncea* which is occupying around 90% of the total area under rapeseed and mustard. The chapter highlights the historical importance of rapeseed-mustard in the global oil economy, its origin, and evolutionary process, floral biology, breeding objectives, progress made in the varietal improvement, and future targets with the strategies to achieve them. Conservation of enormous genetic diversity of the different coenospecies of *Brassica* and its use in the pre-breeding/breeding activities have also been highlighted. A good number of varieties have been developed, and some of these varieties like Pusa Bold, Pusa Jai Kisan, Pusa Agrani, Varuna, RH 30, Laxmi, Pusa Mustard 30, and Pusa Mustard 25 have been the land mark

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_15

varieties contributing significantly to the national edible oil production. Presently more focus is being given in developing hybrids, climate resilient varieties, and in improving the oil/meal quality traits. A series of low erucic acid and canola quality varieties of *B. juncea* and *B. napus* have been developed which meet the global standards for erucic acid and glucosinolates. Short-duration varieties developed during the last decade have impacted the oilseed area expansion in non-traditional areas and also helped in crop diversification in traditional mustard-growing belts. Heat tolerance at seedling and reproductive stages, tolerance to salinity, and drought are the focused areas of research under the changing climatic scenarios, and a good number of climate resilient high-yielding varieties are in the seed chain. Biotic stresses like *Sclerotinia* stem rot, *Alternaria* blight, *Orobanche*, and aphids are required to be addressed on priority in the future programmes through the use of modern biotech tools including genome editing. Marker-assisted selection for introgression of quality and white rust resistance traits is already in progress which needs to be augmented further.

Keywords

Rapeseed-mustard · Breeding objectives · Improved varieties · Genetic resources · *Brassica* species

15.1 Introduction

Rapeseed-mustard group of crops belonging to Brassicaceae (syn. Cruciferae) family, is the third major oilseed crop at the global level, after soybean and palm oil. China, Canada, India, Germany, the UK, France, and Australia are the major producers of rapeseed-mustard. Brassicaceae (syn. Cruciferae) family currently includes 3709 species and 338 genera (Warwick et al. 2006) and is one of the most economically important plant families (Rich 1991). At the end of the fiscal year 2019, India produced more than nine million metric tons of rapeseed and mustard (https://www.statista.com/statistics/769713/india-rapeseed-and-mustard-production-oilseeds; Statista.com). In India, rapeseed-mustard is the second most important oilseed crop after soybean. Rapeseed-mustard commonly falls under the genus *Brassica* which constitute a group of crops that are used as vegetables (*Brassica oleracea* cv. cabbage, cauliflower, and *broccoli*), condiments (*Brassica nigra*, i.e. mustard/rai seeds), and the edible oil (*Brassica juncea*, *Brassica rapa*, *Brassica napus*, and *Brassica carinata*) extracted from their seeds. These crops are the chief sources of edible oil in the Indian diet, and the de-oiled cake with a high concentration of proteins and minerals is a rich source of animal feed. Among these oleiferous *Brassica* spp., *Brassica juncea* (Indian mustard) is a predominant crop, occupying around 90% area of rapeseed-mustard per se.

Though the area in the country has been static with minor fluctuations over the years, there has been a significant 33% increase in the production (from 6.28 million tons in 2014–2015 to 9.34 million tons in 2018–2019) and a 38% increase in the

productivity (from 1083 kg/ha during 2014–2015 to 1499 kg/ha in 2018–2019), due to the modern scientific interventions, concerted efforts of the breeders and other stake holders. During 2018–2019, Rajasthan emerged as major mustard-growing state with a lion's share of ~60% (Agricultural statistics 2019). Mustard has been introduced in several non-traditional states like Assam, Meghalaya, Tripura, Sikkim, and Arunachal Pradesh in northeast and Andhra Pradesh, Telangana, Karnataka, and Tamil Nadu in the south during the last decade. The short duration varieties, viz. Pusa Agrani, Pusa Mahak, Pusa Mustard 25, Pusa Mustard 27, Pusa Mustard 28, Pant Rai 20, etc., have given encouraging performance in these non-conventional mustard-growing areas. With the development and introduction of short duration (100–110 days) Indian mustard varieties, some area of *B. rapa* cv. *toria* has also been replaced by *B. juncea* in plains as well as in northeastern states.

15.2 Origin, Evolution, and Distribution of Species, Forms and Wild Relatives

The tribe Brassiceae can be split into eight distinct lineages or clades (Arias et al. 2014). Three of these clades diverged earlier from the others, while the rest are generally thought of as the core Brassiceae. The genus *Brassica* itself contains three distinct lineages: lineage A comprises species with ten chromosomes (*B. rapa*), lineage B comprises species with eight chromosomes (*B. nigra*), and lineage C (*B. oleracea*) comprises species with nine chromosomes. Some *Brassica* species have also undergone another round of polyploidization and interspecific hybridization, leading to the rise of new *Brassica* allopolyploid species: *B. juncea*, *B. napus*, and *B. carinata*. Their progenitor species all come from different lineages: *B. juncea* ($n = 18$) stems from *B. rapa* (lineage A) and *B. nigra* (lineage B), *B. napus* ($n = 19$) stems from *B. rapa* and *B. oleracea* (lineage C), and *B. carinata* ($n = 17$) stems from *B. nigra* and *B. oleracea*. These relationships were discovered very early on by Morinaga (1934) and Nagaru (1935). These six species have been known as the U's Triangle species ever since Nagaharu (1935) demonstrated that Brassica crop species comprise three diploid species and three amphidiploid species.

It was believed that the three diploid species originated from one common ancestor. However, molecular investigations summarized by Gómez-Campo and Prakash (1999) point to a common origin for *B. rapa* and *B. oleracea*, while *B. nigra* evolved from a separate progenitor. The cytogenetic relationship between the *Brassica* species established by Nagaharu (1935) was later confirmed by chromosome pairing and artificial synthesis (Axelson et al. 2000), nuclear DNA content, DNA analysis, and by the use of genome-specific chromosome markers (Hasterok et al. 2001).

Brassica juncea (L.) Czern. and Coss., known as oriental, brown, or Indian mustard, is believed to be one of the earliest domesticated plants. It is described in Sanskrit texts as early as 3000 BC (Hemingway 1995). *B. juncea* spread to Europe as a medicinal crop during the middle ages. Today, *B. juncea* is used worldwide as an

oilseed, a condiment, and a vegetable (Edwards et al. 2007) crop. Wild forms of *Brassica juncea* have been found in the near east and southern Iran. According to Vavilov (1949), Afghanistan and its neighbouring regions (Central Asia) were the primary centre of the origin of *Brassica juncea*. Multiple centres of origin for *B. juncea* have been proposed by others, where the putative progenitors, *B. campestris* (syn. *rapa*) and *B. nigra*, had geographic sympatry, leading to conflicting views about the origin of *B. juncea* (Bhowmick 2003). The Middle East has been proposed as the most probable place of origin of *B. juncea* as wild forms of its progenitor species *B. rapa* and *B. nigra* occur together in this region (Olsson 1960a, b; Mizushima and Tsunda 1967; Prakash and Hinata 1980; Gómez-Campo and Prakash 1999). The regions of south-western China and North Western Himalayas may constitute two secondary centres where there is enormous diversity in *B. juncea* forms. Biochemical studies support this finding of the diversity in these regions (Vaughan et al. 1963) and further provide evidence for the existence of two geographical races of *B. juncea*, the Chinese pool and the Indian pool (Vaughan and Gordon 1973). This evidence is supported by Song et al. (1988) through RFLP studies which suggest two centres of origin: (a) the Middle East and (b) China. However, Rakow (2004) had opined that China cannot be considered as a centre of origin for *B. juncea* because the two-parent species *B. nigra* and *B. campestris* (syn. *rapa*) were never found as wild species in that country.

B. juncea is an annual crop that grows as cultivated, weedy escapes, or wild forms in coastal lowlands, sandy beaches, plateaus, and mountainous regions. It has a wide geographical range, spanning the continents of Europe, Africa, Asia, America, and Australia. *B. juncea* is closely related botanically to canola (*B. napus*) and turnip rape (*B. rapa*) and has a similar growth habit (Hemingway 1976). The genera *Brassica* display enormous diversity, and a range of wild and weedy species occur in nature which are related to the cultivated genus. However, most of these species in the wild germplasm belong to secondary and tertiary gene pools, reproductively isolated, and invariably show strong incompatibility barriers. A list of economically important species of genus *Brassica* and its close allies along with their uses is presented in Table 15.1, and those grown in India are given in Table 15.2.

15.3 Genetic Resources: Improvement of Cultivated Species

Successful varietal development program needs continuous supply of resource genes for crop yield, quality, and various biotic and abiotic stress tolerance traits. A major shift in yield level of any crop plant with increased tolerance to biotic and abiotic stresses is possible only with the extensive genetic manipulation through breeding. This requires systematic efforts in the management of global crop genetic resources in the face of emerging challenges like climatic change, resource degradation, habitat destruction, species invasion, deforestation, etc. There are many unaddressed issues where genetic solutions are possible by systematic use of available genetic resources. Despite attaining many highs in rapeseed mustard crop improvement with the release of 203 varieties for different situations, still there exists a gap between the achieved

Table 15.1 Economically important *Brassica* species

Botanical name	Common name	Genome	Chromosome no.	Usage
Brassica rapa (syn. B. campestris)		AA	20	
spp. oleifera	Turnip rape			Oilseed
var. *brown sarson*	Brown sarson			Oilseed
var. *yellow sarson*	Yellow sarson			Oilseed
var. *toria*	Toria			Oilseed
spp. *rapifera*	Turnip fodder			Vegetable (root)
spp. *chinensis*	Bok choi			Vegetable (leaves), fodder (head)
spp. *pekinensis*	Chinese cabbage			Vegetable (leaves)
spp. *nipposinica*	–			Vegetable (leaves)
spp. *parachinensis*	–			Vegetable (leaves)
Brassica nigra	Black mustard	BB	16	Condiment (seed)
Brassica oleracea		CC	18	
var. *acephala*	Kale			Vegetable, fodder (leaves)
var. *capitata*	Cabbage			Vegetable (head)
var. *sabauda*	Savoy cabbage			Vegetable (terminal buds)
var. *gemmifera*	Brussels sprouts			Vegetable (head)
var. *gongilodes*	Kohlrabi			Vegetable, fodder (stem)
var. *botrytis*	Cauliflower			Vegetable (inflorescence)
var. *italic*	Broccoli			Vegetable (inflorescence)
var. *fruticosa*	Branching bush kale			Fodder (leaves)
var. *alboglabra*	Chinese kale			Vegetable (stem, leaves)
Brassica juncea	Indian mustard	AABB	36	Oilseeds, vegetable
Brassica napus		AACC	38	
spp. oleifera	Rapeseed, gobhi sarson			Oilseed
spp. rapifera	Rutabaga, swede			Fodder
Brassica carinata	Ethiopian mustard	BBCC	34	Vegetable, oilseed
Eruca sativa	Rocket, taramira	EE	22	Vegetable, non-edible oilseed
Raphanus sativus	Radish	RR	18	Vegetable, fodder
Sinapis alba	White mustard	SS		Oilseed

Table 15.2 Rapeseed and mustard crops grown in India

Botanical name	Common name	Botanical name	Common name
Brassica juncea	Indian mustard, sarson, Rai, Raya, Laha, Rayda, Banga sarson	*B. tournefortii*	Panjabi rai, Jangali rai
B. juncea var. *cuneifolia*	Vegetable mustard, Rai	*B. nigra*	True mustard, black mustard, Banarasi rai
B. rapa spp. *oleifera*	Turnip	*B. pekinensis*	Chinese cabbage-heading
B. rapa var. *brown sarson*	Brown sarson, Kali sarson	*B. napus*	Gobhi sarson
B. rapa var. *yellow sarson*	Yellow sarson, Pili sarson	*B. carinata*	Karan rai, Ethiopian mustard
B. rapa var. *toria*	Toria, Rai, Lahia, Magni achara rai	*Eruca sativa*	Taramira, rocket salad

Source: Mishra and Kumar (2008)

and achievable (Nanjunadan et al. 2020). From 1998 onwards, a good number of genetic resources including genetics stocks, varieties, and germplasm lines for different important traits have been procured, developed, released, and registered with NBPGR. At present, more than 11,119 mustard accessions are being conserved at the National Bureau of Plant Genetic Resources, New Delhi. An equal number is being maintained at the Directorate of Rapeseed-Mustard Research, Bharatpur, and different AICRP centres located at various State Agricultural Universities and ICAR Institutes. Good success has been made in the development of quality Indian mustard cultivars, where stable donors like Heera and EC 597325 for low glucosinolates were used and the first Double Zero variety Pusa Mustard 31 was released from ICAR-IARI in 2016. Later RLC-3 (2017), Pusa Double Zero Mustard 33 (2021), and hybrid RCH 1 were released. Varieties like Pusa Karishma (LES-39), LES-1-27 (Pusa Mustard 21), LET-17 (Pusa Mustard 22), LET-18 (Pusa Mustard 24), LET-36 (Pusa Mustard 29), LET-43 (Pusa Mustard 30), LES-54 (Pusa Mustard 32), RLC 1, and RLC 2 for low erucic acid are currently available for cultivation. As far as abiotic stresses are concerned, a good number of varieties like CS-52, CS-54, CS-56, CS-60 (salt tolerant), Pusa Vijay (NPJ-93), Pusa Mustard 25 (NPJ-112), Pusa Mustard 27 (EJ-17), Pusa Mustard 28 (NPJ-124) (heat tolerant), and RGN-13, RGN-48 (frost tolerant) have been developed and are available in the public domain. Well-established short duration genotypes, viz. Pusa Agrani (SEJ-2), Pusa Mahak (JD-6), Pusa Tarak (EJ-9912-13), Pusa Mustard 25 (NPJ-112), Pusa Mustard 27 (EJ-17), Pusa Mustard-28 (NPJ-124), Kranti, NDRE-4, PR-2006-1, Divya (IC-553910), Pant Rai 19, etc., with 100–120 days maturity have been developed which can be further exploited for breeding early maturing and high-temperature-tolerant *Brassica juncea* varieties. For white rust resistance breeding Bio-YSR, BEC-144, BEC-286, EC-399299, EC-399301, RC-781, JM-1, Heera, etc. are stable sources and are being used widely in the national programme.

15.4 Floral Biology: Emasculation—Pollination Techniques

B. juncea is an annual herbaceous plant. The plants are tall (90–200 cm), erect and branched. The fruits (siliquae) are slender, 2–6.5 cm long, strongly ascending, or erect with short and stout beaks. The colour of the seed is yellow or brown. The seed coat is rough. Figure 15.1a–d provides the structure of flower, inflorescence, and silique of *B. juncea*.

The leaves are alternate (rarely opposite), and maybe coriaceous, very often pinnately incised, and do not have stipules. The inflorescence is corymbose raceme type. Flowering is indeterminate, beginning at the lowest end on the main shoot and continues upward. Flowers are ebracteate, pedicillate, complete, hypogynous, and actinomorphic. Calyx comprises four sepals in two whorls each. Anteroposterior sepals form the outer whorl, whereas lateral ones form the inner whorl. Sepals are pale green in colour. Corolla comprises four cruciform petals. These are clawed and regular. Two functional nectaries are located at the base of the short stamens and two non-functional nectaries are at the base of the long stamens. The androecium is tetradynamous and consists of six stamens arranged in two whorls. The longer four stamens form the inner whorl and are arranged in anteroposterior pairs. The two shorter stamens form the outer whorl and are present in a lateral position. Anthers are bithecus and basifixed. The gynoecium is usually bicarpellary, syncarpus, and superior with carpels transversely placed. It is bilocular due to the presence of a false septum. Placentation is parietal; the ovary is usually sessile with many ovules, short style, and bifid stigma.

The mature bud flowers within 2 h after sunrise. The stigma becomes receptive 2–3 days before the flower opens and thus facilitates selfing by bud pollination (Kumar 2001). The dehiscing side of anther sacs faces the stigma, but as the time of dehiscence approaches, the inner whorl of two anthers undergoes a twist of 60°–180° which results in extrose dehiscence in the case of the self-incompatible types. The dehiscence of all the anthers in self-compatible types is introse. *B. juncea* is a predominantly self-pollinated crop (Labana et al. 1992). However, in some environments out crossing varies from 7.6% to 22% (Dhillon and Labana 1988; Ram et al. 1991; Abharam 1994). Pollen can live up to 4 or 5 days when the

A B C D (brown and Yellow seeded)

Fig. 15.1 (**a**) Flower (**b**) inflorescence (**c**) siliquae (**d**) seeds of *Brassica juncea*

temperature is low and humidity is high. With warm temperatures and low humidity, survival time may drop to 1 or 2 days (Mayers 2006). *Brassica* pollen is viable even after 4 h of stress at 60 °C (Rao et al. 1992) however, under experimental conditions, it has been observed that pollen could remain functional for a year or more in dry storage at −20 °C (Brown and Dyer 1990).

Pollination is carried out both by insects and wind. Wind can carry pollen over long distances as pollen counts of up to 22 pollen grains/m^3 were observed 1.5 km away from the source field and were sufficient to affect the seed set on bait plants (Timmons et al. 1995). The extent of wind pollination was recorded up to 11–17.5% (Singhal et al. 2005). Bees however are the primary pollen vector because the pollen is heavy and sticky and is not carried to great distances in the absence of wind (Labana and Banga 1984). Bees visit flowers for nectar; the positioning of nectaries is such that in self-incompatible types, the body of the bee gets smeared with pollen and in self-compatible types, the bee affects self-pollination by pressing the inner whorl of introrsely dehisced anthers while extracting nectar thus bringing them in contact with the stigmatic surface (Kumar 2001). The stigmas remain receptive 3 days before opening to 3 days after opening of the flowers (Singh and Rai 2004). Bees may carry pollen over long distances and have been found foraging in fields more than 4 km away from the hive (Eastham and Sweet 2002), resulting in an outcross seed set. Besides the physical carrying of pollen grains, bee visitations also cause pollens to become airborne. Airborne pollen grains can then be carried by the wind, leading to cross-pollination. Cross-pollination of nearby plants can also result from physical contact with the flowering racemes.

The extent of wind pollination in *B. juncea* cv. Pusa Bold was studied in New Delhi, India. Dispersal of pollen grains by the wind was noticed up to 35 m from the pollen source (Balasubramanian 2011). Airborne pollen grains may pass through insect-proof nets, and effective pollination may occur. The commonly used method of reproductive isolation in the case of *B. juncea* is spatial isolation. The recommendations made on isolation distance for production of foundation seed and certified seed of 96% purity of self-fertile *B. juncea* are 200 and 50 m, respectively (Anonymous 2013).

15.5 Breeding Objectives

In general, plant breeding has four fundamental steps:

- **Goal setting:** Taking a cue from economic and biological factors, methodologies are selected.
- **Generating new diversity:** Continuous breeding for a particular set of desirable traits has eroded genetic variability. Sufficient genetic variability must be available for any trait and the crop to be improved. If necessary, the genetic base of the breeders' gene pools can be widened through mutation breeding using gamma rays or EMS techniques. Introgression of single genes or traits or by a large-scale

infusion of new germplasm through base broadening/wide hybridization can also be used for generating diversity.

- **Selection:** The improvement of a character or a crop is achieved through selection after the crosses are made between the chosen parents with desirable traits. The selection methods used in plant breeding differ between inbreeding, crossbreeding, and vegetatively propagated crops.
- **Cultivar release:** After the rigourous cycles of selection and testing, the improved genotype is released and marketed (Nanjundan et al. 2020; Tandon et al. 2015).

B. juncea breeders aim to make simultaneous improvements of agronomic performance, disease resistance and quality traits, and developing suitable varieties for non-traditional areas (southern states) and rice-fallows in eastern and northeastern states. Agronomic performance includes yield, lodging, maturity, herbicide tolerance, drought tolerance, shattering resistance, and seed size. Disease resistance efforts may include *Sclerotinia* stem rot (*Sclerotinia sclerotiorum*), white rust (*Albugo candida*), *Alternaria* blight (*Alternaria brassicae*), downy mildew (*Peronospora parasitica*), and powdery mildew (*Erysiphe cruciferarum*) resistance. Improvements in quality traits will depend on whether the aim is to develop canola or conventional mustard varieties. For canola, high oil content, low glucosinolate content (<30 ppm), high protein content, and a fatty acid profile with low erucic (<2%) and low saturated fatty acid content (~7%) are desired. For conventional mustard varieties, high oil content, high glucosinolate content, and a fatty acid profile with a moderate level of erucic acid are aimed commonly (Yadava et al. 2014).

The major breeding objectives for mustard crop are as follows

15.5.1 Increased Seed and Oil Yield

In oilseed crops, oil content determines the economic yield and market rate of the produce. In rapeseed-mustard the oil content ranges from 35% (*E. sativa*/*B. carinata*) to 46% (*B. rapa* spp. *yellow sarson*); thus, despite the fact that the oil content is a complexly inherited trait, there is ample scope for raising its present level in largely cultivated species like *B. juncea* and *B. napus.*

15.5.2 Abiotic Stresses

High-temperature stress at the time of sowing leads to high seedling mortality and thus poor seedling establishment and plant stand. Plants exposed to high-temperature stress at the reproductive stage show high pollen mortality, increased flower drop, decrease in the number of siliquae, and decreased seeds per siliqua. High temperature also affects the translocation of photosynthates; thus plants exposed to high temperature have shriveled/small seeds with reduced oil content. The problem is

more severe in the late sown crops. Frost is another abiotic stress that occurs after every 5−7 years and causes massive losses; hence, breeding for frost tolerance needs to be addressed. Soil with salinity and sodicity is also occupying significant area; hence the ongoing efforts need to be continued on breeding varieties for salinity tolerance too.

15.5.3 Biotic Stresses

Sclerotinea stem rot, white rust caused by *Albugo candida*, and *Alternaria* blight are major diseases and need to be addressed. Likewise, *Orobanche* a parasitic weed creates havoc on this crop in some regions. Breeding for tolerance to *Orobanche* should be undertaken on priority. Mustard aphid (*Lipaphis erysimi*) and painted bug (*Bagrada hilaris*) are insects of economic importance and cause huge losses to the rapeseed-mustard crop at various stages of growth. Breeding for resistance to these two insects also needs to be undertaken.

15.5.4 Seed and Oil Quality

Erucic acid and glucosinolates are two major antinutritional components in the oil and seed meal cake of conventional *Brassica* varieties being grown in India. Low erucic acid with increased oleic acid and a balanced proportion of omega-3 and omega-6 fatty acids and enhanced oil content (>42%) are desirable features in quality Indian mustard varieties. Having achieved the development and release of "00" canola quality (erucic acid < 2% in seed oil and glucosinolates < 30 μmoles/g in defatted seed meal) Indian mustard varieties, the Indian breeding program now has to think of "Triple Zero" quality rapeseed mustard varieties ie. genotypes with high oleic acid (>60%) and low fibre content in addition to the "00" traits.

15.5.5 Early Maturity

Short duration varieties are needed to fit into different cropping systems of the country especially in eastern and northeastern regions. Toria is low yielder and vulnerable to most of the biotic and abiotic stresses; therefore, Indian mustard varieties like Pusa mustard 25 and Pusa mustard 28, that mature in about 100 days, are a good substitute of toria. Indian mustard varieties that can mature in 80 days without compromising the yield should be targeted for fitting this crop in multiple cropping systems and also as a real substitute to *B. rapa ssp* toria.

15.5.6 Plant Type

More concerted efforts are needed to identify suitable plant types for different crop geometries and cropping systems. Dwarf plant type (<120 to 150 cm) with basal branching and amenable to mechanical harvesting is need of the hour for making this crop more competitive and less labour-intensive. To achieve this, the plant type has to be tailored with required component traits including determinate growth habit.

15.5.7 Exploitation of Heterosis and Commercial Hybrid Development

Unlike in most other crops, no natural cytoplasmic male sterility and fertility restoration (CMS-FR) system was available in *B. juncea*. The major emphasis during the initial phase of hybrid breeding was towards the development of CMS-FR systems using alloplasmic variation. Cytoplasmic male sterile lines could be developed by backcross substitution of *B. juncea* genomes in the cytoplasmic background of wild crucifers. CMS lines originating from sexual hybridizations possess unaltered organelle genomes because of exclusive maternal inheritance.

With the availability of a highly effective hybrid seed production mechanism, heterosis is being exploited commercially in oilseed *Brassica*. A number of cytosterility sources from *Brassica* coenospecies, viz. *Diplotaxis siifolia* (Rao et al. 1994), *Raphanus sativus* (Bannerot et al. 1974; Kirti et al. 1995a), *B. tournefortii* (Banga et al. 1994; Rawat and Anand 1979), *B. oxyrrhina (*Prakash and Chopra 1990), *Trychystoma ballii* (Kirti et al. 1995b), *Moricandia arvensis* (Prakash et al. 1998), *D. catholica* (Prakash et al. 2001), *Enarthrocarpus lyratus* (Banga et al. 2003a; Janeja et al. 2003), *Erucastrum canariense* (Prakash et al. 2001; Banga et al. 2003b), synthetic *B. napus* ISN-706 (Sodhi et al. 2006), *D. erucoides* (Bhat et al. 2006), and *D. berthautii* (Bhat et al. 2008), were identified and used for the development of CMS lines and restorers. A number of such CMS systems are now available for use in the active hybrid development (Table 15.3).

Varying degrees of leaf chlorosis were found to be associated with *Raphanus/Ogu*, *Oxyrrhina*, *Tournefortii*, *Moricandia*, and *Enarthrocarpus* systems. Floral abnormalities in male sterile plants included petaloid anthers (*nigra*, *muralis*, *trachystoma*, *raphanus*, *tournefortii*, *canariense*); poor or absent nectarines (*tournefortii* and *raphanus*); crooked style (*tournefortii*, *raphanus*); thick pistil (*raphanus*); and low seed fertility (*raphanus*, *tournefortii*, *enarthrocarpus*, and *trachystoma*). Fertility restorers for *Moricandia*, *Ogura*, *catholica*, and *erucoides* and *lyratus* CMS systems could be developed by introgressing gene(s) for fertility restoration from donor wild species cytoplasm (Prakash et al. 2009). Fertility restorer for the mori CMS could also restore fertility in *eru* CMS system (Bhat et al. 2005).

Mainly, two CMS sources, *Moricandia* and *Ogura*, are being used in the *Brassica* hybrid development programme in India. In addition, two more CMS systems, viz. *erucoides* and *berthautii*, in which fertility is restored by common restorer line with that of *Moricandia* are also being used in IARI, New Delhi. With an objective

Table 15.3 Various CMS systems reported in *Brassica* spp

Cytoplasm donor	Code	Technique used	Restoration status	Crops	References
Diplotaxix siifolia	*sif*	Intergeneric cross	Not available	*B. juncea*	Rao et al. (1974, 1994)
Raphanus sativus	*ogu*	Interspecific cross, protoplast fusion	Available	*B. napus* *B. juncea*	Bannerot et al. (1974), Kirti et al. (1995a), Ogura (1968)
B. tournefortii	*tour*	-	Unstable, genotype-specific partial restoration	*B. napus* *B. juncea*	Banga et al. (1994), Rawat and Anand (1979)
B. oxyrrhina	*oxy*	Interspecific cross, protoplast fusion	Not available	*B. juncea*	Prakash and Chopra (1990, Kirti et al. (1993)
Trachystoma ballii	*trachy*	Protoplast fusion	Incomplete	*B. juncea*	Kirti et al. (1995b)
Moricandia arvensis	*mori*	Protoplast fusion	Available	*B. juncea*	Prakash et al. (1998), Malik et al. (1999), Prakash (2001), Bhat et al. (2006)
D. sietiana	*Sie*	Intergeneric cross	Not available	*B. juncea*	Prakash et al. (2001)
D. catholica	*cath*	Intergeneric cross	Available	*B. juncea*	Prakash et al. (2001), Pathania et al. (2007)
Enarthrocarpus lyratus	*lyr*	Intergeneric cross	Available	*B. juncea* *B. napus*	Deol et al. (2003), Banga et al. (2003a, b), Janeja et al. (2003)
Erucastrum canariense	*can*	Intergeneric cross	Available	*B. juncea* *B. napus*	Prakash et al. (2001), Banga et al. (2003a, b)
Synthetic *B. napus* ISN-706	126-1	Interspecific cross	Available	*B. juncea*	Sodhi et al. (2006)
D. erucoides	*Eru*	Intergeneric cross	Available	*B. juncea*	Malik et al. (1999), Bhat et al. (2006, 2008), Prakash et al. (2001)
D. berthautii	*bar*	Intergeneric cross	Available	*B. juncea*	Bhat et al. (2008)
Brassica fruticulosa	fruticulosa	–	Available	*B. juncea*	Kaur Atri et al. (2016)

Source: Kumar et al. (2020)

of nuclear diversification of different CMS sources into the desired genotype, backcrosses have been attempted at IARI, New Delhi; DRMR, Bharatpur; PAU, Ludhiana, and CCSHAU, Hisar. A total of around 200 CMS lines in different genetic backgrounds have been converted using *mori*, *eru*, *ber*, and *ogu* cytosterlity sources. Heterotic combinations are being evaluated at multiple locations, and potential hybrids are expected to be identified soon.

15.6 Breeding Approaches: Conventional and Non-conventional Including Use of Genomic Tools

Being a heterogeneous group of crops in terms of their pollination control, almost all breeding methods are being employed for the genetic improvement of different *Brassica* spp.

15.6.1 Different Varieties Developed by Following Various Breeding Methods Are Given Below

- ***Pureline Selection***: Gobhi Sarson—GSL-1, Neelam, NUDH-26-11; Yellow sarson—Benoy, Jhumka, Ragini, etc.
- ***Mass selection*****:** Black mustard-Surya; Yellow sarson—YSPb-24; Taramira—ITSA, RTM-314, T-27; Toria—Agrani, Bhawani, TL-15, M-27, PT-303, RAUTS, ITSA, T-9.
- ***Pedigree Method and Its Modifications*****:** *B. juncea*—Aravali, RGN-48, RGN-13, NRCDR-2, CS-54, Pusa Mahak, Jagannath, Pusa Agrani, Pusa Bold, RH-781, RH-819, RL-1359, Vasundhra, Vardan, Vaibhav, Ashirwad, Pusa Mustard 24, Pusa Mustard 25, Pusa Mustard 26, Pusa Mustard 27, Pusa Mustard 28, Pusa Mustard 29, Pusa Mustard 30, Pusa Double Zero Mustard 31, Pusa Mustard 32, Pusa Double Zero Mustard 33 etc.; *B. napus*—GSC-5, GSC-6, GSC-7, GSL-2, Sheetal; Karan rai-PC-5, Pusa Swarnim, Pusa Aditya; Yellow sarson—Subinoy, Gujrat Sarson-1.
- ***Recurrent Selection*****:** Toria—PT-30.
- ***Synthetics and Composites*****:** Composites: Toria—Jawahar toria-1, Panchali, PBT-37, PT-507, TH-68; Synthetics: Toria-Sangam, Brown sarson-Pusa Kalyani.
- ***Backcrossing*****:** *B. juncea*—JM-1, JM-2, JM-3, Pusa Karishma, Pusa Mustard 21, Pusa Mustard 22; *B. napus*—OCN-3. Modifications of backcross methods, such as limited backcrossing followed by pedigree selection, help in defect elimination and generating larger genetic variation simultaneously.
- ***Mutation Breeding*****:** *B. juncea*—Geeta, RLM-619, RLM-514, TM-2, TAM 1028-1, Birsa Bhabha Mustard 1 (BBM1), Trombay Him Palam Mustard-1 (THPM-1); Toria—Anuradha, Parbati; Yellow sarson—Narendra sarson-2.
- ***Hybrids:*** At present, *Raphanus sativus (ogu)*, *Moricandia arvensis (mori)*, and synthetic *B. napus* ISN-706 (126-1) cytosterility systems are being used for the

Table 15.4 Hybrids of rapeseed-mustard released in India

S. No	Hybrid	Year of release	*Brassica* spp	Institute/organization	CMS system
1	PGSH-51	1996	*Brassica napus*	PAU, Ludhiana	*Tournefortii*
2	Hyolla 401 (PAC 401)	1997		Advanta India Ltd.	*Ogura*
3	PGSH 1699 (GSH 1699)	2021		PAU, Ludhiana	*Ogura*
4	NRCHB 506	2008	*Brassica juncea*	DRMR Bharatpur	*Moricandia*
5	Coral 432 (PAC 437)	2010		Advanta India	*Ogura*
6	Coral 437 (PAC 437)	2012			
7	Dhara Mustard Hybrid-I (DMH-I)	2010		NDDB, Delhi University	CMS, "126-1"
8	SVJH 108	2021		Shakti Vardhak Hybrid Seeds Pvt., Ltd., Hisar	*Ogura*
9	RCH 1	2021		PAU, Ludhiana	*Ogura*
10	PHR 126	2021		PAU, Ludhiana	*Ogura*

development of commercial hybrids in *Brassica.* Seven hybrids of *B. juncea* (NRCHB 506, DMH 1, RCH-1, PAC 437, PAC 432, PHR 126, SVJH 108) and three of *B. napus* (PGSH 51, PAC 401, PGSH 1699) have been released for commercial cultivation in India (Table 15.4). Possibility of using genetic male sterile (GMS) lines in GMS Facilitated Recurrent Selection in self-pollinated species can be explored with the availability of stable and good outcrossed seed-producing GMS system.

- ***Tissue Culture*:** Alloploid *Brassica* species were artificially resynthesized by crossing the respective diploid *Brassica* species followed by embryo rescue. This led to development of a short duration variety Pusa Agarni in *Brassica juncea* at IARI, New Delhi. Using synthetic *B. juncea*, short duration varieties, viz. Pusa Mustard 25, Pusa Mustard 26, and Pusa Mustard 28, were developed. Most of the breeding programmes are directed towards improvement of mega varieties, and due to repeated cycles of such breeding, there is great loss of variability, and the gene pool becomes narrow. Somaclones are important source of variation when the genetic variability is limited in the germplasm. Pusa Jaikisan (Bio 902), a somaclone of cultivar Varuna developed at NIPB, New Delhi, released for commercial cultivation is one of the best examples.

15.6.2 Development of Altered Plant Type

To amenable the brassica crop suitable for mechanised harvesting efforts are going on for the development of determinate type *Brassica juncea* and *B. carinata*. Efforts

are also going on for developing newer plant types in these species which are dwarf and possess basal branching.

15.6.3 Embryo Rescue Technique for Interspecific Crosses

Abraham et al. (2000) at BARC, Mumbai, reported somaclonal variants from mesophyll protoplast in *B. juncea* cv. Rai 5 which showed 3–5 days early flowering. Optimization of regeneration protocols has been achieved for most of the *Brassica* species using different explants such as cotyledons, hypocotyls, leaf segments, protoplasts, cotyledonary petiole, and shoot apex (Narasimhulu et al. 1989; Kirti and Chopra 1989). Somaclonal variation as a tool for creating in vitro variability offers a unique opportunity for desirable attributes. A somaclone of Varuna, Bio-902, has been released as a variety which possesses shattering resistance along with high yield (Katiyar and Chopra 1995). Prakash et al. (2004) reported regeneration of normal plants by culturing anthers of CMS line of *B. juncea* carrying *Diplotaxis erucoides* cytoplasm.

Somatic cell fusion of sexually incompatible species has also been made possible through the production of somatic hybrids which have been utilized for the transfer of desirable traits from parents to hybrids. Interspecific hybrids were produced by fusing mesophyll protoplast of *B. juncea* and *B. spinescens* (Kirti et al. 1991). Prakash et al. (1998) developed a male sterility and fertility restoration system in *B. juncea* by protoplast fusion with *Moricandia arvensis*. These CMS lines were found to be chlorotic. Protoplast fusion of chlorotic male sterile *B. juncea* with green male sterile *B. juncea* resulted in green male sterile plants (Kirti et al. 1997). Stable, fertile somatic hybrids between *Sinapis alba* and *Brassica juncea* were successfully developed which show resistance to *Alternaria brassicae* and heat stress (Kumari et al. 2018). Kumari et al. (2020) developed stable allohexaploids of *B. juncea* + *S. alba* and their backcross progeny which provides new insights into the genetic inheritance of traits such as the resistance to *Sclerotinia* stem rot and yellow seed colour. As these allohexaploids have been confirmed for their crossability with diploid and amphidiploids of cultivated *Brassica*, introgression of *Sclerotinia* stem rot and yellow seed colour into other *Brassica* spp. could be exploited. Singh et al. (2021) identified quantitative trait loci governing resistance to *Alternaria* blight introgressed from *S. alba* to the backcross population of stable *S. alba* + *B. juncea* somatic hybrids ($2n = 60$; AABBSS), and the identified QTLs explaining 5.51–10.87% of the phenotypic variations for the resistance to *Alternaria brassicae* in the backcross progeny of *Sinapis alba* + *Brassica juncea* somatic hybrids.

15.7 Precise and High-Throughput Phenotyping Protocols for Key Traits

To combat the increasing challenges of climate change, a highly reproducible and rapid protocol for screening against high temperature at the seedling stage has been standardized which is proving very helpful in the identification of donors for high-temperature tolerance (Singh et al. 2012a). This protocol has helped in the development of high-temperature-tolerant varieties which can establish even under very high temperatures (up to 40 °C) during plant establishment stage. In case of quality breeding programme, a new method for methyl esterification of fatty acids in *Brassica* seed oil has been developed which helps in phenotyping of large number of samples for analysis of fatty acids (Sujata et al. 2008). This new method has revolutionized the quality breeding programme, and as a result series of low erucic acid and double low varieties, viz. Pusa Mustard 29, Pusa Mustard 30, Pusa Mustard 31, Pusa Mustard 32, and Pusa Mustard 33, have been developed.

Sclerotinea stem rot (SSR) caused by *Sclerotinea sclerotiorum* (Lib.) de Bary is becoming a havoc in mustard-growing areas of India. A rapid and reliable screening technique standardized for the first time in *Brassica napus* has demonstrated that a cotyledon assay can be successfully applied to rapidly differentiate the reactions of *B. napus* genotypes against *S. sclerotiorum* (Garg et al. 2008). Mei et al. have reported a "detached stem assay method" under a controlled environment for screening Brassica crops for resistance against *Sclerotinia sclerotiorum*. Gupta et al. (2020) reported a field-based non-injury method of inoculation technique for SSR in oilseed *Brassica*, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary

15.8 Development of Molecular Markers and Linkage Maps

The discovery of high levels of inter as well as intra-specific DNA polymorphism in RFLP profiles, obtained with random genomic DNA clones used as probes, by Figdore et al. (1988) encouraged molecular mapping of *Brassica* genomes and tagging of genes for several useful traits. Different mapping populations at F_2 or later stages (F_3, F_4, or recombinant inbred lines) have been used to construct genetic maps in *B. oleracea* (Slocum et al. 1990; Kianian and Quiros 1991), *B. nigra* (Truco and Quiros 1994), *B. campestris*/*B. rapa* (Chyi et al. 1992; Kole et al. 1997), *B. napus* (Hoenecke and Chyi 1991; Landry et al. 1991), and *B. juncea* (Sharma et al. 1994; Mohapatra et al. 2002; Sharma et al. 2002). Backcross mapping population has been used to generate maps for *B. nigra* (Lagercrantz and Lydiate 1995), and doubled haploid (DH) mapping population has been used to construct maps of *B. oleracea* (Voorrips et al. 1997; Li and Quiros 2001; Saal et al. 2001), *B. napus* (Ferreira et al. 1994; Uzunova et al. 1995; Foisset et al. 1996), and *B. juncea* (Cheung et al. 1997; Axelson et al. 2000). Apart from these, a combination of mapping populations has also been used. Ramsay et al. (1996) and Kearsay et al. (1996) have used backcross individuals of DH lines to generate maps of *B. oleracea*. Some maps were also constructed using RAPD, AFLP, and SSR markers (Sharma

et al. 2002; Pradhan et al. 2003, 2011). Pradhan et al. (2003) constructed a high-density genetic linkage map of *B. juncea* ($2n = 36$) using AFLP and RFLP markers in an F_1-derived doubled-haploid population. The number of publicly available *Brassica* microsatellite primers is increasing as a result of publicly funded international initiatives (http://www.*Brassica*.info/ssr/SSRinfo.htm).

The release of a set of robust, highly polymorphic, mapped SSR markers spanning the entire *B. napus* genome into the public domain greatly assisted in genome mapping and gene tagging. To enrich the SSR resource further, Federico et al. (2008) developed 587 new primer pairs flanking SSRs using sequence information from 3500 genomic clones mainly from *B. oleracea* to identify di-, tri-, tetra-, and pentanucleotide repeats. Yadava et al. (2009) evaluated the cross-transferability and polymorphic potential of the genomic SSRs to assess their utility across *Brassica* species and related genera. The study revealed that the available SSR markers can be used effectively in monitoring gene introgression. Nevertheless, a large set of markers should be used to overcome the low level of intra-specific polymorphism (Koundal et al. 2008). Sun et al. (2007) used sequence-related amplified polymorphism (SRAP) to construct an ultra-dense genetic recombination map for a doubled haploid population in *B. napus*. Gao et al. (2007) constructed a high-density genetic map of *B. oleracea* adding over 1000 new markers to *Brassica* molecular tools. Panjabi et al. (2008) designed and tested the efficacy of PCR-based intron polymorphism (IP) markers to analyse genome-wide synteny between the oilseed *B. juncea* and *A. thaliana* and analysed the arrangement of 24 genomic block segments in the A, B, and C *Brassica* genomes to study the evolutionary events contributing to karyotype variations. These recently reported SRAP and IP markers should find application in tagging of useful genes in oilseed *Brassica* crops.

15.8.1 Molecular Markers Studies in Decoding Genetic Diversity and Assisting Selection of Superior Genotypes

With the development of genomic resources in oilseed *Brassica* species, emphasis had been focused on using DNA/molecular markers for multiple purposes. Several studies have advocated the use genetically diverse genotypes or genotypes of different geographical regions to harvest high heterosis in comparison to the local or closely related genotypes. This in turn emphasized the importance of genetic diversity evaluation in the plant material for the selection of the most appropriate and promising parents for breeding programmes. The molecular/DNA markers have found profound use in genetic diversity analysis because of their reproducibility, abundance, and distribution, and they are free from the environmental factors' influences, proving them to be a better marker system over morphological marker. Molecular marker-based approach is more likely to generate an unbiased picture of diversity than that obtained by employing agro-morphological traits. Genetic diversity analysis using various types of molecular markers, such as randomly amplified polymorphic DNA (RAPDs), inter-simple sequence repeats (ISSRs), restriction fragment length polymorphisms (RFLPs), amplified fragment length

polymorphisms (AFLPs), simple sequence repeats (SSRs), expressed sequence tags (ESTs), and single-nucleotide polymorphisms (SNPs), provides unique opportunities to accurately evaluate the genetic variability present in a given population for various traits. Several studies carried out till date are presented in Table 15.5.

15.8.2 Disease Resistance

Molecular markers have been generated for the genes conferring resistance to *Leptosphaeria maculans*, *Plasmodiophora brassicae*, *Xanthomonas campestris*, *Sclerotinia sclerotiorum*, *Verticillium* wilt, turnip mosaic virus, and white rust in different *Brassica* spp. Cheung et al. (1997) identified one co-segregating dominant RFLP marker x140a, mapped on LG7 in *B. juncea* and designated as *Acr*. In the same year, white rust resistance locus $Ac2_1$ present in an Eastern European source that was effective against a Canadian isolate of the pathogen was mapped using RAPD markers (Prabhu et al. 1998). Mukherjee et al. (2001) mapped a locus designated as *Ac*2(t) effective against Indian isolate of the white rust pathogen. Varshney et al. (2004) developed a tightly linked marker using AFLP, and a PCR-based cleaved amplification polymorphic sequence (CAPS) marker for closely linked RAPD marker for white rust resitance. Two independent loci for conferring resistance to *Albugo candida* in the east European germplasm of Indian mustard were reported (Panjabi et al. 2010). Several transgenic approaches to incorporate resistance have also been applied. Borhan et al. (2010) used WRR4 gene to develop white rust resistance through *Agrobacterium*-mediated transformation. Chhikara et al. (2012) transferred antifungal genes chitinase and ribosome-inactivating gene to develop *Alternaria* blight resistance. Hada et al. (2015) used *Agrobacterium*-mediated transformation for the LETgene thaumatin and developed improved resistance to salinity, drought, and *Alternaria* blight in *Brassica*. Ali et al. (2017) developed enhanced resistance to *Erysiphe cruciferarum* using overexpression of BjNPR1.

15.8.3 Oil Content and Fatty Acid Composition

Both oil quantity and quality traits have been studied using molecular markers. Ecke et al. (1995) mapped three loci on different linkage groups in *B. napus* using RFLP markers. In *B. juncea*, Sharma et al. (1999) identified three loci based on segregation of RAPD markers in a recombinant inbred population. Zhao et al. (2006) reported mapping of QTLs for oil and protein content in rapeseed. Delourme et al. (2006) reported that additive effects are the main factors contributing to variation in oil content. Two RAPD markers, $K\text{-}01_{1100}$ and 25a, were generated and linked to the linolenic acid concentration (Hu et al. 1995; Tanhuanpaa et al. 1995). RAPD markers linked to oleic, linolenic, and linoleic acid were identified in *B. napus* (Hu et al. 1999). Markers linked to genomic regions controlling linolenic acid

Table 15.5 Role of molecular markers in revealing genetic diversity in Indian mustard (*Brassica juncea*)

S. No.	Plant material used	Markers used	Significant results	References
RAPD marker				
1	12 Indian and 11 exotic *B. juncea* genotypes of canola quality	32 RAPD primers	595 total alleles, 500 polymorphic alleles, average of 11.8 polymorphic alleles per primer	Jain et al. (1994)
2	52 *B. juncea* germplasm including 41 accessions from Pakistan, 6 oilseed cultivars, and 5 Japanese vegetable cultivars	30 RAPD markers	198 polymorphic amplicons, a low level of polymorphism between the oilseed accessions collected from Pakistan	Rabbani et al. (1998)
3	30 Indian and exotic germplasm accessions of *B. juncea*	4 RAPD markers	21.54–59.36% level of genetic polymorphism	Ali et al. (2007)
4	45 *B. juncea* genotypes comprising 37 germplasm accessions, 5 advanced breeding lines, and 3 improved cultivars	15 RAPD markers	A total of 92 RAPD fragments, of which 81 (88%) were polymorphic, each primer produced 4–9 amplified products with an average of 6.13 bands per primer	Khan et al. (2008)
5	9 varieties of 4 *Brassica* spp including *B. rapa*, *B. napus*, *B. juncea*, and *B. oleracea*	4 RAPD markers	In total, 59 reproducible DNA bands generated, of which 58 bands were polymorphic with a size range from 212 to 2272 bp. The UPGMA cluster analysis divided all the varieties into two distinct groups	Saha et al. (2008)
6	9 varieties of *Brassica* species	4 RAPD markers	In total, 59 reproducible DNA bands generated, of which 58 (98.03%) bands were polymorphic; cluster analysis divided the 9 accessions into 2 major groups	Ghosh et al. (2009)
7		4 RAPD markers	A high degree of genetic	Khan et al. (2011)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
	15 *B. juncea* lines of exotic and local origin		polymorphism among the *Brassica* lines with average genetic distance ranging from 14.45 to 25.43%; a high level of genetic dissimilarity reported among the 14 genotypes	
8	34 *B. juncea* lines comprising 6 parental genotypes and their 28 F1 hybrids	12 RAPD markers	A total of 57 DNA fragments obtained, out of which 48 were polymorphic showing 84.63% polymorphism; the genetic resemblance ranged from 0.32 to 0.96, showing that significant genetic variation exists among various combinations of mustard lines; the dendrogram grouped all the parental genotypes into one cluster and all F1s in the other cluster	Gami et al. (2013)
9	50 diverse genotypes of *B. juncea* including 12 exotic and 38 indigenous genotypes	10 agro-morphological traits and 11 RAPD markers	100% polymorphism for all the 12 primers; a lack of association between genetic and phenotypic diversity	Singh et al. (2013)
10	30 *B. juncea* lines and varieties	RAPD markers	A total of 104 loci with an average of 8.6 bands per primer and size range between 300 bp and 3 kb; on an average, 84% similarity was observed among all the genotypes	Tahira et al. (2013)
11	5 varieties of *B. juncea*	4 RAPD markers	A total of 20 alleles reported with a genetic polymorphism level of 0–66.66%	Yousuf et al. (2013)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
12	4 *Brassica* cultivars (2 of *B. juncea* and 1 each of *B. nigra* and *B. rapa*)	6 RAPD markers	Out of 43 fragments generated, 38 were found to be polymorphic; cluster analysis grouped *B. juncea* and *B. rapa* cultivars in one group and *B. nigra* cultivars in the second group	Sharma et al. (2015)
13	Seven individuals of introgressed *Brassica* lines (Binasarisha-5/ Daulot) and two of their parental lines	3 RAPD markers	Result of cluster analysis indicated that the nine accessions were classified into two major groups—one consists of only one parent Daulot (*Brassica juncea*) while another consists of Binasarisha-5 (*Brassica napus*) and all introgressed lines of C_6 generation (treated with colchicine in C_1 generation) resulted from the cross *B. napus* and *B. juncea*	
14	Ten mustard varieties	10 RAPD markers	The RAPD cluster pattern showed four major clusters, cluster-1 comprised of Rohini and Varuna, cluster-II Narendra Rai and Maya (EC-98), cluster III Kanti and Urvashi. Similarly cluster IV including Pusa Jaikisan and Pusa Agrani. The varieties Pusa Tarak (EJ9912-13) and GM-3 occupied distinct places in the dendrogram, thereby indicating its	Wani et al. (2017)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
			distinctiveness from other varieties	
15	6 *Brassica* genotypes	20 primers	A total of 231 scored band, generated 87% polymorphic bands. Average PIC, MRP, RP, MI, and EMR values were 0.088, 0.65, 6.7, 0.78, and 8.9, respectively	Raza et al. (2020)
ISSR markers				
1	93 genotypes from 24 wild populations	8 ISSR markers	A total of 86 highly reproducible ISSR bands; most of the variation (54.09%) occurred among the population and the remaining (45.91%) variance was attributed to differences among individuals within populations	Huangfu et al. (2009)
2	30 Indian mustard genotypes	20 ISSR markers	A total of 156 bands with an average of 7.8 bands per primer, out of which 115 were polymorphic	Yadav and Rana (2012)
RAPD markers				
1	Forty-two genotypes of different oilseed *Brassica* spp. including 28 of *B. juncea*, 4 of *B. carinata*, 3 of *B. napus*, 1 of *B. nigra*, 5 of *B. campestris*, and 1 of *Eruca sativa*	RAPD, ISSR, and Anchored-SSR markers	Combination of 11 informative primers belonging to all the 3 DNA marker profiles could precisely identify all the 28 *B. juncea* genotypes. These informative primers can be employed in varietal identification in oilseed *Brassica* species	Kalita et al. (2007)
2	20 varieties of *B. juncea*	RAPD and ISSR markers	Mean PIC value greater for RAPD (0.419) with an average of 9.3 alleles per primer, whereas in the case of ISSR markers, 0.261 as	Ahmad et al. (2012)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
			average PIC value with an average number of 6.8 alleles per primer	
3	15 varieties of *B. juncea*	43 RAPD and 31 ISSR markers	Out of 43 amplified RAPD primers, 42 were found to be polymorphic with an average of 6.09 alleles/primer, while 30 out of 31 ISSR markers resulted in polymorphic amplicons; 2 main clusters of varieties	Sankhla et al. (2015)
RFLP markers				
1	5 accessions of Indian mustard	SDS-PAGE and one RFLP marker	A polymorphism level of 28.57% obtained by protein profiling, whereas RFLPs exhibited a comparatively very high level of polymorphism (87.5%)	Mir et al. (2015)
AFLP markers				
1	21 established and 9 synthetic varieties and lines of *B. juncea* originated from Asia, Australia, Canada, Europe, and Russia	21 AFLP markers	A 62.2% polymorphism; a total of 1251 scorable fragments, of which 778 bands were polymorphic with an average of 37 polymorphic bands per primer pair; 3 distinct clusters	Srivastava et al. (2001)
2	77 breeding lines of *B. juncea* from Canada, Australia, and 15 lines of quality mustard from India, China, Russia, and Australia	10 AFLP markers	A total of 751 scorable fragments with an average of 26 polymorphic bands per primer pair (35%); 5 major clusters	Burton et al. (2004)
3	16 Chinese vegetable mustard (*B. juncea* var. *tumida*) accessions	14 AFLP markers	A total of 66 scorable fragments, of which 29 bands were polymorphic with an average of 21.1%	Qi and Zhang (2008)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
			polymorphic bands per primer combination; 2 main groups	
Cross-transferability studies of SSR markers				
1	All the species of U's triangle, *Eruca sativa* and *Arabidopsis thaliana*	100 genomic STMS markers developed earlier in *B. napus*, *B. oleracea*, *B. rapa*, and *B. nigra*	87.5% cross-transferability in 3 genotypes of *B. juncea*	Yadava et al. (2009)
2	75 germplasm accessions of *B. napus*, *B. rapa*, *B. nigra*, *B. juncea*, and *B. carinata*	25 SSRs derived from *B. napus*, *B. nigra*, *B. oleracea*, and *B. rapa*	A cross-transferability rate of more than 90% obtained in various *Brassica* species	Sadia et al. (2010)
3	11 different species of *Brassica* and allied genera	161 *Brassica species*-derived genomic-SSRs	Out of 161 SSR markers, only 70 of them (43.5%) demonstrated their transferability to at least 1 of the 11 species	Singh et al. (2012b)
4	12 varieties of *B. juncea*	124 SSRs derived from *B. nigra*, *B. rapa*, *B. oleracea*, and *B. napus*	81 cross-transferable SSRs in *B. juncea* background with an average of 2.17 alleles/locus; an overall cross-amplification efficiency of 84.8% of SSR loci	Thakur et al. (2015)
5	All the three primary diploids and three amphidiploids species of U's triangle	124 SSRs derived from *B. nigra*, *B. rapa*, *B. oleracea*, and *B. napus*	A 100% cross-transferability rate obtained for *B. juncea* and three subspecies of *B. rapa*; the average percentage of cross-transferability across all the 7 species was 98.15%	Thakur et al. (2018)
6	21 accessions representing 19 species from 8 different genera of *Brassicaceae* family	460 EST-SSRs	A total of 200 amplicons, of which 150 (75%) exhibited cross-transferability; of which 121 (80.67%) EST-SSRs were found to be	Singh et al. (2018)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
			polymorphic with PIC value ranging from 0.09 to 0.66	
7	An international germplasm collection of 86 *B. napus* and 43 *B. juncea* lines	15 genomic and 10 novel EST-derived SSR markers	Almost similar polymorphism levels found in both the species, with an average of 11.1 alleles/locus (4–31) in *B. napus* and 10.6 alleles/locus (2–24) in *B. juncea*; EST-SSRs showed lesser polymorphism than genomic-SSRs with an average of 6.5 alleles/locus as compared to12.6 alleles/locus with genomic-SSRs	Batley et al. (2003)
8	Resynthesized *B. juncea* (11), diploid progenitor genotypes (*B. nigra*, *B. rapa*, 19) and natural *B. juncea* (4 genotypes)	46 A- and B-genome-specific SSR markers	A total of 198 alleles with 2–12 alleles/ locus and PIC values from 0.2 to 0.89, the dissimilarity coefficient ranging from 0.13 to 0.74	Bansal et al. (2009)
9	120 different accessions of *Brassica* species including *B. napus*, *B. juncea*, and *B. rapa* of Pakistan origin	39 SSR markers	A total of 162 scorable bands, in which 105 were polymorphic	Turi et al. (2012)
10	37 genotypes of Indian mustard for Alternaria blight tolerance	10 *Brassica*-derived SSR markers	41 alleles with 97.56% polymorphism; 5 separate groups	Chandra et al. (2013)
11	44 Indian mustard genotypes including varieties/purelines from India and few exotic genotypes from Australia, Poland, and China	12 morphological traits and 143 SSR markers	134 SSRs reported polymorphism and a total of 355 alleles amplified; 4 clusters	Vinu et al. (2013)
12	27 genotypes of *Brassica* species (23 *B. juncea* varieties, 2 *B. napus*	15 RAPD and 3 EST-SSR markers	RAPD markers resulted in >91% polymorphism percentage with an	Gupta et al. (2014)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
	genotypes, and 1 each of *B. campestris* and *B. oleracea*)		average PIC value of 0.31, 4.44 marker index, and 6.89 average resolving power; for EST-SSRs, 86.66% polymorphism percentage reported along with average PIC value of 0.28, marker index of 0.94 and resolving power of 0.269; all the genotypes of *B. juncea* grouped into one cluster and other *B.* species formed a separate cluster	
13	30 genotypes belonging to *B. juncea*, *B. rapa*, *B. napus*, and *B. carinata*	24 *B. rapa*-derived SSR markers	72% polymorphism with a total of 84 alleles, with 0.933 highest allele frequency and PIC values in the range of 0.12–0.79	Prajapat et al. (2014)
14	A diversity fixed foundation set (DFFS) of *B. juncea* comprising 48 accessions	158 nuclear-SSRs and 9 chloroplast SSR markers	158 nuclear-SSRs amplified 241 alleles, while 9 chloroplast-specific SSRs could amplify 34 cpSSR alleles; the different diversity groups obtained did not show full compliance with structure analysis, especially for the nuclear genetic variation	Akhtar et al. (2015)
15	20 Indian mustard genotypes (14 advanced breeding lines, 4 tolerant germplasm accessions, 1 tolerant check PAB 9511, and a susceptible check Varuna) for *Alternaria* blight tolerance	65 *Brassica*-derived SSRs	25 polymorphic SSRs with 3.52 average number of alleles/ locus and 0.351 average PIC value; 2 distinct clusters separating resistant and susceptible genotypes	Pratap et al. (2015)

(continued)

Table 15.5 (continued)

S. No.	Plant material used	Markers used	Significant results	References
16	25 genotypes of *B. juncea* and wild relatives	25 EST-SSRs	2–5 alleles per locus detected with PIC values ranging from 0.22 to 0.66	Singh et al. (2016a, b)
17	Indian and exotic genotypes of *B. juncea*	32 genomic-SSRs and EST-SSRs derived from *B. rapa*	16 polymorphic markers; 54 alleles with an average of 2.37 alleles per locus and 0.31 as the average PIC value; 3 distinct clusters	Sudan et al. (2016)
18	Indian mustard genotypes	Two CAPS markers	The developed CAPS markers for *FAE1.1* and *FAE1.2* used in *B. juncea* for differentiating between LEA and HEA lines	Saini et al. (2016)
19	27 Indian mustard genotypes	Promoter-based markers developed	The markers based on promoter polymorphism distinctly differentiated the genotypes between LEA and HEA group	Saini et al. (2019)
20.	48 mustard genotypes	20 SSR markers	50% polymorphism was detected. 48 genotypes were divided into 3 major groups, group 'I' contained 17 genotypes, group 'II' hold 24 genotypes, and core group 'III' included 7 genotypes	Baghel et al. (2020)

Source Singh et al. (2020)

concentration in *B. napus* corresponding to *fad3* (omega-3-desaturase) gene in *A. thaliana* (Arondel et al. 1992) were also identified (Jourden et al. 1996a, b; Thormann et al. 1996). In another study, a single QTL containing six markers associated with oleic, palmitic, and linoleic acid content was detected in *B. rapa* (Tanhuanpaa et al. 1996). In *B. juncea*, Sharma et al. (2002) mapped two major QTLs influencing oleic acid level using both single factor analysis of variance and interval mapping. Erucic acid loci have been linked to RFLP markers by Ecke et al. (1995), Thormann et al. (1996), Jourden et al. (1996c), and Barret et al. (1998) in *B. napus*. Two QTLs underlying the variation of seed erucic acid content were assigned to two linkage groups of *B. juncea* map using double haploid mapping

populations derived from high × high erucic acid hybrid (Gupta et al. 2004). Qiu et al. (2006) constructed a genetic linkage map consisting of 277 loci and identified reproducible QTL for seed oil and erucic acid content. The SRAP markers based on *B. napus FAE1* gene developed by Rahman et al. (2008) were expected to be highly useful in MAS for erucic acid content.

Several SNPs have been reported to distinguish *FAE1.1* and *FAE1.2* in LEA and HEA genotypes of *B. juncea* (Gupta et al. 2004). These SNPs were converted into CAPS markers and have been successfully used in marker-assisted breeding of *B. juncea* (Saini et al. 2016). The complete co-segregation between SNPs at position 591 and 1265 in CDS of *FAE1.1* gene and at position 237 in case of *FAE1.2* with erucic acid content was reported by Saini et al. (2016).

The sequence comparison of the promoter region of *FAE1* of different *Brassica* species (Zeng and Cheng 2014; Chiron et al. 2015; Li et al. 2017) provides insight into the differences in regulation of genes controlling the erucic acid content in the seed. Yan et al. (2015) identified the polymorphism in the promoter region of *FAE1.1*. The *FAE1* promoter is a seed-specific promoter (Zeng and Cheng 2014; Chiron et al. 2015), phylogenetically conserved in related species of *Brassica*. The *B. oleracea* and *Capsella rubella* promoter region were found to be 48.7% similar compared to 84.9% between coding regions (Li et al. 2017). The allelic variation based on length polymorphism in the promoter region was exploited to develop the polymorphic markers to be used in the breeding programme to develop the low erucic genotypes in *B. juncea*. These markers were validated in diverse genotypes and are highly efficient (Saini et al. 2019).

A reduced erucic acid in the total fatty acid profile increases the oleic acid (C18:1) fraction, as a malfunction of *FAE1* genes results in the failure of conversion of C18:1 to C20:1 or C22:1 (Yashpal et al. 2020). This in turn results in a better ratio of linoleic and linolenic fatty acids in the oil (Jagannath et al. 2011). A series of hypomorphic and null mutations in the FAD2.A5 isoform were characterized, and four of these were combined with null mutations in the other two isozymes, FAD2. C5 and FAD2.C1, in *Brassica napus*. The resulting mutant lines contained 71–87% oleic acid in their seed oil, compared with 62% in wild-type controls (Shuangyi et al. 2019). In yet another attempt to improve oleic acid content, by performing chemical mutagenesis using ethyl methanesulfonate, mutant winter rapeseed breeding lines were developed that can produce oil with a high content of oleic acid (C18:1, more than 75%) and a low content of linolenic acid (C18:3, less than 3%) (Spasibionek et al. 2020). However, all these studies have been academic exercises and have yet to perform in the field.

15.8.4 Seed Glucosinolate Content

Ripley and Roslinsky (2005) reported an ISSR marker tightly linked to high level of 2-porpenyl glucosinolate. It has a good potential to be used for MAS for low glucosinolate in the canola breeding programme. Ramchiary et al. (2007) used a recurrent backcross selection (RSB) method with a doubled haploid (DH) interspersing backcross generations for the introgression of low glucosinolate

alleles from an east European *B. juncea* line, Heera into an Indian variety, Varuna. A validation study on a population of low glucosinolate DH lines derived from all the backcross generations of the RSB breeding programme revealed that the QTL detected in BC_4DH were the 'true' QTL. This study was extended by Bisht et al. (2009) who mapped a total of six QTLs for this trait. Hasan et al. (2008) reported four genes involved in the biosynthesis of indole, aliphatic, and aromatic glucosinolates that might be associated with known quantitative trait loci for total seed glucosinolate content in *B. napus*. In association with mapping of seed glucosinolate (GS) content using the 60K *Brassica* Infinium single-nucleotide polymorphism (SNP) array in 520 oilseed rape accessions, a total of 11 peak SNPs significantly associated with GS content were detected and validated by the qRT-PCR analysis of their expression profiles (Qu et al. 2015). In another study, the QTLs for 2-propenyl glucosinolates (GSLs) colocalized with the QTLs for 3-butenyl GSLs between At1g26180 and BnapPIP1580 on LG08 and accounted for an average of 42.3% and 42.6% phenotypic variation for 2-propenyl and 3-butenyl GSLs, respectively. Joint QTL mapping and RNA-sequencing analyses revealed one candidate gene of ***IIL1*** (LOC106416451) for GSL metabolism in ***B. juncea*** (Khattak et al. 2019).

15.8.5 Seed Coat Colour

RFLP markers linked to seed coat colour in *B. napus* were identified using the BSA approach (Van Deynze et al. 1995). Seed coat colour trait in *B. campestris* was tagged with RAPD markers using *B. campestris-oleracea* addition lines (Chen et al. 1997). Two RFLP markers flanking one of the interacting loci were identified. Negi et al. (2000) tagged the seed coat colour trait using AFLP markers through BSA in *B. juncea*. Bulked segregant RNA-Seq (BSR-Seq) of BC_9 population of yellow mustard and brown mustard was used to identify the candidate genes controlling the yellow seed colour in *Brassica juncea* L., and the seed coat colour gene was mapped to chromosome A09 (Huang et al. 2020). Twenty thousand nine hundred fifty four DEGs from transcriptome comparisons of seeds sampled from a pair of *B. rapa* accessions with different seed size, seed colour, and oil content at seven seed developmental stages identified a group of cell cycle-related genes whose expression was positively correlated with SZ increase and identified a conserved TT8-involved complex which may determine the seed colour through downregulation of the key TF gene *TT8* and its targets *TT3*, *TT18*, and *ANR* in the flavonoid pathway (Niu et al. 2020).

15.9 Biotechnological Developments in *Brassica*/Genomics-Assisted Breeding

To address the new emerging challenges biotechnological interventions have also been made in the Indian mustard breeding programme with the available tools and techniques. Many attempts have been made by scientists to improve *Brassica* using

molecular markers like amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), single-nucleotide polymorphism (SNP)-based maps (Raman et al. 2014), etc. Molecular markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), AFLP, and SSR have been used for improving selection efficiency and selecting plant genotypes with the desired combinations of traits. Markers linked with white rust resistance (Prabhu et al. 1998), fatty acids, oil content, yellow seed colour, and fertility restoration have been reported. Transgenic approaches have been followed to develop the transgenic for aphid resistance, male sterility, AB tolerance, herbicide resistance, and drought tolerance. Bar, barnase, and barstar based herbicide resistance and genetic male sterility have been used in the development of experimental hybrids (Jagannath et al. 2002). Lectin gene for aphid resistance and DREB gene construct for drought tolerance are being used. Osmotin (from tobacco) for drought and salt tolerance, annexin gene for stress tolerance, chitinase and glucanase (from *Arabidopsis*) for tolerance to *Alternaria* blight disease, and FAE1 gene for low erucic acid mustard cultivar are other transgenes being used in rapeseed-mustard.

15.10 Progress in Genetic Enhancement

Up to 1970, mass and pure line selection were the main breeding methods used in breeding programmes, and 26 varieties were developed. The first variety was released in 1936. After 1980, varieties developed through hybridization increased, and 22 varieties were released in each of the eighth and ninth decade of the twentieth century. This number further increased to 41 during the first decade of the twenty-first century. Simultaneously, 12 varieties have been developed through mutation breeding. It is believed that most of the Indian mustard varieties were the pure line selections derived from a very few common ancestors, and a limited number of donors were utilized in the breeding programme resulting in a narrow genetic base.

Since the early 1980s, systematic and vigorous recombinant breeding has been followed, and a large number of varieties have been identified and released. The first notified variety was ITSA of toria (*B. rapa*) in 1973 after the adoption of official notification of varieties in 1969 under the Seed Act 1966 (Section 5). The major objectives of the varietal improvement programme have been genetic enhancement for seed and oil yield through developing varieties for early, timely, and late sown conditions to cater to the need of diverse agroecological situations of the country, improvement of oil (low erucic acid), and seed meal (low glucosinolate) quality, introgression of resistance/tolerance against major biotic (white rust, *Alternaria* blight, *Sclerotinia* rot diseases, and aphid and painted bug insects) and abiotic stresses (drought, high temperature, frost tolerance, and salinity), many other situation-specific varieties have been developed under the programmes.

A total of 35 novel genetic stocks of rapeseed-mustard with important traits (CMS, restorer, low erucic acid and low glucosinoltes, high oil content, high oleic acid and low linolenic acid, dwarf, earliness, long main shoot, bold seed, yellow seed, tetralocular siliquae, white rust resistance, tolerance to high temperature

and salinity during juvenile stage, high temperature tolerance during terminal stage, and high water use efficiency) have been registered with NBPGR, New Delhi, till January 2021. Several varieties/hybrid of different *Brassica* spp. and *Eruca sativa* have been developed. Among these varieties/hybrids, the historical achievement is the development of first (0) single zero (<2% erucic acid in oil) *B. juncea* variety Pusa Karishma released in 2005, first (00) double low (<2% erucic acid and <30 ppm glucosinolate) *Brassica juncea* Pusa Mustard 31 released in 2016, and first double low (00) *B. juncea* hybrid RCH 1 released in 2021. Ten single zero or low erucic acid (0) varieties, viz. Pusa Karishma (LES-39), LES-1-27 (Pusa Mustard 21), LET-17 (Pusa Mustard 22), RLC 1, RLC 2, LET-18 (Pusa Mustard 24), LET-36 (Pusa Mustard 29), LET-43 (Pusa Mustard 30), and LET-54 (Pusa Mustard 32), and 3 canola quality varieties/hybrid, viz. Pusa Double Zero Mustardd 31, Pusa Double Zero Mustard 33, RLC 3, and RCH 1, have been released (Table 15.6). In addition, ten hybrids of *B. napus* and *B. juncea* have also been released (Table 15.4).

Table 15.6 Donor sources and breeding methods used in the development of quality varieties

S. no.	Variety	Pedigree	Breeding method
***B. juncea* (single low i.e. low erucic acid)**			
1.	Pusa Karishma	Pusa Barani/Zem 1	Backcross method
2.	Pusa Mustard 21	Pusa Bold/Zem-2	Backcross method
3.	Pusa Mustard 24	Pusa Bold/LET 15//LES 29	Pedigree selection
4.	Pusa Mustard 22	Pusa Barani/Zem-2	Backcross method
5.	ELM 079 (RLC 1)	QM-4/Pusa Bold	Pedigree selection
6.	ELM-123 (RLC 2)	QM-4/Pusa Bold	Pedigree selection
7.	Pusa Mustard 29	ZEM-2/Pusa Barani//EC 287711	Pedigree selection
8.	Pusa Mustard 30	Bio-902/ZEM-1	Pedigree selection
9.	Pusa Mustard 32	LES-1-27/EC-597325	Pedigree selection
***B. juncea* (Double low i.e. low erucic acid and low glucosinolates)**			
10.	Pusa Double Zero Mustard 31	LES-1-27 (PM21)/NUDHYJ-3	Pedigree selection
11.	RLC 3	JM06003/JM06020	Pedigree selection
12.	Pusa Double Zero Mustard 33	Pusa Agrani/Heera	Pedigree selection
13.	RCH 1	CMS- ZM 20 × OCRE-4NR	CMS-based hybrid
***B. napus* (Double low)**			
14.	GSC 5	Hyola 401//Agat GSL-8888	Pedigree selection
15.	OCN-3/GSC 6	NECN 13/Tribute//NECN-13	Backcross method
16.	GSC 7	Rivette/RR001	Pedigree selection
17.	PAC-401	44002A/4154 R	CMS-based hybrid
18.	NUDH 26-11	Selection from germplasm	Pure line selection
19	PGHS 1699 (GSH 1699)	CMS-AG7 × FR-ZY 005	CMS-based hybrid

15.11 All India Coordinated Research Project on Rapeseed-Mustard

Systematic research work on rapeseed-mustard was initiated after the constitution of the Indian Central Oilseed Committee. This committee was designated as Oilseed Development Council in 1966. In 1967 an All India Coordinated Research Project on Oilseeds was initiated under the leadership of Project Coordinator which included five crops, viz. groundnut, rapeseed-mustard, sesame, linseed, and castor. In 1981, a separate All India Coordinated Research Project on Rapeseed and Mustard was established at HAU, Hisar, and 14 research centres were established for research work on rapeseed and mustard. On October 20, 1993, National Research Centre on Rapeseed-Mustard was established at Sewar, Bharatpur, in Rajasthan. The Indian Council of Agricultural Research raised the NRC on Rapeseed-Mustard to Directorate of Rapeseed-Mustard Research in February 2009. Presently there are 11 main and 12 sub-centres under the AICRP Rapeseed-Mustard. In addition, there are 22 verification centres also for evaluation of the advanced material in different zones under AICRP trials. The country has been divided into six agro-climatic zones for evaluation and release of the material.

Since the inception of All India Coordinated Research Project on Rapeseed-Mustard (AICRP-RM) in 1967, a total of 212 varieties (Indian mustard-131; toria-28; yellow sarson-16; gobhi sarson-16; brown sarson-6; karan rai-5; taramira-8 and black mustard-1) including 11 hybrids of rapeseed-mustard have been released till now. Details of varieties released since 2010 are presented in Table 15.7. Rapeseed-mustard varieties having tolerance to biotic (white rust, *Alternaria* blight, powdery mildew), abiotic stresses (salinity, high temperature), and quality traits have been recommended for specific growing conditions.

15.12 Future Thrust Areas

- Enhancing level of heterosis of hybrids through proper utilization of genetic resources and focused breeding options for their better commercial adoption.
- Developing early maturing, dwarf, and determinate type varieties for enhancing crop intensity, suitable for different cropping systems and amenable to mechanical harvesting.
- Development of varieties resistant to biotic stresses especially *Sclerotonia* stem rot, white rust, *Alternaria* blight, aphids, and *Orobanche* for stabilizing the productivity.
- Development of varieties with enhanced water and nutrient use efficiency for optimum utilization of the available soil moisture and nutrients.
- Tailoring varieties tolerant to abiotic stresses such as drought, high temperatures at sowing and/or maturity, salinity, and frost in rapeseed-mustard.
- Developing double zero varieties (erucic acid $<2\%$ and glucosinolate <30 μmol/g of defatted seed meal cake) with high oleic acid.
- Improving the oil content of present-day varieties from 40% to 45%.

Table 15.7 Different varieties/hybrids of rapeseed-mustard released during 2010–2021

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
***Brassica juncea* (Indian mustard)**							
Pusa mustard −25 (NPJ-112)	2010	107	ICAR-Indian Agricultural Research Institute, New Delhi	39.6	14.07	Delhi, Haryana, Punjab, Jammu and Kashmir, Rajasthan	Suitable for early sown (September sowing) irrigated conditions, tolerant to high temperature at seedling stage, a potential substitute of toria and wheat can be taken after its harvest
Pusa mustard −26 (NPJ-113)	2011	125	ICAR-Indian Agricultural Research Institute, New Delhi	37.6	16.04	Delhi, Haryana, Punjab, Jammu and Kashmir, Rajasthan	Suitable for late sown (November sowing) irrigated conditions, moderately tolerant to high temperature at seedling and maturity stage
Pusa mustard −27 (EJ-17)	2011	115	ICAR-Indian Agricultural Research Institute, New Delhi	41.7	15.35	Madhaya Pradesh, Uttar Pradesh, Uttarakhand, and Eastern Rajasthan	Suitable for early sown (September sowing) irrigated conditions, tolerant to high temperature at seedling and maturity stage
RH 0119	2011	147	CCS, Haryana Agricultural University, Hisar (Haryana)	40.0	19.00	Timely sown conditions in rainfed areas of Haryana	Erect plant type with long siliqua having thermo-tolerance

(continued)

Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
Coral PAC 437 (Hybrid)	2012	140	Adventa India Ltd., Hyderabad (Telangana)	40.0	25.00	Delhi, Haryana, Punjab, Jammu, and parts of Rajasthan	Hybrid, tolerant to white rust
PBR-357	2012	146	Punjab Agricultural University, Ludhiana (Punjab)	39.7	25.50	Delhi, Haryana, Punjab, Jammu, and parts of Rajasthan	Suitable for timely sown irrigated condition
RH 0406	2012	144	CCS, Haryana Agricultural University, Hisar (Haryana)	40.5	22.50	Delhi, Haryana, Punjab, Jammu, and parts of Rajasthan	Tolerant to high temperature and salinity at seedling stage; suitable for timely sown rainfed condition
Pant Rai-19 (PR 2006-1)	2012	117	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	41.3	20.69	Jammu and Kashmir, Punjab, Haryana, and Delhi	Tolerant to high temperature during early stages, suitable for early sowing
Pusa Mustard 28 (NPJ-124)	2012	107	ICAR-Indian Agricultural Research Institute, New Delhi	41.7	19.93	Rajasthan, Haryana, Punjab, Delhi, Plains of J&K, HP, and Western UP	Suitable for early sown conditions (first week of September), moderately tolerant to high temperature at seedling, tolerant to salinity up to 12 dS/m and powdery mildew
RH 0749	2013	147	CCS, Haryana Agricultural University, Hisar (Haryana)	39.5	26.00	Timely sown conditions in rainfed areas of Haryana, Punjab, Delhi, and parts of Rajasthan	Suitable for timely sown irrigated conditions, bold seeded with more seeds/siliqua

RGN-229	2013	146	Rajasthan Agricultural University, Zonal Research Station, Sriganganagar (Rajasthan)	40.7	23.60	Rajasthan, Punjab, Haryana Delhi, and Jammu	Suitable for rainfed, timely sown conditions tolerance to high temperature as well as salinity
RGN-236	2013	127	Rajasthan Agricultural University, Zonal Research Station, Sriganganagar (Rajasthan)	39.1	16.36	Rajasthan, Punjab, Haryana, Delhi, and Jammu	Suitable for late sown irrigated conditions. Tolerant to high temperature at terminal stage
Pusa Mustard 29 (LET-36)	2013	143	ICAR-Indian Agricultural Research Institute, New Delhi	37.2	21.69	Delhi, Haryana, Jammu, Punjab, and northern Rajasthan	Single zero (<2% erucic acid) variety
Pusa Mustard 30 (LES-43)	2013	137	ICAR-Indian Agricultural Research Institute, New Delhi	38.0	18.24	Uttar Pradesh, Uttarakhand, Madhya Pradesh, and eastern Rajasthan	Single zero (<2% erucic acid) variety
RVM-2	2013	131	Rajmata Vijayaraje Scindia Krishi Viswa Vidyalaya, Zonal Research Station, Morena (Madhya Pradesh)	40.0	17.00	Delhi, Haryana, Jammu, Punjab, and northern Rajasthan	Suitable for rainfed as well as irrigated conditions
Giriraj (DRMRIJ 31)	2013	145	ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur (Rajasthan)	42	25.5	Rajasthan, Punjab, Haryana, Delhi, and Jammu	Timely sown irrigated conditions
Gujarat Dhantiwada Mustard 4	2015	110	Sardar Krushinagar Dantiwada Agricultural University, Sardar Krushinagar (Gujarat)	39	20.4	Gujarat	Timely sown irrigated conditions

(continued)

Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
Albeli	2015	145	Shakti Vardhak Hybrid Seeds Pvt. Ltd., Hisar (Haryana)	40	20.7	Rajasthan, Jammu, Uttar Pradesh, Madhya Pradesh, and Uttarakhand	Timely sown irrigated conditions
Pant Rai 20	2015	124	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	40	19.7	Uttarakhand	Timely sown irrigated conditions
PBR 357	2015	145	Punjab Agricultural University, Ludhiana (Punjab)	39.5	26.6	Punjab, Haryana, Delhi, Jammu and Kashmir, parts of Rajasthan, Western Uttar Pradesh	Timely sown irrigated conditions
RGN 298	2015	143	Rajasthan Agricultural University, Zonal Agricultural Research Station, Sri ganganagar (Rajasthan)	40.0	22.7	Punjab, Haryana, Delhi, Jammu and Kashmir, parts of Rajasthan, Western Uttar Pradesh	Timely sown rainfed conditions
GM-3	2016	107	Sardar Krushi nagar Dantiwada Agricultural University, Sardar Krushinagar (Gujarat)	38.8	19.90 (Irrigated); 12.10 (Rainfed)	Gujarat	Timely sown irrigated and rainfed conditions
Pusa Double Zero Mustard 31 (PDZ-1)	2016	144	ICAR-Indian Agricultural Research Institute, New Delhi	40.56	23.79	Punjab, Haryana, Delhi, Jammu and Kashmir, parts of Rajasthan, Western Uttar Pradesh	Timely sown irrigated conditions. It is the first double zero (low erucic acid <2% and low glucosinolates <30 ppm) Indian mustard variety in the

							country. It is a yellow seeded variety with improved oil and seed meal quality (canola quality) making this variety beneficial for farmers, traders, and consumers
RLC 2 (IC 511615)	2016	149	Punjab Agricultural University, Ludhiana (Punjab)	37.6	21.7	Delhi, Haryana, Punjab, Jammu, and parts of Rajasthan	Timely sown irrigated conditions; low erucic acid (<2%)
PBR 378	2016	146	Punjab Agricultural University, Ludhiana (Punjab)	40.5	24.5	Punjab	Timely sown rainfed conditions
Gujarat Dhantiwada Mustard 5 (GDM 5)	2016	144	Sardar Krushinagar Dantiwada Agricultural University, Sardar Krushinagar (Gujarat)	40.5	22.2	Punjab, Haryana, Delhi, Jammu and Kashmir, parts of Rajasthan, Western Uttar Pradesh	Timely sown irrigated conditions
Raj Vijay Mustard 1	2016	108	Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Zonal Research Station, Morena (Madhya Pradesh)	41.7	17–18	Madhya Pradesh	Early sown, rainfed conditions
JK Samriddhi Gold (JKMS 2)	2016	125–130	JK Agri Genetics Ltd., Hyderabad (Telangana)	39.5	23.00	Uttar Pradesh	Tolerant to salinity and white rust
Bayer Mustard 5450	2016	130–135	Bayer Bio Science Pvt., Hyderabad (Telangana)	39.9	28.00	Uttar Pradesh	Quality oil
RLC 3	2016	149	Punjab Agricultural University, Ludhiana (Punjab)	41.4	18.2	Punjab	Timely sown rainfed conditions, low erucic and low glucosinolate

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Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
CS-58	2017	135	ICAR-Central Soil Salinity Research Institute, Karnal (Haryana)	39	19.5	Punjab, Haryana, Rajasthan, Uttar Pradesh	Suitable for timely sown, irrigated, salinity, conditions
Pant Rai 21 (PRB 2008-5)	2017	122–127	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	40	12.2	Uttarakhand	Timely sown irrigated conditions
RH 725	2018	141	CCS, Haryana Agricultural University, Hisar (Haryana)	40.2	20.0	Punjab, Haryana, Delhi, Jammu and Kashmir, parts of Rajasthan, Western Uttar Pradesh	Timely sown rainfed conditions
CS 60	2018	134	ICAR-Central Soil Salinity Research Institute, Karnal (Haryana)	41	18.0	Punjab, Haryana, Rajasthan, Uttar Pradesh	Suitable for timely sown, irrigated, salinity, and water-logged conditions
RSPR-69 (MCN-04-35)	2019	135–145	Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (Jammu and Kashmir)	39.4	19.9 4	Jammu	Suitable for early sowing under irrigated and rainfed, low fertility areas during rabi season. Resistant to white rust and moderately resistant to *Alternaria* blight and major pests
RH 761	2019	141	CCS, Haryana Agricultural University, Hisar (Haryana)	40.4	28.6	Jammu, Punjab, Haryana, Delhi, and Northern Rajasthan	Suitable for timely sown and rainfed conditions in *rabi* season

SVJ-64	2020	130	Shakti Vardhak Hybrid Seeds Pvt. Ltd., Hisar (Haryana)	39.1	23.2	Haryana	Suitable for irrigated conditions under both high and low fertility conditions
DRMR 1165-40	2020	141	ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur (Rajasthan)	39.8	23.00	Jammu, Punjab, Haryana, Delhi, and Rajasthan	Suitable for timely sown rainfed condition, heat tolerant at seedling stage and moisture stress tolerant
Pusa Mustard 32 (LES 54)	2020	147	ICAR-Indian Agricultural Research Institute, New Delhi	38.0	27.10	Rajasthan (Northern and Western Parts), Punjab, Haryana, Delhi, Western Utter Pradesh, Plains of Jammu and Kashmir, Himachal Pradesh	Suitable for timely sown irrigated, quality mustard (low erucic acid content in oil)
Kesari Gold (31J3403)	2020	100	Bayer Bio Science Pvt., Hyderabad (Telangana)	41.0	17.92	West Bengal	Timely sown irrigated conditions; resistant to lodging; moderately resistant to fungal disease and white rust; resistant to aphid infestation
Kesari 5111 (PCJ03-401)	2020	104	Bayer Bio Science Pvt., Hyderabad (Telangana)	41.5	14.81	West Bengal	Timely sown irrigated conditions, moderately resistant to fungal disease and white rust, and moderately resistant for aphids infestation

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Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
Bayer Mustard 5222	2019	118	Bayer Bio Science Pvt., Hyderabad (Telangana)	39.1	16.78	West Bengal	Timely sown irrigated conditions, resistant to lodging, moderately resistant to white rust and resistant to aphid infestation
TBM-204 (Trombay Bidhan Mustard-204)	2019	110	Bidhan Chandra Krishi Visvavidyalaya, Nadia (West Bengal)	41	13.4	Bihar, Jharkhand, Odisha, Assam, and Manipur	Timely sown irrigated conditions, moderately resistant to *Alternaria* leaf spot
Kesri 5111 (PRO 5111)	2020	115	Bayer Bio Science Pvt., Hyderabad (Telangana)	42	10–12	Uttar Pradesh, Madhya Pradesh, Uttarakhand, and east Rajasthan	Early maturing, dark brown mustard, tolerant to white rust
DRMR 150-35 (Bharat Sarson 7)	2020	114	ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur (Rajasthan)	39.8	18.28	Bihar, Jharkhand, Odisha, West Bengal, Assam, Chhatisgarh, Manipur	Suitable for rainfed condition with protective irrigation during rabi season, moderate resistance to *Alternaria* blight and powdery mildew, moderate resistance to aphid infestation
Azad Mahak [(KMR (E) 15-2)]	2021	122	C.S.A University of Agricultural and technology, Kanpur (Uttar Pradesh)	41.5	20.50	Uttar Pradesh	Suitable for irrigated conditions

Radhika (DRMR 2017-15)	2021	131	ICAR- Directorate of Rapeseed-Mustard Research, Bharatpur (Rajasthan)	40.7	17.88	Jammu, Punjab, Haryana, Delhi and Rajasthan	Suitable under late sown irrigated conditions with tolerance to terminal heat stress
Brijraj (DRMRIC 16-38)	2021	132	ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur (Rajasthan)	39.9	17.33	Jammu, Punjab, Haryana, Delhi, and northern Rajasthan	Suitable under late sown irrigated conditions with tolerance to terminal heat stress
SVJH-108 (Hybrid)	2021	143	Shakti Vardhak Hybrid Seeds Pvt., Ltd., Hisar (Haryana)	41.3	25.5	Haryana	Irrigated conditions under both high and low fertility
Pusa Double Zero Mustard 33	2021	141	ICAR-Indian Agricultual Research Institute, New Delhi	38.0	26.40	Rajasthan (Northern and Western parts), Punjab, Haryana, Delhi, Western Uttar Pradesh, Plains of Jammu and Kashmir, and Himachal Pradesh	A canola quality (low erucic acid content in oil and glucosinolates in defatted seed meal cake) variety with high seed yield. Suitable for timely sown irrigated conditions
RCH 1 (Hybrid)	2021	145	Punjab Agricultural University, Ludhiana (Punjab)	38.5	26.70	Rajasthan (Northern and Western parts), Punjab, Haryana, Delhi, Western Uttar Pradesh, Plains of Jammu and Kashmir, and Himachal Pradesh	First canola quality (low erucic acid content in oil and glucosinolates in defatted seed meal cake) hybrid with high seed yield. Suitable for timely sown irrigated conditions
PHR 126 (Hybrid)	2021	145	Punjab Agricultural University, Ludhiana (Punjab)	40.2	22.70	Punjab	Timely sown irrigated ecologies

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Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
TAM 1028-1	2021	101	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, College of Agriuculture, Nagpur (Maharastra)	40.0	14.00	Maharashtra (Vidarbha Region)	Suitable for timely sown condition in *rabi* season under restricted irrigation. Early maturing variety
Birsa Bhabha Mustard 1 (BBM1)	2021	118	Birsa Agricultural University, Ranchi (Jharkhand)	40.0	15.50	Jharkhand	Suitable for rainfed timely sown condition with tolerance to moisture stress
Trombay Him Palam Mustard-1 (THPM-1)	2021	153	CSK Himachal Pradesh Krishi Visvavidyalaya, Palampur (Himachal Pradesh)	39.9	10.80	Himachal Pradesh	Timely sown, irrigated conditions in low and mid-hill zone
***Brassica rapa* ssp Yellow sarson**							
JK Pukhraj (JKYS 2)	2016	117	JK Agri Genetics Ltd., Hyderabad (Telangana)	45.0	15.5	Uttar Pradesh	Bold seed, high oil content
Pant Sweta (PYS-2007-10)	2017	115	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	45.0	14.5	Uttarakhand	Timely sown irrigated conditions
Pant Girija (PYS-2012-6	2020	110	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	45.3	14.4	Uttarakhand	Timely sown irrigated conditions resistant to lodging; moderately resistant to fungal disease and white rust; resistant to aphid infestation

Sanchita (YSWB-2014/2)	2020	96	Pulses and Oilseed Research Station, Berhampore (West Bengal)	44.5	15.0	West Bengal	Suitable for medium maturity, timely sown irrigated condition
Anushka (YSWB-2011-10-1)	2020	85	Pulses and Oilseed Research Station, Berhampore (West Bengal)	44.5	15.0	West Bengal	Suitable for early maturity, timely sown irrigated condition
***Brassica rapa* ssp Brown sarson**							
HPBS 80	2018	147	CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur (Himachal Pradesh)	40	11.50	Himachal Pradesh	Timely sown, rainfed conditions
Shalimar Sarsaon – 2 (KBS-49)	2019	210	Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar (Jammu and Kashmir)	42.7	16.00	Jammu and Kashmir	Suitable for irrigated conditions of J&K at 1500–1800 m above MSL
Shalimar Sarsaon – 3 (KBS-3)	2019	205	Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar (Jammu and Kashmir)	41.3	16.00	Jammu and Kashmir	Suitable for irrigated conditions of J&K at 1800–2000 m above MSL
***Brassica rapa* ssp Toria**							
Uttara (PT 2002-25)	2011	97	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	41.7	13.30	Uttarakhand	Moderately resistant to WR, DM, and PM diseases

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Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
Sushree	2016	75	Odisha University of Agriculture and Technology, Bhubneshwar (Odisha)	44.1	13.80	Odisha	Late sown conditions, non-lodging type
TL-17	2016	90	Punjab Agricultural University, Ludhiana (Punjab)	42.1	13.00	Punjab	Early maturing and suitable for multiple cropping system
Pant Hill Toria-1 (PT-2006-4)	2017	128	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	42.1	7.50	Uttarakhand	Spring type Toria (Sept-Oct sowing)
Pant Toria 508	2017	93	G. B. Pant University of Agriculture and Technology, Pantnagar (Uttarakhand)	42.1	12.40	Uttarakhand	Suitable for multiple cropping system
Raj Vijay Toria 1	2017	100	Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Zonal Research Station, Morena (Madhya Pradesh)	43.4	12.80	Madhya Pradesh	Early sown, irrigated, and rainfed conditions
Tapeshwari (TK 06-1)	2018	92	CSA University of Agriculture and Technology, Kanpur (Uttar Pradesh)	41.6	14.00	Uttar Pradesh	Early sown, irrigated, and rainfed conditions, drought tolerant
Tripura Toria 1	2018	86	ICAR Research Complex for NEH Region, Tripura Centre, Agartala (Tripura)	42.6	9.500	Tripura	Rainfed, upland, and lowland after *Kharif* rice

RSPT-6 (TCN 13-9)	2019	88	Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (Jammu and Kashmir)	42.6	11.30	Jammu	Early sowing under irrigated and rainfed, low fertility areas during *rabi* season
Raj Vijay Toria 3 (RVT 3) (RTM 08-6)	2020	91	Zonal Agricultural Research Station, Morena (Rajmata Vijayaraje Scinedia Krishi Vishwa Vidyalaya), Madhya Pradesh	42.0	13.88	Madhya Pradesh	Suitable for irrigated and rainfed conditions, resistant to white rust, *Alternaria* leaf blight, powdery mildew, downy mildew, and *Sclerotinia* stem diseases
Jeuti (JT 90-1)	2020	91	Assam Agricultural University, Jorhat (Assam)	42.5	4.76	Assam	Suitable for normal condition during *rabi* season
Azad Chetna (TKM 14-2)	2021	93	C.S.A. University of Agricultural and technology, Kanpur (Uttar Pradesh)	42.0	14.0	Uttar Pradesh	Suitable for early sowing during mid-September
Raj Vijay Toria 2 (RMT 08-2)	2021	108	Zonal Agricultural Research Station, Morena (Rajmata Vijayaraje Scinedia Krishi Vishwa Vidyalaya), Madhya Pradesh	42.0	11.5 (Irrigated); 9.0 (Rainfed)	Madhya Pradesh	Suitable for double cropping systems (Toria-Wheat/Onion; Mung-Toria-Vegetable; Early bajra-Toria-wheat/vegetable)

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Table 15.7 (continued)

Variety	Year of release/ notification	Maturity (days)	Source institute	Oil content (%)	Average yield (q/ha)	Area of adoption	Salient features
***Eruca sativa* (Taramira)**							
Jobner Tara (RTM 1351)	2017	140	SKN College of Agriculture, SKN Agriculture University, Jobner (Rajasthan)	39.7	11.60	Rajasthan, Haryana, Punjab, Uttar Pradesh, Gujarat, Delhi, Uttarakhand, and Maharashtra	Rainfed conditions
Jwala Tara (RTM 1355)	2017	134	SKN College of Agriculture, SKN Agriculture University, Jobner (Rajasthan)	38.9	13.70	Rajasthan and Haryana	Rainfed conditions and marginal lands
***B. carinata* (karan rai or African sarson)**							
BJC 1 (PC 6)	2016	157	Punjab Agricultural University, Ludhiana (Punjab)	40.0	19.00	Punjab	Suitable for mechanical harvesting
***Brassica napus* (Gobhi sarson)**							
RSPN 25	2015	150	Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (Jammu and Kashmir)	39	15.90	Jammu and Kashmir	Subtropical regions of Jammu and Kashmir, moderately resistant to diseases
GSC 7 (GSC 101)	2015	154	Punjab Agricultural University, Ludhiana (Punjab)	40.5	22.20	Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, and Rajasthan	Timely sown irrigated conditions, canola quality variety (<2% erucic and <30 ppm glucosinolate)

PGHS 1699 (GSH 1699)	2021	168	Punjab Agricultural University, Ludhiana (Punjab)	41.9	15.81	Punjab, Himachal Pradesh, Jammu and Kashmir	Timely sown irrigated conditions, canola quality variety (<2% erucic and <30 ppm glucosinolate)
Him Palampur Gobhi Sarson 1 (AKMS 8141)	2021	168	CSK Himachal Pradesh Krishi Visvavidyalay, Palampur (Himachal Pradesh)	40.4	15.60	Himachal Pradesh, Punjab, Jammu and Kashmir	Irrigated, timely sown variety having resistance to white rust

- Use of genomics and gene editing tools as precision breeding methods for the development of improved quality and climate-resilient varieties.

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Groundnut Breeding

16

T. Radhakrishnan, Praveen Kona, B. C. Ajay, and Narendra Kumar

Abstract

Considerable progress has been made in groundnut breeding programmes where 233 varieties were released from the past 25 years. The chapter will throw insights on the heritability, gene action of different traits and their improvement using conventional and modern non-conventional approaches with an aim to reach the ultimate goal of farmers or breeders, i.e. yield. Approaches to overcome problems encountered in resistance breeding are discussed, with particular reference to foliar fungal diseases, aflatoxins, viruses, bacterial wilt, insects, drought, heat, etc. Progress in breeding for confectionery groundnut and biofortification and adaptation is also considered. Different techniques were highlighted such as interspecific hybridization and genetic engineering to transfer useful genes from wild *Arachis* species and other sources into *A. hypogaea* lines.

Keywords

Germplasm · Gene pool · Breeding objectives · Karyotype · Genomics

16.1 Introduction

Groundnut is one of the major oil and protein crops grown globally over more than 100 countries. China is the world leader having the largest production of about 16.6 M tons followed by India (7 M tons) followed by Nigeria (3.4 M tons), while the crop is grown over a total of more than 26 M ha with a production of about 44 M tons. Groundnut yield varies considerably across the groundnut-growing countries with an average of 1655 kg/ha, and the highest average yield of more than 3500 kg/

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_16

ha is realized in the USA. Nearly 60% of the cultivation is Asia followed by Africa (32%). In semi-arid tropics of Asia and Africa, the crop is grown by small and marginal under rainfed conditions with limited or no inputs. Groundnut is a rich source of nutrients like protein (about 25%), oil (45–55%), carbohydrates (10–20%), dietary fibre, vitamins, minerals and antioxidants. Nearly 60% of the groundnut produced globally is used for extraction oil for edible and industrial uses, while about 40% is consumed in food and confectionary uses and as seed for sowing the next season crop (Birthal et al. 2010; Janila et al. 2013). Groundnut oil has a high smoking point which makes it a preferred cooking medium in India, China, Vietnam and Myanmar (Singh and Diwakar 1993).

The de-oiled cake of groundnut is a very rich source of protein, and in India it is mainly consumed as animal feed and in preparing food for children and the aged and as soil amendment, while in Europe and North and South America, nearly 75% of the produce is used directly as raw, boiled or roasted and for culinary purposes. In the USA, Canada, and Australia peanut butter is the most popular groundnut-based product. The groundnut shells are used in particle board industry, as a fuel or filler in fertilizer and feed industry and as a substrate for industrial production of pectinase (Dey et al. 2001). Haulm of groundnut is a preferred fodder for livestock, which has a digestibility of around 53%, and it contains carbohydrate (38–45%), minerals (9–17%), protein (8–15%) and lipids (1–3%). Being a nodulating leguminous crop groundnut helps in improving soil health and fertility by leaving behind the nitrogen fixed by the root nodules (Janila et al. 2013).

Significant improvement in the productivity of groundnut globally has taken place during the last 50 years, and starting from an average productivity of 850 kg/ha, we are now at more than 1655 kg/ha with improved cultivars and the crop management practices. Now with the availability of the modern methods of breeding and the genomic resources, the process of cultivar development will be accelerated further. This chapter summarizes the advances in groundnut breeding process for enhancing the genetic gain and to improve the productivity.

16.2 Origin, Evolution, and Distribution

The genus *Arachis* is native to South America, and the species of Arachis are distributed in the river beds of Amazon in the north, Rio de la Plato in the south, the Andes to the west and the Atlantic to the east. The centre of diversity of the genus *Arachis* is the Mato Grasso, Brazil, from where the majority of the species are reported and the origin of the cultivated groundnut, *Arachis hypogaea*, is believed to be northern Argentina and south Bolivia Krapovickas (1969). The other regions from where large number of *Arachis* species were reported are Bolivia, Paraguay, Argentina and Uruguay. Six gene centres, as indicated in Table 16.1, have been proposed for the cultivated types.

The first five centres in the list are considered as secondary centre of diversity, and North-East Brazil and Africa are considered as a tertiary centre. The genetic variability reported from Africa is resulted from the introduction of cultivated types

Table 16.1 Centre of diversity and distribution of cultivated type

Centre of diversity	Distribution of cultivated type
Guarani region (Paraguay-Parana)	Erect Valencia type, ssp. *fastigiata* and intermediate type between var. *fastigiata* and *vulgaris*
Goias and mina Gerais region (Tocatins, San Frasncisco)	Erect type, ssp. *fastigiata* var. *fastigiata* and races of both var. *fastigiata* and *vulgaris*
Rondonia North-West Mato Grosso (Brazil)	Nambyquarae type of ssp. *hypogaea* and erect type with yellow testa colour
Eastern foothills of the Andes and Bolivia	Subspecies *hypogaea* var. *hypogaea* and few races of ssp. *fastigiata* and intermediate of these two ssp.
Peru (Upper Amazon and West Coast)	Races of ssp. *hypogaea*, with constriction, veins and beak, similar type of ssp. *fastigiata* var. *fastigiata* (var. *peruviana*) and ssp. *hypogaea* var. *hirsuta*
North-East Brazil	All morphological types along with intermediate types between var. *fastigiata* and var. *hypogaea*

ssp subspecies, Source: Singh and Simpson (1994)

from different countries especially from Bolivia, Brazil and China (Smartt 1990; Singh and Simpson 1994).

16.3 Systematics and Species Relationships

Groundnuts belong to the legume family (*Fabaceae*), and the members of genus *Arachis* are distinctly differentiated by the geocarpic fruit development and are well defined morphologically as well. The genus *Arachis* is included in the sub-tribe *Stylosanthinae* of the tribe *Aeschynumeneae* along with *Arthrocarpum*, *Chapmannia*, *Pachecoa* and *Stylosanthes* based on their common morphological characters and floral morphology. The taxonomical hierarchy of the genus is as follows:

Kingdom	*Plantae*
Division	*Tracheophyta*
Class	*Magnoliophyta*
Order	*Fabales*
Family	*Fabaceae*
Subfamily	*Faboideae*
Tribe	*Aeschynomeneae*
Genus	*Arachis*

Till the sixteenth century, the only species known under the genus *Arachis* was *A. hypogaea* (Von Linnaeus 1753) and later on five more species, *A. glabrata*, *A. pusilla*, *A. villosa*, *A. prostrata* and *A. tuberosa*, were described by Bentham (1841). Another 11 new species were added by Valls and Simpson (1997), which are yet to be described formally, totalling the number of species to 80 (Lavia 2000). A subgeneric classification dividing the genus into sections and series (Table 16.2) was

Table 16.2 Taxonomic treatment of the genus *Arachis* (Krapovickas 1969, 1973, 1990; Gregory et al. 1980) and proposed genomes

Section	Series	Genome	Chromosome no. (2*n*)
Arachis	1. *Annuae*	A, B, D	20
	2. *Perennes*	A	20
	3. *Amphiploides*	AB	40
Erectoides	1. *Trifoliolatae*	E_1	20
	2. *Tetrafolilatae*	E_2	20
Procumbensae	–	P	20
Caulorhizae	–	C	20
Rhizomatosae	1. *Prorhizomatosae*	R	20
	2. *Eurhizomatosae*	2R	40
Extranervosae	–	Ex	20
Ambinervosae	–	AM	20
Triseminalae	–	T	20

Table 16.3 Most widely adopted classification of groundnut (*Arachis hypogaea* L.)

Subspecies	Varieties	Botanical type	Branching pattern	Growth habit	Seed/pod
Hypogaea	*hypogaea*	Virginia	Alternate	Prostrate to semi-erect	2–3
	hirsuta	Peruvian runner	Alternate	Prostrate	2–4
Fastigiata	*fastigiata*	Valencia	Sequential	Erect	3–5
	vulgaris	Spanish	Sequential	Erect	2

proposed based on morphology, cross-compatibility and pollen fertility of interspecific hybrids (Krapovickas 1973; IBPGR 1990; Gregory et al. 1973, 1980). The cultivated species have been grouped into two subspecies, with two botanical varieties each as in Table 16.3 (Krapovickas and Rigonii 1960; Krapovickas 1969) and further into six botanical varieties (Krapovickas and Gregory 1994) (Table 16.4). This grouping, which is most frequently used, is on the basis of growth habit, branching pattern, flowering pattern, pod and seed characters, seed dormancy, etc. Due to the vide variations and several intermediate forms observed in the germplasm and breeding populations of cultivated groundnut, mostly the classifications are restricted to the three botanical types erect, semi-spreading and spreading for the sake of practical applications.

16.3.1 Gene Pools of Groundnut

The *Arachis* gene pools have been classified into primary, secondary and tertiary based on the cross-compatibility relationships. The primary gene pool of *Arachis* includes the two tetraploid species, viz. *Arachis hypogaea* and *A. monticola* of

Table 16.4 Botanical classification of *Arachis hypogaea* as proposed by Krapovickas and Gregory (1994)

Varieties	Market type	South American location where it is abundant	Characteristics
Subspecies ***hypogaea***			
No floral axes on main stem; alternating pairs of floral and reproductive axes on branches; branches short; less hairy			
Hypogaea	Virginia runner	Bolivia, Amazon	Less hairy; large seeded
hirsuta	Peruvian runner	Peru	More hairy, small seeded
Subspecies ***fastigiata***			
Floral axes on main stem; alternating pairs of floral and vegetative axes on branches			
Fastigiata	Valencia	Brazil Guranian Goias Minas gerais Paraguay Peru Uruguay	Less branches, long upright branches, hairy leaf
peruviana	Peruvian forms	Peru N.W. Bolivia	Less hairy; deep pod reticulation
aequatoriana	Peruvian forms	Ecuador	Very hairy; deep pod reticulation; purple stems; more branched, erect
vulgaris	Spanish bunch	Brazil Guranian Goias Minas Gerais Paraguay Uruguay	More branched; upright branches

section *Arachis*. All the diploid species of section *Arachis* are grouped under the secondary gene pool. The species under the sections other than *Arachis* are grouped under tertiary.

16.3.2 Evolution of Cultivated Groundnut

The cultivated groundnut, *Arachis hypogaea*, is an allopolyploid with two different genomes 'AABB' (Stalker 1992; Raina and Mukai 1999). Krapovickas and Rigoni (1957) proposed the direct amphidiploid origin, while Smartt and Gregory (1967) suggested its origin from a pre-existing wild allotetraploid. A hybridization between an annual x perennial species within the section *Arachis* is considered as the key event in the evolution of groundnut.

Different species, viz. *A. cardenasii*, *A. chacoense*, *A. correntina*, *A. duranensis nom.nud.* and *A. villosa*, were proposed as putative "A" genome donors and *A. batizocoi* as "B" genome donor of *A. hypogaea* (Stalker and Moss 1987; Singh

and Smartt 1998). However, Paik-Ro et al. (1992) reported that *A. batizocoi* is not closely related to *A. hypogaea* and hence cannot be considered as the "B" genome donor. *A. ipaensis* was proposed as putative "B" genome donor on the basis of restriction fragment length polymorphism (RFLP) analysis (Kochert et al. 1991). Fernandez and Krapovikas (1994) supported *A. duranensis* and *A. ipaensis* as the "A" and "B" genome donors, respectively. Based on crossability and molecular data, scientists at ICRISAT proposed *A. hanoei* as the "B" genome and *A. duranensis* as "A" genome donors of cultivated groundnut (Upadhyaya et al. 2011). However, till date no cross combinations between "A" and "B" species could recreate *A. hypogaea*-like species. Molecular studies (Kochert et al. 1991; Halward et al. 1991a; Stalker 1991) at DNA levels (RFLP, PCR, isozymes, and seed storage proteins) have indicated that a large amount of genetic differentiation had already taken place in 'A' and 'B' genomes as reported earlier by Stalker and Dalmacio (1981) and Singh and Moss (1982) thus making it difficult to predict either one or both the putative genome donors of *A. hypogaea.* More recently, Chen et al. (2019) analysed the evolution of the cultivated groundnut with the support of genome sequence information and concluded that asymmetrical evolution involving several hybridizations A genome progenitor undergoing gene loss and conversions, and rearrangement, pointing to the possibility of more A genome progenitors other than *A. duranensis* or hybridization of *A. ipaensis* with several varieties of *A. duranensis* contributed to the formation of the allotetraploid.

16.4 Karyology of *Arachis*

16.4.1 Chromosome Numbers

The chromosome number of cultivated groundnut *A. hypogaea* is of $2n = 40$ (Husted 1931, 1933, 1936; Ghimpu 1930; Kawakami 1930). The first chromosome count reported for a wild species was $2n = 40$ for *A. glabrata* (section: *Rhizomatosae*) (Gregory 1946). However, majority of the wild species in the genus *Arachis* are diploid ($2n = 20$). Though the tetraploid species in sections *Arachis* and *Rhizomatosae* have the same chromosome numbers $2n = 40$, they are reported to be cross-incompatible indicating the independent evolution of polyploidy in the two sections *Arachis* and *Rhizomatosae* of this genus.

Lavia (1998) reported a chromosome number of $x = 9$ for *A. palustris* and *A. praecox* and in *A. décora* (Penaloza et al. 1996) of the section *Arachis*. Among the two series of chromosome numbers that appear to occur in the genus *Arachis* ($2n = 2x = 20$ and $2n = 4x = 40$), the diploid forms are more predominant, and hence the basic chromosome number is believed to be $x = 10$. The proposed basic chromosome number of $x = 9$ in the species *A. palustris* and *A. praecox* might have originated by the selective elimination of a single chromosome from the other species having $x = 10$. On the other hand, Bera et al. (2002) proposed that reverse may be true and species with chromosome number $x = 10$ might have originated by selective duplication of a single chromosome. The presence of two basic

chromosome number ($x = 9$ and $x = 10$) and less existence of polyploid species in the genus *Arachis* indicate that aneuploidy has played a key role in the evolution and speciation of *Arachis* species rather polyploidization. Therefore, the species diversity of *Arachis* may be mainly due to structural chromosomal rearrangements and thus supports the theory that groundnut is segmental polyploidy and that the section *Arachis* represents the most advanced traits within the genus.

16.4.2 Karyotype and Pairing Behaviour

The chromosomes of groundnut are small ranging from 1.4 to 3.9 μm in length and are predominantly metacentric. Several karyotypic analysis have revealed a pair of a small chromosomes termed as "A" chromosomes and another pair with a secondary constriction, termed as "B" chromosomes in the somatic cells of *Arachis* (Husted 1933, 1936; Stalker and Dalmacio 1981; Singh and Moss 1982; Stalker 1991). However, the "A" chromosome was absent in the species, *A. batizocoi*, *A. cruziana*, *A. magna*, *A. williamsii* and *A. ipaensis* which had the "B" chromosome pair (satellite chromosome) (Smartt et al. 1978). Babu (1955) reported several types of secondary constriction in *A. hypogaea*, and D'Cruz and Tanskasale (1961) and Stalker and Dalmacio (1986) proposed that cultivars could be distinguished based on karyotype differences. On the basis of this, the "A" and "B" genomes designated in groundnut to describe the two cytological groups. Stalker (1991) designated *A. glandulifera* as "D" genome species, and further, the B genome was divided into B, F and K genomes by Seijo et al. (2004) and Robledo and Seijo (2010).

The meiotic chromosomes of *A. hypogaea* pair mostly as 20 bivalents, but a few multivalents occasionally have also been reported (Husted 1936). Husted (1936), Raman (1976) and Stalker (1980) concluded that structural differences in chromosomes exist between the two subspecies *hypogaea* and *fastigiata* and intraspecific hybrids mostly have bivalents at metaphase I, though univalents also exist at a low frequency.

16.5 Plant Genetic Resources

Genetic resources are the reservoir of variability in economically important traits which are invaluable in crop improvement. The largest collection of germplasm (15,445 accessions from 93 countries) is held at ICRISAT, India (Pandey et al. 2012). The ICAR-NBPGR, India, has 14,585 accessions in their gene bank, while the ICAR-Directorate of Groundnut Research, India, holds 9024 accessions. The US Department of Agriculture (USDA) stores 9917 accessions, and the Oil Crops Research Institute (OCRI) of China has 8083 accessions, while the Crops Research Institute of the Guangdong Academy of Agricultural Sciences in China conserves 4210 accessions. In addition to this, small to medium collections are held at the different research organizations across the world.

Though the number of wild species available in the gene banks prior to the 1980s were very few, the explorations in Bolivia, Brazil, Paraguay and northern Argentina have enriched the collection (Stalker 2012). So far, 83 species of *Arachis* have been described, and more than 3400 accessions of *Arachis* species have been documented of which nearly 1300 are available in field gene banks or storages (Stalker et al. 2002). The largest collection of *Arachis* species are with Brazilian Agricultural Research Corporation (EMBRAPA), Brazil and Texas A & M University (1200 accessions each). The USDA holds 607 accessions, and the ICRISAT has 477. More than 400 accessions are being held at the Instituto de Botánica del Nordeste (IBONE) in Argentina and at North Carolina State University (Singh and Simpson 1994; Pandey et al. 2012). About 50% of wild species accessions have <50 seeds in storage, and several are propagated in greenhouses or in field as vegetatively (Stalker 2012).

In order to facilitate the utilization of the large number of germplasm, a representative collection of about 10% of the entire germplasm collection has been identified so as to have a manageable and cost-effective starting point in identifying candidate genotypes with new sources of disease and pest resistance or abiotic stress tolerance (Brown 1989). Such representative collections have been designated as core collection comprising 1704 *A. hypogaea* accessions was developed at ICRISAT, which is similar to the USA. With the increasing number of the accessions, the size of these core collections also has become too large for easy exploitation by breeders. Therefore, "mini-core collections" (i.e. 10% of the core collections and 1% of entire germplasm collection).

In addition to germplasm collections, amphiploids and autotetraploids (Mallikarjuna et al. 2011), chromosome segment substitution (CSSL) lines (Fonceka et al. 2012) and multiparent advanced generation intercross (MAGIC) populations (Janila et al. 2016a) have also been developed to facilitate groundnut breeding.

16.6 Floral Biology: Emasculation—Pollination Techniques

16.6.1 Plant Morphology and Floral Biology

The groundnut seed is dicotyledonous with a stem axis, leaf primordia, hypocotyl and primary root. It is interesting to note that all primordial leaves and above-ground structures appearing within the first few weeks after germination are already present in the seed. Germination is epigeal; hypocotyl is white and very prominent in the early stages of growth but becomes indistinguishable from the roots as the plant matures. The primary root system is tap rooted, but many lateral roots develop about 3 days after germination. Even though roots can reach 135 cm deep, generally they are restricted to 5–35 cm below the soil surface (Intorzato and Tella 1960). Roots do not have typical root hairs, but tuffs of hairs emerge in the axils of lateral roots (Moss and Ramanatha Rao 1995). Although groundnut has a symbiotic relationship with *Bradyrhizobium*, root hairs are not the site of infection as observed in many legumes (Elkan 1995).

Fig. 16.1 Parts of a groundnut flower

Stems are solid, but become slightly hallow when the plant matures. The main stem develops from a terminal bud of the epicotyl, and two cotyledonary laterals develop and grow on the opposite sides near the soil level. The main stem can be upright or prostrate depending on the botanical type. The shape of the leaflets on the main stem and lateral branches may vary. Branching pattern of reproductive to vegetative nodes on the cotyledonary laterals is one of the prime criteria for grouping of the two subspecies.

Groundnut inflorescence is unique among domesticated plants in that it flowers above ground but produces seeds below the soil surface. Flowers are borne on axils of leaves on primary or secondary branches, spike-like, simple or compound monopodia, and each node has up to five flowers. However, three flowers per inflorescence are most common. Only one flower per inflorescence opens at any given time. The flowers are modified sessile and papilionaceous that appear to be stalked due to the presence of a tubular hypanthium or "calyx tube". The flower is subtended by a bract, with a second bract on the inflorescence branch. There are two calyx lobes, an awn-like one opposite the keel (includes one sepal) and a broad one opposite to the back of the standard (includes four sepals-fused). The corolla consists of five petals (one standard, two wing, two keel), and the calyx has five sepals both are borne at distal end of the calyx tube. The colour of the standard petal varies from light yellow to deep orange or rarely white. A central crescent area exists on the face of the standard petal which can be deeper in colour as that of standard or even express a different colour. The colour of the standard and wing is usually the same though there are varietal differences.

The style is enclosed within the calyx tube, and both calyx tube and style elongate rapidly up to 5–7 cm in 24 h prior to anthesis. The androecium is a monoadelphous structure with the staminal tube bearing five oblong and five globular anthers (Fig. 16.1). The filaments are fused for two-thirds of their length. Among the globular anthers, two are sterile. This number usually varies in different varieties. In erect types, the sterile anthers are more common, while it may be absent in spreading types.

The eight fertile normally developed anthers consist of four globose, dorsifixed, uniloculate anthers alternating with four adnate, introse, oblong anthers. Ovary is superior, small and conical with a beak-shaped point at the tip and contains a single

sessile carpel with 1 to 6 ovules; style is glabrous throughout its length and covered with bristles near the club-shaped stigma and is enclosed in a filiform hypanthium.

16.6.2 Flower Emergence and Opening

Groundnut produces a large flush of flowers of which nearly 40% of flowers fail to develop pegs or pegs do to produce mature pods and nearly 20% of flowers produce mature fruits. Genotypes which flower early and produce most of the flowers during first 2 weeks of the flowering period produce greater number of pods. Depending on photoperiod, temperature and genotype, flowering starts at about 25 days after emergence. The number of days required to first flowering increases from 24 to 38 days when the daily mean temperature rises to 20–30 °C in spreading and semi-spreading types, while it drops from 35 to 24 days in Spanish and Valencia types. The most prolific flowering occurs between 5 and 11 weeks after sowing depending on the duration of cultivar and the season with a high degree of first formed flowers producing mature fruits.

Usually four to flushes of flowering can be observed in a groundnut plant. Very few flowers are produced in the initial flush which is followed by a large number of flowers in the second flush. After reaching a peak in the second flush, there will be a gradual decline in the number of flowers in the subsequent flushes. In some genotypes, there may be only two flushes of flowering, and generally the fruits developed from the flowers of the later flushes may not reach maturity at the time of harvest.

16.6.3 Anthesis and Pollination

Anthesis takes place when the flower bud is 50–70 mm long. Flower buds generally open at the beginning of the light period; it may be delayed in cold or wet weather. The pollen matures 6–8 h before anthesis and has two generative nuclei at the time of anthesis. The self-pollination occurs because the stigma and anthers are enclosed by the keel. Cross-pollination (ranging from 0 to 6.16%) also occurs through bees to a very limited extent. The stigma is at the same level or protrudes beyond anthers, papillate type without surrounding hairs (surrounded by many papillae), elongated and strongly curved. Stigma is receptive before anthesis. Pollination takes place at or near the time of anthesis (flower opening). Enzymes associated with pollen germination are produced on the stigmatic surface from 48 h before to 8 h after anthesis.

The ovary is unilocular and has 1–3 ovules, superior with the calyx tube attached to the base of the ovary. Fertilization is complete within 6 h after pollination or before midday. After fertilization, the flower drops, and the hypanthium and the style may remain attached to the base of the ovary for 4–5 days. The ovary at the base of the calyx tube starts growing actively within a week by the activation of the intercalary meristem located below the ovary. The green ovary becomes purplish at its tip, and the developing ovary pierces through the floral parts to produce ageotropically elongating peg (botanically a “carpophore” or “gynophore”) which

carries the ovule at its tip. The pegs stop growing after penetrating the soil. The normal pod-forming zone is 4–7 cm below soil surface, and the optimum temperature in zone is about 31–33 °C. Lower soil temperature around 23 °C increases number of pods and pod weight but increases filling duration thus increasing maturity. It takes about 60 days from the time of fertilization to full maturity.

Pods are elongated with varying degrees of reticulation on the surface. They contain two to five seeds although differences exist among members of the subsp. *hypogaea* and *fastigiata.* Seeds (kernels) may be oval, round or elliptical, have pointed or flattened ends, vary in seed coat colour from off-white to deep purple and may be monochrome or variegated. Seed size ranges from 0.15 to more than 1.3 g/seed, while the seeds of the wild species weigh as low as 0.047 g/seed (Stalker 1997). For further understanding on morphological variations in groundnut readers may refer to IBPGR/ICRISAT (1992); Krapovickas and Gregory (1994); IBPGR (1990); Rao and Murty (1994), and Stalker and Simpson (1995).

16.6.4 Emasculation and Pollination Techniques

Flower buds of appropriate size, which may open the next day, are emasculated in the evening. Different methods are employed for emasculation.

16.6.4.1 Ring Cut Method

Described by Kale and Mouli (1984) where bud was hold between the thumb and the index finger of the left hand, and with the help of a razor blade in the right hand, a superficial circular incision was made with a razor blade in the bud at about two-thirds down from the top or 2 mm above the base. Using a forceps the standard petala and calyx are removed exposing the wing petals. The wing petals are forced back using the forces to expose the keel so as to remove the anthers without damaging the style and stigma.

16.6.4.2 Straw Tube Insertion Method

Described by Reddy et al. (1970), a razor blade is used to make a cut on the depressed side of the bud at two-thirds of its length below the tip so as to cut the standard and a portion of the wing petals. The sepals and petals, except the keel, are removed. Emasculation is carried out with forceps after separating the stamens and the pistil from the keel. Verify with the help of a magnifying glass that all the anthers are removed. A 4–5 cm straw (used for sipping drinks) is then inserved over the calyx tube and close the upper open end with the help of the forceps. The straw has to be removed at the time of pollination and re-instated after pollination.

16.6.4.3 Paper Towel Use Method

Norden and Rodriguez (1971) described a Paper Towel method where the flower bud is emasculated by first removing the lower lip of the calyx and then the wing and keel petals. The standard, which is retained, is held out of the way, while the anthers are removed. The standard returns to its original position and curls over the stigma after emasculation. A paper towel, approximately 12 cm × 12 cm, is dampened with

water and placed around the flower immediately after pollination. A slit is made in the towel on one edge to slide it in between the stem and leaf axil. The wet paper towel provides shade and a favourable environment for the germination and subsequent growth of pollen on the stigma.

The emasculated buds are marked by coloured nylon threads which are date coded. Pollinations are to be done in the early morning hours of the subsequent day of emasculation. Pollens can be dusted directly on the stigma from the detached flowers of the pollen parent of by using a forceps or brush. Pegs will start generally 5–7 days after pollination.

16.7 Genetic Studies of Qualitative and Quantitative Traits

Majority of the traits of agronomic importance in groundnut follow quantitative inheritance and, hence, highly have a high G × E interaction. Additive inheritance was observed as principal component of variance when the parental genotypes are from the same botanical varieties while when crosses involve different botanical varieties non-additive variance also reported (Table 16.5).

16.7.1 Heritability of Various Traits

The heritability estimates reported were dependent on the experimental design, genotypes used and the traits studied, method used to estimate, environmental conditions and the controls used (Table 16.6).

16.8 Breeding Objectives

16.8.1 Breeding for Yield and Yield-Related Traits

Most extensively targeted traits of groundnut are the yield and yield contributing traits in the crop improvement programmes worldwide. High yields in terms of pod and seed are the main goals of a groundnut breeder. Due to large G × E interactions selection for yield per se was low and slow (Nigam et al. 1991a) even though it is a major basis for improving groundnut productivity in the world. The pod yield is a function of crop growth rate, fraction of crop growth rate and the duration of reproductive growth partitioned towards pod yield. Therefore, better understanding physiology of yield is also necessary to better target yield increase. Number of pods per plant, pod yield per plant, 100-seed mass and shelling outturn are the important yield contributing parameters. There exists a wide gap between the realized and potential yields mainly due to rainfed cultivation of the crop with little or no inputs, and recurrent drought coupled with biotic factors reduces yield of groundnut in *Kharif* season. Developing *Kharif* groundnut varieties with stress tolerance along with low inputs response and *rabi*-summer cultivation varieties with response to high nutrient and water management conditions will be fruitful and increase the productivity.

Table 16.5 Genetics/inheritance of various traits in groundnut

S. no.	Trait	Reported inheritance in literature
1.	*Plant type and associated traits*	
	Growth habit—erect/bunch (Valencia and Spanish types), semi-spreading/ spreading/runner (Virginia type)	Complex inheritance—monogenic, digenic, trigenic, tetragenic, gene cytoplasmic and maternal inheritance for growth habit; generally spreading habit dominant over bunch habit but in some cases, the opposite also found; semi spreading habit monogenic dominant to bunch and spreading forms; spreading dominant to bunch with complementary and duplicate effects of two genes
	Plant height	Both additive and on-additive gene effects
	Dwarf plant stature	Different forms of dwarfism are observed: sterile brachytic, fertile dwarf, etc. Sterile brachytic—monogenic recessive in natural and induced mutants; two recessive complementary factors; trigenic complementary; tetragenic inheritance with two sets of factors with complementary-duplicate action; fertile dwarf—normal monogenic dominant over dwarf with cytoplasmic modifiers; more than one gene involved in plant height determination; dominant with variable expressivity and penetrance
	Canopy breadth	Additive gene effects; partial dominance
	Branching vs. non-branching	Branching in Virginia type monogenic dominant over non-branching Valencia type; monogenically recessive/double recessive for suppressed primary branches in induced mutants; quantitative inheritance
	Number of primary branches	Both additive and non-additive gene effects
	Number of secondary branches	Both additive and non-additive gene effects
	Length of primary branches	Both additive and non-additive gene effects
	Reproductive branches on main stem	Absence is dominant to the presence; two sets of duplicate loci with epistatic control, when both loci of each set or all four loci are recessive flowering occurs; two, three or four sets of homozygous recessive loci or modifying factors
	Stem pigmentation	Purple or red pigmentation monogenic dominant/incompletely dominant over light or green colour; monogenic, digenic duplicate and digenic complementary inheritance for purple colour; two sets of

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
		genes of which one responsible for purple and the second for green pigmentation, extra nuclear factors; duplicate recessive for white stem
	Stem pubescence	Stem pubescence monogenic dominant/ incompletely dominant over absence of pubescence; monogenic inheritance with overdominance of hairiness
2.	*Leaf traits*	
	Elliptical shape	Elliptical leaflet shape on the main axis monogenic recessive to elliptical-oblong shape
	Krinkle leaf	Both monogenic dominant and recessive in different krinkle mutants
	Mottled leaf	Monogenic dominant to normal leaf in natural mutants
	Narrow leaf	TMV 2 narrow leaf mutant and Gujarat narrow leaf mutant are genetically different; partial dominant monogenic; monogenic dominant in induced mutant
	Cup leaf	Monogenic recessive in induced mutant
	Flop leaf	Monogenic recessive and digenic recessive in induced mutants
	Curly leaf	Monogenic recessive in a natural mutant
	Corduroy leaf	Duplicate recessive in induced mutant
	Puckered leaf	Recessive with 13 normal: 3 puckered leaf ratio
	Dark green leaf colour	Dark green colour monogenic dominant/ incomplete dominant over light green colour; dark green colour duplicate recessive in radiation-induced mutant; light green colour dominant over dark green colour in crosses involving Chico variety with at least two dominant alleles of one of the two loci or one dominant allele each at both loci necessary for regulation of chlorophyll synthesis
	Lutescent leaf colour	Two duplicate recessive genes
	Golden yellow colour	Two duplicate recessive genes
	Albinism	Duplicate recessive, triplicate recessive, trigenic model in which duplicate loci controlling chlorophyll development epistatic to a third locus governing a zygotic lethal; cytoplasmic factors influencing expression of nuclear genes governing albinism

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Variegated leaf	Monogenic dominant in induced mutant, maternally inherited
	Leaflet size/leaf area	Intra-plant variation in leaflet size common in groundnut. Large leaflet size incompletely/completely dominant over small size; monogenic/duplicate recessive in induced and natural mutants; predominant additive gene effects for leaf length and both additive and non-additive gene effects for leaf width; predominantly non-additive gene effects and reciprocal effects for leaf area
	Petiole length	Both additive and non-additive gene effects important; three recessive genes for short petiole
	Number of stomata	Low number monogenic recessive and digenic recessive
	Number of leaves on main stem	Both additive and non-additive gene effects
	Number of leaves on cotyledonary branches	Predominantly additive gene effects
3.	*Inflorescence*	
	Inflorescence length	Elongated inflorescence dominant over condensed inflorescence with two genes with complementary interaction
	Type of inflorescence	Two complementary genes with 9 simple: 7 compound inflorescences in Valencia (simple) × Virginia (simple)crosses, however, a ratio of 13 compound—3 simple inflorescences observed in Valencia (simple) × Spanish(compound) crosses suggesting presence of two genes inhibiting expression of simple inflorescence in Spanish type
	Colour of standard petal	Five different intensities of standard petal colour observed; in general deep colour dominant over light colours; orange monogenic dominant/incomplete dominant/co-dominant over white; faint orange monogenic recessive to orange; duplicate recessive control of white flower when crossed with lines having yellow flowers; incomplete monogenic dominance of yellow flower over white flower; additive effects of two different genes for yellow colour; lemon yellow dominant to orange flower with presence of transposable elements; two complementary genes for garnet colour which is dominant over orange colour; trigenic control

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Presence vs. absence of standard crescent	Purple crescent dominant to no crescent governed by duplicate genes; single dominant gene for purple crescent
	Number of pegs	Both additive and non-additive gene effects
	Peg strength	Significant SCA
	Peg pigmentation	Monogenic dominant/digenic inheritance for purple pigmentation of pegs
	Brachytic sterility	One, two, three or more recessive genes; two sets of factors with complementary duplicate action condition brachytic character
	Female sterility	Monogenic and trigenic recessive inheritance
	Male sterility	Two recessive genes
4.	*Pod traits*	
	Pod size	Large size dominant over small size and monogenic, digenic, trigenic and multigenic control reported; small size dominant over large size with duplicate gene interaction; predominantly additive gene effects
	Pod length	Normal length dominant over long length with duplicate genes; both additive and non-additive gene effects, maternal effects
	Pod width	Both additive and dominance gene effects, maternal effects
	Pod constriction	Absence of constriction or shallow constriction dominant over deep constriction and controlled by two independent dominant genes; trigenic complementary inheritance for shallow constriction; three unlinked loci and cytoplasmic factor
	Pod reticulation	Monogenic dominant for strong reticulation over shallow or absence of reticulation; at least four factors for deep reticulation; smooth pods dominant over reticulated pods with digenic inhibitory gene action (13 smooth: 3 reticulated types)
	Pod pubescence	Two loci with additive gene action
	Pericarp thickness	Monogenic dominant thin pericarp over thick pericarp; five factors with thin pericarp dominant over thick pericarp; three complementary genes for moderately thick pericarp

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Pod beak	Non-beaked pods monogenic dominant over beaked pods; prominent beak monogenic dominant over non-beaked pods
	Aerial podding	Aerial podding monogenic dominant over normal podding and the opposite also true depending upon the genetic backgrounds of the parents
	One-seed pod	Any two of three duplicate recessive genes control one-seed pod
5.	*Seed characters*	
	Number of seeds per pod	Three or more seeds dominant over fewer seeds with trigenic/monogenic control; fewer than three seeds dominant over three or more seeds with monogenic control
	Seed size	Large seed size monogenic dominant over small size with possible modifiers; five pairs of genes with four having isodirectional effects
	Shrivelled seeds	Single recessive gene
	Seed shape	Round shape monogenic/duplicate genes recessive to elongated seeds; seed length maternally controlled; button type seeds dominant over normal seeds (round or elongated) with digenic ratio of 13:3 indicating influence of inhibitory genes on seed shape
	Seed ends	Flat ends of seed monogenic dominant over smooth ends
	Seed length	Mainly additive gene effects and significant reciprocal/maternal effects; both additive and dominance gene effects
	Seed width	Mainly additive gene effects and significant reciprocal/maternal effects; both additive and dominance gene effects
	Seed length and width ratio	Mainly additive gene effects and significant reciprocal/maternal effects
	Hard seed	Paternal inheritance, both hard and soft seeds are allelic and follow monogenic inheritance in induced mutant; the gene for hard seed has pleiotropic effect
	Rough testa	Duplicate genes with recessive epistasis with 9 rough:7 smooth testa
	Testa colour	Inheritance of testa colour varies from simple to very complex. Seven gene pairs/ nine loci interacting in several ways to produce different testa colour

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	(a) Flesh (rose/pink/russet or tan) testa	Duplicate dominant loci
	(b) White testa	Recessive to coloured tests; two different dominant genes epistatic to red and flesh testa colour in specific genotypes
	(c) Red testa	One dominant gene/partial dominance/ single recessive gene/two duplicate recessive genes/two complementary recessive genes/polygenic control responsible for red testa colour
	(d) Purple testa	Partial/complete dominance over all other colours with inheritance monogenic/ digenic epistatic, duplicate, digenic cumulative, trigenic and tetragenic with complex interactions; duplicate recessive
	(e) Wine testa	Monogenic recessive
	(f) Chocolate testa	Monogenic/digenic recessive in an induced mutant
	(g) Variegated testa	Dominant/partially dominant and recessive to non-variegated testa; monogenic/digenic/two genes with cumulative effects; trigenic inheritance with epistatic effect of purple over red
	Seed coat splitting	Monogenic inheritance with additive effects, duplicate additive and digenic complementary
6.	*Yield and related traits*	
	Pod yield	Both additive and non-additive gene effects
	Number of mature pods per plant	Both additive and non-additive gene effects
	Number of immature pods per plant	Both additive and non-additive gene effects
	Shelling outturn	Governed by a pair of genes without dominance; both additive and non-additive gene effects
	Weight of seed per plant	Both additive and non-additive gene effects
	Seed number per kg	Predominantly additive gene effects
	% sound mature kernels	Predominantly on-additive gene effects
	100-pod weight	Both additive and non-additive gene effects
	100-seed weight	Both additive and non-additive gene effects; mainly additive gene effects and significant reciprocal/maternal effects
7.	*Haulm yield and quality*	
	Green weight/haulm yield	Both additive and non-additive gene effects; both GCA and SCA for biomass

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
8.	*Life cycle*	
	Annual vs. perennial	Perennial growth habit dominant over annual habit
9.	*Crop duration*	
	Early maturity and its components	Late maturity monogenic dominant/ incompletely dominant over earliness; four or five genes with complete dominance of lateness over earliness and absence of reciprocal differences; single gene with additive effect/predominantly additive gene effects for days to flower, three genes with two types of epistasis (dominant recessive, 13 late: 3 early and duplicate dominant, 1 late: 15 early) for days to accumulation of first 25 flowers and absence of reciprocal differences; two recessive genes acting in an additive manner for days from seedling emergence to first flower; significantly higher GCA variance than SCA variance for time of emergence (in h), time to first leaf opening on cotyledonary branch (in h), time to first leaf opening on main stem (in h), days to first flower and number flowers per plant (at 32 days) under controlled environment conditions; significantly higher GCA variance than SCA variance for days to first flower under field conditions; significant additive genetic variance with bidirectional dominance/non-additive gene effects for days to first flower; significant additive genetic variance for pod and seed maturity indices; both additive and dominance genetic effects for maturity index; highly significant additive and significant dominance, additive × additive and dominance × dominance effects with duplicate digenic interaction for days from emergence to first flower, more than two genes or linkage effects between the genes for number of flowers produced during first 4 days of flowering, highly significant additive and significant dominance effects for percentage of ripe pods at 80 days after sowing; predominantly non-additive gene effects for days to maturity
10.	*Biochemical/nutritional traits*	
	Oil content	Both additive and non-additive gene effects; epistatic interaction

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Protein content	Both additive and non-additive gene effects; epistatic interaction
	Oleic acid content	One/two recessive genes for high oleic acid
	Fatty acids (palmitic, stearic, oleic, linoleic, arachidic, eicosenioic, behenic and lignoceric fatty acids, total saturated fatty acids and long chain saturated fatty acids)	Additive gene effects and additive × additive interaction
	Oleic/linoleic fatty acid (O/L) ratio	Single recessive or two recessive genes and some possible modifiers depending upon the parents involved in the crosses; additive gene effects and additive × additive interactions
	Polyunsaturated/saturated fatty acid (PS) ratio	Both additive and dominance gene effects and additive × additive and additive × dominance interactions
	Arginine content	Two major genes with partial dominance for low arginine
	Iodine value	Both additive and dominance gene effects and additive × additive and additive × dominance interactions
	Soluble sugars	Predominantly additive gene effects
11.	*Physiological traits*	
	Iron chlorosis	One basic gene and two or four inhibitory complementary genes for expression of iron chlorosis
	Harvest index	Both GCA and SCA; predominantly GCA; predominantly additive gene effects with additive × additive epistasis
	Leaf chlorophyll content	Quasi-quantitative with modifiers either in positive or negative direction in different genetic backgrounds
	Chlorophyll a, chlorophyll b and total chlorophyll	Non-additive gene effects with significant additive × dominant epistatic interaction
	Carotenoid content	Dominant gene effects with significant additive × dominance and dominance × dominance interactions
	SPAD chlorophyll meter reading (SCMR)	Both additive gene effects and dominance gene effects with duplicate and complementary epistatic and additive × additive interactions
	Specific leaf area (SLA)	Predominantly additive gene effects and additive × additive interactions; predominantly dominance gene effects with duplicate epistasis

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Specific leaf weight	Predominantly non-additive gene effects
	Fresh seed dormancy	Complete/incomplete monogenic dominance for dormancy over nondormancy; multigenic control.
	Apparent photosynthesis	Dominance and overdominance effects
	Response to photoperiod	Mainly GCA, additive gene effects in some cases and partial dominance to dominance in others. Maternal effects
12.	*Nitrogen fixation*	
	Nodulation and nitrogen fixation	Nodulation dominant to non-nodulation; monogenic, digenic and trigenic inheritance; a trigenic model proposed—the first two genes produce nodulation, while the third one inhibits nodulation when dominant and the former two in homozygous recessive condition. Although the presence or absence of nodulation is governed by a few major genes, the intensity of nodulation appears to be controlled quantitatively; predominantly additive gene effects for nitrogen fixation; both additive and non-additive gene effects and additive x additive, additive x dominance and dominance x dominance interactions for nodule number and nodule mass per plant, nitrogenase activity and acetylene reduction; non-additive gene action and reciprocal effects for nitrogenase activity, nodule number, nodule mass and total nitrogen; predominantly SCA for nodule number and weight, specific nitrogenase activity, shoot weight and total plant nitrogen, maternal effects also important
13.	*Disease resistance*	
	Rust	Sexual stage and races in groundnut rust pathogen not yet observed. Monogenic/digenic/trigenic inheritance with resistance being recessive; preponderance of non-additive gene action; greater dominance variance; dominant, partial dominant or additive gene action for resistance; both additive and non-additive gene effects and additive × additive and additive × dominance interactions; significant GCA and SCA for rust resistance; resistance dominant/partial dominant in wild *Arachis* species. The resistance is stable overs years and locations

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Late leaf spot (LLS)	LLS and ELS inherited independently; duplicate complementary recessive in induced mutants; resistance recessive and level of resistance controlled by presence of recessive gene(s) at any or all of the five loci; 4–5 duplicate recessive genes; both additive and non-additive gene effects and additive × dominance epistatic, maternal effect; additive gene effects for components of resistance (lesion number, lesion area, defoliation, latent period and spore production)
	Early leaf spot (ELS)	Chemical-induced physiological races observed which may give differential reaction to resistant sources. Additive and non-additive gene effects and additive × additive gene interaction for resistance with involvement of cytoplasmic factors; duplicate recessive in induced mutants
	Sclerotinia blight	At least two genes involved; quantitative inheritance with involvement of dominance, epistatic and cytoplasmic factors
	Cylindrocladium black rot	Predominant additive gene effects; complex inheritance with resistance delaying the onset of disease
	Aspergillus flavus/*A. parasiticus* and aflatoxin production	No correlation between aflatoxin content and in vitro seed colonization and the population density of *A. flavus* in the soil; three resistance mechanisms—preharvest resistance, seed coat resistance (in vitro seed colonization and cotyledon resistance (aflatoxin production)), all inherited independently; predominance of additive gene effects for seed coat resistance, reciprocal differences observed; predominantly non-additive gene effects for aflatoxin production
	Groundnut rosette disease(GRD)—chlorotic rosette and green rosette	Three agents responsible for expression of disease symptoms – groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV) and satellite RNA (SatRNA); two independent recessive genes for resistance to GRD (effective against GRV and SatRNA but not against GRAV)

(continued)

Table 16.5 (continued)

S. no.	Trait	Reported inheritance in literature
	Peanut bud necrosis disease(PBND)	Three resistance factors inherited additively for reduced disease incidence; highly significant GCA and significant SCA and reciprocal effects; non-additive/additive/dominance/epistatic and additive x additive gene effects in different crosses
	Tomato spotted wilt virus disease (TSWV)	Significant GCA and SCA; transgressive segregation for resistance observed
	Peanut stripe virus disease (PStV)	Significant GCA and SCA
	Bacterial wilt	Partially dominant involving three pairs of major genes and some minor genes; recessive resistance; nucleo-cytoplasmic interaction and both additive and dominant gene actions
14.	*Resistance to nematodes*	
	Meloidogyne arenaria (root-knot nematode) race 1	Resistance conferred by single/two dominant genes (one inhibits root galling and the other inhibits egg production)
15.	*Insect pest resistance*	
	Leaf hopper	Resistance controlled by three recessive genes with additive effects; predominant additive gene effects for long trichomes on mid-rib and petiole and jassid damage; predominant non-additive gene effects for short, medium or long trichomes on adaxial leaf surface, margins, mid-rib and petiole
	Aphids	Single recessive gene for resistance
	Leaf miner	Significant GCA and SCA

Adapted from Nigam (2014)

Recently, physiological traits like harvest index, transpiration-use-efficiency, etc. which are associated with yield are also gaining attention in breeding programmes where necessary infrastructure and resources are available. High peg-strength to reduce harvest losses, ease in shelling, more number of seeds per pod, pod characters like constriction, beak and reticulation, kernel shape and uniformity, testa colour and blachability have also emerged as traits being sought after by farmers and industry. Haulm yield coupled with pod yield is an important consideration for development of dual-purpose varieties. For improved quality of haulm, the traits to be targeted are digestibility and nitrogen content. To tide over the problem of viviparity in Spanish varieties resulting from unseasonal rain, fresh-seed dormancy and short duration (maturing in nearly 90 days) are traits or relevance. Early maturity makes crop suited for multiple cropping systems by evading the drought and frost stress conditions. Lack of fresh seed dormancy results in in situ germination and leads to loss in pod yield and quality in rainfed environments when rain coincide with the maturity stage of the crop. Globally released most of the high-yielding varieties of groundnut were

Table 16.6 Heritability of various traits reported from different studies in literature

S. no.	Trait	Broad sense heritability (H)	Narrow sense heritability (h2)
1.	Height of main axis	33.7–97.2	71.0
2.	Branching pattern	90.4	55.3
3.	No. of primary branches	33.5–94.5	23.2–59.0
4.	No. of secondary branches	12.0–98.9	–
5.	Length of primary branch	28.0	64.5
6.	Length of secondary branch	38.0	36.2
7.	Shoot dry weight	37.0–100.0	–
8.	Plant fresh weight	1.0	–
9.	Total dry matter	38.8–98.0	–
10.	No. of nodules per plant	44.3	–
11.	Single nodule weight	42.9	–
12.	Nodule weight per plant	33.0–100.0	–
13.	Nitrogenase activity	60.8	–
14.	Acetylene reduction	31.0	–
15.	Days to emergence	48.0–83.0	51
16.	Days from emergence to first flower	11.9–96.9	23.0–39.0
17.	Days to 50% flowering	66.5–96.3	60.0
18.	Days from emergence to accumulation of 25 flowers	17.0–61.0	–
19.	Number of flowers produced during first 4 days of flowering	16.0–44.0	9.0–38.0
20.	Peg number	54.2–56.2	11.7–45.7
21.	Peg strength	74.1	–
22.	Peg length	74.0	37.7
23.	Peg diameter	75.0	–
24.	Pod-peg ratio	48.8	–
25.	Days to maturity	91.7–98.6	–
26.	Fruit maturity based on oil pigmentation	69.0–95.0	
27.	Maturity based on hull scrap method	71.0	
28.	Percentage of ripe pods 80 days after sowing	13.0–41.0	24.0
29.	No. of immature pods per plant	3.7–92.7	1.4–5.1
30.	Pod size	–	42.0–50.0
31.	No. of mature pods per plant	26.1–100.0	31.7–32.0
32.	Pod weight	42.0–80.0	–
33.	Pod length	54.0–92.0	–
34.	Pod yield per plant	13.2–98.0	59.8
35.	100-pod weight	75.0–100.0	–
36.	Seed yield	38.3–83.9	31.5
37.	100-seed weight	28.6–100.0	57.3–66.8
38.	Harvest index	27.7–100.0	–

(continued)

Table 16.6 (continued)

S. no.	Trait	Broad sense heritability (H)	Narrow sense heritability (h2)
39.	Shelling outturn	33.3–100.0	10.5–42.0
40.	Oil content in seed	22.0–94.4	29.0
41.	Protein content in seed	43.0–64.0	14.0
42.	Zn content in seed	92.0	–
43.	Fe content in seed	81.0	–
44.	Carbon isotope discrimination (Lab)	53.0	–
45.	Carbon isotope discrimination (Field)	75.0–89.0	–
46.	Transpiration efficiency	34.0–86.0	–
47.	Total transpiration	12.0–70.0	–
48.	SPAD chlorophyll meter reading	9.0–98.0	–
49.	Specific leaf area	5.0–98.0	–
50.	Drought tolerance index	54.0–98.0	–
51.	LLS disease index	–	22.0–27.0
52.	LLS resistance	–	0–13.0
53.	ELS resistance	51.7	–
54.	Resistance to CBR	48.0–65.0	51.7
55.	Resistance to S. miner# of days until plants wilted Disease rating	41.5–50.314-23	−1.0-11.0
56.	Seed coat resistance to A. flavus	78.5	–
57.	Resistance to *A. parasiticus*. Dry seed resistance. ii. Aflatoxin production. iii. Pre-harvest resistance	30.0–65.020.0–65.027.0–33.0	–
58.	Fodder quality traits. (1) Nitrogen content. (2) In vitro organic matter digestibility (OMD) (3) Metabolizable energy content (ME) (4) Digestible haulm yield	0.720.720.670.91	–

Adapted from Murthy and Reddy (1993), Nigam (2014), and others

resulted from the higher harvest index brought about by reduction in the total biomass. Breeding for high harvest index coupled with high biomass can be one of the strategies to further increase groundnut yield.

16.8.2 Breeding for Abiotic Stress Resistance Including Climate Change

In India, 106 million ha of area is completely unirrigated and is under dryland agriculture generating nearly half of total value of agricultural output. In these

regions, around 300 million people depend for their sustenance on dryland agriculture, of whom 30–40% can be classified as poor (Ryan and Spencer 2001). Peanut is an important crop of dryland agriculture wherein it is valued both for its seed and fodder. Groundnut fodder is highly nutritive and forms an important component of cattle feed. Nearly, 85% area under peanut remains rainfed of which approximately 80% comes under dryland where irrigation facilities do not exist at all (Roy and Shiyani 2000). Water stress in dryland is intermittent and unpredictable (Vorasoot et al. 1985) and can occur at any stage of the crop starting from pre-flowering, flowering, pegging and pod development (Jogloy et al. 1996) and causes significant yield loss. Yield losses due to water stress are dependent on crop growth stage, intensity and duration of its occurrence (Nigam et al. 2005). Drought stress at pre-flowering stage increased the yield by 13–19% (Nageswara Rao et al. 1985a), whereas at pod setting stage it caused yield losses up to 88% (Vorasoot et al. 2003). Not only the yield but also the quality of products decreases under drought stress (Rucker et al. 1995; Stansell and Pallas 1985), and the latter was aggravated by the contamination of aflatoxin under drought environment (Sanders et al. 1993).

Drought in rain-dependent cropping system is always associated with heat stress (Nautiyal et al. 2004). In the last few decades, there has been constant increase in global air temperature (Nautiyal et al. 2004) and in the twenty-first century, it is predicted to increase further and associated with frequent warm spells, heat waves and heavy rainfall and a likely increase in the frequency of droughts (Qin et al. 2007). In India, temperature increase is predicted to be 3.5 and 5.5 °C by 2080 (Lal 2001). Studies made by the CGIAR have reported that the high temperature stress (above 30 °C) will be widespread in East and Southern Africa, India, South East Asia and Northern Latin America which are important groundnut-growing areas. Thus, efforts to breed varieties that can thrive and yield under both drought and heat stress need to be intensified (Janila et al. 2013).

Climate change being projected for India will increase the problem of heat and drought stress in groundnut, thus further limiting the production potential (Singh et al. 2014). Temperatures during the crop growing seasons in certain areas of India are already above the upper limit of optimum temperature range (20–30 °C). When climate changes are small, agronomic practices can help farmers to adopt, but when changes are intensive then we need extensive changes like genetic improvement of crop for greater tolerance to high temperature and drought, improved response to rising CO_2 and development of new agronomic practices (Boote et al. 2011). The critical stages to heat stress in groundnut are at the flowering (3–6 days before flowering) and pod formation (Craufurd et al. 2002, 2003).

Apart from low unpredictable water supply and increased temperatures, arid regions are also known for low soil fertility (Peek and Forseth 2003). Due to capillarity in the in drought-affected soils, the nutrient uptake will be low as air-filled capillaries block water diffusion into roots (Baligar et al. 2001; Fageria et al. 2002; Gunes et al. 2006; Ghanbari et al. 2011). Like other agricultural crops, peanut requires essential nutrients during its life cycle. This may further lead to the impaired active transport and membrane permeability (Tanguilig et al. 1987). In peanut water deficit during pod filling stages (Kulkarni et al. 1988) and a long-term

drought period from 14 days after emergence until harvest (Arunyanark et al. 2008) reduced uptakes of N. Likewise, reductions of K, P, Ca and Mg uptake as a result of drought at flowering pegging, pod formation and development stages were also observed (Kolay 2008). Htoon et al. (2014) and Dinh et al. (2014) have proposed nutrient uptake under drought stress as a surrogate trait for drought tolerance.

Though 235 varieties have been developed in groundnut, none of those have been bred with specific objectives to suit them of typical dryland conditions. It has been an ever-increasing challenge for the plant breeders to evolve efficient varieties for dryland conditions because of extreme variability in the agro-climatic conditions of such stress areas. Hence, for groundnut to sustain its yield in the future, it should possess tolerance to drought, heat stress and ability of high nutrient use efficiency under drought conditions. In order to make significant progress in breeding programmes for these stresses, we need to identify sources of resistance/tolerance. Few sources of tolerance to drought, heat and nutrient stresses are available in literature. Genetic variability for thermotolerance of selected peanut genotypes was studied (Nautiyal et al. 2004) using leaf cell membrane thermostability and identified ICGS 44 and ICGV 86031 as tolerant to high temperature and water deficit stress. Calcium enrichment in peanut tissues has shown to improve drought tolerance in peanut (Chari et al. 1986), and genetic variability for Ca uptake was established the genotypes ICGHNG88448, NRCG 7085-1 and NRCG 6155 (Singh et al. 2004). As moisture availability is low under drought conditions, absorption and mobility of nutrients such as phosphorus is also affected.

Singh and Basu (2005) screened peanut genotypes under low P availability and identified genotypes GG 5, NRCG 7085-1, NRCG 6919, NRCG 1308, NRCG 3498 and SP250A as P efficient. Rajgopal and Bandyopadhyaya (1999) screened available germplasms and identified genotypes NRCG 3787, 3778, 1116, 5150, 7627, 5311, 3920, 4481 and 7706 as drought and heat stress tolerant. Vasanthi et al. (2006) have identified genotypes ICGV 8603, CSMG 84-1, ICGS 76 and TAG 24 with most useful traits for drought tolerance. Likewise, several promising genotypes have been identified for drought tolerance such as Dh 3–30, JGN 2, ICGS 1, ICGS 37, JGN 3, GG 5, Kadiri 5, Abhaya, Kadiri 9, BG 3 and ICGS 5 (Rathnakumar et al. 2013).

Transgenic approach has been used to develop drought-tolerant genotypes using *AtDREB1A* (Sarkar et al. 2014, 2016, 2019; Bhalani et al. 2019) and *mtlD* genes (Bhauso et al. 2014; Patel et al. 2017). Salinity-tolerant genotypes have also been developed using transgenic approaches in peanut by deploying the *AtDREB1A* (Sarkar et al. 2014) and *mtlD* (Bhauso et al. 2014; Patel et al. 2016) genes. Breeding programmes are underway to develop either heat stress-tolerant, drought-tolerant or nutrient use-efficient genotypes. But in actual field conditions drought stress, heat stress, nutrient use efficiency and other abiotic stresses are interrelated. Hence, to increase productivity under dryland conditions, it is important to develop a cultivar which is drought and heat stress tolerant and also take up and utilize nutrients efficiently.

16.8.3 Breeding for Quality Characters

Quality traits vary from the consumer demand in groundnut. Different end users have different prerequisites in terms of quality traits. Groundnut is categorized as oil types and confectionary types according to its utilization pattern.

16.8.3.1 Breeding for High Oil Content and Quality

Wide variation exists for oil content in groundnut germplasm. It ranged from 46.5 to 63.1% in cultivated types, while the range observed in wild species was from 43.6 to 55.5% (Nordan et al. 1982). In few wild *Arachis* species, oil content up to 60% has also been reported by the Directorate of Groundnut Research, Junagadh, Gujarat. Few promising high oil lines (>52%) have also been reported by Directorate of Groundnut Research, Junagadh, Gujarat and ICRISAT. Oil content and yield have been reported to be independent thus suggesting possibilities of breeding varieties with high yield and oil content. Narrow-sense heritability has been worked out to be high (Martin 1967) for oil content. Inheritance of oil is governed by two pair of alleles with non-additive genetic component being predominant (Basu et al. 1988). Selection should be postponed to later generations to eliminate the undesirable recombinants.

The oil content, fatty acid composition, iodine value, ratio of oleic to linoleic acid (O/L) and stability or shelf life are factors to be considered in oil quality of groundnut. Genetic manipulation of fatty acid composition has been reported by few workers. The Virginia types generally have higher oleic acid content, while Spanish-Valencias have higher linoleic acid. This results in a lower iodine value for oil of Virginia types and indicates that these types will become rancid through auto-oxidation more slowly than the Spanish-Valencias. The groundnut breeder is faced with a paradox when breeding for oil quality. Consumers prefer to have oils both with low iodine (long shelf-life) and high iodine value (to have high level of unsaturation from the health point of view). Crosses between all the four habit groups have shown that a wide range of iodine values can be obtained through recombination of genes from different parents and that the iodine value in groundnuts is highly heritable (Bovi 1982).

High oleic acid groundnut is one of the most sought types by the processing industry especially the confectionaries. High oleic groundnut has better keeping quality (O'Keefe et al. 1993; Braddock et al. 1995), nutritional quality and better flavour. It is reported to have a gain of tenfold in shelf life. High oleic groundnut has a mutation in the fatty acid dehydrogenase (FAD) gene which is responsible for adding the second double bond to oleic acid resulting in linoleic acid. By preventing this conversion, the oleic acid content can go up to 80% while keeping linoleic acid content around 2–5%. Since cultivated groundnut is an allotetraploid, there are two homeologous gene sequences, FAD2A and FAD2B, believed to have originated from the two progenitor species genomes, *Arachis duranensis* and *Arachis ipaensis* (Bertioli et al. 2019; Chen et al. 2019; Zhuang et al. 2019), and a mutation in either one of these genes result in an enhanced oleic acid content (Nawade et al. 2016; Nawade et al. 2018). However, to achieve more than 75% of oleic acid, mutations in

both alleles are essential (Pandey et al. 2014a, b) which is further confirmed by Janila et al. (2016a, b) in introgression line developed using allele-specific markers and also by Nawade et al. (2019). At ICAR-DGR using marker-assisted introgression, two new varieties were developed, Girnar 4 and Girnar 5, with having >78% of oleic acid content.

16.8.3.2 Breeding for Confectionery Groundnut

There are important trade attributes for confectionary-type groundnut, and the groundnut cultivars should possess certain physical attributes and chemical composition and maintain some definite processing and end-use characteristics to be acceptable to traders, manufacturers and ultimate consumers. Among the physical quality requirements for confectionery groundnuts, size, shape and high sound mature kernel (SMK) are important. High SMK of >80%, 100-seedmass (HSM), kernels with elongated shape, tapering ends and pink to light brown testa colour and large seed size are desirable traits (Nigam et al. 1989). Though no parameter has been fixed for chemical properties, low (<1%) free fatty acid (FFA), high sugars (>6%) and high protein (>30%) along with nutritional qualities like high O/L and low oil (<45%) are preferred traits for confectionery groundnuts (Kona et al. 2019, 2020).

Indian Hand Picked Select (HPS) peanuts have a strong demand in Southeast Asia and countries neighbouring India. The Agricultural and Processed Food Products Export Development Authority (APEDA) and the Indian Oilseeds and Produce Export Promotion Council (IOPEPC) are jointly working towards the increase of international awareness of Indian groundnut offer and addressing quality-related concerns. APEDA has issued export guidelines for groundnuts and groundnut products and provides information on registration of groundnut processing units and/or warehouse, as well as on the issuance of export certificates by IOPEPC.

In the past, for confection, protein content of 20%, >55 g of HKW and low oil content (42%) were preferred (Ramanathan 2004). With the evolving market and industry requirement, the traits for confectionary uses now are greater proportion of sound mature kernels (SMK), free from aflatoxin contamination, attractive seed size and shape, pink or tan seed colour, flavour, 100-seed weight exceeding 55 g, >6% of sugar content, >24% of protein content, blanchability (>60%), low oil content (<45%) and high oleic/linoleic (O/L) ratio (Nigam et al. 1989; Dwivedi and Nigam 1995; Kona et al. 2019, 2020) (Table 16.7). Seed mass is an important attribute to confectionary quality; however, like yield and yield parameters, it is highly influenced by environment. The carbohydrate components of the kernel determine the taste and sensory attributes of roasted groundnuts (Pattee et al. 2000). Seed colour and shape and flavour are the other important confectionary attributes. Blanchability is removal of testa or seed coat (skin) from raw or roasted groundnuts, and this attribute is of economic importance in processed groundnut food products, which include peanut butter, salted groundnuts, candies and bakery products and groundnut flour. Breeding programme for confectionery quality trait improvement especially for protein and sugar requires non-destructive estimation

Table 16.7 Standards for confectionery groundnut

Traits	Desirable aspects
Seed size	155–170 seed/100 g >55 g 100 seed mass
Seed shape	Round or elongated with tapering ends
Colour	Pods; light golden-yellow Kernels, with tan rose and pink testa
Flavour (roasted)	Almond, coffee, nutty popcorn, smoky and sweet
Texture (roasted)	Firm and crispy
Biochemical and nutritional	Low oil content (<45%), high protein content (>24%), high O/L ratio (>60% oleic acid), high vitamins B1, B2, E, high in minerals like Ca, Mg, Fe, low in anti-nutritional compounds like oxalic and phytic acid, blanchability (>60%), high sugar content (>6%)
Aflatoxin	Free from aflatoxin

Nigam et al. 1989; Dwivedi and Nigam 1995; Basu et al. 2003; Kona et al. 2019, 2020; Aman et al. 2020; Sushmita et al. 2020

procedures and molecular markers linked to them so that selection in segregating generations will be easy and reduces the cumbersome process. Both nutritional and food processing quality traits are gaining importance in the breeding programmes to meet various uses as well as consumer preference.

Many varieties having large seed size were developed in India (Table 16.8) of which very few genotypes have export potential (GAUG 10, M 13, TMV 10, ICGS 76, GG 20, BAU 13, B 95, HNG 10, Girnar 2, Mallika (ICHG-00440), GJG 22, RG 559–3 (Raj Mungfali 3)).

The cultivation of specifically developed confectionery groundnut cultivars to meeting the international standards using their production technologies are most vital to increase the export. Some of the regions in India are suited for the cultivation of confectionery groundnut; however, it cannot be grown on all soils with full potential. Most of the production of export quality groundnut is obtained from Gujarat and Tamil Nadu and now from Rajasthan. Bold-type groundnut is mostly obtained from Rajasthan, Gujarat, Madhya Pradesh, Punjab and Haryana states which are grown from light textured to heavy soil with FYM. The other states mostly produce Java-type groundnut. Indian groundnuts in the Far East are preferred over others origins such as China because of the high oil content. The Saurashtra region of Gujarat and other part of Gujarat produce both Java and bold type of export quality. Raising of good-quality groundnut requires a high standard of crop husbandry which should not suffer from diseases, insect pests and moisture and nutritional stresses. Further, in the context of India, the crop duration should be short as in long duration crop the management becomes difficult. Therefore, cultivation of large seeded and confectionery groundnut for export promotion should preferably be entrusted to resource-rich farmers capable of affording good management practices.

Table 16.8 Varieties released in India having combination of traits suitable for confection or table purpose

S. no.	Variety	Season	Area of adoption	Pod yield (kg ha^{-1})	Duration (days)
Spanish bunch					
1	TKG-19A (TG-19A)	Rabi-summer	Konkan region of Maharashtra	2260	107
2	TPG-41	Rabi-summer	All India	2088	122
3	TLG-45 (Trombay-Latur Groundnut-45)	Kharif	Maharashtra	1506–2000	115
4	RARS-T-1	Kharif, Rabi-summer	Andhra Pradesh	2500–4000	115
Valencia					
1	Gangapuri	Kharif	Madhya Pradesh	2000	95–105
Virginia bunch					
1	Vikram (TG-1)	Kharif	Maharashtra	2695	120
2	Kadiri-2	Kharif	Andhra Pradesh	1800	115–120
3	BG-1 (Birsa Groundnut-1)	Kharif	Bihar	2200	120–125
4	BG-2 (Birsa Groundnut-2)	Kharif	Bihar	2200	120–125
5	BAU-13 (Birsa bold-1)	Kharif	Bihar	2191	125–135
6	ICGS-76	Kharif	Southern Maharashtra, Karnataka	1300	115–125
7	GG20	Kharif	Gujarat	1960	109
8	Koyana (B-95)	Kharif, Rabi-summer	Southern Maharashtra	3345	115–125
9	M-522	Kharif	Punjab	2525	110–120
10	Ak-303	Kharif	Maharashtra	2100	125
11	TBG-39 (TG-39)	Kharif	Rajasthan	3154	118
12	Girnar-2	Kharif	Uttar Pradesh, Punjab, northern Rajasthan	2907	130
13	Kadiri-7 (K-7)	Kharif	Andhra Pradesh	1643	120–125
14	Kadiri-8 (K-8)	Kharif	Andhra Pradesh	1523	120–125
15	Mallika (ICHG-00440)	Kharif	All India	2579	125–130
16	TGLPS-3 (TDG-39)	Kharif	Karnataka	2500–3000	115–120
17	Gujarat Groundnut HPS 2 (GG HPS 2)	Kharif	Gujarat	2835	121
	GJG 22	Kharif	Tamil Nadu	1914	125–130

(continued)

Table 16.8 (continued)

S. no.	Variety	Season	Area of adoption	Pod yield (kg ha^{-1})	Duration (days)
Virginia runner					
1	M-13 (Moongphali No.13)	Kharif	All India	2750	135
2	Chandra (Ah-114)	Kharif	Uttar Pradesh	2500	130
	M-335	Kharif	Sandy soil areas of Punjab	2300	120–125
4	Somnath (TGS-1)	Kharif	Gujarat and Rajasthan	1900	110–125
5	GJG-HPS-1 (JSP-HPS-44)	Kharif	Gujarat	2125	110–120
6	Raj Mungfali 1 (RG 510)	Kharif	Rajasthan and Punjab	2558	112–138
7	RG 559–3 (Raj Mungfali 3)	Kharif	Rajasthan, Uttar Pradesh and Punjab	3173	120–125

Zinc and Iron Biofortification

Micronutrient deficiencies have increased over recent decades due to a generalized decrease in the quality of poor people's diets both in developed and developing countries and even in areas where food is not a limiting factor (Welch and Graham 1999; Graham et al. 2001). Micronutrient malnutrition affects more than one-half of the world's population, especially women and pre-school children (UNSCN 2004). Furthermore, micronutrient deficiencies are more widespread than deficiencies caused by inadequate consumption of energy or protein. Breeding crop plants for higher micronutrient concentration, an approach termed as biofortification, has become an active goal of plant breeding programmes in the developing world at both the international and national agricultural research centres (Welch 2002; Bouis 2003). It aims on the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology.

Staple diet is the prime sources of the total intake of zinc for the people in developing countries. Biofortification, wherever possible, is a cost-effective and sustainable solution for tackling the micronutrient deficiencies as the intake of micronutrients is on a continuing basis with no additional costs to the consumer in the developing countries (Kumar et al. 2011). It has the potential to help to alleviate the suffering, death, disability and failures to achieve human potential, which results from micronutrient deficiency-related diseases. In comparison to other strategies, it provides a truly feasible means of reaching out to remote and rural areas where people has limited access to diverse diets, commercially fortified foods and supplements, to deliver naturally fortified foods (Bouis et al. 2011). Results from germplasm screening suggest that the iron and zinc concentration of staple foods can be doubled through conventional breeding. This result, in turn, implies that iron and zinc intakes in poor people's diets can be increased by 50 per cent. This should result in an appreciable improvement in nutrition and health even for those whose intakes remain below recommended daily intakes.

Groundnut is valued as a rich source of energy contributed by oil (48–50%) and protein (25–28%) in the kernels. In addition, groundnut kernels also contain antioxidants and vitamins and are rich in mono-unsaturated fatty acids (Janila et al. 2013). Those contain vitamin E and many important B-complex group of vitamins like thiamin, pantothenic acid, vitamin B-6 and niacin. Of the 20 minerals necessary for normal body growth and maintenance, seven, including iron and zinc, are present in peanut. Groundnut is a dietary source of biologically active polyphenols, flavonoids and isoflavones but lacks completely in vitamin A (Misra 2006). Developing countries, where micronutrient deficiencies are widespread, contribute world's maximum peanut area and production (FAOSTAT 2011).

Thus, peanut can contribute significantly towards reduction of protein-energy and micronutrient malnutrition (Janila et al. 2014). If there is sufficient genetic variation for the density of micronutrients in edible parts of the crop, biofortification can be achieved through plant breeding (Mayer et al. 2008). In groundnut genetic variability was reported for iron and zinc concentration (Upadhyaya et al. 2012; Janila et al. 2014), and thus biofortification is possible.

16.9 Breeding for Major Biotic Stresses

Groundnut is a mainly grown as a rainfed crop in India which accounts about 84% of the total area. Many biotic stresses are known to limit groundnut productivity during *Kharif* and *rabi*-summer season, but their severity and distribution vary with prevailing environmental conditions. These biotic stresses reduce the quality (nutritional, appearance, sensory attributes) and quantity in terms of pod and haulm yield of groundnut. Groundnut is attacked by several diseases caused by fungi, virus, nematodes and insects and pests. Early leaf spot (ELS) caused by *Cercospora arachidicola* Hori, late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) Van Arx and rust caused by *Puccinia arachidic* Spegazzini are among the major foliar fungal diseases worldwide (Gajjar et al. 2014; Bala et al. 2016). Stem and podrot, caused by *Sclerotium rolfsii*, collar rot caused by *Aspergillus niger* van Tiegham and dry root rot caused by *Macrophomina phaseolina* are major soilborne diseases of groundnut production in many warm, humid and dry areas (Thirumalaisamy et al. 2014; Bosamia et al. 2020). Aflatoxins are carcinogenic substances produced by the fungi *Aspergillus flavus* and *A. parasiticus*, and the contamination of export consignments by this toxin had forced several importing countries to impose strict regimes in place on permissible levels of aflatoxins (Dodia et al. 2014; Singh et al. 2015a, b). The major viral diseases of groundnut are peanut bud necrosis disease (PBND) in India and peanut, groundnut rosette disease (GRD) in Africa, tomato spotted wilt virus (TSWV) in the USA, peanut stripe potyvirus (PStV) in East and Southeast Asia, peanut stem necrosis disease (PSND) in pockets in Southern India and peanut clump virus disease (PCVD) in West Africa and some parts of India (Nigam et al. 2012). Bacterial wilt of groundnut caused by *Pseudomonas solanacearum* is prevalent in Southeast Asia, the Far East and Uganda (Hayward 1991).

Aphids (*Aphis craccivora* Koch), several species of thrips (*Frankliniella schultzei*, *Thrips palmi* and *F. fusca*), leaf miner (*Aproaerema modicella*), red hairy caterpillar (*Amsacta albistriga*), jassids (*Empoasca kerri* and *E. fabae*) and *Spodoptera* are the major insect pests in groundnut, among which aphids, thrips and *Spodoptera* have worldwide distribution and cause serious damage (Wightman and Amin 1988). Termites and white grubs are major soil arthropods causing damage to groundnuts. Groundnut bruchid beetle (*Caryedon serratus (Olivier)*) are the major storage insect pests in groundnut. Breeding for resistance to insect pest has been limited as only moderate degrees of resistance to the sucking insects like aphids, jassids and thrips and leaf miner are available in the cultivated germplasm, and the transfer of these into high yielding backgrounds has not been very successful.

Root-knot diseases caused by *Meloidogyne* species of nematode are widely distributed in Asia, Australia and North America (Sharma et al. 1990). Globally, nematodes cause nearly 11.8% yield loss in groundnut. The root-knot nematodes, *Meloidogyne* spp., and the lesion nematodes, *Pratylenchus* spp., are important (Sharma and McDonald 1990a), while *Meloidogyne arenaria* and *M. javanica* are predominant species causing economic loss of nearly 21.6% in India (Khan et al. 2010).

16.9.1 Breeding for Foliar Diseases Resistance

Among foliar fungal diseases, early leaf spot (*Cercospora arachidicola* Hori), late leaf spot [*Phaeoisariopsis personata* (Berk. & Curt.)Van.Arx.] and rust (*Puccinia arachidic* Speg.) are widely distributed and economically important worldwide. These diseases together can cause more than 70% loss in yield besides adversely affecting the quality of the produce (pods, seeds and haulm) (Aruna et al. 2005). Late leaf spot is the major and widely distributed disease, which can cause defoliation and reduce pod and fodder yields about 50% and adversely affect quality of produce (Subrahmanyam et al. 1984). Rust is also economically important causing yield losses ranging from 10 to 52% and affecting seed and fodder quality (Subrahmanyam et al. 1995). Foliar diseases can be controlled by chemical measures, but they increase costs of production thus beyond the reach of small and marginal farmers and also pollute the environment. Therefore, development and growing of resistant cultivars are the best viable option to minimize economic losses of farmer and to maintain the quality of the produce.

Foliar disease resistance breeding has started in the early 1970s, and high levels of resistance or immunity in wild *Arachis* species for early leaf spot in *A. chacoense* and late leaf spot in *A. cardenasii* were reported by Abdou et al. (1974). However, foliar disease resistance was found to be liked to undesirable pod and seed characters, low shelling outturn and long duration. By utilizing resistant source NCAc-17,090, first-generation foliar disease-resistant varieties, viz. ICGS (FDRS) 10 and Girnar 1, were released in India. Later on, advanced resistant breeding lines were used to develop new resistant cultivars with desirable agronomic characters, and the resistant cultivars ICGV 86590 and ALR 2 were released in India. These

cultivars also suffered the drawbacks associated with the linkage drag of traits like long duration and lower partitioning and with undesirable pod (highly reticulated, constricted, prominently ridged and conspicuously beaked pods with thick shells) and seed (purple or blotched seed colour) characteristics (Wynne et al. 1991; Singh et al. 1997). An interspecific derivative, GPBD-4 (KRG 1 x ICGV 86855), which combined early maturity, high yield potential and high shelling outturns with minimum reduction in yield due to high level of resistance to rust and late leaf spot (Gowda et al. 2002). Many high-yielding groundnut varieties with resistance of multiple foliar diseases (leaf spot and rust) have been released for commercial cultivation to groundnut farmers in India, viz. AK 265, PhuleBharti (JL 776), PhuleMorna (KDG 123), PhuleWarna (KDG 128), GJG 32 (ICGV 03043), PhuleUnnati (RHRG-6083).

Modern crop breeding approaches like marker-assisted selection improve the precision of or targeted breeding (Bosamia et al. 2015). Development of tightly linked markers for LLS and rust resistance would make breeding programmes more efficient in production of resistant cultivars in shorter time. Recently, many DNA markers have been found to be putatively linked to rust and LLS resistance genes, and many markers were recently found to be associated with QTLs for rust and late leaf spot (Mondal and Badigannavar 2010, 2018; Sujay et al. 2012; Mishra et al. 2015; Zhou et al. 2016; Ahmad et al. 2020). The molecular markers validated for LLS and rust resistance (Sukruth et al. 2015; Yeri and Bhat 2016) were used to improve LLS and rust-resistance of the cultivars TAG 24, ICGV 91114 and JL 24 through MABC (Varshney et al. 2014). In the same lines, the cultivars GJG 9, GG 20 and GJG-HPS 1 have also been improved for their resistance to foliar disease resistance along with high oleic acid (Shasidhar et al. 2020).

Alternaria leaf blight is a minor disease but becoming a severe in *rabi*-summer season as compared to *Kharif* season groundnut-growing states of India. Therefore, very little breeding work has been done to identify resistance sources and to develop resistant varieties. The genotypes PI 405132, PI 259747, PI 215696, NcAc 17,132, NcAc 17,133 RF and NcAc 17,135 were reported to be resistant to *A. alternata* (Muthuswamy et al. 1991). Five multiple disease-resistant germplasm accessions, viz. NCAc17149, NCAc927, NCAc17133 (RF), PI 393646 and PI 341879 have been identified resistant to early leaf spot, rust and *Alternaria* leaf spot (Ghewande et al. 1992).

Bera et al. (2011a, b, c, d, e, f, g, h) reported eight groundnut germplasm, viz. NRCGCS 77, NRCGCS 83, NRCGCS 85, NRCGCS 86, NRCGCS 21, NRCGCS 124, NRCGCS 180 and NRCGCS 222 having multiple disease resistance to PBND, stem rot, late leaf spot, early leaf spot, rust and *Alternaria* leaf blight. It has been observed that cultivars TG-37A, ICGS-37, JL-24, AK-159, DRG-12 and TPG-41 were susceptible to *Alternaria* leaf blight (Kumar et al. 2012). Three cultivars, viz. Kaidiri 9, KadiriHaritandra and ICGV 00348; four advanced breeding lines, viz. PBS 12190, PBS 22131, PBS 22132 and PBS 22133; and four interspecific derivatives, viz. NRCGCS 176, NRCGCS 180, NRCGCS 196 and NRCGCS 298 found resistant to *Alternaria* leaf blight. Therefore, these varieties may be used directly in *rabi*-summer groundnut areas of India (Anonymous 2019).

16.9.2 Breeding for Soilborne Diseases

Stem rot caused by *Sclerotium rolfsii* Sacc. and collar rot caused by *Aspergillus niger* van Tieghem are prevalent in almost all groundnut-growing areas of India especially in medium black and sandy loam soils, respectively. Stem rot pathogen is prevalent in warm temperate and sub-tropical regions of the world and has a host range of over 500 plant species (Harlton et al. 1995; Dodia et al. 2016, 2019). It is reported from India, Thailand, Indonesia, Taiwan and the Philippines where yield losses were ranging from 10 to 25% (Mayee and Datar 1988). In India, it is widespread in Gujarat, Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh causing 10 to 40% yield losses (Akgul et al. 2011; Bera et al. 2014).

Several germplasm accessions and breeding lines were screened for their resistance to stem rot. Among them, nine breeding lines (ICGV 86034, 86,124, 86,252, 86,388, 86,590, 86,606, 86,635, 87,160 and 87,359) showed low susceptibility to stem and/or pod rot (Mehan et al. 1995). The disease can be managed through cultural practices together with resistant cultivars (Shew et al. 1984). Most sources of resistance to soilborne fungi reported in groundnut show low levels of resistance or tolerance. Such partial resistance is presumably governed by polygenes and is assumed to be similar to horizontal resistance (Fry 1982). There are practical difficulties in incorporating this type of resistance from germplasm with desired agronomic traits. The strategy has been to breed for a low level of host pathogen coexistence that is stable, environmentally balanced and economically useful (Wynne et al. 1991).

Although several advanced breeding lines, cultivars and wild species have been screened for resistance against stem rot by several workers, absolute resistance for stem and collar rot is not present in available groundnut germplasm, only partial resistance has been identified in groundnut which is being utilized currently for cultivar development. Currently interspecific derivatives, viz. NRCGCS 19 and NRCGCS 319, and one breeding line PBS 18037 have resistance to stem rot and are being utilized breeding programme widely.

With the advancement of molecular marker technology, various genomic tools have been utilized to identify specific DNA regions tightly linked to stem rot resistance in groundnut. Bera et al. (2016) developed SSR-based genetic map and identified 12 SSR polymorphic marker loci with one major QTL (QTL qstga01.1) for stem rot disease resistance in groundnut. Dodia et al. (2019) reported development of high-quality genetic map with 585 SNP loci with spanning the distance of 2430 cM with an average inter-marker distance of 4.1 cM. She identified 44 major epistatic QTLs with phenotypic variation explained ranging from 14.32 to 67.95%.

Collar rot is an important disease caused by *Aspergillus niger* van Tieghem and is a filamentous fungus growing aerobically on organic matter. Collar rot can be rotting of seed, pre-emergence soft rot of hypocotyls and post-emergence collar rot of seedlings (Mehan et al. 1995). Collar rot is prevalent in almost all groundnut-growing states of India, viz. Rajasthan, Gujarat, Punjab, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Maharashtra, Orissa, Karnataka and Madhya Pradesh particularly in sandy loam and medium black soils. In Gujarat the losses in terms of

mortality of plants due to collar rot range from 28 to 50 per cent (Ghewande et al. 2002). In India, the breeding work on resistance of collar rot was not much due to non-availability of stable source of resistance to collar rot. The cultivars J11, JCG 88 and OG 52-1 were reported to be moderately tolerant to collar rot (Ghewande et al. 2002). Palaiah et al. (2019) found 37 germplasm lines were resistance and cultivars, viz. KRG-1, R-2001-3, Kadiri-9, ICGV-00350, ICGV-00351, TG-37A, GPBD-4, GPBD-5, KDD-128, Dh-101, Dh-216, G2–52, Ch-2, TDG-51, S-230, DSG-1, Chintamani-2 and J-11, were moderately resistant to collar rot. Divya Rani et al. (2018) reported following breeding lines having less than 15% disease incidence, viz. ICGV 00202, ICGV 00211, ICGV 86590, ICGV 91114, ICGV 05155, ICGV 00350, ICGV 93261, ICGV 92195, ICGV 92035 and ICR 48. Most of resistant sources are from the wild species which are generally cross-incompatible with cultivated genotypes and linked with undesirable agronomic traits. While selecting for better agronomic traits, the levels of resistance often diluted. Therefore, breeding strategy should be developing cultivars with low level of host pathogen interaction for durable resistance.

16.9.3 Breeding for Reduced Aflatoxin Contamination

Aflatoxins are one of the most potent toxic and extremely carcinogenic secondary metabolites produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus.* There are four major aflatoxins that occur in crops, including B1, B2, G1 and G2, and sum of these four is referred to as the total aflatoxin. The dominant aflatoxins produced by *A. flavus* are B1 and B2, whereas *A. parasiticus* produces two additional aflatoxins, G1 and G2 (Payne 1998). Aflatoxin B1 is considered to be the most important of the four because it is the most toxic and has been classified by the International Agency for Research on Cancer as a probable human carcinogen (IARC 1987).

In groundnut, based on the site of action of the aflatoxin-producing fungi, there are three types of host-pathogen resistance mechanisms: pre-harvest aflatoxin contamination (PAC) at pod wall; in vitroseed colonization (IVSC) at seed coat; and resistance to aflatoxin production (AP) at cotyledons level (Nigam et al. 2009). Mixon and Rogers (1973) were the first to suggest the use of resistant cultivars to contain the problem of aflatoxin contamination in peanut. They reported two Valencia genotypes, PI 337394 F and PI 337409, as having high levels of resistance to in vitro seed colonization (IVSC) by both pathogens (*A. flavus* and *A. parasiticus*). Genotypes PI 337409, PI 337394F and UF 71513 were reported to be resistant to seed invasion and colonization, the cultivars Doran and Shulamit to be resistant to pod infection and U-4-477, 55–437, 73–30 and J 11 resistant to seed infection in the field and U 4–7-5 and VRR 245 with low production of aflatoxin B1 (Mehan 1989). However, the aflatoxin content and seed colonization could not be correlated with the population of *A. flavus* in the soil (Will et al. 1994). Nigam (2002) has reported that preharvest seed infection and aflatoxin production are influenced significantly by the G × E interactions. Relationships between in vitro seed colonization (IVSC)

and natural seed infection and aflatoxin production in the field and their contribution in reducing aflatoxin contamination are not understood clearly (Xue et al. 2004). The resistance breeding programme requires stable resistance source and a reliable, efficient and reproducible screening technique. Lack of high levels of resistance and limitations of screening techniques place severe constraints on the progress in resistance breeding to eliminate aflatoxin (Nigam et al. 2009). Further, it is suggested that genetic resistance alone cannot solve the problem of aflatoxin contamination in groundnut until unless other cultural management practices such as soil and water management, bio-control, soil pest management and postharvest management practices should be followed for managing this disease.

However, the advancement of genomic tools may provide an opportunity to achieve stable genetic resistance by combining the three mechanisms of aflatoxin resistance. Currently resistant genotypes are being deployed for breeding aflatoxin-resistant lines and to develop different types of genetic populations such as association mapping panels, bi-parental and multi-parent populations (Pandey et al. 2016). ICRISAT, together with its NARS partners, are working on such an integrated approach wherein a MAGIC population and bi-parental populations are planned to be used for genetic mapping and QTL discovery, while a mini core collection will be used for association analysis to discover marker-trait associations (MTAs). These studies are likely to facilitate the identification of genomic regions controlling aflatoxin resistance. At the same time, a transcriptomic approach has been deployed to study the functional genomics of resistance mechanisms IVSC, PAC and AP by conducting separate RNAseq experiments. These integrated approaches comprising genetics, structural genomics and functional genomics together with next-generation sequencing and comprehensive analysis will provide precise information on candidate genes to facilitate the development and validation of genetic markers for use in molecular breeding (Pandey et al. 2019).

16.9.4 Breeding for Virus Diseases

About 31 viruses representing 14 genera are reported to naturally infect groundnut worldwide, although only few are economic importance. Viral diseases are very difficult to manage because most of those are transmitted through vectors, and there are no viricides available in the market for control of viruses within plants. Most of the chemicals are used for controlling virus vectors. Therefore, integrated approach including growing resistant cultivars with suitable cultural practices is the most effective approaches to reduce yield losses due to viral diseases.

Peanut bud necrosis disease (PBND) was first noticed in farmers' fields in Punjab during 1958–1959 and is one of the deadliest diseases of peanut (Patil et al. 2017). Reddy et al. (2000) found that two cross compatible wild species of three accessions of *A. cardenasii*, viz. ICG 11564, 13,164 and 13,165; and the two accessions of *A. villosa* ICG 8144 and 13,168 are free from virus in the field. Dwivedi et al. (1995) reported that several breeding lines with vector resistance, ICGV 86031 and 86,388, showed resistance to PBNV. Gururaj et al. (2002) screened 172 genotypes of

cultivated groundnut for 3 years. Among them seven genotypes, DRG 18, ICG 7812, ICG(FDRS) 10, ICGV 80325, JSSP 3, KNG 22 and PI 393516 were highly resistant to PBND (up to 1% disease incidence). Several high-yielding cultivars with field resistance to PBND have been released in India. These are CO 3, ICGS 11, ICGS 44 (ICGV 87128), ICGS 37 (ICGV 87187), R 8808 (KRG 2), R 9251, K 134, DRG 12, RSHY 1, Kadiri 4, JCC 88, GG 7 and DRG 17 (Basu et al. 2002). Other cultivars reported with field resistance to PBND in India are Kadiri 3, ICGS 5, RS 138, CSMG 881, CSMG 888 and CSMG 892 (Singh et al. 1994); ICGS 1 (Nigam et al. 1991b); ICGV 87141 (ICGS 76) (Nigam et al. 1991c); ICGV 86699 (Reddy et al. 1996); ICGV 86325 (Dwivedi et al. 1996); GPBD 4, JSSP 9 and Dh 53 (Nagaraja et al. 2005); PratapMungphali 1 (Nagda and Joshi 2004); and PratapMungphali 2 (Nagda and Dashora 2005). The breeding approach for resistance to PBND should be improving the levels of resistance to the vector and the virus into superior agronomic backgrounds.

Peanut stem necrosis disease (PSND) caused by tobacco streak virus (TSV) has been epidemic in Anantapur district in Andhra Pradesh in 2000, affecting 2,25,000 ha and causing an economic loss of US$ 65 million (Reddy et al. 2002). PSND has been reported from parts of Andhra Pradesh (Anantapur, Kurnool, Cuddapah and Chittoor or districts) and adjoining areas in Karnataka (Raichur district). It remains a potential threat to peanut in southern states in India (Nigam et al. 2012). Kalyani et al. (2007) screened 56 germplasm accessions from 20 wild *Arachis* species belonging to *Arachis*, *Erectoides*, *Procumbente* and *Rhizomatosae* sections. Among them, eight accessions, ICG 8139, 8195, 8200, 8203, 8205 and 11,550, belonging to *A. duranensis*, ICG 8144 belonging to *A. villosa* and ICG 13210 belonging to *A. stenosperma* were found to be free from virus on mechanical inoculation. As the level of resistance to TSV is not available in the cultivated germplasm, interspecific hybridization programme utilizing available *Arachis* accessions should be started to transfer in cultivated groundnut genotypes in superior agronomic background. Besides this, high levels of resistance for thrips vectors are available in cultivated germplasm and should also be utilized in breeding programmes. Marker-assisted backcrossing has been reported to be successful in improving resistance to tomato spotted wilt virus (TSWV) (Holbrook et al. 2017).

16.9.5 Breeding for Insect Pest Resistance

More than 360 soil and foliage inhabiting arthropod pests of groundnut have been reported in the literature. However, only a few are economically important to the crop either because they cause significant direct yield loss or acting as vectors of virus diseases. These include aphids, thrips, jassids, leaf miner, *Spodoptera* and white grub in Asia and aphids, jassids, *Spodoptera*, Hilda, millipedes, termites and white grub in Africa (Nigam et al. 1991a). Aphids (*Aphis craccivora* Koch), some species of thrips (*Frankliniella schultzei*, *Thrips palmi* and *F. fusca*), leaf miner (*Aproaerema modicella*), red hairy caterpillar (*Amsacta albistriga*), jassids (*Empoasca kerri* and *E. fabae*) and *Spodoptera* are the major insect pests in

groundnut. Aphids, thrips and *Spodoptera* have worldwide distribution and cause serious damage (Wightman and Amin 1988). Patel and Vora (1981) reported jassids as serious pests of groundnut in India. They can cause about 9% reduction in pod yield and 18% reduction in haulm weight.

Chemical control of insects is possible in groundnut. But development of insecticide resistance in the insects, insecticide residues in the food products and adverse effects of insecticide use on the environment have received considerable attention. Host plant resistance is one of the most economical and eco-friendly approach to manage pest populations in groundnut. Several sources of resistance to insect pests, particularly thrips, jassids and termites have been identified in groundnut germplasm.

A Spanish-type groundnut variety ICGV 86031 (PI 561917) was released in 1991 as a source of resistance to *S. litura*, *Thrips palmi*, *Empoasca kerri*, *Aproaerema modicella* and bud necrosis virus (Dwivedi et al. 1993). Several groundnut genotypes were screened for *Helicoverpa armigera* and *S. litura.* Only BG 2, a Virginia bunch groundnut variety, was found resistant to both pests (Singh et al. 1993).

Bruchid beetle (*Caryedon serrate* Olivier) is the only primary pest of stored groundnut causing both quantitative and qualitative losses. The pod damages in groundnut varied according to the storage period, and it may vary from 19 to 60%, when the groundnut was stored for 5 months (Matokot et al. 1987). If the groundnut is stored about 90 days, the damage of seven groundnut varieties ranged from 64 to 93% (Ghorpade et al. 1998). Prasad et al. (2012) screened cultivars for resistant to bruchid beetle. The cultivar CO-2 recorded least susceptible with growth index value of 0.079 followed by VRI 3, R 8808 and GG4 indicating their resistance to *C. serratus* infestation. Most of the germplasm accessions of *A. hypogaea* are susceptible to lepidopterous pests (Wightman et al. 1990), and wild relatives possess high levels of resistance to insects feeding on groundnut (Sharma et al. 2003).

Stevenson et al. (1993) reported that more than 90% mortality of larvae of *S. litura* fed on the excised leaves of *A. batizogaea* (ICG 8901), *A. kempff-mercadoi* (ICG 8959 and ICG 13159), *A. appressipila* (PI 2261877), *A. paraguariensis* (ICG 8964) and *A. villosa* (ICG13169) compared with less than 20% mortality on the cultivar TMV 2. He reported that accessions belong to *A. cardenasii* (ICG 8216), *A. duranensis* (ICG 13242, except for leaf feeding), *A. ipaensis* (ICG 8206), *A. paraguariensis* (ICG 8130) and *A. appressipila* (ICG 8946) showing resistance to leaf feeding and antibiosis to *S. litura* under no-choice tests in the greenhouse conditions. Lack of a high level of resistance to *S. litura*, *Helicoverpa armigera* and sucking pests in cultivated groundnut and reliable screening technique under field conditions are the main reasons for the slow progress of breeding for resistance to insect pest in groundnut.

16.9.6 Breeding for Nematode Resistance

The root-knot nematodes, *Meloidogyne* spp., and the lesion nematodes, *Pratylenchus* spp., are important in groundnut (Sharma and McDonald 1990a). Based on the worldwide survey of nematologists, annual losses caused by all the nematodes to groundnut were estimated at 12% (Sasser and Freckman 1987). Root-knot disease is a serious problem in groundnut-growing areas of the world and also economic important in India. Estimated crop losses due to *Meloidogyne* spp. range from 5 to 43% (Sasser 1980). In India, the estimated yield loss of groundnut was reported 21.60% due to *M. arenaria* and *M. javanica*. In Gujarat, *M. arenaria* in Saurashtra and *M. javanica* in middle Gujarat infect groundnut and cause yield losses up to 31–38% in groundnut (Khan et al. 2010).

The cultivated groundnut has no resistance to *M. arenaria*, but resistance has been reported from several wild *Arachis* spp. (Baltensperger et al. 1986; Holbrook and Noe 1990; Nelson et al. 1989). Nelson et al. (1990) reported that resistance to *M. arenaria* in *A. cardenasii*. Stalker et al. (1995) identified *M. arenaria*-resistant genotypes (TxAG-6, TxAG-7) where resistance was introgressed into *A. hypogaea* from *A. cardenasii* using a hexaploid pathway. TxAG-6 was developed from a complex cross of *Arachis batizocoi* × (*A. cardenasii* × *A. diogoi*) (Nelson et al. 1989; Starr et al. 1995). Second germplasm line TxAG-7 which is resistant to *M. arenaria*, *M. javanica* and an undescribed *Meloidogyne* sp. from Texas was developed from a backcross of *A. hypogaea* ('Florunner') × TxAG-6 (Simpson et al. 1993).

The root-knot nematode resistance from TxAG-7 was introgressed in to breeding populations through backcross breeding (Starr et al. 1995). The first root-knot nematode (M. arenaria)-resistant peanut cultivar "COAN" with the resistance gene introgressed from A. cardenasii was released in the USA (Simpson and Starr 2001). Another groundnut variety "Tifguard", bred for resistance to both root-knot nematode and TSWV (Holbrook et al. 2008). Currently, there are six registered interspecific germplasm lines with resistance to *M. arenaria*: TxAG-6 and TxAG-7 (Simpson et al. 1993), GP-NC WS 5 and GP-NC WS 6 (Stalker et al. 2002) and NR 0812 and NR 0817 (Anderson et al. 2006). Ravindra et al. (2013) screened 71 groundnut genotypes for resistance to *M. arenaria* and *M. javanica*, and the cultivar TAG 24 was found to be highly resistant. The other cultivars ICGV 86590, JL 24, Dh- 2000-1 and TMV 2 were resistant, while Dh-3-30, Dh 86, JSP 39, GPBD 4 and Dh 101 were moderately resistant to root-knot nematodes.

In Kalahasti and Nellore district of Andhra Pradesh in India, a severe nematode disease caused by *Tylenchorhynchus brevilineatus* of groundnut reported in 1976 which has been later known as "Kalahasti malady". The reduction in the yield due to this disease was up to 50% (Vasanthi et al. 2003). Mehan et al. (1993) screened 1599 groundnut germplasm accessions and breeding lines for resistance to the nematode disease "Kalahasti malady". Of those TCG 1518 (Tirupti 3), a high-yielding breeding Virginia bunch line was found resistant to Kalahasti malady and released for disease-affected areas of Andhra Pradesh. But it was less popular among the farmers due to long duration (125–130 days). Therefore, a breeding programme was initiated

Table 16.9 Resistant/tolerant germplasm lines of groundnut for various biotic factors

Biotic factors	Genotypes
Diseases	
Early leaf spot	ICGs 6902, 11,476, 8298, 6284, 6902, 7878, NRCG Nos. 5201, 6900, 6935, 6947, 6985, 7020, 7066, 7216, 7326, 7345
Late leaf spot	ICGs 2716, 6330, 7888, 10,035, NRCG Nos. 10,121, 10,123, 10,125, 10,183, 10,427, 11,108, 11,616, 12,488, 12,338, 12,565, 12,652, 12,900, 12,925, 12,929, 2375, 2392, 5292, 7041, 7353
Rust	ICGs 1697, 2716, 4746, 7296, 7893, 7899, NRCG Nos. 10,121, 10,123, 10,950, 11,072, 11,108, 11,597, 12,487, 12,510, 12,565, 12,566, 12,652, 12,718, 12,889, 12,900, 12,912, 12,915, 12,925
Stem rot	NRCG Nos. 10,181, 10,527, 10,733, 10,751, 10,965, 11,073, 11,494, 12,488
Limb rot	NRCG Nos. 7065, 7170, 7247, 7410, 7480, 7548, 7587, 7597
PBND	NRCG Nos. 10,121, 10,123, 10,125, 10,143, 10,181, 10,233, 10,427, 10,950, 11,001, 11,069, 11,108, 6508, 7162
PSND	NRCG Nos. 12,283, 12,295, 12,316, 12,392, 12,394, 12,406, 12,408, 12,527, 12,706, 12,878
Insect pests	
Leaf miner	ICGVs 87,237, 86,709, 87,157, 87,160, 85,011, 86,162, 86,031, 87,194, 87,242, 86,601, 87,326, 87,327, 87,339, 87,341, 87,165, 86,598, 86,027, NRCG 5001, 6701, 6704
Leaf hoppers	NRCG 9773, 9767, 6688
Spodoptera litura	ICGVs 86,029, 86,030, 86,031, 86,590, NRCG 5724, 2615, 9773, 8313, 8673
Ash-weevil	NRCG 5014, 9465, 8938
Jassids	ICGs 2271, 2036, 2307, 5040, 5041, 5043
Thrips	ICGs 2271, 2307, 5037, 5040, 5041, 5042, 5043, 5044, 5045, 799
Aphids	ICG 5240

Source: ICRISAT Information Bulletin Nos. 47 and 50; Basu 2003

during 1988–1889 utilizing TCGS 1518 as source of resistance to Kalahasti malady. A breeding line TCGS 320 was released in 2002 as "Kalahasti" is a short-duration (105–110 days), high-yielding, Spanish bunch variety resistant to Kalahasti malady. The source of resistance to Kalahasti malady is available in the cultivated groundnut germplasm as well as cultivars which can be easily utilized breeding programme to develop superior cultivars for disease-prone areas. However, the sources of resistance are available, but levels of resistance to root-knot nematode in cultivated groundnut germplasm are quite low, and wild species possess high levels of resistance to root-knot nematodes.

Many resistance sources for various biotic stresses were identified (Table 16.9), and at ICAR-DGR, Junagadh, many advanced breeding cultures were developed which possess resistance/tolerance to one or more stresses (Table 16.10). So many varieties with disease resistance/tolerance have been reported (Table 16.11).

Table 16.10 Advanced breeding cultures developed at ICAR-DGR possessing resistance/tolerance to one or more stresses

Stress component	Genotypes
ELS and LLS	PBS 12,024, 12,031, 12,144, 12,032, 12,034, 12,038, 12,127, 12,048, 12,050, 12,056, 12,059, 12,077, 12,079, 12,080, 12,081
ELS	PBS 12,060, 12,038, 22,020
LLS	PBS 22,012, 22,017, 22,018, 12,097, 12,137
Alternaria blight	PBS 23,017, 13,013
Aspergillus flavus	PBS 19,003, 21,076, 23,017, 23,007, 23,019, 29,027, 29,031
Helicoverpa	PBS 23,007, 23,010 (moderately resistant)
Spodoptera I instar	PBS 13,010, 23,010, 23,011, 23,017, 23,018, 21,062, 11,050 (all highly resistant)
Spodoptera III instar	PBS 23,007, 23,010, 23,017, 23,018, 23,019, 21,062, 11,050 (all moderately resistant)
Spodoptera V instar	PBS 12,025, 12,144, 12,034, 12,039, 12,047, 12,048, 12,066, 12,067, 12,085, 22,011, 22,013, 22,020, 22,025, 22,035 (moderately resistant)
Thrips	PBS 13,010, 23,003, 24,005, 24,006, 24,030, 24,040
Leaf miner	PBS 11,050, 21,062
Alternaria, leaf hoppers and thrips	PBS 12,026, 12,039, 12,069, 12,086, 23,009

Source: Basu 2003

Table 16.11 Disease-resistant/disease-tolerant groundnut cultivars

Disease	Resistant/tolerant cultivars
Early leaf spot, late leaf spot, rust	ALR 1, ALR 2, ALR 3, Girnar 1, ICGV 86590, ICGV 87160, ICGV 86325, CSMG 84–1, OG 52–1, RSHY 1, DRG 12, DRG 17, TAG 24, BSR 1, VRI 5, Co4
Collar rot and aflaroot	OG 52–1, JCG 88, and J 11
Stem rot	OG 52–1, Dh 8 and ICGV 86590
Peanut bud necrosis disease (PBND)	ICGS 11, ICGS 44, ICGS 37, Kadiri 3, ICGV 86325, K 134, DRG 12, R 8808, JCG 88, CSMG 884, Chandra

Source: Ghewande et al. (2002)

16.10 Breeding Approaches-Conventional and Non-Conventional

The breeding methods employed in self-pollinated crops such as mass selection, pedigree, bulk, single seed descent and backcross methods are used in groundnut breeding. Introduction and mass selections played a pivotal role in the beginning, but later, hybridization followed by selection in segregating generations adopting different methods was predominantly practiced in breeding improved groundnut varieties. Cumbersome process of emasculation and pollination procedures with low success

rate of making crosses and huge time lag (8 years or more) for release as variety are the major limitations in breeding programmes of groundnut.

In most of the crossing programmes in groundnut, only two parents were used, whereas double or convergent crosses which could create form variability for selection are sparingly used. Pedigree method has been the most used one for highly heritable traits like plant type, pod size, seed shape and size, test colour, etc. and for quantitative trails like yield and seed quality pedigree method with delayed selection. For traits with low heritability, bulk-pedigree is the most used approach. When resources and space are a constraint, single seed decent method also is being resorted to (Isleib et al. 1994). Though limited use has been made of the recurrent selection procedures (Wynne and Gregory 1981), owing to space and hybridization requirements, it has been used for continued genetic enhancement in groundnut (Guok et al. 1986; Halward et al. 1991b).

Backcross breeding methods have not been used extensively as most of the economically important traits in groundnut are quantitatively inherited (Wynne and Gregory 1981; Knauft and Wynne 1995). With the increased emphasis on multiple resistance breeding, emphasis is now focused on complex crosses followed by inter-crossing of segregants to bring the desired improvement into breeding populations. While selection for resistance to insect pests and diseases is practiced in early generations, selection for yield and yield component traits is delayed to later generations (Dwivedi et al. 2003). Mutation breeding in groundnut has led to the development of several improved varieties (Janila et al. 2013). With the advent of molecular markers linked to the target trait and quantitative trait locus (QTL), marker assisted backcross breeding is now being used frequently in breeding programmes aimed at trait based crop improvement.

Groundnut is not native to India, and it was introduced in the sixteenth century; the systematic attempts for its improvement were initiated in twentieth century. In 1884, the' Mauritius' variety was introduced to Pondicherry and Madras from Mauritius, 'Spanish' and 'Virginia' from the USA and 'Small Japan' and 'Large Japan' from Japan in 1901–1902. Pedigree followed by mass selection, pureline selection, bulk-pedigree, modified bulk-pedigree, mutation and introduction are the breeding methods which are frequently used in groundnut breeding (Table 16.12). Currently, 233 groundnut cultivars have been released till 2021 for commercial cultivation in India. Groundnut varieties released for commercial cultivation in India during the last 10 years was given in Table 16.13. New breeding programme like 'Speed breeding' especially uses single seed descent method by rapidly advancing the generations by altering photoperiod, and temperature during the growth of the crop is also now being resorted.

By the enhanced availability of genomic resources, use of genomic tools also has been employed in groundnut breeding, which could accelerate the process and enhance the efficiency. Diagnostic molecular markers linked to the major effect of QTLs or other traits of breeding interest like disease resistance reported. Markers linked with root-knot nematode (Choi et al. 1999; Chu et al. 2007; Church et al. 2000; Garcia et al. 1996; Simpson and Starr 2001), rust (Khedikar et al. 2010; Mondal et al. 2012a, b), and LLS (Kolekar et al. 2016; Sujay et al. 2012), nutritional

Table 16.12 Breeding methods employed for developing groundnut varieties in India

S. no.	Breeding method	Varieties developed (no.)
1	Introduction	3
2	Mass selection	32
3	Pureline selection	23
4	Pedigree	130
5	Bulk-pedigree	20
6	Modified bulk-pedigree	14
7	Single seed descent	1
8	Mutation	8
9	Marker assisted backcrossing	2
	Total	233

quality traits (Chen et al. 2010; Chu et al. 2009; Sarvamangala et al. 2011; Wilson et al. 2017), TSWV (Tseng et al. 2016) and growth habit (Li et al. 2017). Such validated molecular makers were deployed in marker assigned breeding programmes, and the approach was successful in pyramiding nematode resistance and high oleic acid (Chu et al. 2011).

The validated markers from the rust-resistant cultivar GPBD were used to improve the disease resistance of the popular cultivars ICGV 91114, JL 24 and TAG 24 (Varshney et al. 2014). Besides, MAS and MABC were used to improve the oil quality traits in three groundnut varieties ICGV 06110, ICGV 06142 and ICGV 06420 by transferring FAD2 mutant alleles from SunOleic 95R. A large number of lines with increased oleic acid in the range of 62%–83% were identified (Shasidhar et al. 2017). At Dharwad University of Agricultural Sciences in India, MABC was used to improve JL 24 with GPBD 4 as donor parent (Yeri and Bhat 2016). Similarly, MABC was employed to improve TMV 2 for LLS and rust using GPBD 4 where two backcross lines showed enhanced resistance to LLS and rust along with 71.0% and 62.7% increase of pod yield over TMV 2 (Kolekar et al. 2017). A genome-wide association study reported by Pandey et al. (2014a, b) on 50 agronomic traits involving 300 genotypes of a "reference set" could identify 524 highly significant MTAs for 36 traits pointing to the complex genetic control. Though marker-assisted recurrent selection and genomic selection are the preferred approaches for introgression of a larger number, small-effect QTLs of such approaches have not yet been widely used in groundnut.

16.10.1 Precise and High-Throughput Phenotyping Protocols for Key Traits

The conventional phenotyping methods are laborious, time-consuming and less efficient, when a large number of genotypes are to be handled. Hence, it is time to resort to novel methodologies of high-throughput phenotyping that connects extensive genotypic data to phenotypic characteristics in a field context (Furbank and

Table 16.13 Groundnut varieties released for commercial cultivation in India during last 10 years

Cultivar	Habit group	Year	Area of adoption	Production system	Pedigree	Special characters
JL 501	SB	2010	Gujarat and southern Rajasthan	Kharif	Selection from TAG 24	Comparatively lowest intensity of LLS was observed in case of JL-501 as against higher in variety JL-24; suitable for early as well as late sown conditions
Vijetha (R 2001–2)	SB	2010	W.B., Orissa, Jharkhand, Assam, Maharashtra, Karnataka, A.P. and, T.N.	Kharif	ICGS 11 × ICG 4728	Resistant to PBND
GPBD-5	SB	2010	Jharkhand and Manipur	Kharif	TG-49 × GPBD-4	Resistant to LLS and rust
Girnar 3 (PBS 12160)	SB	2010	W.B, Orissa, Manipur	Kharif	Girnar 1 × ICGS 11	Tolerant of leaf miner and thrips
Kadiri Haritandhra (K 1319)	SB	2010	Karnataka and Maharashtra	Rabi-summer	91/57–2 X PI 476177	Multiple diseases (rust, ELS, LLs, stem rot, PBND) and insect pests (thrips, *Spodoptera litura*, jassid, *Helicoverpa*) resistant
HNG 69	VB	2010	U.P., Punjab and northern Rajasthan	Kharif	CSMG 84-1 X PG 1	Tolerant to collar rot, stem rot and ELS
GJG-HPS-1 (JSP-HPS-44)	VR	2010	Gujarat	Kharif	JSP 21 X VG 5	Tolerant to PBND
Divya (CSMG 2003–19)	VR	2011	UP and Rajasthan	*Kharif*	Amber × ICG-1697	Tolerant to PBND
RARS-T-1	SB	2011	Andhra Pradesh	Kharif and rabi-summer	TAG-24 X TG-19	Tolerant to leaf spots and rust, sucking pests (thrips and jassids)
RARS-T-2	SB	2011	Andhra Pradesh	Kharif and rabi-summer	Tirupati-4 X TIR-45	Moderately tolerant to *Spodoptera litura* and leaf hoppers

Pratap Raj Mungphali	SB	2011	Rajasthan	Kharif and summer	Selection from ICGV-98223	Tolerant to ELS, LLS, PBND, jassids, thrips and leaf miner and *Spodoptera litura*; early maturity
RG 425	VB	2011	Rajasthan	Kharif	ICG 5013 X RG 340	UP, RJ under timely sown irritated condition
ICGV 00350	SB	2012	TN and AP	Rabi-summer	ICGV 87290 X ICGV 87846	Resistant to LLS and rust, tolerant of stem rot
HNG 123	VB	2012	Rajasthan, UP and Punjab	*Kharif*	Chandra X RSB-87	Tolerant to collar rot, stem rot, LLS, *Spodoptera litura* and leaf miner
Raj Mungfali-1 (RG 510)	VR	2012	Rajasthan and Punjab	*Kharif*	RG 318 X RG 340	Tolerant to collar rot, stem rot, LLS, peanut stem necrosis diseases, thrips, jassids and grass hopper
GJG 31 (J 71)	SB	2012	Gujarat	Kharif	GG 5 X ICGV 90116	Tolerant to stem rot
GJG 9 (J 69)	SB	2012	Gujarat	Summer	GG 2 X PBS 21065	GJ under rabi-summer irrigated condition
CO 6	VB	2012	Tamil Nadu	Kharif	CS-9 X ICGS-5	Resistant to LLS and rust
Dharani (TCGS 1043)	SB	2013	Andhra Pradesh	Kharif and summer	VRI 2 × TCGP 6	Tolerant to stem rot, dry root rot and drought tolerant
GJG-22 (JSSP 36)	VB	2013	Gujarat	Kharif	JSSP 17 X GG 20	Tolerant to collar rot
GJG-17 (JSP-48)	VR	2013	Gujarat	Kharif	JSSP 11 X GG 6	Tolerant to stem rot
Phule Bharti (JL 776)	SB	2015	Maharashtra and Madhya Pradesh	*Kharif*	Selection from (ICGV 92069 × ICGV 93184) × ICGV 98300	Resistant to *S. litura* and rust in field condition
Raj Mungfali-2 (RG 578)	VB	2015	Odisha, WB and Manipur	Kharif	ICG 5013 × RG 141	Resistant to LLS, dry root rot, ELS and rust; tolerant to S. litura, thrips, jassids. Leaf miner

(continued)

Table 16.13 (continued)

Cultivar	Habit group	Year	Area of adoption	Production system	Pedigree	Special characters
GJG 18 (JSP 49)	VR	2015	Odisha, WB, Jharkhand and Manipur	Kharif	JSSP 12 × LGN 2	OD, WB, JH, MN in timely sown rainfed condition
Groundnut Co 7	SB	2015	Tamil Nadu	*Kharif* and summer	Cross derivative from ICGV 87290 × ICGV 87846	Resistant to rust
Birsa Groundnut 4 (BAU 25)	VB	2015	Jharkhand	*Kharif*	Robut × M-13	Resistant to LLS
Raj Mungfali 3 (RG 559–3)	VB	2016	Rajasthan, UP and Punjab	*Kharif*	(TKG 19A × Kadiri 3) × TKG 19A	Tolerant to *S. litura*, leaf miner and thrips
Phule Warna (KDG 128)	VB	2016	Tamil Nadu, Andhra Pradesh, Karnataka and southern Maharashtra	*Kharif*	Selection from ICGV-020059-SSU-SSD-P 37-B; (ICGV 87121 × ICGV 87853) × 92023) × ICGV 98300)	Moderately resistance to rust and leaf spot
Phule Moma (KDG 123)	VB	2016	Gujarat and Rajasthan	*Kharif*	Secondary selection from line ICGV-04168; (ICGV 96050 × ICGV 96239)	Moderately resistance to rust and leaf spot
GJG 19 (JSP 51)	VR	2016	Odisha, West Bengal, Jharkhand and Manipur	*Kharif*	JSSP-12 × LGN-2; K-99-13-B-1-2-B-B	Tolerant to stem rot, dry root rot and rust as compared to check (KDG 123)
G 2–52	SB	2016	Karnataka	*Kharif*	Gamma rays (200 Gy) induced mutant of GPBD 4	Foliar disease resistant
KCG 6 (CTMG 6)	SB	2016	Karnataka	*Rabi-summer*	(BPI PnG × ICGV 95172) × ICGV 96234	Moderately resistance to rust and late leaf spot
GKVK 5		2016	Southern Karnataka	*Kharif and Summer*	NRCG 11915 × NRCG 12326	Tolerant to rust and LLS
Central Groundnut ALG −06-320	SB	2017	Tamil Nadu and Andhra Pradesh	*Rabi-summer*	(J 11 × CG 52) × ICGV 86015	Tolerant to rust, LLS and peanut bud necrosis disease (PBND), *S. litura*, leaf miner and thrips

VRI 8 (VG 09220)	SB	2017	Tamil Nadu	*Kharif and Summer*	R 3 × AK 303	Moderately resistant to sucking pest (jassids and thrips), moderately resistant to LLS and rust
Kadiri Amaravathi (K 1535)		2017	Andhra Pradesh		Kadiri 6 × NCAc 2242	Field tolerance to PBND
GJG 32 (ICGV 03043)	SB	2018	Tamil Nadu, Andhra Pradesh, Karnataka, southern Maharashtra and Telangana	*Kharif*	[(F 334 A-B-14 × NCAc 2214) × ICG 2241) × (ICGMS 42 × Kadiri 3) × ICGMS 28 × (F 334 A-B-14 × NCAc 2214)] × LI × (Robut 33–1–1-5)	Tolerant to stem rot, colour rot and rust
GJG 33 (ICGV 07222)	SB	2018	Tamil Nadu, Andhra Pradesh and Telangana	*Rabi-summer*	[(ICGV-92069 × ICGV-93184) SIL-4 × (ICGS 44 × ICGS 76)]	Tolerant to colour rot and rust
VRI 8 (VG 09220)	SB	2017	Tamil Nadu	*Kharif and Summer*	ALR 3 × AK 303	Moderately resistant to sucking pest (jassids and thrips), moderately resistant to LLS and rust
DH-232	SB	2018	Karnataka	*Kharif*	GPBD 4 × TG 37A	Resistance to foliar diseases, 'High Shelling (78.7%)
DH-245	SB	2018	Karnataka	*Kharif*	Mutation of GPBD 4	Resistance to foliar diseases, 'High oleic acid (>70%)
Avtar (ICGV 93468)	SB	2018	Uttar Pradesh	*Rabi-summer*	ICGV 86015 × ICGV 86155	Tolerant to BND, fungal diseases, jassid and pod borer, 'Early maturity
TMV 14	SB	2019	Tamil Nadu	*Kharif*	VRI Gn. 6 × R2001–2	Tolerant to *S. litura*, thrips, leaf minor; moderately resistance to LLS and rust, 'Early maturity
Phule Chaitanya (Central- KDG 160)	SB	2019	Tamil Nadu, Telangana and Andhra Pradesh	*Rabi-summer*	Selection from [(ICGV 92069 × ICGV 93184) × (ICGV 87121 × ICGV 87853) × ICGV 92023]	Moderately resistance to stem rot and LLS, 'High Oil content (51.6%)

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Table 16.13 (continued)

Cultivar	Habit group	Year	Area of adoption	Production system	Pedigree	Special characters
Konkan Bhuratna (RTNG-29)	VB	2019	Maharashtra	*Kharif*	PBS 24030 × GPBD 4	Resistance to ELS, LLS, rust, PBND, thrips, jassids and leaf miner, 'High Oil content (50.1%)
Gujarat Groundnut HPS 2 (GG HPS 2)	VB	2019	Gujarat	*Kharif*	JVB HPS 2 × GG 20	Large seeded
AK 335 (PDKVG-335)	SB	2019	Maharashtra	*Kharif*	Selection from TG 36B	Moderately resistance to tikka, collar rot, stem rot, jassid, thrips and aphids
Phule Unnati (RHRG 6083)	SB	2019	Maharashtra	*Kharif and Rabi-summer*	(ICGV 92069 × ICGV 93184) × (ICGS 44 × ICGS 76)	Resistance to LLS, stem rot, rust, *S. litura*, and thrips, 'High Oil content (52%)
Phule Dhani (JL 1085)	SB	2019	Tamil Nadu, Andhra Pradesh and Karnataka	*Kharif*	JL 24 × ICGV 03061	Resistance to LLS and rust
Gujarat Groundnut-34 (GG 34) (AG-2012-06)	SB	2019	Gujarat	*Summer*	TG 26 × ICGV 00380	High oil content (52.8%)
Dheeraj (TCGS 1073)	SB	2019	Andhra Pradesh	*Kharif* and *Summer*	JAL 30 × Narayani	Possesses heat tolerance and high water use efficiency
BSR 2 (BSG 0912)	SB	2019	Tamil Nadu	*Kharif* and *Rabi-summer*	VRI 2 × TVG 0004	Moderately resistant to rust, LLS, jassid, thrips and aphids
Central-Pragati (TCGS 894)	SB	2019	Tamil Nadu, Telangana and Andhra Pradesh	*Rabi-summer*	Selection from sp. Mutant 1	
Dh 256	SB	2019	Tamil Nadu, Andhra Pradesh, Karnataka and Telangana	*Kharif*	R 2001–2 × GM 4–3-12	Tolerant to *S. litura*, thrips and leaf miner and leaf hopper, tolerant to mid-season drought

Girnar 4 (ICGV 15083)	VB	2020	Rajasthan, Gujarat, Karnataka, Tamil Nadu and Andhra Pradesh	*Kharif*	[ICGV 06420 × (ICGV 06420 × Sun Oleic 95R)]	Recorded 78.5% oleic acid and 4.8% linoleic acid. Tolerant to late leaf spot, rust, stem rot and peanut bud necrosis disease, leaf hopper, leaf miner, thrips and *Spodoptera litura*
Girnar 5 (ICGV 15090)	VB	2020	Rajasthan, Gujarat, Karnataka, Tamil Nadu and Andhra Pradesh	*Kharif*	[ICGV 06420 × (ICGV 06420 × Sun Oleic 95R)]	Recorded 78.4% oleic acid and 4.6% linoleic acid. Tolerant to late leaf spot, rust, stem rot and collar rot, leaf hopper, leaf miner, thrips and *Spodoptera litura*
Pratap Mungphali 3 (UG 116)	SB	2020	Rajasthan	*Kharif and Summer*	Selection from ICGV 03063	Moderately tolerant to early leaf spot (ELS), late leaf spot (LLS), rust, collar rot and dry root rot; moderately resistant to *Spodoptera litura*, leaf miner, defoliators, jassids, thrips and leafhopper
K 1719 (Kadiri Chithravati)	SB	2020	Andhra Pradesh, Telangana and Tamil Nadu	*Rabi-summer*	Kadiri 7 Bold × TAG 24	Large seeded. Moderately resistant to thrips; collar rot and PBND
K 1812 (Kadiri Lepakshi)	SB	2020	Tamil Nadu, Andhra Pradesh Karnataka and Telangana	*Kharif*	((ICGV 92069 × ICGV 93184) SIL4 × ICGV 98300)	Tolerance to leaf miner, PBND, ELS
J 87	SB	2020	Uttar Pradesh and Punjab	*Rabi-summer*	JVR- 244 × JB-866	Moderately resistant to leaf miner and ELS
Dh 257	SB	2020	Karnataka and Maharashtra	*Rabi-summer*	ICGV 07211 × ICG 2381	Tolerant to mid-season drought. Tolerance to *Spodoptera litura*

Tester 2011) and overcomes the defects of manual techniques and provides a multi-trait assessment. This approach using the automated readings and data recording reduces considerably the time required and gives the advantage of precision in readings, non-destructive samplings, high throughput with large number of data points and direct storage of the data.

It provides quantitative and non-destructive analysis of crops, automated plant handling, image-based characterization of plant growth, structure and composition, daily data acquisition and analysis for pipeline screening, gathers many data points on a large number of plants in relatively short times and acquisition of high-quality digital phenotypic data with explicit metadata and has high computing capacity for image analysis. Automation enables flexible growth conditions to elicit and measure stress responses, enables automated statistical analysis and reports integration with corporate data systems.

Ground-based camera has been used to measure the leaf water potential in groundnut effectively where random plants were selected in plots to classify and differentiate young and old leaves and subjecting the images to principal component analysis (Zakaluk and Sri Ranjan 2008). The other important trait is stomatal conductance, chlorophyll content, etc. which also have been estimated through automated setup. Chlorophyll estimates using SPAD have been widely used as rapid tool for rapid assessment of relative chlorophyll status especially under stress conditions (Arunyanark et al. 2009). Hyperspectral sensing technologies involving point spectroscopy and thermal imaging can profitably be used in estimating the incidence foliar diseases in endemic areas (Omran 2017). A basic parameter for the interpretation of remotely sensed data is the spectral reflection of the disease.

Reflectance data helps in detecting changes in the biophysical properties of plant canopy and leaf associated with pathogens. The spectral reflectance factors differ significantly according to the health condition of the plant. The leaves of the healthy peanut show a decreasing reflection in 1015 nm, whereas the heavily diseased leaves show an increasing reflection. In the thermal infrared range, diseased plants show higher temperature than healthy ones. The temperature difference allows the discrimination between the infected and healthy leaves before the appearance of visible necrosis on leaves. Two simple indices, early leaf spot index (ELSI) and late leaf spot index (LLSI), allows early prediction of the peanut disease severity. The disease severity estimation using ELSI and LLSI have an overall accuracy of 78% and 89%, respectively.

Screening a large set of germplasm at early stages of growth will reduce the load of field evaluation for plant breeders and plant genetic resource managers. Precise phenotyping of germplasm enables breeders to use these readily in their breeding activity. In the present situation, it is necessary for the breeders to be equipped with the emerging technologies to develop crop varieties to suit changing climate and cropping patterns.

16.11 Modernization of Crop Improvement Programme in Groundnut

Though significant contributions have been made by the conventional breeding approaches, to break the existing yield barriers to exploit the targeted approaches for breeding the use of genomic tools is profitable and will accelerate the cultivar development. Technologies such as next-generation sequencing (NGS) and genotyping have facilitated the discovery of functional genes and functional markers that would enhance the momentum of cultivar development (Varshney 2016). Most of the progress in the generation of genetic information of groundnut has now been made possible in recent advancements, whereby new molecular markers have been developed, refined expressed sequence tags (EST) identified, genetic and physical maps that are dense generated, important genes discovered and chromosomal regions (QTL) linked to stresses of economic importance identified (Table 16.14). This has further led to the application of molecular markers in breeding (MAB) to complement conventional methods, culminating into the release of superior groundnut varieties.

16.12 Future Thrusts

Considerable improvement has been made in the cultivar development globally leading to the release of a good number of varieties with improved productivity and quality. Several sources of variability for economically important traits in groundnut were identified or developed. A good number of new germplasms also has been registered with the national repositories. Though there was an initial lag, a large volume of genomic resources was added during the last decade, and genomic tools like MAS, MABC, etc. have been applied in groundnut breeding. Further advancement resulted from the availability of the genome sequence of cultivated groundnut, and its progenitors have facilitated more precise approaches of genome-assisted breeding. The methodologies of high-throughput genotyping and phenotyping also have accelerated the process or trait-oriented breeding. The low-cost genotyping services have added an additional impetus to the progress accelerated breeding. Thus, with these emerging technologies and developments, we look forward for more improved cultivars with higher productivity and quality.

Table 16.14 The QTLs reported in groundnut

QTL for the trait	Number	References
Leaf spot resistance	28	Wang et al. (2013) and Liang et al. (2017)
Late leaf spot resistance	45	Agarwal et al. (2018) and Kolekar et al. (2016)
Thrips resistance	3	Wang et al. (2013)
Total number of holes on pods (bruchid)	3	Mondal et al. (2014a)
Per cent pod weight loss (bruchid)	1	Mondal et al. (2014a)
Total development period (bruchid)	4	Mondal et al. (2014a)
Per cent adult emergence (bruchid)	4	Mondal et al. (2014a)
Susceptibility index	4	Leal-Bertioli et al. (2015)
Rust resistance	51	Kolekar et al. (2016), Leal-Bertioli et al. (2015) and Mondal et al. (2014b)
Total number of lesions/ leaf area	3	Leal-Bertioli et al. (2015)
Log incubation period	3	Leal-Bertioli et al. (2015)
Number of sporulated lesions/leaf area	2	Leal-Bertioli et al. (2015)
Lateral branch length	5	Leal-Bertioli et al. (2016) and Wang et al. (2018)
Number of lateral branches	2	Leal-Bertioli et al. (2016)
Specific leaf area_40 days	5	Leal-Bertioli et al. (2016)
Root galling index	5	Leal-Bertioli et al. (2016)
Eggs per gram of root_2011	3	Leal-Bertioli et al. (2016)
Root-knot nematode resistance	17	Burow et al. (2014) and Leal-Bertioli et al. (2016)
Linoleic acid	52	Sarvamangala et al. (2011), Hu et al. (2018) and Pandey et al. (2014a, b)
Palmitic acid	31	Wang et al. (2015)
Oil content	27	Selvaraj et al. (2009), Sarvamangala et al. (2011), Huang et al. (2015) and Pandey et al. (2014b)
Oleic acid	27	Sarvamangala et al. (2011) and Hu et al. (2018)
SPAD chlorophyll meter reading	12	Faye et al. (2015)
SPAD chlorophyll meter reading_40 days	7	Leal-Bertioli et al. (2016)
Peg length	11	Fonceka et al. (2012)
Pod beak	10	Fonceka et al. (2012)
Pod constriction	14	Fonceka et al. (2012) Leal-Bertioli et al. (2015)
Hundred pod weight	6	Fonceka et al. (2012) and Wang et al. (2018)
Pod width	21	Fonceka et al. (2012), Wang et al. (2018) and Chavarro et al. (2020)
Pod length width ratio	4	Wang et al. (2018)

(continued)

Table 16.14 (continued)

QTL for the trait	Number	References
Protein content	8	Sarvamangala et al. (2011)
Log root dry weight	2	Leal-Bertioli et al. (2016)
Seed length	18	Selvaraj et al. (2009), Fonceka et al. (2012) and Wang et al. (2018)
100-seed weight	5	Selvaraj et al. (2009) and Fonceka et al. (2012)
Seed size index	3	Chavarro et al. (2020)
Kernel percentage	3	Chavarro et al. (2020)
Single kernel pod	4	Chavarro et al. (2020)
Double kernel pod	2	Chavarro et al. (2020)
Pod area	3	Chavarro et al. (2020)
Pod density	3	Chavarro et al. (2020)
Weight of 10 seeds	2	Leal-Bertioli et al. (2015)
Log weight of 10 seeds	4	Leal-Bertioli et al. (2016)
Seed width	13	Fonceka et al. (2012) and Chavarro et al. (2020)
Stress tolerance index, hundred pod weight	2	Fonceka et al. (2012)
Stress tolerance index, seed number	2	Fonceka et al. (2012)
Stress tolerance index, total biomass	2	Fonceka et al. (2012)
Stress tolerance index, hundred seed weight	2	Fonceka et al. (2012)
Stress tolerance index, haulm weight	2	Fonceka et al. (2012)
Stress tolerance index, pod number	3	Fonceka et al. (2012)
Resistance to tomato spotted wilt virus (TSWV)	18	Qin et al. (2012) and Wang et al. (2013)
Tomato spotted wilt virus resistance	12	Agarwal et al. (2018)
Total biomass	5	Fonceka et al. (2012) and Leal-Bertioli et al. (2016)
Haulm weight	16	Fonceka et al. (2012), Leal-Bertioli et al. (2016) and Faye et al. (2015)
Main stem height	11	Fonceka et al. (2012) and Leal-Bertioli et al. (2015)
Log_Main stem height	3	Leal-Bertioli et al. (2015)
Plant weight	1	Selvaraj et al. (2009)
Log weight ratio root/ aerial part	3	Leal-Bertioli et al. (2016)
Growth habit	6	Fonceka et al. (2012)
Harvest index	2	Fonceka et al. (2012) and Faye et al. (2015)
Shell weight	1	Fonceka et al. (2012)
Pod weight	7	Fonceka et al. (2012) and Faye et al. (2015)
Number of pods per plant	6	Selvaraj et al. (2009) and Chen et al. (2019)
Pod number	2	Fonceka et al. (2012)

(continued)

Table 16.14 (continued)

QTL for the trait	Number	References
Seed weight	6	Fonceka et al. (2012), Dodia et al. (2019) and Chavarro et al. (2020)
Seed number	8	Fonceka et al. (2012), Leal-Bertioli et al. (2016) and Chen et al. (2019)
Log seed number	3	Leal-Bertioli et al. (2015)
Fruiting branches	5	Wang et al. (2018)
Total number of branches	4	Wang et al. (2018)
Internode number	4	Wang et al. (2018)
Stem rot resistance	43	Bera et al. (2016), Dodia et al. (2019) and Luo et al. (2020)
Root hairiness	3	Dodia et al. (2019)
Leaf shape	2	Dodia et al. (2019)
No of primary branches	1	Dodia et al. (2019)
No of secondary branches	1	Dodia et al. (2019)
Plant height	11	Dodia et al. (2019), Wang et al. (2018) and Faye et al. (2015)
Pod length	4	Dodia et al. (2019) and Wang et al. (2018)
Shelling percentage	5	Dodia et al. (2019), Faye et al. (2015) and Huang et al. (2015)
Per cent seed infection index (aflatoxin)	2	Yu et al. (2019)
Aflatoxin B1 content	7	Yu et al. (2019)
Aflatoxin B2 content	5	Yu et al. (2019)
Aflatoxin resistance	3	Pandey et al. (2014a, b) and Zuang (cited from Soni et al. 2020)

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Soybean Breeding

17

Anita Rani and Vineet Kumar

Abstract

Unique confluence of oilseed, leguminous and nutraceutical properties in soybean seed has made this crop the leading oilseed, the major animal feed and a much sought-after health food of this century. Undoubtedly, sustained effort to breed soybean varieties to enhance the yield is the major objective; however, there is a tremendous scope for genetic improvement to meet the requirement of each segment of the soy industry. To begin with, the chapter briefs about the historical account of soybean across the world and the morphological phenotypes important from the point of view of soybean breeder. Subsequently, the major breeding methods employed in soybean, the genetics of the important qualitative and quantitative traits and the interventions required on the improvement of the yield components, resistance against major diseases, enhancement of oil and protein, improvement of oil quality, elimination/reduction of anti-nutritional and undesirable factors such as Kunitz trypsin inhibitor and off-flavour generating lipoxygenases are discussed. Conventional, molecular and transgenic approaches employed for achieving the breeding objectives are highlighted.

Keywords

Glycine max · Breeding methods · Maturity · Disease resistance · Speed breeding · Yield components · Kunitz trypsin inhibitor · Off-flavour · Marker-assisted breeding · Oleic acid and phytic acid · Transgenic

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_17

17.1 Introduction

Globally, soybean [*Glycine max* (L.) Merr.] is the leading oilseed crop with a production of 363.27 million tonnes, accounting for 60.43% of the world oilseed production (601.14 million tonnes) (United States Department of Agriculture 2021). Brazil is the leading country in soybean production with a share of 37.71% in the world's soybean production, followed by the United States of America (30.97%), Argentina (12.66%), China (5.39%), India (2.87%), Paraguay (2.72%) and Canada (1.74%) (United States Department of Agriculture 2021). India ranks fifth position in the world soybean production. Soybean oil is the most consumed vegetable oil worldwide after palm oil. The global soybean oil production in 2020–2021 was 59.74 million tonnes, which accounted for 28.85% of total vegetable oil (207.01 million tonnes) produced worldwide (United State Department of Agriculture 2019). China is the leading soybean oil-producing country with a share of 28.20% followed by the United States of America (18.97%), Brazil (15.06%), Argentina (13.55%), the European Union (5.12%) and India (2.82%). Deoiled cake obtained after crushing soybean for extraction of oil fraction is termed soymeal and is a protein-rich commodity animal feed and processing soy products for human consumption. A total of 249.75 million tonnes soybean meal is produced worldwide in 2020–2021, which is 71.24% of total protein meal (350.55 million tonnes) produced.

China is the leading soybean meal-producing country with a share of 29.80%, followed by the United States of America (18.40%), Brazil (14.51%), Argentina (12.59%), the European Union (5.09%) and India (3.01%). Besides being a rich source of oil (average 18–20%) and protein (about 40%) which determine the market value of soybean crop, soybean is a rich source of several nutraceutical molecules such as isoflavones, tocopherols, essential fatty acids, and phosphatidylcholine (lecithin) which provide special health benefits to the consumers (Messina et al. 1994; Potter et al. 1998; Nordentoft et al. 2008; Kumar et al. 2010c). Besides, low glycemic index of soybean makes it an ideal food ingredient for diabetic patients. Utilization of this golden bean in food uses is by and large confined to southeast nations where soybean constitutes the staple diet of the masses. In the wake of expanding awareness about the special health benefits soybean provides the consumer, apart from a rich source of plant-based protein, efforts are being made to incorporate soybean in a regular diet in the countries beyond its traditional bastion of Southeast Asia. This has necessitated the development of specialty soybean suiting the local mode of incorporation of soybean in regular diet.

17.2 Historical Account (Origin, Evolution and Expansion of Soybean)

There is a common concurrence that soybean cultivation originated in China about 3000–3700 years ago based upon the carbon dating of the soybean seed remains obtained in excavation. Theories do exist that the crop originated in northeast, Huang-Huai Valley (HHV) and southern region of China. However, Lu (1978)

opined that soybean originated from several regions of China as indicated by the geographical distribution of short-day character of wild soybean. During the rule of Zhou dynasty in China (1050 BC–250 BC), it was one of the five sacred grains along with wheat, barley, rice and millet, and used to be known by the name *shu*. Modern cultivated soybean was domesticated from wild soybean (*Glycine soja*) about 6000–9000 years before. Han et al. (2016) sequenced more than 50,000 targeted genomic regions of 404 accessions of *Glycine max*, 72 accessions of *Glycine soja* and 36 accessions of *Glycine gracilis* and land races. *Glycine gracilis* is considered as an evolutionary product of domestication of soybean. The authors reported that there was no gene flow from *Glycine max* to *Glycine gracilis* or *Glycine soja*, but significant gene flow was observed from *Glycine soja* to *Glycine gracilis* and from *Glycine gracilis* to *Glycine max*. A moderate gene flow from *Glycine soja* to *Glycine max* was also observed.

This substantiated the theory that *Glycine soja* is the progenitor of both *Glycine max* and *Glycine gracilis*. Far greater genetic introgression from *Glycine soja* in *Glycine max* accession from the Huang-Huai Valley (HHV) region of China than from the accessions from other geographical regions of China also substantiated that HHV of China is the most likely centre of domestication of soybean (Sedivy et al. 2017). During the period between 200 BC and 200 AD, soybeans from North China came to Japan via Korea. In the third century, there is a mention of soybean varieties in Korean literature. Further, soya sauce and *meso* as soya food are mentioned in Korean literature in 638 AD. The earliest Japanese reference to soybean is in the classic Kojiki in 712 AD. In the early eighteenth century, Europeans started raising soybean in their botanical gardens; however, commercial cultivation of the crop in this continent commenced in 1875. In 1829, US farmers raised the soybean variety for soy sauce. The earliest known reference of soybean in India was in 1832 by Roxburgh who described a soybean variety grown in Calcutta Botanic Garden; however commercial cultivation of soybean in the country commenced in the late 1960s as an economical source of plant-based protein to combat malnutrition. However, it transformed as a major oilseed crop of the country. In Brazil, soybean arrived around 1882 to the state of Bahia where Gustavo Duttra evaluated soybean cultivars (Gavioli 2013). In Brazil, immigration of Japanese people in the first decade of the twentieth century led to the promotion of soybean in this country as soybean was in the staple diet of the immigrants. Presently, Brazil is the leading producer which grows soybean in 38 million ha, followed by the United States of America (33.6 million ha), Argentina (16.4 million ha), China (9.3 million ha) and India (12 million ha).

17.3 Taxonomy, Floral Biology and Hybridization

Soybean falls in the order Fabales, family Fabaceae, tribe Phaseoleae, subfamily Papilionoideae, genus *Glycine* and subgenus *Soja*. Cultivated soybean *Glycine max* is considered to be domesticated from its wild annual soybean *Glycine soja* ($2n = 40$) and on crossing with the latter produce fertile F_1 hybrids. Besides, the

subgenus *Glycine* consists of 26 wild perennial species, which are native to Australia and replete with genes for resistance against insect-pests and developing climate-smart genotypes.

Both the main stem and the branches bear axillary buds which blossom into racemes, containing 8–16 or more number of flowers depending upon the genotype. Soybean has a typical papilionaceous flower, with tubular calyx of five unequal sepals, corolla of five petals, androecium of ten stamens and a gynoecium. The flower is white or purple. Five petals are arranged in one large petal in the posterior region, two lateral wing petals and two anterior keel petals. The keel surrounds the androecium, which has nine fused and one free posterior stamen filament. Gynoecium has one ovary with four ovules and one style which terminates into a capitate stigma tilting towards the free stamen filament. Growth of the style and stamens is synchronous which facilitates shedding of the pollens at maturity directly on the stigma. As a result, the floral biology of the soybean flower makes soybean largely a self-pollinated crop. This necessitates the removal of anthers for crossing between the desired parents. For crossing, if the days-to-flowering information of the parents is not known, the parents are planted on staggered dates to synchronize the appearance of buds of the female parent with the opening of flowers for the availability of pollens from the male parent to affect the hybridization. In general, both emasculation and pollination are done in the morning hours using forceps and needles, and in 1 h, a skilled person can affect 25 crossing events, comprising of both emasculation and pollination procedures. True F_1 plants can be identified by dominant morphological markers such as hypocotyl pigmentation, flower colour, glabrous nature of the pod and stem and pubescence colour on the stem and pods. The absence of these contrasting morphological features in female and male parent, necessitates the deployment of co-dominant molecular markers or trait gene-specific markers for the identification of the true F_1 plants (Fig. 17.1).

17.4 Breeding Methods

Soybean breeding, like the breeding of other self-pollinated crops, involves development of variability for desired traits, generation advancement for achieving homozygosity and identification of superior genotypes. To achieve sufficient variability selection of parents is a very important step. Parents are selected on the basis of their genotypic diversity and the traits desired in the final variety. Progenies of these crosses segregate genetically during generation advancement; most of the loci get fixed after six to seven generations of selfing, and new recombinants are thus formed. The selection methods used for identification of genotypes with most useful combinations of the desired traits are pedigree method, single-seed descent method, backcross method, recurrent method and bulk method.

Pedigree selection involves visual selection of the families with high-yielding capacity in each generation followed by within-family selection of one or more plants to advance to the next generation. This increases the frequency of lines with desirable traits. Single-seed descent (SSD) method involves advancing one seed

Fig. 17.1 Confirmation of true F_1 plants using null KTI allele-specific marker (**a**) and KTI-linked SSR marker, Satt228 (**b**), in NRC7-derived KTI-free line×NRC109. Lane nos. 2, 4, 5 and 8 correspond to true F_1 plants. P1 and P2 correspond to NRC7 and PI542044 (donor of null *KTI* allele), respectively. Lane L corresponds to 50 bp DNA ladder

from each progeny plant to the next generation to develop nearly homozygous lines that still preserve most of the original genetic variation in a population. Single-pod method is also used instead of single-seed descent method, for the added convenience of collecting pod from each plant and maintaining the variability during generation advancement in case one seed from the pod fails to establish a plant due to any unfavourable condition. SSD method of selection in combination with speed-breeding method of multiple generations in a single year is a very effective method of breeding soybean varieties with desirable trait. But there are certain shortcomings as a large number of progenies have to be maintained until the production of a generation in which uniform lines are selected for yield testing.

In bulk method of breeding, the population is advanced in bulk with no artificial selection until later generations, when nearly homozygous lines are selected for yield testing (Orf et al. 2004). Bulk method is simple method, but there are certain shortcomings like a steady reduction of genetic variability in each generation due to inadequate sample size and the natural selection within the population moving in an undesirable direction. However, the population exhibited a higher genetic gain from selection and higher mean values than populations developed by the other two methods (pedigree and single-seed descent), when natural selection within the population favoured high-yielding genotypes. Backcross breeding method is mainly used to introgress disease resistance or quality trait governed by major genes in popular and adapted varieties lacking few desirable traits. Molecular markers are

very helpful in introgression of desirable trait in adapted varieties. In India, resistance to yellow mosaic disease was introgressed in the most popular soybean variety JS335 to make it resistant to the disease, and null allele of Kunitz trypsin inhibitor (KTI) was introgressed in soybean cultivar with multiple disease resistance variety JS97-52 using marker-assisted backcross selection. Mass selection (Burton and Brim 1981; Tinius et al. 1991), selection among half-sib families (Burton and Carver 1993), selection within half-sib families (Burton et al. 1983) and the selection from S1 (or S2) families (Brim and Burton 1979; Kenworthy and Brim 1979; Sumarno and Fehr 1982; Rose et al. 1992) are other alternate breeding methods used in soybean cultivar development. The traditional pedigree method and the single-seed descent method (SSD) are most often used in soybean breeding (Goulden 1939; Grafus 1965; Cooper 1990). The selection of breeding method depends on the breeding objective, available variability and availability of resources. Breeding objectives depend on the local ecological conditions, diseases and insect-pest pressure and the market demand. The efficiency of various methods of selection failed to show significant differences among them (Raeber and Weber 1953; Torrie 1958; Luedders et al. 1973; Boerma and Cooper 1975; Degago and Caviness 1987; Byron and Orf 1991; Bravo et al. 1999; Cober and Voldeng 2000). The highest genetic gains in seed yield from selection were most frequently found in populations developed by the pedigree method, while the highest mean values were found in populations developed by the SSD method.

17.5 Speed Breeding

Cross breeding in soybean is time-consuming as the crop life cycle takes 3–4 months for completion. Therefore, genetic improvement rate in soybean is slow. Shuttle breeding is the regular strategy to increase the generation turnover to accelerate the breeding programmes in soybean. Ghosh et al. (2018) reported the speed-breeding platform developed by scientists at the John Innes Centre, University of Queensland and University of Sydney. This uses a glasshouse or an artificial environment with enhanced lighting to create intense day-long regimes to speed up the breeding programmes, which greatly shortens generation time and accelerates breeding and research programmes. An economical method of speed breeding has also been lately suggested by Rani et al. (2020), which comprises of simple net house instead of compact growth chamber, and does not deploy fluorescent lamps or carbon dioxide (CO_2) supplementation.

The authors shortened the generation time by combining two approaches. The first approach was to shorten the photoperiod by covering the pots with black bags in the evening and their removal in the morning. Soybean is a short-day plant, and shortening of the photoperiod accelerates the flowering and reproductive growth. This is attributed to the fact that short inductive period causes initiation of flowering 10–15 days earlier than in the normal/longer photoperiod. Shortening of photoperiod also reduces the lag phase of around 10 days between the flower-opening and the pod initiation. A number of flowers and pods decrease due to acceleration of the growth

induced by short photoperiod; however, fewer flowers or pods produced under short photoperiod do not matter as single-seed descent (SSD) method is the most common breeding approach in soybean. The second approach was to harvest immature pods (30 days after flowering), air-dry them for 7–8 days and sow them. The generation time combining these two approaches was reduced to 70 days which is significantly lower than 90–130 days taken under longer photoperiod. Using these approaches, the authors could advance F_2 generation of a cross to F_6 generation within a time span of 1 year. The authors took four generations in a year instead of five possible generations due to the low temperature experienced during December–January months, which delayed the flowering and extended the life cycle as the experiment was performed under ambient conditions. The authors suggested that the use of temperature-controlled glasshouse with openable roof to control the photoperiod would allow rapid generation cycling through single-seed descent (SSD) for large crop improvement programmes.

17.6 Genetics and Breeding for Qualitative and Quantitative Trait

17.6.1 Qualitative Traits

17.6.1.1 Plant Growth Habit

Soybean crop plant may be determinate, semi-determinate and indeterminate in terms of plant growth habit. In determinate type, plant vegetative growth terminates with the completion of flowering, while in indeterminate type, vegetative growth and flowering occur simultaneously. In semi-determinate, plant growth does occur for a limited duration after flowering. Two loci Dt1 and Dt2 govern these growth pattern determinates (Bernard 1972). Determinate type is governed by recessive allele dt1, while the indeterminate type by Dt1. Dt2 controls semi-determinate type growth, as the locus reduces the effect of Dt1.

17.6.1.2 Seed Coat Colour

Soybean can be in yellow, green, black, brown and red in its seed coat colour (Fig. 17.2). It is an important seed attribute that determines that end use of soybean. It is the yellow seed coat colour soybean which is used as raw material for extraction of oil. Green, brown, black or any bicolour soybean is primarily used for processing food products or in soy-based food recipes. They vary in their antioxidative and nutraceutical properties, with black soybean possessing the highest antioxidative and nutritional value (Kumar et al. 2010b). Classic genetic studies revealed primarily five loci I, R, T, W1 and O that determine the seed coat colour appearance (Yang et al. 2010). I locus has four alleles, namely, I, i^i, i^k and i. Dominant I allele produces complete colourless soybean, while i produces coloured seed coat (Senda et al. 2004), and i^i and i^k determine pigmentation on the hilum and saddle regions of the seed coat. R and T determine the type and extent of seed coat colour. R, T and *i* result in complete black; I, R and t generate imperfect black; i, r and T produces brown; and

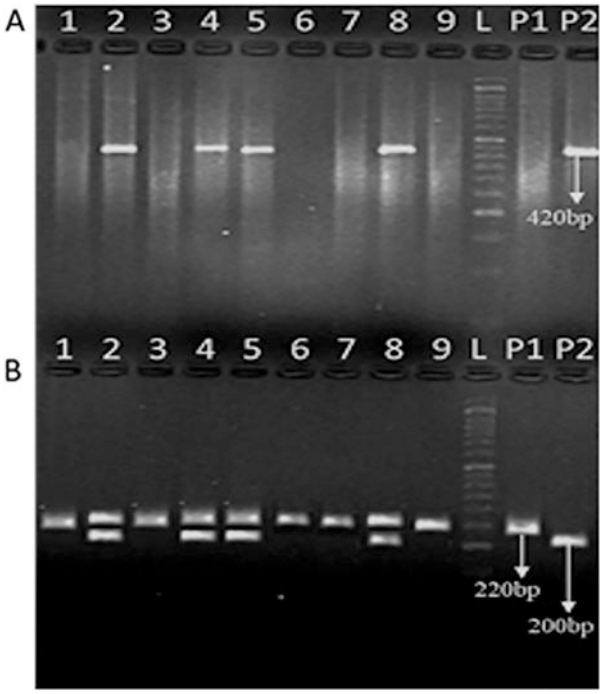

Fig. 17.2 Soybean of varying seed coat colour

i, r and t cause buff seed coat colour. Genetic background of irT (brown colour) is affected by O locus, as recessive o allele produces red-brown colour, while I,R, t background is affected by W1 locus, as recessive w1 allele generates buff colour. Green cotyledon phenotype has been reported to be governed by the nuclear as well as cytoplasmic genes (Terao 1918). D1 (Glyma.01 g214600 in W82.a2.v1 assembly) and D2 (Glyma.11 g027400) are two unlinked, paralogous nuclear genes, whose double-recessive mutant (*d1d1d2d2*) results in chlorophyll retention, called 'stay-green' (Fang et al. 2014). D1 and D2 are homologs of the STAY-GREEN (SGR) genes from other plant species and were duplicated as a result of the most recent whole genome duplication in soybean. Transcriptional analysis by Fang et al. (2014) showed that both D1 and D2 were more highly expressed in older tissues, and chlorophyll degradation and programmed cell death-related genes were suppressed in a d1d2 double mutant, indicating that these genes are probably involved in the early stages of tissue senescence. Double variants of the D1 and D2 gene result in the stay-green phenotype, including delayed yellowing of leaves during senescence, with green seed cotyledons (Guiamet et al. 1991; Fang et al. 2014). Cytoplasmic inheritance of green cotyledon is governed by 5-bp insertion in the soybean chloroplast genome resulting in a frameshift in PsbM, which encodes one of the small subunits of photosystem II (Kohzuma et al. 2017). The genotyping of D1, D2 and PsbM from 212 soybeans with green cotyledons revealed that all lines carry either d1d2 or PsbM with the known mutations (Kohzuma et al. 2017).

17.6.1.3 Maturity Gene(s)

Soybean is a typical short-day (SD) plant, i.e. the plant enters the reproductive phase once the day length becomes shorter than the critical length. Soybean plant flowering under short-day conditions would revert back into vegetative phase if shifted to long-day (LD) conditions (Washburn and Thomas 2000; Wu et al. 2006). GmFT2a and GmFT5a genes have been reported to promote flowering in *Arabidopsis* and soybean (Kong et al. 2010; Sun et al. 2011; Cai et al. 2018), while GmFT4 gene is induced by LD conditions and was reported to function in delaying flowering when transformed and expressed in *Arabidopsis* (Zhai et al. 2014; Cao et al. 2016). Samanfar et al. (2017) reported that GmFT4 is the most likely candidate gene at a newly identified maturity locus E10.Hitherto, 13 major genes/loci, namely, E1 (Cober and Voldeng 2001; Molnar et al. 2003), E1La and E1Lb (Xia et al. 2012), E2 (Cregan et al. 1999), E3 (Molnar et al. 2003), E4 (Abe et al. 2003; Molnar et al. 2003), E6 (Li et al. 2017b), E7 (Cober and Voldeng 2001; Molnar et al. 2003), E8 (Cober et al. 2010), E9 (Kong et al. 2014), E10 (Samanfar et al. 2017) and J and GmAGL1 (Zeng et al. 2018) affecting flowering and maturity period have been mapped. Barring E6, E9, J and GmAGL1, the dominant allele of all of these genes, delay flowering.

With regard to maturity, nine loci have been identified as *E1–E8* and *J*, and these loci are strengthened and weakened by long-day length condition (LD) and short-day length condition (SD), respectively (Wang et al. 2008). Furthermore, four maturity loci have been characterized at the molecular level. *E1* gene encodes a transcription factor which functions as a flowering repressor with a putative nuclear localization signal and a B3-related domain (Xia et al. 2012), while *E2* is an orthologue of *Arabidopsis* flowering gene *GIGANTEA* (Watanabe et al. 2011). Two E1-L genes, E1 La and E1Lb (*Glyma04g24640.1*/*Gm18g22670*), have been reported to have an expression pattern similar to E1 (Xia et al. 2012). E1Lb retards flowering under long-day conditions by repressing the expression of FT2a and FT5a independently of E1 (Zhu et al. 2018). *E3* and *E4* are phytochrome genes *GmPhyA3* (Watanabe et al. 2009) and *GmPhyA2* (Liu et al. 2008), respectively. Besides, two homologs of soybean *flowering locus T* (*FT*) genes, *GmFT2A* and *GmFT5A*, coordinately regulate flowering. Four identified maturity genes *E1*, *E2*, *E3* and *E4* delay flowering and maturity under LD through downregulating *GmFT2A* and *GmFT5A* (Kong et al. 2010; Watanabe et al. 2011; Xia et al. 2012).

17.6.1.4 Pod Shattering

Loss of pod shattering function in low humid and high-temperature condition was one of the key functions during soybean domestication. Two major genes Pdh1 and SHAT1–5—a NAC gene—govern pod dehiscence in soybean. According to Dong et al. (2014), NAC gene triggers the deposition of the secondary walls of the lignified fibre cap cells (FCC) in the pod ventral suture and determines the binding strength of pods. Conversely, functional product of *Pdh*1 gene triggers the dehiscence of pods under low humidity conditions (Funatsuki et al. 2014). Zhang and Singh (2020)

reported a novel locus NST1A, apart from Pdh1 and NAC gene, associated with pod shattering and its interaction with Pdh in determining the pod shattering.

17.6.1.5 Breeding for Biofortification

Soybean seed possesses a unique seed composition. Besides being rich in protein, it does suffer from the shortfall of the presence of undesirable components which affect protein digestibility and generate off-flavour, thereby limiting its bioavailability. The concentration of these biomolecules in soybean seeds may vary depending upon the location and the environment they have been raised (Kumar et al. 2006a, b). However, the genetic inheritance of some of the undesirable biomolecules is well established, and these undesirable molecules can be genetically eliminated from soybean genotypes through conventional and marker-assisted breeding.

Breeding for Improved Protein Digestibility Through Genetic Elimination of Kunitz Trypsin Inhibitor

Despite being the rich source of quality seed protein, soybean does suffer from the shortfall of poor protein digestibility due to the presence of trypsin inhibitor present in its seeds. Trypsin inhibitor activity in soybean is a function of genotype (Kumar et al. 2001; Kumar et al. 2019), the growing location and environment (Kumar et al. 2003; Kumar et al. 2006a, b). Kunitz trypsin inhibitor (KTI-a 20kDa polypeptide), and Bowman-Birk inhibitor (BBI-a smaller 8 kDa polypeptide) both contribute to trypsin inhibitor activity. The former is primarily responsible for total trypsin inhibitor activity, and its contribution to trypsin inhibitor activity is genotype-dependent (Peric et al. 2014; Kumar et al. 2019), present in immature pods also (Kumar et al. 2006c), and affects human health (Liener 1994). KTI is relatively more thermolabile due to the presence of only two disulphide linkages compared to the seven disulphide bonds present in BBI. However, minimum 15–20 min boiling of soybean seeds is required for its complete inactivation (Chen et al. 2014). Residual activity of KTI in the soy food and feed products is ascribed to faulty processing such as insufficient temperature and duration of the heating (Brandon et al. 1991). Moreover, heat treatment is not only cost-ineffective but also results in approximately 20% decline in protein solubility (Anderson 1992).

KTI in soybean seed is governed by a single gene and is controlled by multiple alleles (Hymowitz 1973; Zhao and Wang 1992). The four electrophoretic forms of soybean KTI are controlled by co-dominant multiple allelic series (Ti^a, Ti^b, Ti^c and Ti^d). A fifth form lacking Kunitz trypsin inhibitor activity is controlled by a recessive allele *ti* (Orf and Hymowitz 1979). The gene has been located on the linkage group (LG) A2, corresponding to chromosome 8 of soybean genome of the soybean molecular linkage map (Cregan et al. 1999). Several studies have shown the tight linkage of three SSR markers, namely, Satt409, Satt228 and Satt429, with *ti* locus (Kim et al. 2006; Rani et al. 2011). A KTI null allele-specific marker has also been designed from genotype PI157440 (de Moraes et al. 2006) which has been deployed in identification of plants carrying the null allele of KTI derived from PI542044 (Kumar et al. 2013b). In India, KTI-free genotypes NRC101 and NRC102 have been developed using PI542044 as the donor of null KTI allele through

marker-assisted forward breeding (Rani et al. 2010), and both these advanced breeding lines were commercialized to private soy food industries in India. Further, KTI was genetically eliminated from elite soybean varieties, viz. NRC7, JS97–52, MACS450, JS93–05 and S97–12, through marker-assisted breeding (Kumar et al. 2011a, b, 2012, 2015). The country released its first Kunitz trypsin inhibitor-free soybean variety NRC127 developed through marker-assisted backcrossing (MABC) for the farmers of Central India for the entire state of Madhya Pradesh, Vidharbha and Marathwada region of Maharashtra and Bundelkhand region of Rajasthan, Uttar Pradesh and Gujarat state. In Serbia, two KTI-free soybean genotypes, viz. 'Laura' and 'Launa', have been developed (Peric et al. 2014).

Breeding for Improved Flavour and Fragrance

Grassy and beany flavour associated with the soy products constrains human consumption of soybean in the countries where people are not accustomed to it. This off-flavour in soy food products is generated by the catalytic oxidation of unsaturated fatty acids by lipoxygenases Lox1, Lox2 and Lox3 present in the soybean seed (Gerde and White 2008; Wilson 1996; Axelrod et al. 1981). Lox2 is the principal contributor to the off-flavour developed in soy products (Davies et al. 1987). The absence of each lipoxygenase isozyme in soybean seed is monogenically controlled by three null alleles, *lox1*, *lox2* and *lox3*, which are inherited as simple recessive alleles (Davies and Nielsen 1986; Hildebrand and Hymowitz 1982; Kitamura et al. 1983, 1985). The *Lox1* and *Lox2* loci are tightly linked and are present on chromosome 13 (LG F). *Lox3* locus is present on chromosome 15 (LG E), and its segregation is independent of *Lox1* and *Lox2* (Kitamura et al. 1985; Davies and Nielsen 1986; Hajika et al. 1992). Therefore, it was possible to easily breed double-null lipoxygenases lx1lx3 and lx2lx3 soybean genotypes. However, the repulsion-phase linkage present between *lox1* and *lox2* recessive alleles was the major impediment in development of triple-null lipoxygenase (lx1lx1lx2lx2lx3lx3) soybean genotypes (Davies and Nielsen 1986; Hildebrand and Hymowitz 1982; Kitamura et al. 1985). However, the use of irradiation helped in breaking of repulsion-phase linkage between mutant alleles at *Lox1* and *Lox2* loci, resulting in a coupling-phase linkage which made possible to develop a triple-null lipoxygenase (lx1lx1lx2lx2lx3lx3) genotype (Hajika et al. 1991; Kitamura 1991).

Genetic basis of mutation in Lx1, Lx2 and Lx3 is known. In null *lox2* genotype, T2849A is the missense mutation, which caused the substitution of glutamine for histidine in a highly conserved histidine-rich motif (Wang et al. 1994), thereby causing loss of function of Lox2. Reinprecht et al. (2011) and Shin et al. (2012) also reported missense mutation (single point mutation T-A) causing conversion of histidine codon to glutamine codon leading to loss of function of Lox2 in OX948 and Jinpumkong. Lenis et al. (2010) reported a 74 bp deletion in exon 8 in PI 408251 while Reinprecht et al. (2011) reported this deletion in OX948, responsible for the premature truncation of the Lox1 protein. In PI 133226, a nonsense mutation C2880A relative to the start codon was observed in *lox1* allele (Lenis et al. 2010). For the loss of Lox3 function in PI 20585 and PI 417458, Lenis at al (2010) attributed loss of function of Lox3 to a single base deletion of a guanine in

a run of five guanine nucleotides, within exon 1, from position 97 to 101 relative to start codon. Gene specific and simple sequence repeat (SSR) markers linked to *Lx1*, *Lx2* and *Lx3* have been developed through mapping and characterization of *Lx1*, *Lx2* and *Lx3*, which have been deployed in development of lipoxygenase-free soybean varieties in several countries (Lenis et al. 2010; Reinprecht et al. 2011; Kumar et al. 2012; Rani et al. 2013). In India, ICAR-Indian Institute of Soybean Research has developed lipoxygenases free advanced breeding lines through marker assisted breeding (Rawal et al. 2020; Kumar et al. 2013a) and commercialized lipoxygenase-2-free soybean advanced breeding line NRC109 to private soy food industries using these markers (Kumar et al. 2013a). Recently, the country released its first ipoxygenase-2-free soybean variety NRC132 developed through marker asssisted breeding for the southern zone. In the United States of America, there are seven lipoxygenase-free soybean varieties, viz. IA1008LF, IA2053LF, IA2076LF, IA2104LF, IA3027LF, IA3045LF and IA3051LF, for commercial cultivation. In Canada, Agriculture and Agri-Food Canada (AAFC), Greenhouse and Processing Crops Research Centre (GPCRC) at Harrow, Ontario, has developed and released lipoxygenase-free food-grade soybean germplasm line, HS-151, in 2015 (Yu et al. 2016).

Further, immature pods of a special kind of genetic stock of soybean is harvested at R_6 stage (when the pod cavity is completely filled, but has not started turning yellow) of soybean consumed as snack or vegetable (Shanmugasundaram et al. 1991). These special genotypes which are 'vegetable or edamame or green soybean' produce highly sweet and organoleptically good-flavour immature seeds, which is largely due to higher accumulation of sucrose and sweetness-imparting amino acids, and bear larger pods with bold size compared to the grain-type soybean (Kumar et al. 2006, 2011a, b). The distinct fragrance in the seeds of vegetable soybean emanates from volatile compound 2-acetyl-1-pyrroline (2AP) (Fushimi and Masuda 2001), which is also present in basmati rice. A single recessive gene controls the fragrance in vegetable soybean. This recessive mutation causes elevated 2AP biosynthesis that results in a fragrant aroma (Niu et al. 2008). Juwattanasomran et al. (2011) reported a major QTL contributing to fragrance which is in the proximity of betaine aldehyde dehydrogenase 2 (GmBADH2). Sequence of gene coding this enzyme in fragrant and non-fragrant soybean genotypes revealed a non-synonymous SNP in exon 10, resulting in the change of glycine to aspartic acid. The authors developed PCR-based allele-specific SNP markers for marker-assisted breeding of fragrance trait in soybean. Juwattanasomran et al. (2012) discovered a new fragrance allele, which has a 2-bp (TT) deletion in exon 10 of GmBADH2 in another fragrant soybean cultivar Chamame.

Breeding for Improved Shelf Life of Oil Fraction by Genetically Elevating Monounsaturated Fatty Acid (Oleic Acid) and Reducing α-Linolenic Acid

Oil fraction of soybean seed which constitutes about 20% of the total mass is composed of five major fatty acids, palmitic (10–13%), stearic (2–4%), oleic (20–25%), linoleic (50–55%) and α-linolenic acid (7–8%). Palmitic and stearic acids are saturated fatty acids, while oleic, linoleic and α-linolenic acids are

unsaturated fatty acids. Based upon the unsaturation in hydrocarbon chain, fatty acids have been categorized as monounsaturated or polyunsaturated. Oleic acid (C18:1) is a monounsaturated fatty acid (MUFA) with single unsaturation. Linoleic (C18:2/omega 6) and α-linolenic acid (C18:3/omega3), the polyunsaturated fatty acids (PUFA), have two and three unsaturation, respectively, across the fatty acid hydrocarbon chain. The higher unsaturation level in linoleic and α-linolenic acid results in 10.0- and 21.2-fold faster oxidation in these polyunsaturated fatty acids (PUFA) than oleic acid, respectively. Susceptibility to fast oxidation and development of fishy smell in the stored soybean oil is attributed to the high PUFA. To improve shelf life of soya oil, industries employ partial hydrogenation leading to generation of trans fats, which trigger diabetes, atherosclerosis and cancer (De Souza et al. 2015). In several countries, food safety regulatory bodies have made it mandatory to declare the level of trans fats on the nutrition facts label in commercial edible oils and the processed food products containing edible oil as the major ingredient (Food and Drug Administration 2003; Food Safety and Standards Authority of India 2018; Ratnayake et al. 2014). Soybean oil with high oleic acid and low α-linolenic acid possesses improved oxidative stability, flavour and storability, thereby obviating the need of partial hydrogenation which incurs cost and generate health hazardous trans fats. Therefore, development of high oleic acid and low α-linolenic acid soybean genotypes is one of the most important breeding objectives for soybean-growing and soybean oil-consuming countries (Kumar et al. 2004; Kumar et al. 2010a).

During soybean seed development, oleate fatty acid desaturase catalyses the conversion of oleic acid (C18:1) into linoleic acid (C18:2) by inserting a double bond at the twelfth carbon from the carboxyl end of fatty acid hydrocarbon chain, while linoleate desaturase acts upon linoleic acid to produce α-linolenic acid. Omega 6 fatty acid desaturase activity, which is governed by two candidate genes, namely, *FAD2-1A* (*Glyma10g42470*) and *FAD2-1B* (*Glyma20g24530*) (Schlueter et al. 2007), determines the accumulation of oleic acid in soybean. Pham et al. (2011) reported 82–86% oleic acid in soybean genotypes carrying mutated alleles of both FAD2-1A and FAD2-1B. Recently, Rani et al. (2019) reported genomic regions associated with other than candidate genes for the biosynthesis of oleic acid. Low α-linolenic acid soybean genotypes can be developed by modulating the activity of desaturase which inserts a double bond at the fifteenth carbon from carboxyl end, thereby converting linoleic to α-linolenic acid. The activity of this desaturase is determined by at least three loci, namely, *FAD3A/fan1* (*Glyma.14 g194300*), *FAD3B/fan2* (*Glyma.02 g227200*) and *FAD3C/fan3* (*Glyma.18 g062000*) present on LGp B2/chr14, LGp G/chr18 and LGp D1b/Chr2, respectively (www.soybase.org). Deletions, insertions and nonsense mutation in *FAD3A* (Bilyeu et al. 2005), *FAD3B* (Reinprecht et al. 2009) and *FAD3C* (Bilyeu et al. 2005) have been reported to reduce α-linolenic acid content. Besides, Thapa et al. (2018) reported three novel point mutations in *FAD3A* gene responsible for low α-linolenic acid content. Bilyeu et al. (2018) successfully combined mutations in *FAD2* and *FAD3* genes to produce soybean genotypes with high oleic and low α-linolenic acid soybean using functional markers. Haun et al. (2014) developed transgenic plants homozygous for the

cleaved conserved sequences in *FAD2-1A* and *FAD2-1B* with elevated levels of oleic acid.

Breeding for Improved Bioavailability of Minerals

Phytic acid, 1,2,3,4,5,6-inositol hexaphosphoric acid, is a heat-stable anti-nutritional factor present in soybean seeds. It is the principal source of phosphorus in soybean seeds and is present in much higher concentration in soybean seeds compared to other legumes (Chitra et al. 1995). It binds with nutritionally important metals, especially zinc, calcium and magnesium, forming phytic acid-metal complexes (phytin), that are not absorbed readily in the intestine and hence largely excreted by humans and non-ruminant animals that have either no or limited phytase activity (O'Dell 1982; Forbes et al. 1983; Solomon 1982). This may cause deficiency of important nutrients. Being heat stable in nature, phytic acid remains active even after cooking. At alkaline pH, phytic acid binds with negatively charged protein molecules, and at pH values below isoelectric point, it binds with positively charged protein molecules. Therefore, phytic acid not only inhibits the action of a number of enzymes involved in digestion (Vaintraub and Bulmaga 1991) but also affects the isoelectric point, solubility and functionality of soy proteins (Chen and Pan 1985) which are vital in processing quality soy products. In tofu manufacturing, a relatively large amount of coagulants, namely, $CaSO_4$ and $MgCl_2$, is required to offset the effect of phytic acid on *tofu* quality (Schaefer and Love 1992). Further, hard-to-cook phenomenon of soybean and legumes has also been associated with phytic acid (Bernal-Lugo et al. 1991; Jones and Boulter 1983). Further, the undigested phytin excreted through non-ruminants pollute soil and water causing eutrophication (Raboy 2001).

Myoinositol-1-phosphate synthase (MIPS) is the key enzyme which catalyses the conversion of Glc-6-P to myoinositol-1-phosphate (MIP), which is in turn converted to 1,2,3,4,5,6-hexakis (dihydrogen phosphate) (phytic acid) by subsequent phosphorylations in phytic acid biosynthesis during soybean seed development. Hitz et al. (2002) discovered a missense mutation in soybean (*Glycine max*) MIPS structural gene (*GmMIPS1*) responsible for 50% reduction in seed phytic acid, while Wilcox et al. (2000) identified a mutant soybean line CX1834 with reduced phytic acid content without any change in total seed phosphorus. Walker et al. (2006) identified recessive mutations at two interacting unlinked loci responsible for low phytic acid trait of CX1834. Maroof et al. (2009) identified a nonsense mutation within a candidate lpa1 homolog present on chr 3, Glyma03g32500, for the low phytic acid phenotype in soybean. Gillman et al. (2009) identified a novel missense mutation in a conserved portion of the other lpa1 homolog, Glyma19g35230, in CX1834 and developed high-throughput molecular marker assays to directly select for the alleles that control the soybean low phytic acid phenotype.

The authors also reported a novel lpa2-b allele in low phytic acid soybean line M766. Gillman et al. (2009) developed molecular markers which would facilitate in combining nonsense lpa2-b allele from M766 with the nonsense lpa1-a allele from CX1834 to produce soybeans with much lower levels of phytic acid and increased available phosphate levels. Nunes et al. (2006) tried to reduce phytic acid in soybean

seed by downregulating MIPS by RNA interference (RNAi) technology; however complete RNAi knockdown of GmMIPS1 expression resulted in aborted soybean embryos. Bilyeu et al. (2009) developed a soybean line CAPPA, in which an *Escherichia coli* periplasmic phytase, the product of the appA gene, was expressed resulting in 90% reduction in seed PA with concomitant increase in total free phosphate.

Pyramiding the Desirable Quality Traits

As mentioned in Sects. 17.6.1.5.1 and 17.6.1.5.2, specialty soybean genotypes genetically free from Kunitz trypsin inhibitor and off-flavour generating lipoxygenase isozymes have been developed in several countries. However, soybean breeders felt the need of bringing the null allele of Kunitz trypsin inhibitor and lipoxygenases in the same genetic background which would be the most ideal raw material for processing soy products. Kumar et al. (2021) recently stacked null alleles of Kunitz trypsin inhibitor and lipoxygenase-2 through marker-assisted backcrossing (Fig. 17.3). In India, soybean genotype NRC142 carrying null alleles of Kunitz trypsin inhibitor and lipoxygenase-2, which is the principal contributor to the off-flavour, has been developed through marker-assisted pyramiding of null alleles of KTI and lipoxygenase-2 by employing null allele-specific markers and

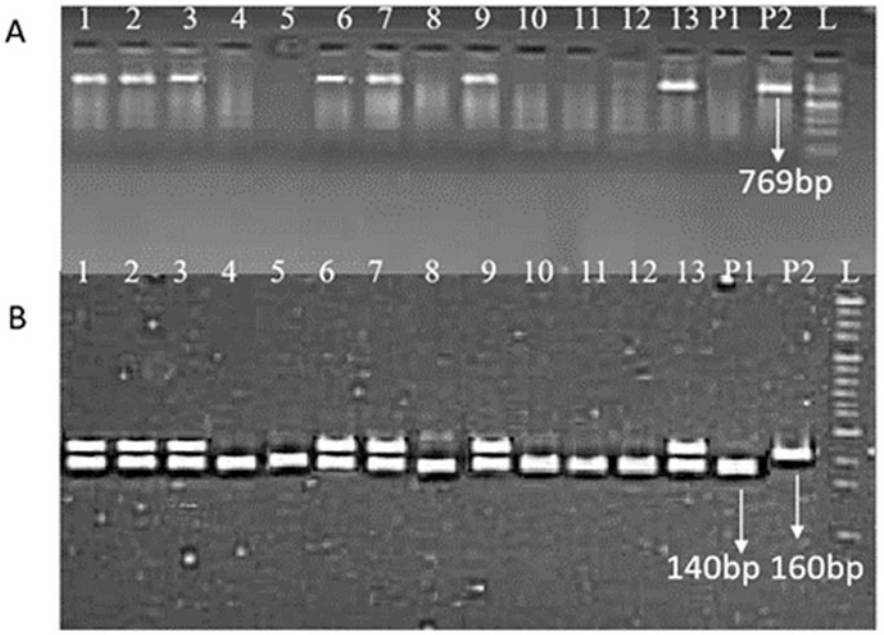

Fig. 17.3 Amplification of null lox2 allele-specific marker (**a**) and lox2-linked SSR marker, Satt656 (**b**), in true F_1 plants of NRC7-derived KTI-free line × NRC109. Lanes 1, 2, 3, 6, 7, 9 and 13 correspond to true F_1 plants. P1 and P2 correspond to NRC7 and NRC109, respectively; lane L corresponds to 50 bp ladder

Fig. 17.4 NRC142 double-null (KTI null and off-flavour generating lipoxygenase-2 null) Indian soybean variety developed through marker-assisted forward breeding (JS97–52 × PI542044 × PI596540)

SSR markers linked to both Ti and Lox2 locus (Rani and Kumar 2018) and released for commercial cultivation for central and southern agroclimatic zones. Average productivity of this variety is 1999 kg/ha and 2200 kg/ha in central and southern zone of the country which is significantly higher than the average productivity of soybean (1300 kg/ha) in the country. A field photograph of NRC142 is presented in Fig. 17.4. Oliveira et al. (2007) also developed soybean lines pyramided for null alleles of Kunitz trypsin inhibitor and lipoxygenase-2. Lately, Kumar et al. (2022) reported improved sprouting and tocopherols contents in introgressed lines for null *Lox2* while improved protein digestibility in introgressed lines for null *KTI* compared to the recurrent parent.

17.6.1.6 Breeding for Disease Resistance

Resistance to Rust

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd., can impact the yield losses up to 80% (Li et al. 2012). *P. pachyrhizi* was first identified in Japan in 1902 (Hennings 1903) which gradually spread to soybean-growing countries around the world (Bromfield 1984; Pretorius et al. 2001; Rossi 2003; Wang and Hartman 1992; Yorinori et al. 2005). Some of *P. pachyrhizi* races have developed increased tolerance to the certain fungicides available to control the disease (Godoy 2009). Development of soybean varieties possessing genetic resistance to this disease is the most effective sustainable measure to control this fungus. Screening of soybean accessions for resistance or tolerance to rust across the world (Miles et al. 2008; Pham et al. 2010) has led to the identification of five different loci carrying dominant alleles: Rpp1 identified in PI 200492 (Mclean and Byth 1980), Rpp2 from (PI 230970) (Bromfield and Melching 1982), Rpp3 (PI 230970) (Bromfield and Hartwig 1980), Rpp4 (PI 459025) (Hartwig 1986) and Rpp5 (PI 200487 and PI 471904). Calvo et al. (2008) identified recessive genes controlling SBR resistance. Brogin et al. (2004) identified simple sequence repeat (SSR) markers linked to rust resistance present in the variety FT-2 in the linkage group (LG)-C2 of the previous soybean consensus map reported by Cregan et al. (1999)

Monteros et al. (2007) mapped a SBR resistance gene from the variety Hyuuga at 3 cM interval between Satt134 and Satt460 on LG-C2. Hyten et al. (2007) recently mapped the Rpp3 locus at the same interval as reported by Monteros et al. (2007). The Rpp1 locus has been mapped to a 1 cM interval on LG-G between Sct_187 and Sat-064 LG-G. Bhor et al. (2015) identified two genes, namely, Rpp1b-like loci linked to SSR marker Satt 191 and Rpp2 loci linked to SSR marker Satt 215 in soybean rust-resistant exotic genotype EC 241780. Khanh et al. (2013) introgressed Rpp genes into a premium soybean variety HL203 in Vietnam. Yamanaka et al. (2015) pyramided Rpp genes in lines No6–12-B, Oy49–4 and No6–12-1 containing two (Rpp4 + Rpp5), three (Rpp2 + Rpp3 + Rpp4) and three (Rpp2 + Rpp4 + Rpp5) genes using molecular markers for durable resistance against SBR.

Resistance to Soybean Mosaic Virus

Soybean mosaic virus (SMV) is the most prevalent and destructive viral pathogen in soybean production worldwide (Hill and Whitham 2014). Seven distinct strains (G1 to G7) in the United States of America (Cho and Goodman 1979) and 21 strains (SC1–SC21) in China have been classified (Wang et al. 2003; Guo et al. 2005; Li et al. 2010) based on their differential responses of susceptible and resistant soybean cultivars. A number of independent loci governing SMV resistance have been reported. Rsv1 was the first SMV resistance gene identified in the soybean line PI 96983 (Kiihl and Hartwig 1979), which confers extreme resistance to SMV-G1 through G6 (Chen et al. 1991; Hajimorad and Hill 2001). Thereafter, a series of multiple Rsv1 alleles including Rsv1-y, Rsv1-m, Rsv1-t, Rsv1-k and Rsv1-r have been identified from different soybean cultivars with differential reactions to SMV G1–G7 strains (Chen et al. 2001). Rsv1 was mapped on chromosome 13, and 3gG2

was found to be a strong candidate for Rsv1 (Hayes et al. 2004). Rsv3 was identified in 'L29', a 'Williams' isoline derived from Hardee (Bernard et al. 1991; Gunduz et al. 2000). This locus gives resistance to SMV G5 through G7, but not G1 through G4 (Jeong et al. 2002).

Jeong et al. (2002) mapped Rsv3 between markers A519F/R and M3Satt on chromosome 14. Fine mapping led to identification of two closely linked SSR markers, namely, Sat_424 at a distance of 1.5 cM and Satt726 at a distance of 2.0 cM from Rsv3 locus (Shi et al. 2008). NBS_C, NBS_D and NBS_E in this genomic region may be the functional alleles of the Rsv3 locus that confer resistance to SMV (Suh et al. 2011; Redekar et al. 2016; Ma et al. 2017). Rsv4 which confers resistance to all seven SMV strains (Chen et al. 1993; Ma et al. 1995) was identified in soybean cultivars V94–5152 and mapped to a 0.4 cM interval between the proximal marker Rat2 and the distal marker S6ac, in a 94-kb haplotype block on chromosome 2 (Hayes et al. 2000; Saghai Maroof et al. 2010; Ilut et al. 2016). Two resistance genes Rsc-8 and Rsc-9, which confer resistance to strains SC-8 and SC-9, respectively, have been mapped to soybean chromosome 2 (Wang et al. 2004). Wang et al. (2011) reported Glyma02g13310, 13,320, 13,400, 13,460 and 13,470 as the probable candidate genes for Rsc-8 based on their predicted functions and expression patterns (Wang et al. 2011). Yang and Gai (2011) mapped resistance gene Rsc-15 between Sat_213 and Sat_286 on chromosome 6, while Fu et al. (2006) identified the resistance gene Rsc-7 in the soybean cultivar Kefeng No. 1 and mapped to a 2.65 mega-base (Mb) region on soybean chromosome 2. Shi et al. (2011) developed an 11 SNP/InDel multiplex assay to investigate the mode of inheritance in a SMV-resistant soybean line carrying Rsv1, Rsv3 and/or Rsv4 through a segregating population with phenotypic data and to select a specific gene or pyramid two or three genes for SMV resistance through MAS in soybean breeding programme. The assay is a very useful tool in marker-assisted development of SMV-resistant soybean varieties. Saghai Maroof et al. (2008) and Shi et al. (2009) successfully pyramided soybean mosaic virus resistance genes using marker-assisted selection.

Resistance to Yellow Mosaic Disease

Yellow mosaic virus causes yellow mosaic disease (YMD) in soybean (*G. max*) and legumes such as blackgram [*Vigna mungo* (L.) Hepper], mungbean [*Vigna radiata* (L.) R. Wilczek] and cowpea [*Vigna unguiculata* (L.) Walp.] (Varma et al. 1992). The virus is transmitted by the white fly (*Bemisia tabaci* Genn.) (Nariani 1960; Nene 1972, 1973). Two distinct begomoviruses, mungbean yellow mosaic India virus (MYMIV; Mandal et al. 1997) and mungbean yellow mosaic virus (MYMV; Morinaga et al. 1990) have been suggested to be associated in the aetiology of YMD in legumes in India and South Asia based on nucleotide sequence data of the genomic components of yellow mosaic viruses. Mungbean yellow mosaic India virus has been reported to infect soybean in India, Vietnam and Indonesia (Nene 1972, 1973). YMD resistance genes were reported in PI171443 by Singh and Mallick (1978) and in *G. soja* accession PI 393551. Yadav et al. (2009) reported accumulation of late viral transcripts and DNA replication in a susceptible cultivar

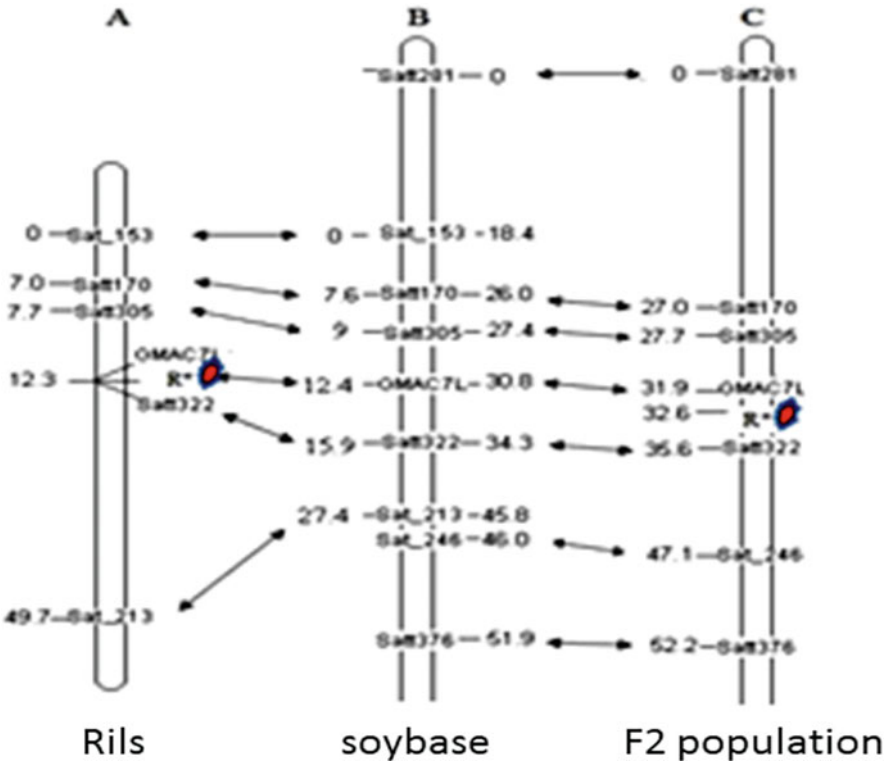

Fig. 17.5 Map position of MYMIV resistance gene on C2 linkage group in F2 population and RILs derived from JS335 × PI171443 [Source: Rani et al. (2017) Breeding Science 67:95–100

and rapid degradation of early viral RNAs in resistant cultivars. This rapid degradation of the early viral transcripts, possibly through a small interfering RNA mechanism, could be a mechanism of natural resistance against geminivirus. There are several reports on the inheritance of MYMIV resistance in these donors.

Rani et al. (2017) reported a single recessive gene, while Singh and Mallick (1978) reported double-recessive genes controlling MYMIV resistance in PI171443. A single dominant gene controlling MYMIV resistance in *G. soja* PI 393551 was reported by Bhattacharyya et al. (1999). Rani et al. (2017) mapped MYMIV resistance gene on chr 6 (LG C2) within a 3.5-cM genome region between two SSR markers GMAC7L and Satt322 whose size was estimated to be 77.115 kb (position of 12,259,594–12,336,709 bp) in PI171443 (Fig. 17.5). Deploying these molecular markers, the authors developed NRCSL1, the first MYMIV-resistant soybean variety for southern agroclimatic zone using marker-assisted forward breeding and later NRCSL2, essentially derived variety (EDV) of JS335, the most popular variety of India, through marker-assisted backcross breeding. In the backdrop of the fact that MYMIV-resistant soybean varieties of India carry resistance gene from the same donor PI171443, it is important to pyramid resistance gene from

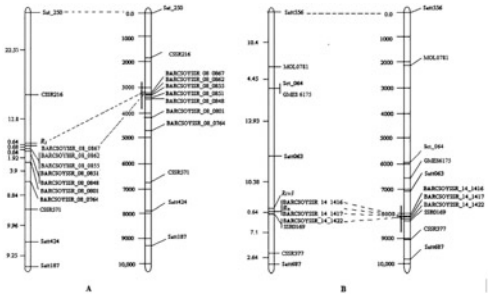

Fig. 17.6 The map positions of MYMIV resistance genes on A2 and B2 linkage in F2 population derived from cultivar JS335 (*Glycine max*) × PI 393551 (*Glycine soja*) [Source Rani et al. (2018) Crop Science 58(4): 1566–1567]

other sources for sustained resistance to MYMIV in the event of breakdown of resistance to single resistance derived from PI171443. Rani et al. (2018) identified SSR marker BARCSOYSSR_08_0867 (15,434,295 bp) on chromosome 8 and BARCSOYSSR_14_1416 (47,686,933 bp) and BARCSOYSSR_14_1417 (47,738,940 bp) on chromosome 14 tightly linked to MYMIV resistance genes in *G. soja* (Fig. 17.6), which are being used to pyramid resistance genes from both *G. soja* and *G. max*.

Resistance to Charcoal Rot

Charcoal rot caused by *Macrophomina phaseolina* can cause yield loss to 100%. The fungus is both soil and seed borne and has a wide host range. Breeding for charcoal rot resistance is difficult as sufficient information at the genome level imparting resistance not available. da Silva (2018) genotyped 140 F_2 individual derived from a biparental cross PI567562A x PI567437 (susceptible) with 5403 single nucleotide polymorphism markers and phenotyped for resistance to charcoal rot resistance through cut-stem inoculation technique in greenhouse and reported one QTL on chromosome 15 explaining 29.4% of phenotypic variation and two QTLS on chr 16 explaining 25.4 and 8.4% of phenotypic variation for resistance to the disease.

17.6.2 Breeding for Quantitative Traits

17.6.2.1 Protein and Oil

Seed oil and protein content are two most valuable quality traits controlled by multiple genes in soybean. The phenotypic range of protein content of soybean has been reported to be 34.1–56.8% of seed dry mass, and oil content ranged from 8.3% to 27.9% (Wilson 2004). The genetic variability available in soybean germplasm suggests that there is a great potential for genetic improvement of soybean seed protein and oil content. It has been reported in various studies that seed protein content is negatively correlated to seed oil and sucrose content in soybean (Nichols et al. 2006; Sonah et al. 2015). Breeding soybean varieties with high protein and high oil are extremely important for value addition. The negative correlation between protein and oil content makes improvement of both traits simultaneously

a challenging task using conventional breeding (Hwang et al. 2014; Bandillo et al. 2015; Chung et al. 2003; Kim et al. 2016). Moreover, protein and oil content in soybean seed are quantitatively inherited and governed by multiple genetic loci subject to genotype × environment interactions (Akond et al. 2014; Li et al. 2019; Patil et al. 2017).

As the differences in seed composition are also affected by epigenetic variation, expression profile of the genes involved in fatty acid biosynthesis, carbon partitioning, seed development and possibly many other unknown regulators (Kim et al. 2016; Nichols et al. 2006; Sebolt et al. 2000), the task of improving oil and protein content simultaneously becomes more challenging. As the protein and oil components of soybean seed are very valuable trait, many QTLs controlling these two seed traits have been reported (Chung et al. 2003; Diers et al. 1992; Nichols et al. 2006; Panthee et al. 2005; Pathan et al. 2013; Wang et al. 2015; Warrington et al. 2015). There have been few studies on identification of candidate genes governing oil and protein content in soybean seed.

Zhang et al. (2020) mapped major soybean protein and oil QTLs on chromosome 15 to a sugar transporter gene (GmSWEET39). The authors reported that a two-nucleotide CC deletion truncating C-terminus of GmSWEET39 was strongly associated with high seed oil and low seed protein, suggesting its pleiotropic effect on protein and oil content. GmSWEET39 was predominantly expressed in parenchyma and integument of the seed coat and likely regulates oil and protein accumulation by affecting sugar delivery from maternal seed coat to the filial embryo. The authors demonstrated that GmSWEET39 has a dual function for both oil and protein improvement and undergoes two different paths of artificial selection. A CC deletion (CC-) haplotype H1 has been intensively selected during domestication and extensively used in soybean improvement worldwide (Zhang et al. 2020). The studies on the molecular basis underlying the major QTL and GmSWEET39 haplotypes associated with seed quality components would be helpful in designing new strategies for soybean seed quality improvement using molecular breeding and biotechnological approaches.

17.6.2.2 Breeding for Yield Contributing Components

Biotic and abiotic factors are known to affect several plant architecture traits such as plant height, number of branches, number of nodes, pods per node, number of seeds per pod and seed traits such as seed size (100-seed weight).

Number of Branches

Branch number is one of the important determinants of yield. Branching in soybean depends upon environmental factors such as plant density and light quality (Agudamu and Shiraiwa 2016; Board 2000). Lower plant density generally used to avoid lodging and the incidence of diseases results in increased branch number on the main stem as the plant compensates for the lower seed rate. However, phenotypic data from Germplasm Resources Information Network (GRIN) shows genetic differences for the branch development as indicated by the higher number of branches in the Japanese/Korean cultivars than the American soybean varieties.

Several studies have shown QTLs associated with the branch number in soybean in F2 or RIL mapping population. Shim (2019) identified a candidate gene GmBRC1 for branching in soybean.

Pod Numbers and Number of Seeds per Pods

The number of pods per plant is another important parameter that determines the seed yield of genotype. Keeping in view the importance of the total number of pods per plant, several studies identified QTLs associated with the total number of pods with the aim to increase the efficiency of breeding for higher yields (Zhang et al. 2010; Rodrigues et al. 2016; Liu et al. 2017). However, vertical distribution of pods on soybean is uneven as more number of pods are distributed in the upper and the central region than in the lower region (Liu et al. 2010). Ning et al. (2018) identified QTLs associated with one-seeded, two-seeded, three-seeded and four-seeded pods in the three different regions, i.e. upper, middle and lower region, of the soybean plant in two associated recombinant inbred lines. Similarly, the number of seed per pod is equally important determinant of yield of any genotype. In general, soybean plant bears three, two and one seeded. Four-seeded pods are also not rare, and occasionally five-seeded pods are also available. The number of seed per plant has been suggested to be tightly associated with LN gene (Domingo 1945). Weiss (1970) attributed the greater frequency of four-seeded pods to the pleiotropic effect of the gene that causes narrow leaf. Jeong et al. (2011) reported a candidate gene Glyma20g25000.1 that is associated with the LN encoding gene. Li et al. (2021) finely mapped QTL locus QNSPFSP07–1 associated with four-seeded pod in soybean.

100-Seed Weight

100-SW is important determinant of seed size of grains so much so that the terms have been used interchangeably in literature. Small-size grains are sought for a better quality of soybean sprouts, *natto* and *miso*, whereas bold-seeded grains make excellent raw material for making soy nuts, *tofu*, *edamame* (Kato et al. 2014). Seed size is determined by the onset of cellularization in developing endosperm. Premature cellularization of the endosperm results in smaller seed size, while its delayed initiation causes increased seed size in soybean. Rani et al. (2021) identified Satt684 (Chr05: start 1,800,423–end 180,073) for 100-seed weight in the proximity of functional gene *Glyma05g02470* (Chr05: start 1,817,656–end 1,822,298) for serine-threonine protein kinase which has been reported to regulate cell cycle growth and may have a role in the cellularization of endosperm (Zhang et al. 2020). The authors also identified two SSR markers, namely, Sat_263 (LGp C2, 118.78 cM, LOD-2.39, R^2–8.83) and AI856415 (LGp D1b,50.11 cM, LOD-2.76,R^2–10.28) in the proximity of QTL, namely, Staga001 (LGp C2, 119.85 cM) and Satt296 (LGp D1b, 52.61 cM), respectively, reported to be associated with seed size by Yang et al. (2017) in chromosome substitution lines derived from N24852 × NN1138–2. Further, Satt181 (LGpH, 91.12 cM), which in our study showed slightly lower LOD value (1.65), was approximately 13 cM distant from QTL qSW12.1 (104.37 cM) identified with 100-SW in an earlier study (Liu et al. 2018). Yu et al. (2018) reported four candidate genes, namely,

Glyma.01G158700, *Glyma.01G156800*, *Glyma.01G125400* and *Glyma.01G147800*, related to 100-SW in 147 RILs from cross Charleston × Dongnong 594 were not found to be significantly associated with this trait in our results.

17.7 Transgenic Development

Transformation in soybean was first reported in 1988 by Christou et al. (1988) and Hinchee et al. (1988), and genetically modified soybean was first introduced commercially in 1996. Transgenic soybean plants have been obtained by two predominant methods for plant transformation, i.e. particle bombardment-based method and the *Agrobacterium*-mediated transformation method (Hinchee et al. 1988; McCabe et al. 1988). *Agrobacterium*-mediated gene transfer method is preferred over particle bombardment-based method due to requirement of minimal equipment costs, possibility of transferring relatively large segments of DNA, lower number of transgene copy integration into plant genomes, rare transgene rearrangement, lower frequency of genomic DNA interspersion and reduced abnormal transgene expression (Gelvin 2003). Particle bombardment method involves the use of complicated and expensive equipment (McCabe et al. 1988) and results in complex integrations, fragmentation and reconstitution of transgenes that may lead to transgene silencing). *Agrobacterium*-mediated transformation method attributes about 85% of the transgenic plant production (Yu et al. 2010). This method has been extensively used to introduce agronomical important traits like herbicide tolerance (Padgette et al. 1995), amino acid modification (Falco et al. 1995), virus resistance (Di et al. 1996), insect resistance (Stewart et al. 1996) and nematode resistance (Yamada et al. 2012) in soybean cultivar. A detailed list of traits introduced in soybean through transgenic method is given in Table 17.1.

Despite production of fertile transgenic plants through *Agrobacterium*-mediated transformation, reported transformation efficiencies are generally low in soybean (Somers et al. 2003; Rani et al. 2012; Verma et al. 2014). The transfer of T-DNA and its integration into the plant genome is influenced by several plant tissue-specific factors. These factors include plant genotype, explant vigour, *Agrobacterium* strain, vector-plasmid and selection system including selection agent and method (Cheng et al. 2004; Shukla et al. 2020). Additionally, inoculation and co-culture media composition, osmotic treatments, vir-gene-inducing synthetic phenolic compounds, tissue damage, suppression and elimination of *A. tumefaciens* infection after co-cultivation also affect the transformation efficiency (Klee 2000; Cheng et al. 2004). To develop an efficient genotype-independent *Agrobacterium*-mediated transformation system and high efficiency, modification should be done in factors affecting transformation.

Although a number of factors that affect transformation efficiency have been studied and manipulated that includes sonication-assisted *Agrobacterium*-mediated transformation (Trick and Finer 1997), the use of cystine, dithiothreitol and thiol compounds (Olhoft et al. 2007), co-cultivation at 22°C and use of Silwet-77 as

Table 17.1 Agronomically important genes transferred into soybean

Target tissue	Gene	Selectable marker	Phenotype	References
Half seed	*AtABF3*	Phosphinothricin (PPT)	Drought tolerance	Kim et al. (2018)
Half-seed cotyledonary explant/ cotyledonary nodes	Bar gene	PPT	Resistance against herbicide	Li et al. (2017a)
Cotyledon	γ-TMT	*PAT*	41-fold increase in α-tocopherol	Lee et al. (2011)
Cotyledon	CPs	*NPTII*	Enhanced accumulation of isoflavones in seed	Marra et al. (2009)
Cot-node	*FAD3*	*PAT*	Significant reduction in linolenic acid (18:3) content, ranging from 1.0% to 3.1%	Flores et al. (2008)
Embryonic axes	*Cry1Ac*	*NPAT*	Resistance to cotton bollworm	Dang and Wei (2007)
Cotyledon	*SbDV-CP*	*CP4 EPSPS and NPTII*	Protection against *soybean looper*, *soybean podworm* and *velvet bean caterpillar*	Miklos et al. (2007)
Somatic embryo	*CP-SMV*	*HPT*	Resistance against SMV	Furutani et al. (2006)
Somatic embryo	*SMV-HC-Pro*	*HPT*	Exhibited resistance response against SbDV	Tougou et al. (2006)
Immature cotyledon	*SMV-CP-3'-UTR*	*HPT*	Bioassay not done	Lim et al. (2005)
Somatic embryo	Bean-chitinase gene (*chi*) and ribosome in activating protein gene (*rip*)	*NPTII*	Bioassay not done	Li et al. (2004)
Cotyledon	*CRC*	*HPT*	Enhanced accumulation of isoflavones in seed	Yu et al. (2003)
Hypocotyl	SMV-CP-3'-UTR	*NPTII*	Resistance against SMV virus	Wang et al. (2001)
Somatic embryo	Maize 15 kDa zein protein gene	*HPT*	Increased methionine and cysteine content	Dinkins et al. (2001)
Somatic embryos	β-casein	*HPT*	Expression of a milk protein in soybean	Maughan et al. (1999)

(continued)

Table 17.1 (continued)

Target tissue	Gene	Selectable marker	Phenotype	References
Cotyledonary nodes	BPMV-CP-P	*NPTII*	Resistance phenotype against BPMV	Di et al. (1996)
Somatic embryo	*Cry1Ac*	*HPT*	Resistance against corn earworm (*Helicoverpa zea*), soybean looper (*Pseudoplusia includens*), tobacco budworm (*Heliothis virescens*) and velvet bean caterpillar (*Anticarsia gemmatalis*)	Stewart et al. (1996)

surfactant (Liu et al. 2007), use of antioxidant during co-cultivation (Wang and Xu 2008), 4-day co-cultivation time period (Ko and Korban 2004) and selection by direct placement of explant at low concentration of antibiotic (Yan et al. 2000). Successful transformation using *Agrobacterium* depends not only on the efficiency of the plant regeneration systems but also on the subsequent elimination of this bacterium from transformed cells. The elimination of *Agrobacterium* is usually achieved by adding one or more antibiotics to the culture medium and is quite important because the continued presence of *Agrobacterium* can present a problem for identifying transformants or interfere with the growth and development of the transformed plant cells or cause the death of the cultures (Horsch et al. 1985; Matzk et al. 1996). Carbenicillin and cefotaxime are the most commonly used antibiotics for this purpose.

An effective and foolproof selection strategy is very important for successful transformation. Five different selection markers have been utilized in soybean transformation. They include *cat*, *npt II*, *hpt*, *bar* and *man*A and neomycin phosphotransferase II (*npt II*). The most successful and popular selection marker is the *bar* gene, derived from *Streptomyces hygroscopicus* which encodes the enzyme phosphinothricin acetyltransferase (PAT) conferring resistance to the herbicide phosphinothricin (PPT) or its analogues *Basta* (with its active ingredient glufosinate ammonia) or bialaphos (Harshavardhan et al. 2003).

A new technology for genome editing—the CRISPR (clustered regularly interspaced short palindromic repeat)/Cas (CRISPR-associated) system—has been successfully used for genome engineering in many important crops in recent years. Since 2015, CRISPR/Cas9-mediated genome editing in soybean has shown an initial success. This technology provides a powerful tool for accurate genetic modification and gene function identification, but it also relies on transformation efficiency (Chen et al. 2018). Soybean genetic transformation is limited to few laboratories due to low transformation and regeneration efficiencies.

17.8 Conclusions

Progress made in soybean breeding through conventional, molecular and transgenic approaches has been tremendous. Yield-related traits and maturity duration have been the most important breeding objectives. Breeding for disease resistance has been gaining importance due to appearance of new diseases and continuous evolution of pathogens threatening soybean crop. As soybean is a very important source of edible oil and one of the most economical sources of plant protein, continuous efforts are being made to improve quantity and quality of both oil and protein fractions. Genetic improvement in oleic acid in soybean oil has been taken on a priority basis due to increasing awareness on ill effects of trans fats on human health. Genetic elimination of Kunitz trypsin inhibitor, a factor responsible for low digestibility of soy protein, and lipoxygenases responsible for generating off-flavour is gaining importance.

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Castor Breeding

18

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Abstract

Castor, a non-edible, commercial oilseed crop of Euphorbiaceae family, is a monotypic genus. It is a highly cross-pollinated crop and amenable to all plant breeding techniques. The present chapter is a comprehensive summary of the transformation of a wild, tall, shattering, perennial plant type to domesticated, medium-to-tall, non-shattering, annual plant type grown under rainfed or irrigated castor-growing regions of India. About 38 high-yielding genotypes including 18 varieties and 20 hybrids are in the seed chain with an average productivity of 1.9 t/ha. The chapter deals with the basic information of the crop, viz. genetics, floral biology, sex expression and different approaches followed for crop improvement.

Keywords

Genetic resources · Breeding strategies · Hybrid production · Genomics · Improved varieties

18.1 Introduction

Castor (*Ricinus communis* L.) is grown for its seed oil in more than 30 countries. India, Mozambique, Brazil and China are the major producers. As per FAOSTAT (2018), it is cultivated in an area of about 1.3 m ha across the globe with the production of around 2.8 mt. India ranks first in both area (0.99 m ha) and production (1.96 mt). It can be grown even in sub-optimal soil conditions with minimal

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_18

management. In general, the commercial cultivars contain 48–50% oil, whereas in germplasm collections, the oil content ranges from 37 to 60% (Wang et al. 2010). Castor oil is the only known source for ricinoleic acid, an unusual fatty acid. The higher proportion (>80%) of ricinoleic acid (12-hydroxyl-cis-9-octadecenoic acid) in the fatty acid composition makes the castor oil unique among the vegetable oils.

The oil and its derivatives are important raw materials for several industries such as soap, nylon, lubricants, plastic, paper, cosmetic, paint, pharmaceutical, etc. More than 400 products are manufactured using castor oil and its derivatives. Castor has a huge potential for production of bioenergy as the oil yield (1250–2500 L/ha) is higher than any other potential crops for biodiesel. It could supply up to 60% of the non-edible oil needed to produce biodiesel (Osorio-González et al. 2020). Comparative advantage of castor lies in its ability to adapt to different weather conditions, possibility of growing even in marginal soils and high ricinoleic acid content providing characteristics desirable for biodiesel production such as high viscosity, high miscibility, low iodine content and low freezing point.

Before industrialization, castor was used for varied purposes. The oil was used for lighting, hairdressing, skin conditioning and as medicine. As medicine, the oil is mainly used as laxative and to relive the pain caused by sprain. In North Eastern parts of India and Myanmar, castor leaves are fed to eri silk worm. In some places, especially Japan, it is used for ornamental purposes. Castor cake is an excellent source of organic fertilizer.

18.2 Origin, Evolution and Distribution of Species

Castor belongs to Euphorbiaceae family, which contains more than 280 genera. Under the genera *Ricinus*, *communis* is the single species. However, three separate species, namely, *Ricinus communis*, *Ricinus macrocarpus* and *Ricinus microcarpus* and a few sub-species such as *persicus*, *chinensis*, *africanus*, *mexicanus*, etc. have been reported in literature (Kulkarni and Ramanamurthy 1977; Moshkin 1986; Weiss 2000). However, none of these is botanically qualified as 'species' or 'sub-species' because they do not differ in the chromosome number (all have $2n = 20$ chromosomes), and they cross easily with each other (Kulkarni and Ramanamurthy 1977; Atsmon 1989). They are actually different morpho-types adapted to specific regions.

Even though 'Eastern Africa' is considered as the most probable origin of castor (Weiss 1971), polyphyletic origins cannot be ruled out. Moshkin (1986) suggested four centres of origin, namely, Iran-Afghanistan, Palestine-Southwest Asia, Indo-China and Arabian Peninsula regions. It is found across tropical and sub-tropical regions of the world. In Ancient Egypt, castor seeds were found in tombs dating to 4000 BC. Wild castor plants are found throughout the African continent. India has a rich history of its cultivation and use. An ancient literature *Susruta Atharvaveda* (1000 BC) refers castor as an indigenous plant of India. The earliest castor seeds (125 BC) were found during the excavation in Maharashtra. In China, castor has been in use as medicine for centuries.

At present, castor is cultivated on a commercial scale in countries such as India, China, Brazil and Mozambique. In Brazil, about 80% of castor area is concentrated in the state of Bahia. In India too, more than 80% of area is in the western parts (states of Gujarat and Rajasthan) under irrigated conditions, while traditional rainfed castor -growing area is limited to Southern India.

18.3 Plant Genetic Resources

There are at least 30 institutes/organizations worldwide involved in conserving castor genetic resources. Of these, six major institutions, namely, National Bureau of Plant Genetic Resources under Indian Council of Agricultural Research (ICAR-NBPGR); Institute of Crop Germplasm Resources under Chinese Academy of Agricultural Sciences (ICGR-CAAS); US Department of Agriculture-Agricultural Research Service (USDA-ARS); *Centro Nacional de Pesquisa do Algodao* (CNPA), Brazil; NI Vavilov All-Russian Institute of Plant Genetic Resources (VIR), Russia; and Institute of Biodiversity Conservation (IBC), Ethiopia, conserve between 510 and 4307 accessions (Anjani 2012). ICAR-NBPGR has the largest collection (>4307 accessions) followed by China (~2111 accessions) as per the FAO's Second Report on the State of the World's Plant Genetic Resources for Food and Agriculture published during 2010.

Global efforts in characterization of germplasm collections have shown tremendous variation for morphological traits in castor (Webster 1994; Anjani 2012). However, the entire genetic diversity is limited to intraspecific diversity mostly created by hybridizations between local germplasm with exotic lines. Exchange of breeding materials, breaking of linkages through breeding and independent inheritance of morphological characters played a great role in generating diversity. Molecular marker analyses have revealed only low to moderate level of DNA polymorphism (Allan et al. 2008; Qiu et al. 2010; Foster et al. 2010; Senthilvel et al. 2017). In the pursuit to generate additional diversity, intergeneric hybridization with *Euphorbia lathyris* (Moshkin 1986), *Jatropha* (DOR 2003) and *Manihot esculenta* (Gedil et al. 2009) was attempted but remained unsuccessful.

For effective utilization of germplasm, a core set of 165 accessions was identified from the original germplasm collection of more than 3000 accessions maintained at ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad, India. The accessions for the core set were selected based on agro-morphological traits. The core set represented almost the entire variability present in the whole collection (Sarada and Anjani 2013).

Systematic screening of the germplasm for several biotic stresses led to the identification resistance sources to *Fusarium* wilt, *Macrophomina* root rot, reniform nematode, leafhopper, capsule borer and serpentine leaf miner. Accessions showing tolerance to drought (Parvathaneni et al. 2017), early maturity and high ricinoleic acid content have also been identified (Anjani et al. 2018). A few of the germplasm accessions carrying useful traits are listed in Table 18.1.

Table 18.1 Trait-specific germplasm accessions of castor

S. no.	Traits	Germplasm accessions/breeding lines
1	Resistance to *Fusarium* wilt	48-1, DCS-107, RG-43, RG-72, RG-111, RG-2819 and many more germplasm and improved lines
2	Resistance to root rot	RG-111, RG-2722, RG-2818, RG-2819, RG-2822
3	Moderate resistance to gray mold	ICS-324, 48-1, DPC-9, RG-1963
4	Resistance to reniform nematode	RG-43, JC-12
5	Resistance to leafhopper	RG-631, RG-1621, RG-2661, RG-3037, RG-3067
6	Resistance to leaf miner	RG-1930, RG-1771
7	Resistance to capsule borer	RG-898, RG-2774, RG-2800
8	Tolerance to drought	RG-27, RG-72, RG-1494, RG-2139
9	Tolerance to salinity	48-1, DPC-9, GC-2
10	Early maturity	RG-18, RG-19, JI-258
11	High ricinoleic acid content	RG-57, RG-66, RG-226, RG-3477

18.4 Floral Biology: Emasculation-Pollination Techniques

The inflorescence is a raceme and popularly called as 'spike'. Separate male (staminate) and female (pistillate) flowers are located in the same inflorescence (monoecious). Male flowers appear in the basal and median portions of the spike, while female flowers are found in the apical part of the spike. Very rarely, one or two bisexual flowers (hermaphrodite) appear in a spike. Male and female flowers open asynchronously. Even though the plant is predominantly monoecious, polymorphism for sex expression is observed with spike containing only pistillate or pistillate with interspersed staminate flower (ISF) forms. Sex expression is highly influenced by environmental conditions (Shifriss 1960). In general, low temperature (<30 °C), early stage of development and high nutrition promote female flowers, and high temperature (>30 °C), old plants and sub-optimal nutrition promote male flowers on a spike (Lavanya 2002). The role of exogenous and endogenous growth hormones like gibberellic acid, silver nitrate and ethylene in shifting the female and male tendency has been well documented (Ramesh et al. 2000; Lakshmamma et al. 2002; Murthy et al. 2003).

Botanically, both the flowers are described as bracteate, ebracteolate, pedicellate, actinomorphic and incomplete. Male flowers are apetalous with a simple perianth consisting of five sepals enclosing a stamen cluster. Each stamen cluster has 1000–1500 anthers borne terminally on branched filaments. Stamens are polyadelphous, and filaments branched and united to form five branches. Anthers are dithecous, globose, basifixed, introrse and dehiscing by longitudinal slits. The

ovary is superior, tricarpellary, syncarpous and trilocular with one ovule in each locule on axile placentation. There are three styles with bifid and feathery stigma.

Due to monoecious nature of the spike, mixed pollinations (both self- and cross-pollinations) occur in nature. Cross-pollination to the extent of 36 to 76% has been reported. Upon anthesis, male flowers produce abundant pollen. Due to lightweight, wind carries the pollen and aids in cross-pollination to a great extent. It has been observed that pollen can travel to a distance of even 1 km under clear sky and with normal wind velocity. Bagging the inflorescence with butter paper bags or muslin cloth bags ensures self-pollination. For attempting crossing between two lines, the inflorescence of female parent is covered with a paper bag after removing the male flowers and any opened female flowers in the previous day. For pollination, the male flowers from the covered inflorescence of male parent are collected in a Petri plate and kept under the sun for about 30 min for dehiscence of the anther. Then, the pollen is rubbed on the stigma of female flowers of the designated female parent using a paint brush and covered with a paper cover. The pollination is done every alternate day for 10–15 days. The pollen grains are viable for 3–4 days, and the stigma remains receptive for a period of 5–10 days depending on environmental conditions.

18.5 Cytogenetics

Castor is a diploid with 20 somatic chromosomes ($2n = 2x = 20$). However, Richharia (1937) considered castor as secondary polyploid based on secondary associations observed during metaphases. The chromosomes are small making cytogenetic studies difficult. The mean chromosome sizes range from 1.19 to 2.12 μm, and the total length of diploid set is 32.15 μm on average (Vasconcelos et al. 2010). Jakob (1956) studied the pachytene of meiosis and reported that the macro chromomere pattern of the chromatic zone is apparently distinct in each of the ten chromosome pairs. Each chromosome pair was morphologically distinguishable by the regions, which stained deeply with acetic orcein. He designated the chromosomes by capital letters on a temporary basis. Later, Jelenkovic and Harrington (1973) provided a more accurate description of the pachytene complement. Each bivalent can be recognized by the presence of characteristic heterochromatic knobs. Paris et al. (1978) constructed idiogram for the ten pachytene bivalents showing chromosome length, centromere position as well as lengths and positions of heterochromatic regions and constrictions. There are five metacentric chromosomes, four submetacentric chromosomes and one subterminal chromosome in the castor complement. Chromosomes 2 and 7 contain nucleolar organizer regions (NOR). Chromosome 3 contains proximal heterochromatic region. Chromosome 4 is the second longest after chromosome 1. Chromosome 5 is metacentric. In chromosome 6, centromere is flanked by one large chromomere in the long arm. Chromosome 8 is more or less similar with chromosome 4. Chromosomes 9 and 10 are the shortest.

Recently, molecular tools were used to study the chromosome structure and organization. Vasconcelos et al. (2010) evaluated mitotic chromosomes using

standard staining, fluorochrome staining (CMA/DAPI), fluorescent in situ hybridization (FISH) and silver impregnation. In contrast to pachytene, more symmetrical karyotype was observed in mitotic metaphases with all chromosomes displaying metacentric morphology except for the submetacentric pair D. Existence of at least 14 45S rDNA sites was noted. Molecular cytogenetic analysis revealed knowledge on the repetitive elements in the genome. Alexandrov and Karlov (2016) studied the chromosomal organization and structure of repeats in mitotic and pachytene chromosomes using FISH, PCR analysis and bioinformatic approaches.

Only a very few cytogenetic stocks are available. A naturally occurring haploid plant was used to generate euploid series consisting of haploid, diploid and tetraploid individuals (Timko et al. 1980). Artificial induction of polyploidy has been attempted. Narain and Singh (1968) induced polyploidy by treating the apical meristem of castor seedlings with colchicine and reported chromosomal interchanges. Baghyalakshmi et al. (2020) generated tetraploid castor plants by treating the seeds with colchicine. In the tetraploid plants, the pairing of chromosomes was abnormal with univalent to octavalent configurations during meiosis I, but the later parts of meiosis were normal. Variable levels of pollen fertility were noticed in tetraploid plants depending on the season. The tetraploid plants were phenotypically comparable with their diploid counterparts but produced substantially bigger seeds.

18.6 Genetic Studies

18.6.1 Stem Colour

The basic stem colour is green. During the development of plants, the presence and intensity of anthocyanin pigments turn the stem colour into different shades of red such as green with reddish-bluish tinge, carmine or rose red, mahogany red, etc. The intensity of anthocyanin pigmentation varies with sunshine, presence and intensity of bloom and age of the plant. Earlier genetic studies indicated predominantly monogenic inheritance for stem colour and dominance or incomplete dominance of coloured stem over green stem (Solanki and Joshi 2001; Lavanya and Gopinath 2008). A purple morphotype collected in wild showing maternal inheritance has been reported (Anjani et al. 2007). A spontaneous mutant with yellow stem colour was identified by Prabakaran and Balakishan (2012).

18.6.2 Waxy Coating

Castor plants differ for the presence of waxy coating, popularly called ‘bloom’ on different parts of the plants. It serves as a natural protection against extremes of weather and infestation of insect-pests. Cold injury, thrips and leafhopper incidence are higher in plants without bloom than in plants with bloom, while it is vice versa

for whiteflies. Plants are classified into four categories (zero bloom, single bloom, double bloom and triple bloom) based on the presence and distribution of bloom. 'Zero bloom' type is devoid of visible waxy coating in all parts of the plant. In 'single bloom' type, waxy coating is found in all plant parts (stem, petiole and inflorescence) except on leaves. In 'double bloom' type, waxy coating is found in all plant parts (stem, petiole, inflorescence, lower surface of the leaf) except on the upper surface of leaves, whereas 'triple bloom' types carry waxy coating in all parts of plants including the upper surface of leaves. However, there is a wide variation for the intensity of bloom on different plant parts and among different lines.

The presence of bloom inherits as dominant or partially dominant over the absence of bloom (Kulkarni and Ramanamurthy 1977; Lavanya and Gopinath 2008). Single bloom was monogenic and dominant over zero bloom while double bloom had a dominant digenic complementary action of 9 double: 3 single: 4 zero bloom in a cross between double bloom and zero bloom (Peat 1926). Double bloom was controlled by two complementary genes B and C, where B alone expresses single bloom, while C can express double bloom only in the presence of B. The variation in the intensity of bloom was also controlled by another dominant gene D. Triple bloom was always dominant to other bloom variations (Narain 1961).

The intensity of bloom within the plants varies with the age of the leaves: mild in the youngest to traces in matured, senescing leaves but highest in the physiologically active leaves. In triple or double bloom types, the presence of bloom on the upper or lower side of the leaf is mostly confined to the latest emerged leaf indicating the role of penetrance and expressivity of the genes controlling the bloom character or the role of multiple alleles.

18.6.3 Plant Height

The height of the main stem varies depending on soil type and availability of moisture. It generally ranges from 45 to 240 cm. However, perennial plants reach up to 12 m. The inheritance studies on plant height in a cross indicated the involvement of three non-allelic recessive genes causing dwarfness.

18.6.4 Nature of Spike

Based on the density of capsules, spikes are classified as loose, compact and semi-compact types. Compactness of spikes influences the pest and disease development. Compact and semi-compact spikes are highly susceptible to mould and capsule borer. Compact spikes appear to be completely or incompletely dominant over loose spike (Solanki and Joshi 2001; Lavanya and Gopinath 2008).

18.6.5 Capsule Characteristics

Capsules with purple, mahogany, sulphur white and green colours have been recorded in germplasm collection. The green colour of capsule was controlled by a single dominant gene (Patwardhan 1931).

18.6.6 Wilt Resistance

Majority of the published reports indicate that *Fusarium* wilt resistance in castor inherits as either single or two genes, and two gene situations are predominant. Both dominant and recessive expressions were observed. Shaw et al. (2018) noted different modes of inheritance when different susceptible parents were crossed with the same resistant source. Two susceptible parents, namely, JI-35 and JC-12, were crossed with the common resistant parent (48–1). In both the crosses, the F_1 showed susceptible reaction, but the F_2 population showed different modes of inheritance, namely, monogenic [3 (susceptible), 1 (resistant)] in the cross JI35 × 48–1 and digenic with complementary gene interaction [9 (susceptible), 7 (resistant)] in the cross JC12 × 48–1.

18.6.7 Sex Expression

Three types of femaleness have been recorded and designated as N, S and NES. The N type carries a recessive sex-switching gene (Katayama 1948). The homozygous genotype will produce female plants, whereas the plants are monoecious under heterozygous condition. The pistillate system is maintained by sib-mating. The hybrid seed production using this system is tedious because the progeny of seeds produced from female plants segregates for female and monoecious plants in 1:1 ratio and the monoecious plants are to be removed before anthesis. Nevertheless, several commercial hybrids have been developed especially in the USA using N-type pistillate lines.

The S type is governed by the mechanisms of sex reversal and expression of interspersed staminate flowers (ISF). The 'sex reversals' are plant variants, which start out as female and then revert to monoecism (Shifriss 1956). This reversion may occur at any time after the first inflorescence. Such pistillate lines are developed and maintained by selection of females of late-reverted type in every generation. Another group of non-reverted females carries sensitive interspersed gene at a high frequency. The penetrance and expressivity of interspersed female state are determined by the environment. VP-1, the widely used pistillate line in Indian breeding programmes, carries both the systems of sex reversals and environmentally sensitive gene for the expression of staminate flowers.

An NES-type pistillate line CNES-1 was derived by backcrossing an N-type pistillate line (N145–4) to a variety 'Cimarron' and selecting for ISF (Zimmerman and Smith 1966). This line is homozygous for N pistillate gene and contains

environmentally sensitive gene for ISF. The NES is advantageous over S type due to the single recessive gene for femaleness, whereas the S pistillate genes behave like polygenic complex with both dominance and epistasis.

18.7 Breeding Objectives

The breeding efforts in castor have transformed the tall, highly monoecious, shattering, wild type into a medium tall, high-yielding, non-shattering commercially viable crop. Systematic breeding efforts in castor date back to 1800 AD. Extensive breeding activities were undertaken predominantly by the USA and erstwhile USSR. In India, crop improvement research was initiated during the 1930s as selections from populations with an emphasis on seed yield and branching habit. After that, non-shattering character and oil content were given importance during the 1950s. Later, development of cultivars with high seed yield and wilt resistance was the focus.

At present, important breeding objectives include development of short duration genotypes with plant type suitable for high-density planting and mechanical harvesting and development of cultivars resistant to biotic stresses (mould, capsule borer, leafhopper, thrips and whitefly). Among the abiotic stresses, drought tolerance assumes significance as the crop is cultivated under rainfed situations or with limited irrigation. In addition, attempts are being made to remove or reduce the ricin content in castor seeds.

18.8 Breeding Approaches

Castor is a unique crop in breeding perspective. The plant is largely monoecious with some exceptions. Both self- and cross-pollinations occur under natural conditions. The considerable level of heterosis is observed without any marked inbreeding depression. Heterosis has been commercially exploited in castor as early as 1960. However, there is no CMS system reported so far in castor. Hybrid development relies on the use of lines producing predominantly female flowers as female and the normal monoecious lines as male parents.

All the breeding methods suitable for self- and cross-pollinated crops are amenable for use in castor. During 1900, pure line selection was attempted to develop high oil lines in the USA. Mass selection is used mainly for selection of female plants, long primary spike and reduced plant height. Due to minimum inbreeding depression on selfing, inbreeding was used to develop and maintain varieties and parental lines.

Hybridization followed by selection is the most widely followed method. Following hybridization, the pedigree selection is used to select simultaneously several heritable and morphological traits. The possibility of recombinants is usually high, when parents are genetically diverse. A large (>1000 individuals) F_2 population is desired because the generation of diversity is limited to the initial population size.

Bulk method of selection is more successful for selecting segregating generations under abiotic and biotic stress conditions.

Recurrent selection has been successfully used for altering the plant stature for mechanical harvest (Auld et al. 2009). To combine high seed yield and oil content, both non-additive and additive gene actions are to be exploited simultaneously. To achieve such a goal, parental mating and recurrent selection approach may be followed. Chen et al. (2016) found that recurrent selection was an effective method to improve oil content in castor. They could raise the mean oil content of a variety, Impala, from 50.3 to 54.5% by two cycles of selection. The oil content in the base population ranged from 40.1 to 57.5% with an average of 50.3%.

Mutation breeding was successful in castor in inducing variability for morphological characters, sex expression and resistance to *Fusarium* wilt. Even though both physical and chemical mutagens have been used, physical mutations were found more successful. Irradiation of HC-6 with thermal neutron treatment led to the isolation of a short duration mutant, NPH-1, which was later released as Aruna variety. Gamma ray irradiation of VP-1, a wilt-susceptible stable S-type pistillate line led to several wilt-resistant pistillate lines like M-574 and M-619 (Lavanya et al. 2003).

To generate novel variability, distant hybridization is a potential approach. Since castor belongs to a monotypic genus, interspecific hybridization is not feasible. Therefore, intergeneric hybridizations were attempted by crossing castor with its close relatives such as cassava (*Manihot esculenta* Crantz) and jatropha (*Jatropha curcas* L.). Laosatit et al. (2017) performed intergeneric hybridization between jatropha and castor in both directions. The pollen tube grew normally and reached the style base within an hour after pollination, but the embryo aborted a few days later indicating post-fertilization barriers. They cultured the excised ovules in vitro and obtained one intergeneric hybrid plant. Similarly, Premjet et al. (2019) generated intergeneric hybrid between jatropha and castor through embryo rescue and doubled the chromosome number of the hybrid using colchicine to improve fertility.

Modern scientific tools are being harnessed for addressing the formidable issues in castor breeding. As there are no resistant sources available in the germplasm for lepidopteron pests, incorporating resistance through transgenic approaches were attempted. Transgenic events carrying *Bacillus thuringiensis* (*Bt*) cry1Aa gene showing resistance to castor semilooper and *Spodoptera* have been developed and characterized at ICAR-IIOR. Transformation of decotyledonated embryo axes through *Agrobacterium tumefaciens*, particle gun bombardment and *in planta* methods resulted in transformation frequencies of 2.4%, 1.1% and 2.1%, respectively. Eight events showing Mendelian segregation were advanced. The laboratory insect bioassays showed up to 80% of larval mortality and up to 87% of weight loss among *Spodoptera litura* and *Achaea janata* larvae. In field bioassays, the event AMT-894 was the most promising with 43% of plants showing less than 25% leaf damage (Muddanuru et al. 2019).

The desirable level of resistance for mould is not found in germplasm. Therefore, transgenic approach is being followed to impart resistance to mould in castor using multiple genes imparting resistance to fungal pathogens. Two polygene cassettes

were developed using genes that impart partial resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. In the first construct, three genes *ERF*1 (ethylene response factor 1), *BIK*1 (botrytis-induced kinase 1) and *AtEBP* (*Arabidopsis thaliana* ethylene-responsive element binding protein), involved in signal transduction during plant-pathogen interaction of necrotrophic fungi, were used. These genes were cloned under three independent promoters with known inflorescence-elevated expression pattern, and the resultant cassettes were cloned in tandem within a single T-DNA of the binary vector so that the transgenic plants realized with this vector will express all the three gene cassettes independently. In the second polygene construct, three genes *RsAFP*2 (*Raphanus sativus* – antifungal protein 2), chitinase and *AceAMP*1 (*Allium cepa* – antimicrobial peptide 1) were cloned under the same promoter (35S, a constitutive promoter) and are separated by 2A signal peptide sequence so that three genes are expressed as a single polycistron and subsequently as a self-cleaving polyprotein (Prasad et al. 2016).

Efforts to breed for low ricin types have met with limited success. Varieties with 70–75% lesser ricin content have been developed, but they are still very toxic to mammals. As per genome sequence data, there are several putative genes in the ricin family, including potential pseudogenes or gene fragments, forming clusters. Therefore, generating detoxified genotypes using classical mutation techniques is not feasible. Sousa et al. (2017) explored the RNA interference (RNAi) concept to silence the ricin gene in castor seeds. RNAi is a post-transcriptional gene silencing mechanism that regulates the expression of protein-coding genes. Constructs to express self-complementary RNA transcripts form a dsRNA, which is processed into small interfering RNAs (siRNAs). These siRNAs trigger a sequence-specific mRNA degradation, leading to gene silencing. Using this technique, a bio-detoxified line TB14S-5D has been developed, which is free from ricin (Sousa et al. 2017).

18.9 Precise and High-Throughput Phenotyping Protocols for Key Traits

18.9.1 Screening for Fusarium Wilt Resistance

Wilt caused by the fungus *Fusarium oxysporum* f. sp. *ricini* is the most important soil-borne disease of castor. It occurs in all castor-growing areas across seasons. Historically, screening for wilt resistance is done based on disease incidence in wilt sick plots under field conditions, which had been very effective but with limitations in terms of the number of lines that can be screened. Shaw et al. (2016) described a high-throughput screening method for large-scale phenotyping of castor genotypes for resistance to *Fusarium* wilt disease. As per this method, the screening is done in plastic pots (30 × 15 × 13 cm) filled with 4 kg of sterilized potting mixture (red soil, black soil and farmyard manure in the proportion of 5:3:1). The pathogen is mass-multiplied on sorghum (*Sorghum bicolor*) grains as substrate. Semi-cooked sorghum grains (100 g in 250 ml of conical flask) are sterilized by autoclaving at 15 psi for 20 min at 121 °C. The flasks are inoculated by actively growing fungal mycelial

culture grown on PDA and incubated at 28 ± 2 °C for 15 days. The flasks are hand shaken daily to ensure complete fungal colonization on the sorghum grains. The 15-day-old fungal culture is added to the potting mixture at the rate of 3 g/kg and thoroughly mixed. The pots are watered and kept for incubation for 24 h before sowing. Control pots are maintained with only sterile soil. The test lines are sown along with standard resistant (48–1) and susceptible (JI-35) checks. The plants are observed for disease reaction up to 75 days after sowing. Genotypes are categorized on the basis of 'days to wilt'. For each genotype, the days to wilt is recorded when 80% of the plants died. The genotypes are scored on a 1 (susceptible) to 4 (highly resistant) scale based on 'days to wilt': susceptible (<30 days), moderate (31–50 days), resistant (51–65 days) highly resistant (>65 days).

18.9.2 Screening for Resistance to Gray Mold Disease

Gray mold in castor is caused by the fungus *Botryotinia ricini* (Godfrey) Whetzel. Screening for mould disease is tricky because it is highly weather dependent and affects only the developing inflorescence. Several methods of screening under field and glasshouse conditions such as detached spike technique, detached capsule technique, field fogging technique and poly-house screening have been tried (Prasad et al. 2016). The detached spike technique involves spraying the spore suspension of pathogen (10^6 conidia/ml) on 15–20-day-old spikes cut from the plants and kept in conical flask containing 2% sucrose solution. The flasks with spikes are kept in glasshouse, where a humidity of 80%, temperature of 25–27 °C and continuous capsule wetness through fogging are maintained. Initial symptoms of mould infection appear 5 days after inoculation. At the seventh day, the disease severity is scored based on percent capsule damage. In the case of detached capsule technique, 15–20-day-old capsules are detached from the spike of castor plants, and surface sterilized and dipped in spore suspension of pathogen (10^6 conidia/ml). Inoculated capsules are kept in growth chamber at 20 °C and 90% humidity. Wetness on capsules is maintained by spraying water at 8 h of interval. Symptoms will appear on capsules at 3–4 DAI. By the sixth day, capsules are fully covered with mycelium. The disease severity is scored at 6 DAI.

18.9.3 Screening for Root Rot Disease

Root rot disease is caused by the fungus *Macrophomina phaseolina*. Screening against root rot is done using sick plot under field conditions and stem tape inoculation technique under greenhouse conditions. Screening of genotypes are done in the permanent root rot sick plot along with susceptible (GCH-4) and resistant (JI-357) checks planted after every five test entries. The pathogen isolated from naturally infected castor plants and grown on sorghum sand medium for 14 days is applied in furrows at the time of sowing. Root rot incidence at 120, 150 and 180 days after sowing is recorded. In the case of 'stem tape inoculation', plants are maintained in

pots containing sterilized soil under greenhouse conditions for 20 days. The hypocotyl region of the test plants are superficially wounded by peeling the epidermis at 2–3 cm above the soil surface. An agar disc (4 mm in diameter) containing mycelium with abundant sclerotia replaced on the wound and covered with cellophane tape. In control, plants are inoculated with a sterile PDA disc. The inoculated plants are observed for root rot symptoms up to 20 days after inoculation (DAI). The percentage of dead plants and length of lesion on stems are recorded.

18.9.4 Screening for Resistance to Leafhopper

The leafhopper (*Empoasca flavescens* F.) is one of the major pests of castor across growing seasons. The screening for resistance to leafhopper is done under field conditions as culturing the insect in laboratory for artificial screening is not feasible. The susceptible plants of DPC-9 are grown 15 days before sowing of test entries in order to build up the leafhopper population naturally. The test entries are sown during the second fortnight of October along with susceptible check (DPC-9) placed after every two rows of test entries. DPC-9 plants (70- to 75-day-old) from the infester block are cut at the base, removed and distributed in the screening plot uniformly near the base of the test plants at the rate of one infester plant per metre. The test entries were scored for the extent of hopper burn and number of nymph and adults when the susceptible check is completely dried up. Leafhopper counts (nymph) will be recorded on three leaves in each plant selecting one leaf from top (excluding two topmost leaves), middle (medium maturity) and bottom (leaving one or two bottom-most leaves) on the main shoot. Population will be reported as the number of leafhoppers/three leaves per plant. The hopper burn is scored on a 0–4 scale based on leaf area burnt per plant (average of five plants): 0, no injury; 1, hopper burn up to 10%; 2, hopper burn 11 to 25%; 3, hopper burn 26 to 50%; and 4, hopper burn above 50%.

18.10 Emerging Challenges at the National and International Level

The use of pistillate mechanism in hybrid development has been successful since the 1970s. However, maintaining genetic purity in hybrid seed lot in commercial production is becoming a challenge. Variations in weather parameters induce development of male flowers in pistillate line leading to a considerable proportion of selfed seeds of female plant in the hybrid seed lot. A clean genetic or cytoplasmic genic male sterility (GMS) system is essential to overcome the issues related to the stability of pistillate lines. There were a few isolated attempts to identify sources of male sterility through interspecific crosses and induced mutations, but no male sterility system yet available in castor for use in hybrid seed production.

Cultivation of castor is labour intensive. Labour scarcity is a major issue faced by the castor farmers. The nature and growth of castor make it unsuitable for

mechanization. There is an immediate need to restructure the plant so that the harvesting can be done through machines.

18.11 Breeding Progress/Varietal Development

18.11.1 Conventional Breeding

Systematic breeding efforts in castor started during the 1920s in the USA and erstwhile the USSR. The major breakthrough is the identification of dwarf-internode mutants followed by identification of pistillate-type plants facilitating hybrid breeding (Claassen and Hoffman 1950). Castor was the first oilseed crop in which variability for duration, plant height and oil content were induced using ionizing radiations, viz., X-rays, gamma rays and chemical mutagens.

In India, organized breeding work was initiated under the All India Coordinated Research Project (AICRP) on castor during the 1960s. AICRP breeding programme was highly successful in increasing the seed yield, reducing duration and plant height and improving the sex expression. Heterosis has been well exploited in castor. With the introduction and development of pistillate lines, hybrid development took a momentum. The first castor hybrid in the world, GCH-3, with high-yielding ability (88% yield increase over the variety S-20), drought resistance, medium maturity (140–210 days) and high oil content (46.6%) was released during 1976. Since then, several hybrids suitable for specific locations have been developed by IIOR and AICRP centres. The introduction of hybrids resulted in a spectacular rise in production and productivity. The productivity at the national level has increased from 270 kg/ha during the 1970s to 1900 kg/ha at present.

Major threat to castor cultivation was the wilt disease. The release of GCH-7, a high-yielding (3000 kg/ha) hybrid, resistant to wilt-nematode complex, leafhopper and root rot, made a dramatic impact in irrigated castor-growing regions of Gujarat and Rajasthan during the last decade. Extensive cultivation of GCH-7 in Gujarat state led to the decrease of wilt incidence from about 50% to 10%.

Castor oil is being considered as a potential source for biodiesel production. However, the high viscosity due to methyl ester of ricinoleic acid is a hindrance for biodiesel production. A line with more than 78% of oleic acid content has been identified (Rojas-Barros et al. 2004), which can be used for developing cultivars suitable as biodiesel feedstock.

18.11.2 Genomics-Assisted Breeding

In contrast to the major food crops, the genomic resources are lacking in castor. As a consequence, progress in molecular breeding is very limited. Even though a draft genome assembly in the form of 25,828 scaffolds accounting 92.8% of the genome was published way back in 2010 (Chan et al. 2010), no further efforts were made to improve the assembly further. A skeletal linkage map with 331 markers, mostly SSR

was constructed (Liu et al. 2016). So far, QTLs linked to plant height (Chen et al. 2014), *Fusarium* wilt (Tomar et al. 2016), root rot (Tomar et al. 2017) and seed size and weight (Yu et al. 2019) have been mapped. Very recently, genome data were generated by 'whole genome sequencing' of 14 diverse castor genotypes with an average of 34X coverage through next-generation sequencing (NGS) technologies. Further sequence analyses resulted in detection of a total of 2,179,759 putative SNPs; of which, 6000 high-quality SNPs were used to design a genotyping array (Infinium BeadChips). Using this genotypic array, a consensus linkage map consisting of 1978 SNP loci was constructed with an average inter-marker distance of 0.55 cM using two recombinant inbred line (RIL) populations produced from the crosses: JC12 × 48–1 and DCS9 × RG1139 (Senthilvel et al. 2019). The SNP array provides a valuable tool for high-throughput and cost-effective genotyping and mapping applications in castor. The genotyping array will provide the required resolution for discovery of marker-trait association through linkage and association analysis, evaluation of genetic variations, unravelling the genetic architecture of key quantitative traits and exercising genomic selection in castor.

18.12 Status of Varietal Development

In India, the release of cultivars is governed by two entities, namely, Central Varietal Release Committee (CVRC) and State Variety Release Committee (SVRC) under Seed Act 1966. The CVRC and SVRC notify the varieties/hybrids of agricultural crops recommended by research systems of ICAR. After notification, the commercial seed production of varieties and hybrids is allowed for popularization among the farmers for cultivation.

About 26 varieties with long duration (240–270 days) and tall plant type like HC 1 to HC 8, EB 16 A, S-20, Junagadh 1, Punjab castor 1, EB 31, Rosy, MC-1, etc. were developed by hybridization and selection method prior to inception of AICRP (Kulkarni and Ramanamurthy 1977). Under AICRP, 18 varieties and 20 hybrids have been developed till 2020. The state-wise varieties and hybrids notified under seed act from 1976 to 2020 are given in Table 18.2.

18.13 Maintenance Breeding

Due to cross-pollinating nature, complexity of sex and high sensitivity to genotype-environment interaction, degeneration of genetic stocks happens very often. If seed multiplication is done without required selection pressure, the parental lines degenerate very quickly. Since pollination occurs mostly by wind, genetic purity should be maintained by maintaining an isolation distance of at least 1000 m from other castor plants.

The initial source of seed for nucleus seed multiplication of male parental line should be from 50 to 100 selected and selfed progenies of preceding crop season. Selfed seeds of 200–300 selected plants are to be planted in progeny rows with at

Table 18.2 Varieties and hybrids of castor notified for cultivation in India

Name	Year of release/ notification	Developer	Recommended areas/ situations	Seed yield (kg/ha)	Oil content (%)	Salient features
Varieties						
GAUC-1	1976	Oilseeds Research Station, Junagadh, under Gujarat agricultural university, Gujarat	Irrigated and rainfed areas in Gujarat	1200 (Rainfed), 1500 (irrigated)	46–47	–
TMV-5	1985	Oilseeds Research Station, Tindivanam under Tamil Nadu agricultural university, Tamil Nadu	Rainfed areas of Tamil Nadu	920	47–49	Resistant to leafhopper
GC-2 (SKI-273)	1993	Castor and Mustard Research Station under S. K. Nagar, Sardarkrushinagar Dantiwada agricultural university, Gujarat	Irrigated areas of Gujarat	2165	47–49	Resistant to *fusarium* wilt
Jyoti (REC-9/ DCS-9)	1994	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	Rainfed areas of Andhra Pradesh, Telangana, Tamil Nadu and Karnataka	1030	48	Resistant to *fusarium* wilt, early maturity
Kranthi (PCS-4)	1996	Regional agricultural Research Station, Palem, under prof. Jayashankar Telangana state agricultural university, Telangana	Rainfed areas of Andhra Pradesh and Telangana	1365	48–50	Drought tolerant
TMV-6	1997	Oilseeds Research Station, Tindivanam under Tamil Nadu agricultural university, Tamil Nadu	Rainfed areas of Tamil Nadu	930	52	Resistant to wilt, moderately resistant to *Alternaria* leaf spot and rust

AKC-1	1998	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra	Rainfed areas of Vidarbha region of Maharashtra	1200	45	Easy threshability
Kiran (PCS-136)	2002	Regional agricultural Research Station, Palem, under prof. Jayashankar Telangana state agricultural university, Telangana	Rainfed and late-sown conditions of Andhra Pradesh and Telangana	1200–1500	48–51	Moderately tolerant to gray mold and drought; suitable for rice fallows
Haritha (PCS-124)	2002	Regional agricultural Research Station, Palem, under prof. Jayashankar Telangana state agricultural university, Telangana	Light soils of southern Telangana, Rayalaseema and Prakasam districts of Andhra Pradesh	1400–1600	48–51	Resistant to *fusarium* wilt
Jwala (48–1)	2007	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	All castor-growing regions of India	1100 (rainfed), 2000 (irrigated)	48	Tolerant to gray mold, saline and drought conditions
MCI-8 (RCV-1)	2008	Agricultural Research Station, Jodhpur, under Mandore agriculture university, Rajasthan	Irrigated areas of Rajasthan	3327	47.9	Resistant to *fusarium* wilt and root rot
DCS-107	2011	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	All castor-growing regions of India	1200–1430 (rainfed), 2140–2510 (irrigated)	45–47	Resistant to *fusarium* wilt
GC-3 (JI-273)	2012	Main oilseeds Research Station, Junagadh under Junagadh agricultural university, Gujarat	Irrigated areas of Gujarat	2310	49.7	Resistant to *fusarium* wilt and root rot
Pragathi	2016	Regional agricultural Research Station, Palem, under prof. Jayashankar Telangana state agricultural university, Telangana	Rainfed areas of Telangana	1500–1800	47–49	Resistant to *fusarium* wilt

(continued)

Table 18.2 (continued)

Name	Year of release/ notification	Developer	Recommended areas/ situations	Seed yield (kg/ha)	Oil content (%)	Salient features
Jawahar Castor-4 (JC-4)	2018	Zonal agricultural Research Station, Chhindwara under Jawaharlal Nehru Krishi VishwaVidyalaya, Jabalpur, Madhya Pradesh	Irrigated areas of Madhya Pradesh	2640	46–47	Resistant to *fusarium* wilt and root rot
Jawahar Castor-24 (JC-24)	2018	Zonal agricultural Research Station, Chhindwara under Jawaharlal Nehru Krishi VishwaVidyalaya, Jabalpur, Madhya Pradesh	Sole crop-irrigated and intercropping-rainfed conditions	2745	45–46	Resistant to *fusarium* wilt and root rot
YTP-1	2019	Tapioca and Castor Research Station, Yethapur under Tamil Nadu agricultural university, Tamil Nadu	Rainfed and irrigated perennial ecosystem	1450 (annual), 3110 (perennial)	49	Non-lodging, non-shattering, perennial
GAC-11	2019	Regional Research Station, Anand and Agricultural Research Station, Sansoli, under Anand Agricultural university, Gujarat	Irrigated and rainfed areas in middle Gujarat	2360 (rainfed), 3230 (irrigated)	48	
Hybrids						
GAUCH-1 (VHB-44)	1973	Oilseeds Research Station, Junagadh, under Gujarat agricultural university, Gujarat	Irrigated and rainfed areas of Gujarat	1520	46–47	–

GCH-2	1984	Oilseeds Research Station, Junagadh, under Gujarat agricultural university, Gujarat	Irrigated and rainfed areas of Gujarat	1750	47–49	Resistant to root rot
GCH-4 (SHB-18)	1986	Main Castor and Mustard Research Station, S.K. Nagar, under Gujarat agricultural university, Gujarat	All castor-growing regions of India	1985	48–50	Resistant to leafhoppers
GCH-5 (SHB-145)	1995	Main Castor and Mustard Research Station, S.K. Nagar, under Gujarat agricultural university, Gujarat	All castor-growing regions of India	2800	50	Resistant to wilt and tolerant to root rot, jassids, whitefly, capsule borer and water stress
GCH-6 (JHB-665)	1997	Oilseeds Research Station, Junagadh, under Gujarat agricultural university, Gujarat	All castor-growing regions of India	1300 (rainfed), 2300 (irrigated)	48	Tolerant to *fusarium* wilt, resistant to *Macrophomina* root rot
Deepti (DCH-32)	1998	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	All castor-growing regions of India	1030 (rainfed), 2460 (irrigated)	48	Resistant to leafhopper, suitable for early sown conditions
TMVCH-1	1998	Oilseeds Research Station, Tindivanam under Tamil Nadu agricultural university, Tamil Nadu	Rainfed areas of Tamil Nadu	1180	47–49	Resistant to leafhoppers and moderately resistant to gray mold
Deepak (DCH-177)	1999	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	Rainfed areas of Telangana, Andhra Pradesh, Tamil Nadu and Karnataka and irrigated areas of Maharashtra	1550 (rainfed), 2130 (irrigated)	49	Resistant to *fusarium* wilt and whitefly, resistant to lodging, non-shattering, moderately tolerant to botrytis gray mold

(continued)

Table 18.2 (continued)

Name	Year of release/ notification	Developer	Recommended areas/ situations	Seed yield (kg/ha)	Oil content (%)	Salient features
RCH-1 (RHC-1)	2000	Agricultural Research Station, Mandore under Rajasthan agricultural university, Rajasthan	Irrigated areas of Rajasthan	3000–3200	49	Resistant to leafhopper
DCH-519	2006	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	All castor-growing regions of India	1740 (rainfed), 2130 (irrigated)	46–51	Resistant to *fusarium* wilt, leafhopper
GCH-7	2007	Main Castor and Mustard Research Station, S. K. Nagar under Sardarkrushinagar Dantiwada agricultural university, Gujarat	Irrigated and rainfed areas of Gujarat	3000	48–49	Resistant to nematode-wilt complex
YRCH-1	2010	Tapioca and Castor Research Station under Yethapur, Tamil Nadu agricultural university, Tamil Nadu	Rainfed and irrigated areas of Tamil Nadu	780	49	Moderately resistant to capsule borer
PCH-111	2012	Regional agricultural Research Station, Palem under prof. Jayashankar Telangana state agricultural university, Telangana	Rainfed areas of Andhra Pradesh and Telangana	1400–1500 (rainfed), 2200–2500 (irrigated)	49	Resistant to *fusarium* wilt and moderately tolerant to sucking pests, semilooper and *Spodoptera*
PCH-222	2012	Regional agricultural Research Station, Palem under prof. Jayashankar Telangana state agricultural university, Telangana	Rainfed and irrigated areas of Andhra Pradesh and Telangana	1600–1800 (rainfed), 3500–2800 (irrigated)	47–48	Resistant to *fusarium* wilt

HCH-6	2016	Zonal agricultural Research Station, Hiriyur under University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka	Central dry zone of Karnataka	1830	50	Resistant to *fusarium* wilt and whitefly
GNCH-1	2017	Oilseeds Research Station, Navsari under Navsari agricultural university, Gujarat	Irrigated castor-growing regions of Gujarat	2500	47–48	Resistant to wilt and leafhopper
YRCH-2	2018	Tapioca and Castor Research Station, Yethapur under Tamil Nadu agricultural university, Tamil Nadu	Rainfed and irrigated areas of Tamil Nadu	2089	49	Resistant to wilt, tolerant to leafhopper
GCH-8 (SHB-896)	2018	Main Castor and Mustard Research Station, S. K. Nagar under Sardarkrushinagar Dantiwada agricultural university, Gujarat	All castor-growing regions of India	1895 (rainfed), 3000 (irrigated)	48–49	Resistant to wilt and root rot
GCH-9 (JHB-1018)	2019	Oilseeds Research Station, Junagadh under Junagadh agricultural university, Gujarat	Irrigated areas in Gujarat	3820	48	Resistant to wilt and root rot
ICH-66	2019	ICAR-Indian Institute of oilseeds research, Hyderabad, Telangana	Rainfed areas of Telangana, Andhra Pradesh, Karnataka, Tamil Nadu, Odisha and Maharashtra	1560	49	Resistant to wilt, root rot and leafhopper

least 30 plants per progeny row, and 100–200 uniform looking progenies are to be selected. In each selected progeny, five to ten representative plants which have same node number and conform to all other morphological characteristics of the genotype are to be labelled, and three to four spikes other than primary are to be selfed. At maturity, spike length, number of productive capsules, yield, 100-seed weight and oil content are measured in the open-pollinated primary spike. The progenies exceeding the general mean by one standard deviation and showing least intra-progeny variations are selected. About 50 seeds from each selected progenies are used for further maintenance, and the remaining portion of seeds are bulked and used as source for breeder seed production.

The pistillate lines need to be raised in ideal location and season so as to assess the sex expression. The selfed seeds of individual plants obtained from 6^{th} or later order spikes of preceding crop season are to be sown in progeny row ensuring a minimum of 30 plants per progeny. At the stage of primary spike initiation, about 50 plants may be selected based on the uniformity for various morphological characters and femaleness, and all other plants are to be removed. Within selected progeny, plants confronting to the desired node number may be retained, and all other plants may be removed. The environmentally sensitive interspersed staminate flower should serve as pollen source. Care should be taken to observe all the plants regularly for reversion to monoecious status. The plants showing monoecious spike before the sixth order should be destroyed. The remaining plants are selfed to provide seed for breeder seed production.

18.14 Coordinated System of Testing

In India, applied research on castor is undertaken by a network of research centres spread across different agro-climatic regions of the country under All India Coordinated Research Project (AICRP) administered by the Indian Council of Agricultural Research (ICAR). The project started in 1967 with four centres, viz., Rajendranagar (Andhra Pradesh), Vijapur (Gujarat), Raichur (Karnataka) and Salem (Tamil Nadu). At present, AICRP on castor is being operated at nine centres, namely, Ananthapuramu (Andhra Pradesh), Bawal (Haryana), Bengaluru (Karnataka), Bhawanipatna (Odisha), Junagadh (Gujarat), Mandore (Rajasthan), Palem (Telangana), Sardarkrushinagar (Gujarat) and Yehtapur (Tamil Nadu) under the coordination of ICAR-IIOR. The new cultivars developed by different institutions are being tested across these centres for 3 years to test their performance against the existing cultivars as checks.

18.15 Future Thrust Area

The following are the major thrust areas of research on castor in the coming years:

- Bridging the knowledge gaps in the understanding of many issues related to castor growth and development, which is essential for breeding of high-yielding varieties adapted to each growing environment.
- The genome assembly is highly fragmented, which needs to be improved, and chromosome-scale assembly is required for furthering the genomic research.
- Understanding the mechanism of resistance for sucking pests, capsule bore and mould disease and identifying reliable resistant sources.
- Improving oil quality to increase wider adaptability and uses, viz., reduction of ricin in castor.
- Development of cultivars suitable for high-density planting and mechanical harvesting.
- Development of gray mold-resistant castor lines through genetic engineering and marker-assisted breeding.

18.16 Conclusions

The present chapter summarizes the overall improvement of castor as a perennial, wild-type plant to an annual, high-yielding plant with inbuilt resistance to wilt and leafhopper. India still retains the *number one* position with the highest productivity (1.97 t/ha) and is the major source of supply of castor oil to the USA, Europe, Japan, China etc. with a net revenue worth of US$ 400 million in 2020. A high genotype × environment interaction in castor necessitates the development of several locally adaptable and high seed/oil yielding genotypes with a major emphasis on production conditions. Further enhancement of productivity levels up to 5–6 t/ha under irrigated conditions needs to be explored with intensified efforts on genetic enhancement of parental lines through biparental mating and recurrent selection methods. While the traditional rainfed castor area needs early maturing, drought-resistant castor hybrids with an emphasis on resistance to mould, capsule borer and sucking pests. AICRP network needs to be strengthened with additional manpower and facilities for full-proof evaluation and screening for major pests and diseases.

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Sunflower Breeding

19

H. P. Meena and M. Sujatha

Abstract

Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops cultivated worldwide for its oil and confectionary purposes. Among the crops where heterosis has been successfully exploited, sunflower assumes importance as that of maize. Identification of the PET-1 cytoplasm along with the complementary fertility restoration system is a landmark achievement which paved the way for transformation of an ornamental crop to a commercial oil yielding crop. Breeding objectives are directed towards development of cultivars with high seed yield, early maturity, resistance to diseases (downy mildew, powdery mildew, rust, necrosis disease, *Alternariaster* leaf spot), insect pests (*Helicoverpa*, sucking pests) and tolerance to herbicides, besides improved content and quality of seed oil and protein. Genetic enhancement for widening the trait base exploited traditional breeding methods, mutation breeding and interspecific gene transfer. Interspecific hybridization was adopted as one of the key tools by various research groups due to the existence of a rich repertoire of genes in the wild *Helianthus* species, and several economically important traits such as cytoplasmic male sterility, resistance to biotic and abiotic stresses, herbicide tolerance and seed quality traits were successfully introgressed. The past two decades witnessed advancements in molecular marker technology and genomics which have been successfully used in marker-assisted breeding for simple inherited traits, while traits governed by quantitative trait loci still remain a challenge for the breeders. Despite the availability of genes in wild *Helianthus* species, successful transfer to cultivar germplasm is hampered by crossability between cultivated sunflower (annual diploid) and *Helianthus* species varying in habit (diploid perennial) and

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_19

ploidy (tetraploids, hexaploids) warranting the use of genetic engineering approaches. This chapter presents a comprehensive account of the history, botany, the extent of genetic diversity in cultivated and wild *Helianthus* species. Strategies are adopted for development of inbreds and hybrids, seed production methodology and progress with regard to exploitation of molecular marker and genomic resources in marker-assisted breeding.

Keywords

Hybrids · Breeding techniques · Oil content · Breeding approaches · Genetic engineering · Genomics

19.1 Introduction

Sunflower is an important annual edible oilseed crop of Asteraceae family grown globally over an area of 27.36 m. ha with a production of 56 mt (FAOSTAT 2019) and world average yield of 2048 kg/ha. Because of its wide adaptability to diverse agro-climatic situation, it is grown in all the countries crossing climatic and geographical boundaries. Being photo-insensitive and day neutral, it can be grown throughout the year, i.e. *kharif*, *rabi* and *spring*/summer, and it is an ideal crop for contingency cropping plan. Being a short duration crop, it can fit into various inter- and sequence cropping systems. Leading producing countries are Ukraine, Russia and Argentina, while countries like Belarus, France, Hungary, Romania, Kazakhstan, Turkey, Tanzania, China and India produce relatively smaller quantity. The highest-yielding countries are Hungary (3025 kg/ha), China (2847 kg/ha), Turkey (2794 kg/ha), Romania (2782 kg/ha), Ukraine (2560 kg/ha), Bulgaria (2375 kg/ha), France (2149 kg/ha) and Argentina (2039 kg/ha) with an average yield of >2.0 t/ha as against the lowest-yielding countries like India and Myanmar with <1.0 t/ha. The crop has become one of the important oilseed crops in India with an area of 2.4 lakh ha and a production of 2.6 mt of seed (2019–2020).

In India, sunflower is mostly grown in the Southern and Central Peninsula comprising Karnataka, Andhra Pradesh, Telangana, Maharashtra and Tamil Nadu. The highest (>50%) area of sunflower is covered by only a single state (Karnataka) with productivity of 785 kg/ha. It has spread to non-traditional states like Punjab, Haryana and Uttar Pradesh as an important oilseed crop and is grown in West Bengal, Bihar and Odisha as well. The productivity (891 kg/ha) of sunflower in India at present is far lower than the world average (2048 kg/ha). Despite high productivity in Punjab (1950 kg/ha), Gujarat (1820 kg/ha), Haryana (1743 kg/ha) and Telangana (1698 kg/ha), area under sunflower has declined drastically due to unavailability of better hybrids that can give more than 3.0 t/ha seed yield, price fluctuations, shift in cropping pattern, profitability of other crops compared to sunflower, withdrawal of private players from sunflower research, bird damage and vulnerability to several biotic and abiotic stresses during various stages of crop growth.

19.2 Economic Importance

In pre-historic times, the North American Indians for the first time found that the seeds of wild sunflowers were a rich source of food and domesticated the plant. Different colour dyes like purple, black and yellow extracted from ray florets of wild sunflowers were used to dye basketry materials. The Hopi Indians obtained a purple dye from deep purple achenes (Heiser Jr 1976) to decorate their bodies. In the southwest, people used it as an antidote to snake bites and to cure rattlesnake bites by chewing the fresh or dried root followed by sucking the snake bite wound (Camazine and Bye 1980). The Europeans also used oil as a remedy for heart diseases, pulmonary infections, cold and whooping coughs (Heiser Jr 1976). It was cultivated as an ornamental or garden plant, where the blooms were cherished for their beauty and the seeds were eaten by both humans and wildlife. The oil is used for human consumption, salad oil, making paints, soaps, as biofuel and candles. Sunflower oil has potential applications in the cosmetic industry (Oliveira et al. 2019). Sunflower meal is an excellent source of protein for human consumption and as a supplementary diet which enhances the animal growth and milk production. Sunflower cake is used in South Africa and Tanzania as the main component of livestock feeds. Some species of sunflowers are grown for fodder or silage.

19.3 Origin and History

The genus *Helianthus* is derived from the Greek word *helios* meaning sun and *anthos* meaning flower and belongs to the subdivision Tubiflorae and tribe *Heliantheae*. It is a member of the Asteraceae (Compositae) family and has a typical composite flower. The cultivated sunflower originated in North America from a diploid ($2n = 34$) annual wild *H. annuus* species (Smith 2014). Sunflower is native to North America, but commercialization of the plant took place in Russia. The American Indians were the first to domesticate the multi-headed ornamental plant into a single-headed plant with a variety of seed colours.

The beginning of domestication and the first steps of sunflower breeding date back to the time when it was cultivated by native Americans over 4000 years ago (Seiler et al. 2017). It was used in food, to obtain oil, for medical purposes, and as an ornamental plant. From its wild weedy forms, the crop has spread to Europe during the sixteenth century and later to former USSR wherein the crop was domesticated. Sunflower as a cultivated plant was reintroduced to North America from Europe in the late nineteenth century. After the Second World War, the introduction of Russian varieties into Europe and America had a decisive impact on the development of sunflower as a commercial oilseed crop. Sunflower oil on commercial scale was first produced in Russia between 1830 and 1840.

19.4 Floral Biology

In sunflower, the inflorescence which is a capitulum or head is most prominent because of its large size and conspicuous yellow colour of the ray florets. The capitulum is made up of two distinct flower types: outer single row of ray florets which are male sterile and the inner disc florets which are hermaphrodite and fertile. The disc florets are arranged in arcs radiating from the centre of the head. Each disc floret is made up of inferior ovary, two pappi (modified sepals) and a tubular corolla formed by the fusion of five petals except at the tip. The five anthers are also fused to form a tube (syngenesious) with filaments attached independently at the base of the corolla tube. The style is inside the anther tube with stigma divided at the tip. When the flower is fully developed, the style elongates and the bifid stigma curls outward.

19.5 Pollination Mechanism

The unfolding of outer ray florets indicates the beginning of flowering in sunflower. Opening of disc florets follows from the periphery proceeding gradually towards the centre of the head at the rate of one to four whorls per day. The flowering in the capitulum is completed in 7–10 days depending upon the size of the capitulum and prevailing weather. Anthesis takes place in the morning between 6:00 and 8:00 AM on warm sunny days. Anthesis is delayed if weather is cool, cloudy or wet. The staminal filaments rapidly elongate and exert the anther tube from the corolla. Pollen is dehisced within the anther tube. The style elongates and forces the two lobed pubescent stigmas up the anther tube. The stigma is not receptive at this stage because the two lobes are held together covering the inner receptive surface. The stigma pushes the dehisced pollen through the upper end of the anther tube, and the lobes separate exposing inner receptive surface. The sunflower to a large extent is protandrous as anthesis takes place first, accompanied by a time lag of 10:00–12:00 h in the maturation of male and female elements. Because of this, cross-pollination is favoured as a rule, and insects, particularly bees, play an important role in pollination. The pollen is spiny and well adapted for transmission by insects mainly by honey bees. Pollination and fertilization occur when the spiny viable pollen is transferred to the stigmatic surface. The achene is the fruit of the sunflower that consists of outer pericarp (hull), thin and papery inner seed coat and embryo (kernel). The achene is attached by a funiculus, but the seed coat is free from the inner wall of the pericarp. Seed is nonendospermic, and the embryo is the major portion and is made up of mostly cotyledons. The endosperm is largely made up of a single layer of aleurone cells coalesced with the seed coat.

19.6 Genetic Resources

Availability of appropriate genetic resources with wide diversity is the key to any crop improvement programme. Several germplasm collections have been stored in different gene banks across the world. The highest number of collections are

maintained at USDA, USA (total 5217 accessions, of which 2616 are cultivated *H. annuus* accessions, 2597 are wild accessions including 1693 annual and 904 perennial and 4 are diverse CMS sources) (Terzić et al. 2020). The France collection at INRA maintains 3933 accessions, 2703 cultivated *H. annuus* accessions, 10 diverse CMS sources and 1214 wild accessions of which 804 accessions are annual and 410 are perennial species. In India, ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad gene bank maintains 3102 sunflower accessions. The collection includes exotic collection, augmented germplasm, inbreds, populations, genetic stocks, gene pools for high oil, high seed yield, autogamy, backcross-derived lines and wild species including their derivatives. Other countries that maintain sunflower germplasm accessions are Russia (1210), Argentina (922), Romania (681), Serbia (593), Germany (503) and Bulgaria (423). The lowest numbers of accessions are maintained in Spain (196). The collection may help improve economical traits related to yield and quality and also serve as the source for biotic and abiotic resistance genes. Further, the elite germplasm may help initiate sunflower breeding programmes in many countries. The details of germplasm collections in gene banks in different countries are presented in Table 19.1.

Table 19.1 Sunflower germplasm resources maintained in gene banks of different countries

S. no.	Country	No. of genetic resources	Type of material
1.	USA	5217	Cultivated lines, CMS, wild *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp. and other genera
2.	France	3933	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp., other genera
3.	India	3102	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp.
4.	Russia	1210	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp.
5.	Argentina	922	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp.
6.	Romania	681	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp.
7.	Serbia	593	Wild *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp.
8.	Germany	503	Cultivated lines, OPV and other populations, CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp.
9.	Bulgaria	423	CMS, wild annual *H. annuus*, other annual *Helianthus* spp., perennial *Helianthus* spp., other genera
10.	Spain	196	Open-pollinated varieties and other populations

Source: Modified from Terzić et al. (2020)

19.7 Wild *Helianthus* Species

Cultivated sunflower is diploid with chromosome number 2n = 2x = 34. The genus *Helianthus* includes 53 species, of which 39 are perennials and 14 annuals being maintained at the USDA-ARS, North Central Regional Plant Introduction Station (NCRPIS) in Ames, IA (Seiler and Jan 2014; Seiler et al. 2017). The 14 annual species are diploid (2n = 2*x* = 34), and the 39 perennial species include 26 diploids, three tetraploids (2n = 4*x* = 68), seven hexaploids (2n = 6*x* = 102), one mixoploid of either diploid or tetraploid and two mixoploids of tetraploid or hexaploid (Liu et al. 2017). Some species occur in dual ploidy series, such as *H. ciliaris* L., which displays both teraploid and hexaploid states, and *H. decapetalus* L., which exists in diploid and tetraploid forms (Atlagic 2004).

19.7.1 Utilization of *Helianthus* Species

The wild *Helianthus* species exhibit high variability for several agronomic attributes. Sunflower is one of the few crops where the use of wild *Helianthus* species in sunflower breeding programmes has produced significant results. Genes for disease (Seiler 2010) and insect resistance (Thompson et al. 1981), oil (Jovanka 2004) and protein content and quality (Miller et al. 1992), cytoplasmic male sterility (Horn 2002), herbicide tolerance (Al-Khatib et al. 1998; Al-Khatib and Miller 2000) or agronomic and physiological traits (Seiler et al. 2017) have been identified in wild *Helianthus* species and transferred into cultivated lines in the USSR, the USA and many East European countries. Interspecific hybridization paved the way for hybrid breeding in sunflower wherein several CMS and fertility restorer gene sources were derived from wild *Helianthus* species (Horn and Friedt 2006). The single most important breakthrough has been the discovery of cytoplasmic male sterility (CMS) via interspecific hybridization and *Rf* genes involving *H. petiolaris*, which allowed practical use of heterosis and development of hybrids worldwide (Leclercq 1969; Kinman 1970). In India, the sunflower variety LSF-8 with tolerance to downy mildew, rust and *Alternariaster* was derived from the cross involving *H. tuberosus*. The variety CO-5 (COSFV-5) with moderate resistance to *Alternariaster* leaf spot, rust and necrosis disease was derived from the gene pool of *H. annuus x H. praecox* (Sujatha et al. 2019). Using conventional methods of crossing, several prebred lines with altered plant architecture, high yield and oil content, maturity duration and inbuilt tolerance to major biotic stresses have been developed from crosses involving diploid annuals (Sujatha et al. 2008). Prebred lines derived from *H. annuus* × *H. argophyllus* crosses were found resistant to leafhopper (unpublished). Jacob et al. (2017) identified one sulfonylurea-based accession of *H. praecox* (PRA-1823) resistant to herbicides as well as to powdery mildew. Different diploid annual wild species like *H. debilis*, *H. petiolaris*, *H. argophyllus*, *H. praecox* and wild *H. annuus* are being utilized for broadening the genetic base of cultivated sunflower in India. Concerted efforts are warranted to overcome the deleterious effects of distant hybridization and combine desirable traits with high seed and oil

yield. Information on the global scenario of the progress made in augmentation and maintenance of wild *Helianthus* sources and utilization for introgression of gene (s) for resistance to biotic and abiotic stresses and oil content and quality is reviewed in the article of Seiler et al. (2017).

19.8 Evolution of High Oil Sunflower

Transformation of an ornamental sunflower into a high oil yielding crop occurred in the USSR by 1769 and by 1830, and the manufacture of sunflower oil was done on a commercial scale. During the early nineteenth century, the Soviet plant breeder, Vasilii Stepanovich Pustovoit, in 1860, initiated selection for high oil content from local varieties which contained 30–33% oil content, and concerted efforts at several agricultural stations resulted in the gradual stepping up of oil content in the cultivars from 33 to 50%. Consequently, the seed oil content increase was 21%. In 1927, V.S. Pustovoit bred a new sunflower variety with 35% oil content. High oil sunflower varieties such as VNIIMK-3519, VNIIMK-6540, VNIIMK-8931, Peredovik, Armavirsky-3497 and VNIIMK-309 developed by Pustovoit and his associates enabled the spread of sunflower as an oilseed crop (Pustovoit 1964). Much of the change was achieved by breeding for thin hulls surrounding the kernels. Following Pustovoit's method (Fig. 19.1), new early, broomrape-resistant, high oil content (45–48%) and high seed yielding varieties such as Krasnodarets, Armavirets, Chrnyanka-66 and Enissei were developed. These high oil selections found their way later on to all the continents, and most of the recently bred varieties owe their origin directly or indirectly to the materials bred in the USSR.

19.9 Inbreeding, Heterosis and Development of Hybrids

Inbreeding as a method for improving sunflower was used as early as 1922. Cardon (1922) described the available variation in sunflower varieties and attempted to isolate different types by self-pollination. Inbreeding was used in the Soviet Union during the 1920s to develop lines with improved oil percentage, strong single stems and resistance to diseases and insect pests (Calson et al. 1972; Jagodkin 1937; Voskoboinik and Soldatov 1974). Unrau and White (1944) recorded a 35% decline in seed yield after one generation of inbreeding in the cultivar Mennonite. Breeders soon realized that the value of inbreeding was to develop inbreds with certain desirable characteristics for subsequent crossing to produce synthetic cultivars or interline hybrids. Attia et al. (2014) observed 2.25% decrease in oil content due to inbreeding. Some of the results involving hybridization of inbred lines showed heterosis for plant height, head diameter, seed size and seed yield. To reduce inbreeding depression, Kovacik and Skaloud (1975) recommended sib cross in early inbred generation. Shvetsova (1979) observed no substantial differences between fertile lines and their male sterile counterparts for oil content in inbreeding. Schuster (1980) studied inbred lines for 25 generations and revealed mean

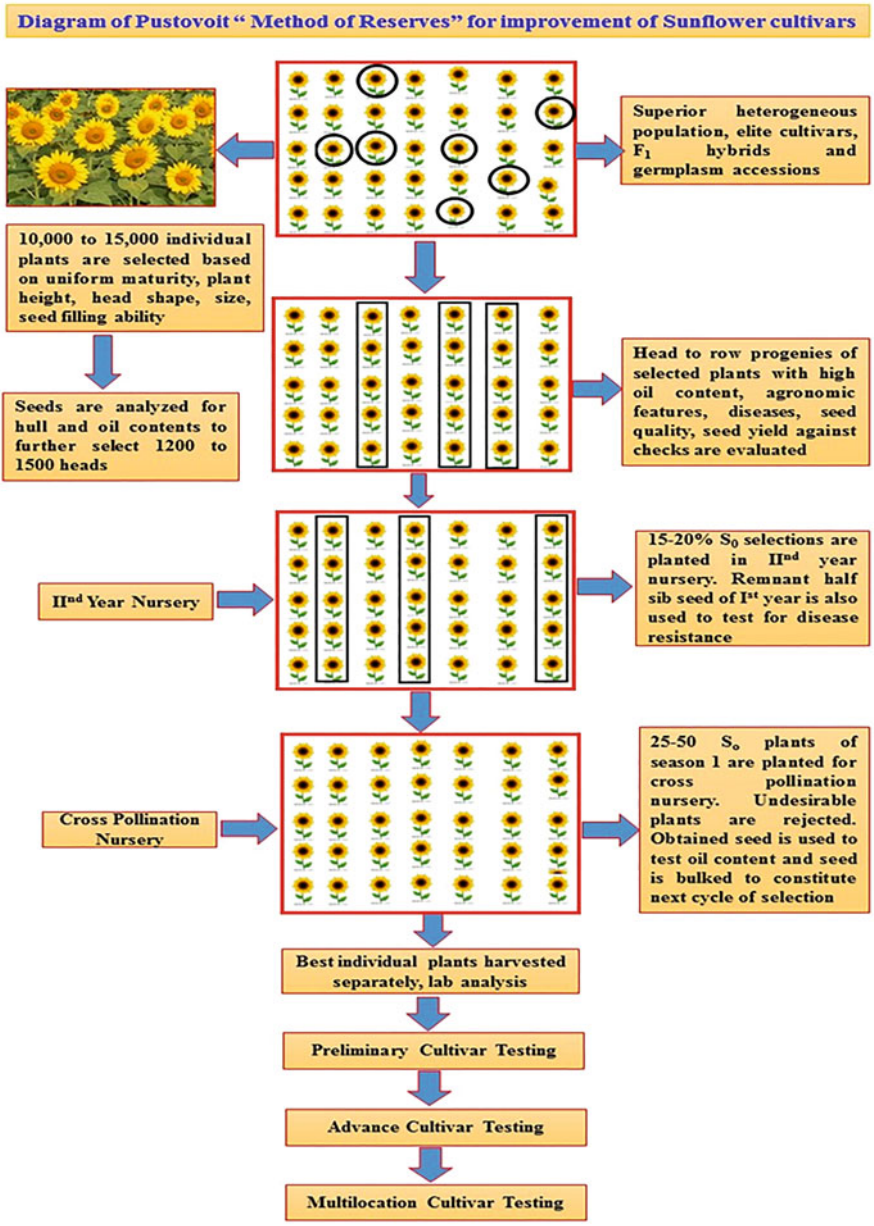

Fig. 19.1 Pustovoit method for developing high oil content material

inbreeding depressions for seed yield (61%), plant height (20%), capitulum diameter (21%), kernel/husk ratio (3%), crude oil content of achene (7%) and 1000 seed weight (22%). Tuberosa (1983) observed 41.7% decrease in achene yield, 4.2% in

oil content and 44% in oil yield in S_4 generation due to inbreeding. Gurev and Osipova (1986) studied a number of varieties and hybrids for the degree of inbreeding depression and found that the yield decreased by 33.0% in I_1, 53.5% in I_2, 46.5% in I_3 and 45% in I_4 generations.

As early as 1940, Putt in Canada carried out preliminary studies on heterosis breeding. Unrau and White (1944) reported 60.8% increase in seed yield and great uniformity in a hybrid compared to unimproved Mennonite. Heterosis studies carried out in sunflower have been presented for interline, inter-varietal and top-cross hybrids involving genetic and cytoplasmic male sterility. In a study of inter-varietal crosses, Kovacik (1959, 1960) observed good response with an increase in seed yield to the extent of 1 to 20% over the parents. While comparing progenies from selfed and crossed seeds, Kurnik and Zelles (1962) observed 18.7% more height, 4.2% higher seed yield, 10.8% higher 1000 seed weight and 3 days of delayed flowering in the plants from crossed seeds. Putt (1964) reported highly significant values of heterosis for seed yield and plant height in a diallel study involving ten inbred lines. In eight sunflower hybrids, Putt (1966) observed heterosis for plant height and seed yield. Reports from various countries indicate (Gundev 1966; Fick and Zimmer 1974; Putt and Dorrell 1975) heterosis to an extent of 61% in the crosses of inbred lines for seed yield and much greater uniformity in hybrids. Kolczowskii (1971) observed an average 10.4% heterosis in the F_1s from 20 crosses among varieties of diverse origin in comparison with the better parent. Stoyanova et al. (1971) studied heterosis in 140 F_1 hybrids obtained from crosses among 192 stabilised inbred lines and reported a heterosis of 25 to 29%. Ge (1971) observed heterosis of 15.9% for head diameter and 47.0% for a total number of seeds per head in certain sunflower hybrids. Heterosis of 12 to 21% for seed yield is reported by Klimov and Ermoshin (1977) in certain top-cross hybrids developed from 29 Soviet varieties and 3 testers. In India, Seetharam et al. (1977) studied the performance of hybrids produced by crossing four CMS and two fertility restorer lines and observed a significant positive heterosis for days to flowering, plant height, head diameter, test weight, oil content and seed yield. Sudhakar and Seetharam (1980) reported heterosis for seed yield up to 41.31% over the mid-parent and up to 5.24% over the better parent in 27 top-cross hybrids. Choudhary and Anand (1984) observed 62.8% heterosis for seed yield, 23.1% for oil content and negative heterosis (7.7%) for days to flowering over better parent. Singh et al. (1984) in a study on performance of variety × inbred crosses observed heterosis for yield to an extent of 47.0–206.0%. The studies of Shivaraju (1984) on ten F_1 hybrids indicated an average heterosis to an extent of 175.0% for seed yield, 129.0% for the number of filled seeds and 6.87% for oil content. Giriraj et al. (1986) observed 15.2% heterosis for head diameter, −7.7% for days to flowering and 37.7% for the number of filled achenes.

The advantages of maize and other crops stimulated sunflower researchers to work towards hybrid development in the crop. Introduction of male sterility using GA_3 tried on a large scale in many countries had inherent problems in exploiting heterosis satisfactorily in sunflower. Several workers (Luciano et al. 1965; Putt 1962) tried to develop hybrids based on the self-incompatibility mechanism. The occurrence of a higher proportion of selfs in hybrid seed plots came in the way of

large-scale seed production of hybrids based on this system. Before the discovery of cytoplasmic male sterility and the corresponding fertility restoration system, genetic male sterility discovered as early as 1934 was tried to produce hybrids (Leclercq 1966; Putt and Heiser 1966). This system was widely explored in France and Romania despite the major disadvantage of removal of 50% plants in seed production plots and higher costs associated with seed production.

The most significant development leading to the large-scale exploitation of heterosis was the discovery of cytoplasmic male sterility (CMS) and restorer system first developed by Leclercq (1969) working at the French National Institute for Agricultural Research (INRA) from the interspecific cross *H. petiolaris* Nutt. × *H. annuus* L. (the variety Armavirsky-9345) back crossed to *H. annuus* L. and identification of fertility restorer lines RHA-265 and RHA-266 derived from a composite cross by Kinman (1970) in the USA. After this discovery there has been a shift in the breeding emphasis from open-pollinated varieties (OPV) to development of single- and three-way cross hybrids. The first hybrid using CMS and genetic restoration system was available as early as 1972, and within a span of 5 years, the hybrids spread to many countries in Europe and America replacing the open-pollinated varieties. Today hybrids are predominantly cultivated worldwide, and > 95% of the area is covered by them.

19.10 Development of Sunflower Hybrids in India

The commercial cultivation of sunflower in India started in 1972 with the introduction of five Russian varieties [VNIIMK-8931 (EC-68413), Peredovik (EC-68414), Armavirskii-3497 (EC-68415), Armaverta (EC-69874) and Vashod (Sunrise)]. The value of hybrids and importance of heterosis breeding were recognized early with the inception of the All India Coordinated Research Project (AICRP) on Oilseeds in 1972–1973. A special project on 'Promotion of Research and Developmental Efforts on Hybrids in Selected Crops-Sunflower' was launched in 1989 as a thrust programme to develop hybrids for diverse situations. Experimental hybrids were developed at Bangalore in 1974–1975 using four CMS lines, namely, CMS-2A, CMS-124A, CMS-204A and CMS-234A, and two restorer lines, viz. RHA-266 and RHA-274, introduced from the USA. Based on seed yield, oil content, yield stability and synchronization of flowering in male and female parents, the first public sector hybrid BSH-1 (CMS-234A × RHA-274) was released for commercial cultivation from public sector in 1980 (Seetharam 1980). Since then, the hybrid base has been further widened in the country through AICRP centres. Till today, a total of 30 hybrids and 19 varieties were released in India from public sector (Sujatha et al. 2019). The salient features of hybrids released since 1980 are given in Table 19.2.

Table 19.2 Salient features of sunflower hybrids released from 1980 to 2020 in India (by public sector)

S. no.	Hybrids	Pedigree	Year of release	Maturity	Yield (kg/ha)	Oil content (%)	Salient features
1	BSH-1	CMS-234A × RHA-274	1980	85–90	1200–1300	41	Resistance to rust and downy mildew
2	LSH-1	CMS-338A × MRHA-II	1990	85–90	1000–1100	38	Resistance to downy mildew
3	LSH-3	CMS-207A × MRHA-1	1990	90–95	1200–1300	39	Resistance to downy mildew
4	KBSH-1	CMS-234A × RHA-6D1	1992	90–95	1300–1500	43	High yield with high oil content
5	PKVSH-27	CMS-2A × AK-1R	1996	85–90	1300–1400	39	Moderate resistance to downy mildew
6	DSH-1	DSF-15A × RHA-857	1997	85–88	1200–1300	38–40	Resistance to downy mildew
7	TCSH-1	CMS-234A × RHA-272	2000	–	–	–	–
8	KBSH-41	CMS-234A × RHA-95-C-1	2001	90–92	1300–1500 (R) 2500–3000 (I)	39–41	Tolerant to moisture stress
9	KBSH-42	CMS-851A × RHA-95-C-1	2001	90–92	1300–1500 (R) 2500–3000 (I)	38–41	Tolerant to moisture stress
10	NDSH-1	CMS-234A × RHA-859	2002	88–90	1400	40	Early hybrid
11	KBSH-44	CMS-17A × RHA-95-C-1	2002	95–98	1400–1600	36–38	High yield
12	LSFH-35	CMS-234A × RHA-1-1	2003	Medium	1400–1500	39–41	Resistance to downy mildew

(continued)

Table 19.2 (continued)

S. no.	Hybrids	Pedigree	Year of release	Maturity	Yield (kg/ha)	Oil content (%)	Salient features
13	PSFH-118	CMS-10A × P-61-R	2004	85–88	1400	40	Resistance to basal stem rot and head rot
14	HSFH-848	CMS-91A × RHA-298	2005	90–95	1800–2400	41–42	Resistance to *Alternaria* leaf spot and *Rhizoctonia*
15	DRSH-1 (PCSH-243)	ARM-243 × RHA-6D-1	2005	95–98	1300	43	High oil hybrid
16	TUNGA (RSFH-1)	CMS-103A × R-64-NB	2005	95–100	1300–1500	39–41	–
17	PSH-996	CMS-11A × P-93R	2012	96–100	1957	37–38	Suitable for late sown conditions
18	RSFH-130 (Bhadra)	CMS-104A × R-630	2008	95–100	1800–2000	39–42	Tolerant to necrosis
19	KBSH-53	CMS-335A × RHA-95C-1	2008	95–100	750–1250	38	Tolerant to powdery mildew
20	KSFH-437 (Phule Raviraj)	CMS-17A × R-437	2009	90–95	1800–2000	34	–
21	CO-2	COSF-1A × CSFI-99	2010	85–90	1900–2200	38–40	Early
22	LSFH-171	CMS-17A × RHA-1-1	2016	90–95	2000–2400	34–35	Resistance to downy mildew
23	PSH-1962	CMS-67A × P-93R	2015	99	2050	41.9	High yield and high oil content
24	RSFH-1887	CMS-38A × R-127-1	2016	95–100	1800–2500	38–40	Tolerant to necrosis and *Alternaria* leaf blight
25	NDSH-1012 (Prabhat)	NDCMS-30A × R-843	2016	90–95	2000–2500	40–41	Moderately resistant to downy mildew

26	PDKVSH-952	CMS-302A × AKSF-6R	2016	90	1800–2000	36.8	–
27	COH-3 (CSFH-12205)	COSF-6A × IR-6	2018	90–95	2200–2400	42	–
28	KBSH-78	CMS-1103A × RHA-92	2018	82–85	1700–2300	39–41	Short duration and medium height
29	DSFH-3	CMS-234A × RHA-IV-77	2018	90–95	2000–2500	37–39	High seed yield
30	PSH-2080	CMS-67A × P-160R	2019	97	2441	43.7	High seed and high oil content

Sources: Meena et al. (2013), Sujatha et al. (2019)

19.11 Major Breeding Objectives

19.11.1 Seed Yield

The main goal of plant breeding is to increase yield. Seed yield is a complex and polygenic controlled trait and strongly influenced by environmental conditions, and hence both additive (Sheriff and Appandurai 1985; Singh et al. 1989; Petakov 1992) and non-additive (Bajaj et al. 1997; Rether et al. 1998; Kumar et al. 1998; Goksoy et al. 2000; Cecconi et al. 2000) genetic effects play an important role in the inheritance of seed yield. Because of the importance of environmental effects, the heritability for seed yield is relatively low compared with other agronomic traits. Recurrent selection for general combining ability (gca), reciprocal recurrent selection, which capitalize on additive genetic variance, and recurrent selection for specific combining ability (sca), which capitalize on the non-additive portion of genetic variance, would be more effective breeding methods in improving seed yield. Seed yield consists of three main components: number of plants per hectare, seeds per plant and 1000 seed weight. Yield per unit area can be increased in a number of ways. One of the main approaches is to increase the seed number and seed size per head while maintaining or increasing plant number per unit area (Merrien 1992). To realize high seed yield in sunflower, parents that have good *gca* and *sca* for most yield components can be used in sunflower breeding (Tyagi 1988). Another most effective way of increasing the yield of sunflower is to exploit heterosis through hybrids. Sunflower seed yield may be increased significantly by breeding for seed size. According to Morozov (1947), the increase in 1000 seed mass by only 1.0 g brings an increase in seed yield of 40.0 kg/ha. To achieve high seed yield per unit area, many breeders consider it essential to develop a genotype capable of providing more than 1500 seeds per capitulum. A head size of 20 to 25 cm and flat shape are important in attaining this goal.

19.11.2 Oil Content and Quality

Sunflower seeds are mainly used for oil extraction, which is predominantly used for human nutrition. The oil concentration in sunflower may be reaching a plateau, but most breeders believe that selecting for higher oil content and oil quality in seed is still a very important and realistic objective when breeding for high oil and quality sunflower varieties and hybrids. According to Borodulina and Khachenko (1976), oil accumulation in sunflower seed begins on the first day after flowering and continues until physiological maturity. The period of most intensive oil accumulation takes place between the 15th and 22nd day after the beginning of flowering. It is a quantitatively inherited trait, and genetic variation is affected by additive genes. The heritability of this trait is relatively high to medium (Mokrani et al. 2002), and progress has been made in increasing oil content in sunflower. Hence, it can be improved through breeding methods like simple recurrent selection or through population improvement. Miller et al. (1987) reported that oleic acid was controlled

by a major gene with partially dominant gene action. Hence, it is clear that breeding for high oleic content is possible in sunflower. Wild *Helianthus* species may also be utilized for increasing oil, protein content and quality in cultivated sunflower. Thompson et al. (1978, 1981) reported that *H. niveus* (Benth.) and *H. salicifolius* are potential sources for oil content. 'Pervenets' breeding lines contain a dominant mutation, which increases oleic acid content to more than 89% in the sunflower oil. Several QTLs have been identified on various linkage groups for seed oil content and altered fatty acid composition of sunflower oil. These QTLs had additive to dominant effects and closely related to domesticated related traits in sunflower (Leon et al. 2001; Burke et al. 2005; Ameena et al. 2016).

19.11.3 Diversification of Cytosterility System

Although more than 72 diverse CMS systems have been reported in sunflower, until now only one system based on PET-1 cytoplasm has been exploited commercially due to the non-availability of proper restorers and maintainers for other CMS lines. Such type of dependency on a single source of male sterility could lead to a narrow genetic base leading to a potential risk and high degree of genetic vulnerability in hybrid sunflower cultivation which can predispose the crop for some unforeseen situations of biotic and abiotic stresses in future years. Sunflower yields have plateaued with the currently available genotypes. Hence, it is essential to diversify the parental material (CMS and restorer base) to develop hybrids and varieties with high yield potential. Development of newer CMS sources should be complemented by identification of suitable restorers for effective hybrid seed production. Among several strategies available to overcome this problem, diversification of CMS sources itself is possibly the inexpensive and most effective method.

19.11.4 Biotic Constraints

Biotic stresses cause significant losses in crop plants and management of biotic stresses (diseases and pests) not only increases the cost of production but also has implications on environment and ecology (Bainsla and Meena 2016). The important diseases that cause significant yield losses in sunflower are *Alternaria* leaf spot [*Alternariaster helianthi* (Hansf.) Tub. and Nish.] (Carson 1985), powdery mildew (*Golovinomyces orontii* (Castagne) V.P. Heluta), downy mildew [*Plasmopara halstedii* (Farl.) Berl. & De Toni] (Gulya et al. 2013), sunflower necrosis disease (SND) (Bhat and Reddy 2016) and rust (*Puccinia helianthi* Schwein.) (Shtienberg and Zohar 1992). Sunflower production in India is constrained by the susceptibility of the released varieties/hybrids to a wide array of biotic stresses. In India, the major diseases and insect pests prevalent in sunflower-growing areas are SND, *Alternariaster* leaf spot, powdery mildew, downy mildew, *Spodoptera litura* and leafhoppers (Basappa and Santhalakshmi Prasad 2005). Sunflower leaf curl virus (SuLCV) also has become severe in India, and there reports on resistance sources for

Table 19.3 Disease resistance genes identified in wild *Helianthus* species

S. no.	Biotic stresses	Resistant/tolerant wild species	References
1.	*lternariaster* leaf spot	*H. tuberosus*	Skoric (1988)
		H. maximiliani, *H. mollis*, *H. divaricatus*, *H. simulans*, *H. occidentalis*, *H. pauciflorus*, *H. decapetalus*, *H. resinosus*, *H. tuberosus*	Sujatha et al. (1997)
		H. occidentalis, *H. tuberosus*	Madhavi et al. (2005)
		H. tuberosus, *H. resinosus*	Sujatha and Prabakaran (2006)
		H. tuberosus, *H. maximiliani*, *H. strumosus*	Santhalakshmi Prasad et al. (2017)
2.	Rust	Wild *H. annuus*, *H. argophyllus*, *H. petiolaris*	Quresh et al. (1993)
		Wild *H. annuus*	Gong et al. (2012)
		Prebred (PS-1089) derived from *H. argophyllus* × cultivated sunflower	Sujatha et al. (2003)
3.	Downy mildew	AD-66 derived from wild *H. annuus*	Vranceanu and Stoenescu (1970)
		H. argophyllus	Qi et al. (2019)
4.	Powdery mildew	*H. tuberosus*, *H. praecox*, *H. bolanderi*	Acimovic (1998)
		H. decapetalus, *H. divaricatus*, *H. laevigatus*	Dedic et al. (2012)
5.	Sucking pests	*H. argophyllus*	Seetharam and Ravi Kumar (2003)
	Tobacco caterpillar	Backcross inbreds derived from *H. argophyllus*, *H. petiolaris*	Sujatha and Lakshminarayana (2006)
	Sunflower pests	*H. tuberosus*, *H. maximiliani*	Seetharam and Ravi Kumar (2003)
	Leafhoppers	Prebred lines derived from *H. argophyllus*	Unpublished

SuLCV from wild species are not available (Govindappa et al. 2011). Insect pests of sunflower are different in tropical and temperate countries. Crop losses due to insect pests in sunflower vary from place to place. Continuous cultivation of the crop subjected it to disease pressure with overlapping disease cycles. Host plant resistance is a major objective in most of the breeding programmes across the crop species including sunflower and is considered as one of the most viable options for enhancing the productivity. The level of resistance available in cultivated species/primary gene pool for some of the diseases and insect pests is rather low, and only a limited number of resistance sources are available. This limited resistance is inadequate to manage more virulent pathogenic strains of diseases that arise during intensive crop production. Therefore, the discovery and incorporation of new genes from wild species provide a means of complementing crop improvement programmes (Table 19.3). The chances of finding dominant genes containing resistance to economically important diseases are higher in wild species which if deployed

effectively using new breeding tools including molecular and biotechnology can be an effective means of combating virulent pathogens.

19.11.5 Abiotic Constraints

Sunflower is generally grown in marginal lands, often in semiarid conditions, where abiotic stresses always act as a major limiting factor for its production and productivity in many parts of the world (Škorić 1987). Therefore, development of a heat-resistant sunflower breeding population or hybrid or breeding for resistance to drought, high temperature and salinity assumes priority for sustainable yield under abiotic stress conditions.

Drought is considered as the single most devastating environmental stress, which decreases crop productivity more than any other environmental stresses (Lambers et al. 2008). Chimenti et al. (2002) reported 5–56% yield reduction in sunflower if drought occurs immediately prior to anthesis. An appropriate strategy and criteria of selection are essential in selection for drought resistance. Restorer gene pool lines RGP-46-P_3 and RGP-60-P_1 developed through simple recurrent selection at ICAR-IIOR, Hyderabad, recorded low drought susceptibility index and good seed yield under moisture stress and were found promising for drought breeding. The use of landraces, cultivated hybrids and varieties has produced some positive results, but not to the extent that would secure stable sunflower production under drought conditions. The best results in enhancing drought resistance of cultivated sunflower have been achieved using wild *Helianthus* species. Some wild sunflower species have been reported as drought-tolerant species, and the introgression of traits from these species is expected to increase drought tolerance in cultivated breeding lines (Saucă et al. 2014). Among the related species, *H. argophyllus* was identified as particularly drought tolerant (Belhassen et al. 1996; Jan et al. 2014; Saucă et al. 2014; Hussain et al. 2017).

Seiler (2012) indicated that *H. anomalus* Blake and *H. deserticola* Heiser are highly adapted to desert and sandy areas and could be used as germplasm source for heat stress studies. Sideli et al. (2013) reported *H. cusickii* to be a potential source for genes for drought resistance.

Salinity is also another factor reducing sunflower yield in many countries including India. Identification of resistance sources from cultivated sunflower is very important for improvement of salinity tolerance of sunflower. Based on plant growth and survival, hybrids CSFH-12205, CO-2, KBSH-44 and DRSH-1 and inbreds, viz. COSF-1A and CMS-103A, were found tolerant at Gangavathi, and CSFH-12205, CO-2, KBSH-44, COSF-6A and COSF-7A were found tolerant at Machilipatnam (Anonymous 2018). Seiler et al. (1981) and Rogers et al. (1982) suggested that *H. paradoxus* would be a likely candidate for salt-tolerant genes. Miller and Seiler (2003) transferred salt-tolerant genes from *H. paradoxus* into cultivated sunflower and released two salt-tolerant maintainer lines, HA-429 and HA-430. Hajjar and Hodgkin (2007) suggests that *H. paradoxus* has a great potential to breed more salt-tolerant cultivated sunflowers, with hybrids developed using this trait potentially

providing a 25% yield premium in saline soils. Shtereva et al. (2015) suggested that the diploid perennial species *H. mollis* could serve as an excellent candidate of salt-tolerant genes. Breeders should apply effective screening methods to identify the donor wild species that possess genes useful in breeding for abiotic stresses and equally effective breeding methods to transfer these genes into cultivated sunflower genotypes.

19.12 Breeding Approaches

19.12.1 Conventional Approaches

Sunflower is a highly cross-pollinated (allogamous) crop, and therefore breeding procedures suitable for cross-pollinated crops are utilized for improvement of sunflower. In general, introduction, mass selection, pedigree method, backcross breeding, recurrent selection and Pustovoit method of reserves and mutation breeding were used for sunflower improvement. The choice of the suitable breeding programme for the development of tolerant cultivars to a defined abiotic stress depends upon a number of factors: screening techniques, sources and mechanism (s) of tolerance, modes of gene action and heritability and their relationship to agronomic traits (Meena et al. 2016).

19.12.1.1 Introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not grown earlier. In India, the first sunflower variety 'EC-68415' was developed in 1976 by AICRP (Sunflower) Centre, University of Agricultural Sciences, Bengaluru, and released for Karnataka state through an introduction. Another short-duration (80–82 days) popular variety 'Morden' was developed through introduction from Canada in 1978. It was released for all India cultivation. Morden was very popular among sunflower growers due to its short stature, short duration and high seed yield.

19.12.1.2 Mass Selection

Mass selection is a method of selection of desirable plants from a population based on their phenotypic characteristics. This method has been used for cultivar improvement in sunflower for many years and was effective in developing cultivars with early maturity, higher oil percentage and resistance to diseases. The efficiency of mass selection depends on gene effects of the selected traits, their heritability, sample size and genotype × environment interaction. Mass selection is effective for characters controlled by additive genes (Gowda and Seetharam 2008). Many varieties like Fuksinka-3, Fuksinka-10, Omskiy Skorospeliy, etc. were developed in the Soviet Union through this method (Skoric 1992). Varieties like Guayacan INTA, Cordobes INTA, Manfredi INTA, etc. were also developed using mass selection in Argentina (Luciano and Davreux 1967). In India, the variety Surya (PKV-SUF-72-37) was developed by mass selection from Latur bulk and released for Maharashtra

state in 1982. Still, this is one of the important breeding methods for sunflower improvement for many traits.

19.12.1.3 Pedigree Method

Pedigree selection is the most common procedure used to develop sunflower inbred lines. The procedure involves self-pollination of phenotypically desirable plants within existing cultivars, breeding populations or segregating generations of planned crosses. Fick and Swallers (1974) used this method to develop the first downy mildew-resistant restorer lines, viz. RHA-271, RHA-273 and RHA-274. Hulke et al. (2010) also developed new rust and downy mildew-resistant restorer line RHA-464 (Reg. No. GP-325; PI-655015; experimental '05187'), using pedigree method. In India, a large number of fertility restorer lines have been developed using the pedigree method (Meena et al. 2013). Jaffar et al. (2019) developed high oleic lines through pedigree method using PI-1806 × B-124 parents. In India, this is the only breeding method utilized by breeders for development of restorer lines and inbreds.

19.12.1.4 Backcross Method

Backcross selection refers to a form of breeding that uses a superior inbred that may nevertheless lack a particular trait. It is mainly used for the transfer of disease-resistant traits in superior inbred lines. But the fertility restorer lines are also developed by incorporating dominant restorer gene(s) by backcrossing using inbred lines of proven performance as the recurrent parent (Vranceanu and Stoenescu 1976). Backcross is also used for the transfer of disease resistance from related species to cultivated species. This method is also employed to transfer male sterility trait from a donor. New CMS lines are mostly developed in sunflower through the backcross method. High oleic acid (>90%) was identified in AOP-l and was used as a donor of this character in a backcross programme to incorporate the trait into commercial material. Liu et al. (2013) introgressed fertility restoration gene Rf_6 for CMS-514A from an interspecific amphiploid (Amp) of *H. angustifolius*/P-21 (2n = 68) into cultivated sunflower by backcross method.

19.12.1.5 Recurrent Selection

Recurrent selection refers to the method of selecting special gene traits from the best sunflower family. Presently, recurrent selection appears to be the most promising method of increasing the frequency of desirable genotypes in a source population, thus enhancing the chance of isolating superior inbred lines. It is a useful method in sunflower breeding, especially for the establishment of the gene pool for different purposes, primarily of increased productivity and resistance to diseases, insects, drought and other stresses. The recurrent selection method has not been exploited in breeding programmes in India even though considerable progress has been achieved using this method in other countries like the USA and France (Virupakshappa 1987). Miller et al. (1977) obtained an increment of 12.2% of oil content after three cycles of simple recurrent selection. Miller and Hammond (1985) reported an increase in seed yield by 6.3% after three cycles of selection. Resistance for *Sclerotinia*

sclerotiorum was obtained by Vear and De Labrouhe (1984) through three cycles of recurrent selection. Dr. Pustovoit in the USSR was a pioneer to practically utilize recurrent selection for oil improvement in which selection was based on progeny performance and subsequent cross-pollination is allowed only among superior progenies. Pustovoit (1964) was highly successful in improving oil content from about 30% in the early 1920s to over 50% in the present-day varieties. Shobha Rani and Ravikumar (2006) also improved partial resistance to *Alternariaster* leaf blight through sporophytic and gametophytic recurrent selection. Vear et al. (2007) reported significantly improved *Sclerotinia* head rot resistance after 15 cycles of recurrent selection. Harinarayana et al. (1980) noticed an improvement in seed yield, seed set and oil content through intra- and inter-population improvement. Powdery mildew-resistant restorer inbreds RGP-21-P_4-$S_{1–3}$ and RGP-50-P_2-S_1 were developed through population improvement after three cycles (Anonymous 2018). A wide range of variability was observed by Seneviratne et al. (2004) for plant height, days to maturity, head diameter, number of filled seeds and seed yield in C_3 cycle (Anonymous 2018). Emphasis has to be laid on using recurrent selection for population improvement to act as a source for development of new parental lines, and the improvement of existing parental lines should receive more attention to achieve a real breakthrough in yield.

19.12.1.6 Mutation Breeding

This method was mostly used in cultivated sunflower for creating genetic variability through irradiation with gamma rays or chemical mutagens. Seed treatment with gamma irradiation has been extensively used to increase variability for several characteristics, such as days to flowering, seed weight, seed coat colour and oil content (Giriraj et al. 1990; Jambhulkar and Joshua 1999; Encheva et al. 2003) in cultivated sunflower. Genetic variability for resistance to *Alternariaster* leaf spot disease was induced by radiation or chemical mutagens (De Oliveira et al. 2004). *Orobanche*-resistant lines were developed through mutation breeding by Encheva et al. (2008, 2012, 2014). Cvejić et al. (2014) found changes in fatty acid and tocopherol content and composition in sunflower oil. Lofgren and Ramaraje-Lers (1982) isolated plants with resistance to sunflower rust in M_2 and M_3. Herbicide-resistant sunflower mutants were generated by induced mutagenesis (Bervill et al. 1992; Sala et al. 2008). Drumeva (2012) developed 159 new downy mildew, *Phomopsis* and *Alternaria*-resistant fertility restorer lines with desirable breeding characteristics by using the gamma ray-induced parthenogenesis method. Jan and Rutger (1988) reported induced male sterility in M_1 heads of HA-89 treated with mitomycin C and streptomycin. Soldatov (1976) produced high-oleic sunflower variety 'Pervenets' by treating the seed of variety VNIIMK-8931 with 0.5% solution of dimethyl sulphate. In India, the sunflower variety Gujarat Sunflower-1 (GAUSUF-15) was developed through mutation breeding and released in 1993 all over India. Another variety CO-3 (TNAUSUF-10) was developed from CO-2 using 5KR gamma rays and released for Tamil Nadu state in 1995. The variety TAS-82 (TAS-82-8-1/3) was developed from Surya variety through mutation breeding in 2006 and recommended for Vidarbha region of Maharashtra state. Readers

interested in additional information on mutations can refer to the article of Vasko and Kyrychenko (2019) on induced mutagenesis for the creation of new starting material in sunflower breeding.

19.12.2 Hybrid Seed Production

19.12.2.1 Development of A, B and R Lines

Hybrids developed using the CMS fertility restorer system require three parental lines, viz. CMS or 'A' line, fertility maintainer or 'B' line and a restorer line or 'R' line (male line). In hybrid development programme, inbred lines are developed by selfing or sib mating from adapted varietal populations, gene pools, local varietal populations, inter-varietal hybrids, composites, synthetics, single-cross and multiple-cross hybrids and interspecific hybrids, etc., over the generations and essentially evaluated for combining ability by inter-crossing among the best by following standard genetic model. Once the best combinations are identified based on high combining ability and per se performance, the female line is converted into cytoplasmic male sterile line (A line) with the original inbred as the isogenic maintainer line (B), and the male inbred line is converted into restorer line (R line). CMS lines are developed by repeated backcrossing using known CMS lines as a non-recurrent parent (female parent). About five to six backcrosses are required to derive a new CMS line genetically similar to the recurrent parent. The inbred, which was used as a recurrent parent (male parent), serves as a maintainer (B line). Derived CMS line will be employed as the female in seed production of hybrids along with the selected male line based on the combining ability and productivity. Wherever the hybrids show 100% fertility in F_1, the 'R' lines may be used directly as the male parent. If the fertility restoration is partial or low in the F_1 plants, fertility restorer lines are developed by crossing the selected best combiner as a recurrent parent to a known restorer line of proven performance. By six to seven backcrosses, R gene(s) may be transferred to the selected male line.

19.12.2.2 Maintenance of Parental Lines

Two methods of planting, viz. row method and block method, are followed for maintenance of the 'A' line (Fig. 19.2). The A × B crosses are used to maintain the 'A' line following separate row and block method of sowing. To take up the breeder/foundation seed production of female (A) line, the planting ratio of 'A' and 'B' is 3:1. To facilitate better pollination, mark the first two rows and last two rows of the seed plot and subsequently every fourth row with a wooden peg to plant these lines with 'B'. The sowing of 'A' and 'B' lines should be taken up by engaging labourers separately. A and B lines in breeder/foundation stages are planted in 3:1 proportion in separate blocks. During anthesis, the pollen is collected from the 'B' line and pollinated on to 'A' line in respect of breeder/foundation seed production. This method ensures the production of high-quality hybrid seeds and meets the genetic standards.

Row method

BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB
BB AAA B AAA B AAA B AAA BB

Block method

Breeder/Foundation seed production	
Block-I	**Block-II**
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB
AAAAAAAAAAAA	BBBB

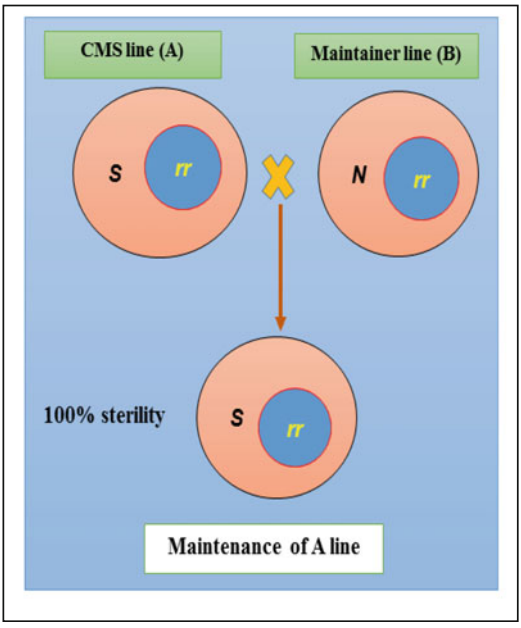

Fig. 19.2 Maintenance of 'A' line through row and block method

19.12.2.3 Certified Hybrid Seed Production

To realize high productivity levels in the commercial scale, the supply of quality hybrid seed assumes importance. The production of high-quality seed of parental lines and the certified seed of hybrid requires systematic planning and management on the part of the seed producers (Fig. 19.3). As in the production of 'A' line, two methods, viz. row method and block method, are being followed to produce hybrid seed in sunflower (Fig. 19.4). The proportion of female to male is 3:1, i.e. three rows of female (seed parent) to one row of male (pollen parent). In recent times, the seed production agencies have faced serious seed quality problems as the seed lots of hybrids containing a high amount of 'R' line plants are contaminated during harvesting/drying in addition to different stages of post-harvest operations followed in the above 3:1 method.

In the suggested block system, A and R lines are planted in separate bocks in 3:1 ratio. At the time of anthesis, the pollen is collected separately from R lines and pollinated on to 'A' line in respect of certified seed production. This method ensures the production of high-quality hybrid seed and meets the genetic standards.

19.12.3 Limitations of Conventional Breeding

Traditional approaches to breeding crop plants with improved abiotic stress tolerances have so far met with limited success (Richards 1996). This is due to several contributing factors including the following: (1) the focus has been on yield rather than on specific traits; (2) the difficulties in breeding for stress tolerance traits,

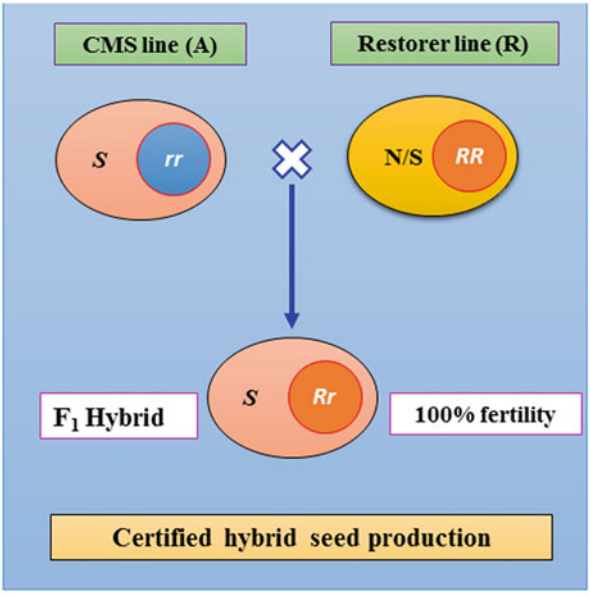

Fig. 19.3 Procedure of certified hybrid seed production

Breeder/Foundation seed production
Row method
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR
RR AAA R AAA RAAA R AAA RR

Certified seed production	
Block-I	Block-II
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR
AAAAAAAAAAAA	RRRR

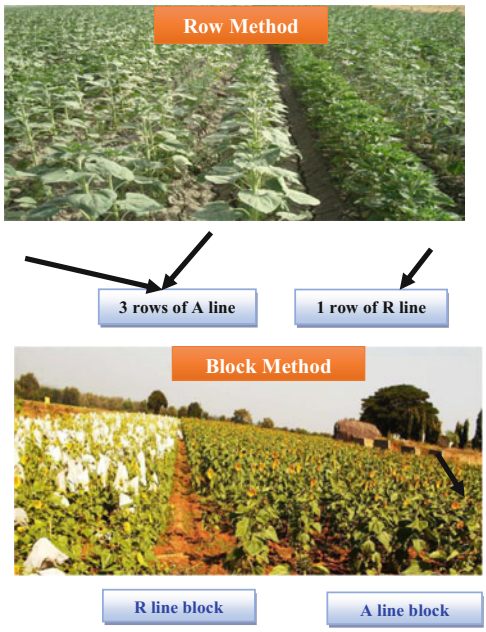

Fig. 19.4 Certified hybrid seed production through row and block method

which include complexities introduced by genotype by the environment, or G × E interactions and the relatively infrequent use of simple physiological traits as measures of tolerance; and (3) desired traits can only be introduced from closely related species.

19.12.4 Molecular Breeding

Despite the large genome size (2871–3189 Mbp) of cultivated sunflower, concerted efforts led to the genome assembly up to the chromosome level with a total length of 3010 (Mb), protein count of 75,695 and GC content of 38.8% (PRJNA396063). Molecular markers have played a significant role in accelerating sunflower breeding programmes through marker-assisted selection (MAS) by virtue of their abundance, technical ease and detection at any development stage of the plant besides being not affected by environmental factors. To facilitate MAS, a number of molecular marker systems, namely, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), sequence characterized amplified region (SCAR), cleaved amplified polymorphic sequence (CAPS), insertion/deletion (INDEL), single nucleotide polymorphism (SNP) and target region amplification polymorphism (TRAP) markers, have been developed and used for various molecular applications in sunflower (Berry et al. 1995; Gentzbittel et al. 1995, 1998; Jan

et al. 1998; Flores Berrios et al. 2000; Burke et al. 2002; Mokrani et al. 2002; Bert et al. 2003, 2004; Langar et al. 2003; Yu et al. 2003; Rachid Al-Chaarani et al. 2004; Lai et al. 2005; Hu et al. 2007; Poormohammad Kiani et al. 2007; Yue et al. 2008). These markers have been extensively used in several applications like assessment of genetic relationships among genotypes, construction of linkage maps, tagging and mapping genes/QTLs of agronomic interest, development of genomic and cDNA libraries, map-based cloning and marker-assisted selection.

In sunflower, the first map was developed using RAPD markers (Rieseberg et al. 1993), followed by RFLP markers (Berry et al. 1995; Gentzbittel et al. 1995; Jan et al. 1998), AFLP markers (Gedil et al. 2001), SSR markers (Tang et al. 2003; Yu et al. 2003) and SNP markers (Lai et al. 2005; Bowers et al. 2012; Talukder et al. 2014; Celik et al. 2016; Livaja et al. 2016) covering the 17 linkage groups. These maps were further enriched with gSSRs, EST-SSRs, INDELs, TRAPs and SNPs (Hu et al. 2007; Heesacker et al. 2008), and a consensus map with 10,080 loci was constructed (Bowers et al. 2012). Recently, studies on large-scale SNP detection and generation of large sets of markers through genotyping by sequencing have been undertaken by several groups (Baute et al. 2016; Celik et al. 2016; Talukder et al. 2016; Mangin et al. 2017; Ma et al. 2017; Qi et al. 2017;). For more information of molecular markers and marker-assisted breeding, readers may refer the articles of Dimitrijevic and Horn (2018).

Molecular markers for simple inherited traits like fertility restoration, herbicide tolerance, resistance to downy mildew, *Orobanche cumana*, rust, high stearic and oleic acid contents (β and γ tocopherols) have been successfully deployed in marker-assisted breeding programmes (Sujatha and Sujatha 2013). QTLs have been identified, but genome-wide association studies are required. However, for investigating complex traits controlled by polygenes, such as seed yield, oil content, resistance to *Sclerotinia* mid-stalk rot, black stem and abiotic stresses like drought, salinity and chilling. Of the various traits that have been improved through MAS, resistance to downy mildew was highly successful as the resistance was dominantly inherited (Dimitrijevic and Horn 2018).

With regard to the genetic resources, initial studies focused on development of biparental populations based on crosses involving elite breeding and introgressed lines (Berry et al. 1995; Horn et al. 2003; Livaja et al. 2016), land races (Kim and Rieseberg 1999), wild *Helianthus* species (Quillet et al. 1995; Ma et al. 2017) or the recombinant inbred lines (Tang et al. 2006; Talukder et al. 2016). These populations were used to map genes conferring resistance to downy mildew, QTLs governing *Sclerotinia* stalk rot resistance and seed quality traits, but owing to the disadvantages of the time and costs involved for deployment of individual populations, low resolution of mapping, mortality of the populations besides evaluation of only two alleles per locus alteration strategies were adopted. Understanding the need for assembling the association panels, researchers have directed their efforts towards this goal. Accordingly, association panels consisting of 433 cultivated accessions from the USA and Europe maintained at USDA and INRA, respectively (Mandel et al. 2011); 170 accessions representing Argentinian collections maintained at INTA (Filippi et al. 2015), which distinguished the maintainer and restorer gene

pools; and 196 Spanish confectionery accessions maintained at CRF-INIA (Velasco et al. 2014) were characterized which disclosed large genetic variation for seed oil content, test weight and seed quality traits.

19.12.5 Trait Improvement Through Genetic Engineering

Despite the successes with traditional breeding methods and interspecific gene transfer for several traits such as cytoplasmic male sterility, fertility restoration gene(s), seed quality traits, resistance to biotic and abiotic stresses, biotechnological interventions are required for introgression of beneficial traits into cultivated sunflower. Direct and callus-mediated shoot organogenesis from different tissues such as cotyledons, hypocotyls, petioles, leaves and immature embryos is reported (Witrzens et al. 1988; Knittel et al. 1991; Pugliesi et al. 1991; Sujatha et al. 2012a). Regeneration through somatic embryogenesis using immature zygotic embryos, mature seeds and seedling tissues is also reported (Finer 1987; Mc-Cann Wilcox et al. 1988; Espinasse and Lay 1989; Sujatha and Prabakaran 2001). The key factors favouring organogenesis include genotype, explant type, age and physiological state of the cultured tissue, exogenous growth regulator combinations and their balance with endogenous growth regulators, etc. Shoot regeneration and quality of shoots are reported to be enhanced through the use of ethylene inhibitors like cobaltous chloride ($CoCl_2$) and silver nitrate ($AgNO_3$) molecules (Chraibi et al. 1992), preconditioning of explants with 5 mg/l BAP (Zhang and Finer 2015) and short-pulse treatments coupled with micrografting (Zhang and Finer 2016). Precocious flowering, hyperhydricity, poor rooting and abnormal morphogenesis are few other problems encountered in sunflower shoot cultures (Lupi et al. 1987; Freyssinet and Freyssinet 1988). Even after four decades of research carried out at several laboratories, availability of a reliable, efficient, reproducible and genotype-independent tissue culture-based regeneration system still continues to be a major limitation for application of genetic engineering tools in sunflower.

Genetic transformation experiments were largely through *Agrobacterium*-mediated techniques using explants with pre-existing meristems as target tissues such as shoot tips, mature embryos, immature embryos and cotyledons from mature seeds. The initial studies were chiefly aimed at optimization of variables for enhancing the transformation efficiency and mostly confined to characterization of primary transformants and the T_1 generation plants. Owing to the amenability of sunflower to *Agrobacterium tumefaciens* infection, high frequency of transient expression is observed in most of the transformation experiments. However, conversion efficiency of transient to stable integration was rather limited besides leading to chimeras (Schrammeijer et al. 1990; Malone-Schoneberg et al. 1994; Grayburn and Vick 1995; Weber et al. 2003). Further, formation of chimeric shoots, low regeneration rate from transformed cells/calli, low *Agrobacterium* virulence, bacterial strain, extreme sensitivity to antibiotics, genotype dependence and lack of stable transmission of the introduced gene are major factors limiting the overall efficiency of the transformation systems reported so far. Attempts made at enhancing transformation

efficiency by imposing wounding treatments using glass beads (Grayburn and Vick 1995; Alibert et al. 1999); sonication at 50 MHz (Weber et al. 2003); vacuum infiltration (Hewezi et al. 2003); digestion with macerating enzymes like cellulose, pectinase and macerozyme (Alibert et al. 1999; Weber et al. 2003); incubation with acetosyringone (Laparra et al. 1995); co-transformation with cytokinin synthesis (*ipt*) gene (Molinier et al. 2002); and dehydration and rehydration of target tissues (Hewezi et al. 2002) met with limited success. *Among the commonly used reporter genes f*or selection of putative transformants (hygromycin, kanamycin and basta), kanamycin is used widely as the plant selection agent in sunflower due to ease of identification of green shoots (putatively transformed) from bleached shoots (untransformed). The problem of premature flowering has been overcome to some extent by incorporation of cytokinins like 2-isopentenyl adenine (2-iP) and kinetin, incubating the tissues at a temperature of 20 °C with 8/16 h light/dark photoperiod cycle or grafting of in vitro recovered putative transformants onto healthy root stocks (Weber et al. 2003; Sujatha et al. 2012b). Step-wise protocols for sunflower transformation are described by Lewi et al. (2006), Radonic et al. (2015) and Manavella and Chan (2009) for development of transformants and for transient expression analysis which can be used for gaining valuable insights of several biological processes through functional validation of genes.

While most of the genetic transformation studies undertaken in sunflower are aimed at establishment of the transformation system, transgenic development for agronomically desired traits was mainly for incorporation of resistance to biotic stresses (*Sclerotinia sclerotiorum*, *Alternariaster helianthi*, necrosis disease) and resistance to abiotic stresses (drought, salinity). *S. sclerotiorum* is an economically important disease in the temperate regions causing root rot, mid-stalk rot and head rot, and oxalic acid has been identified as the key component in *Sclerotinia* infection. Genetic engineering for enhanced resistance to *S. sclerotiorum* is aimed at strategies to degrade oxalic acid and was through deployment of candidate genes like wheat germin oxalate oxidase (OXO) (Lu et al. 2000; Scelonge et al. 2000); coumarin phytoalexins (ayapin and scopoletin) (Urdangarin et al. 1999); and antifungal genes like glucanase, chitinase, osmotin gene and a ribosome inhibitor protein (Radonic et al. 2008). For conferring resistance to *A. helianthi*, transgenic lines harbouring β-1,3-glucanase (Manoj Kumar et al. 2011) and *TVD1* gene (Sirisha et al. 2011) were developed. Sunflower necrosis disease (SND) incited by *Tobacco streak virus* of *Ilarvirus* group accounts for yield losses ranging from 10 to 80% in the tropics and sub-tropics (Jain et al. 2003). Deployment of *TSV-CP* gene in sense and antisense directions was undertaken for conferring resistance to SND (Pradeep et al. 2012; Singareddy et al. 2018; Sunderesha 2017).

Abiotic stresses like drought, salinity and heat have their effects not only on seed yield but also on the oil content. Candidate genes were deployed to increase tolerance to abiotic stresses such as suppression of proline dehydrogenase (*ProDH1*) gene for drought and salinity (Tishchenko et al. 2014). Studies at metabolic engineering towards improving oil stability and nutritional quality are targeted at development of high-oleic sunflower by knocking out delta-12-desaturase gene

which encodes linoleic acid and lines with increased linoleic acid using PTGS technology (Lacombe et al. 2009; Chen et al. 2010).

Genome editing through CRISPR/*cas9* technology is still in its infancy in case of sunflower. Innovative Genomics Institute, California, is at establishing tools for genome editing in sunflower (https://innovativegenomics.org/projects/establishing-tools-sunflower-genome-editing/).

19.13 Conclusions

One of the major challenges in sunflower would be to enhance productivity to the level of the world's average. To achieve this, populations and hybrids superior to the presently grown cultivars need to be bred which should combine high seed yield (>3.0 t/ha), high oil content (>42.0%) and resistance to major pests like leafhopper and diseases like *Alternariaster* leaf spot, powdery mildew, downy mildew, necrosis, rust, stem rot, etc. Another important area of breeding research would be to enhance tolerance/resistance to abiotic stresses. Intensive and monocropping of sunflower has been the major cause for outbursts of diseases like rust, leaf spots, powdery mildew, sunflower necrosis disease, leaf curl virus and downy mildew which require immediate attention. Root rots are becoming severe in some parts of the country. Hence, development of hybrids with multiple resistance(s) will have to be the major thrust areas in the years to come. Public-private partnership is also very important for area expansion and development of high heterotic hybrids. Utilization of information being generated through genomic resources and tools assumes importance in tackling problems such as abiotic stresses and identification of physiological traits contributing to seed yield through genomic selection and GWAS. Wild *Helianthus* species displayed extensive variability for various qualitative and quantitative traits including tolerance/resistance to biotic and abiotic stresses which can be incorporated in cultivated sunflower through introgressive breeding and novel breeding techniques (cisgenics, genome editing).

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Chickpea Breeding

20

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Abstract

Chickpea is the major pulse crop of India, and it accounts for about 45% of the total area and production of pulses grown in the country. Impressive progress has been made in development of cultivars suited to rainfed ecology. This has helped India in expanding chickpea area in central and southern India and compensating the loss in chickpea area that occurred earlier due to expansion of wheat in irrigated areas of northern India. The genetic variability available in the germplasm, particularly in wild species, should be exploited for broadening the genetic base of varieties and introgressing useful traits, such as resistance to insect pests and diseases. The barriers to interspecific hybridization have restricted utilization of several wild species, and, therefore, dedicated efforts are needed to access genes from these species. High-throughput precision phenotyping protocols need to be developed and used for screening of germplasm and breeding materials for different traits related to stress tolerance and nutritional quality. Rapid advancements in development of chickpea genomic resources during the past decade have made it possible to initiate genomics-assisted breeding in chickpea improvement. Molecular markers associated with several useful traits have been identified. Some of these markers have been validated and are being used in the breeding programmes. Efforts should be made on increasing the number of validated/diagnostic markers, so that genomics-assisted breeding becomes an

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_20

integrated approach in chickpea breeding programmes. Marker-assisted selection can accelerate breeding process and facilitate combining different desired traits. Integration of these approaches would be important for improving precision and efficiency of chickpea breeding programmes. In this paper, we have reviewed the status of current research efforts and advancements in Indian and future research priorities to tackle newer challenges.

Keywords

Breeding · Chickpea · Genetic improvement · Improved varieties · Research strategies

20.1 Introduction

Chickpea (*Cicer arietinum* L.) is cultivated in almost all parts of the world covering more than 50 countries spread over Asia, Africa, Europe, Australia, North America and South America continents and is the second most important food legume crop after common bean (*Phaseolus vulgaris* L.). In India, chickpea is grown almost in all parts of the country mainly as a rainfed crop (68% area). There has been an impressive growth in area, production and productivity of chickpea in India during the past decade. During 2019–2020, chickpea production has been estimated to be about 10.90 million tonnes, which is about 47% of the total pulse production (23.01 mt) in India. Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka, Chhattisgarh, Bihar and Jharkhand contribute more than 95% of the total chickpea production in the country. In India, both *desi*- and *kabuli*-type chickpea varieties are grown. India continued to remain the major importer of desi chickpea but has emerged as a major exporter of *kabuli* chickpea during the past decade. India has made remarkable progress in expanding chickpea area and production. Among pulses, chickpea has the longest history of research in India.

It started as early as 1905 when Imperial Agricultural Research Institute, Pusa, made a modest beginning by taking up breeding work on chickpea. Breeding programme to select high-yielding types and its purification from the existing heterogeneous land races were initiated mainly at Kanpur (Uttar Pradesh), Lyallpur (now in Pakistan), Bombay (Maharashtra) and central India (Madhya Pradesh). With the establishment of the All India Coordinated Pulses Improvement Project (AICPIP) in 1967, a systematic research on chickpea in India started in the disciplines of plant breeding, agronomy, plant pathology, entomology, microbiology, and plant physiology. Research activities carried out in these disciplines helped in the development of high-yielding chickpea varieties and matching production and protection technologies for different agro-ecological zones. Realizing the importance of crop and providing focussed attention on every aspect of chickpea, a separate All India Coordinated Research Project on Chickpea was established in 1993.

20.2 Origin, Evolution and Distribution of Species and Forms: Wild Relatives

Chickpeas are extensively cultivated in India, Mediterranean area, the Middle East and Ethiopia since antiquity. During modern years, they are also significant in Mexico, Argentina, Chile, Peru, Australia and the USA. Chickpeas were first domesticated in the Middle East as early as 12,000–10,000 years past along with other crops of wheat, barley, rye, peas, lentil, flax and vetch (Harlan 1971) in the Fertile Crescent (Zohary and Hopf 1973; Bar-Yosef 1998). van der Maesen (1972) believed that the chickpea is originated in the southern Caucasus and northern Persia. However, reported the centre of origin to be southeastern Turkey. Later on van der Maesen (1987) recognized the southeastern part of Turkey adjoining Syria as the possible centre of origin of chickpea based on the presence of the closely related annual species, *C. reticulatum* Ladizinsky and *C. echinospermum*. However, the present distribution of wild relatives may not reflect distribution at the period of domestication (Diamond 1997). The crop diversity may have evolved outside of the centre of origin (Harlan 1971).

Cicer, which was classified under Vicieae Alef., was later reported to belong to the monogeneric tribe, Cicereae. The genus was revised by van der Maesen (1972), who largely adhered to the classification of Popov (1929). The species is divided into two subgenera, *Pseudononis* Popov and *Viciastrum* Popov; four sections, *Monocicer* Popov, *Chamaecicer* Popov, *Polycicer* Popov and *Acanthocicer* Popov; and 14 series. The major taxonomic divisions were made on the basis of flower size, life span, growth habit, whether the plants are woody or herbaceous and form of leaf apex, terminating in a tendril and spine or resulted in definite improvement (van der Maesen et al. 2006).

Wild species of chickpea were most abundant in Turkey, Iran, Afghanistan and Central Asia (Duke 1981). Recent discoveries have raised the number of *Cicer* spp. to 44, with 34 perennials and 9 annuals (van der Maesen 1987). Using the Harlan and deWet (1971) gene pool concept, the gene pool is grouped into:

GP1 a: *Cicer arietinum*.
GP1 b: *C. echinospermum*, *C. reticulatum*.
GP 2: *C. bijugum*, *C. judaicum*, *C. pinnatifidum*.
GP 3: Other *Cicer* species.

The list of known species of genus *Cicer* is furnished below:

20.2.1 Annual Species

1. *C. arietinum** L.
2. *C. judaicum** Boiss.
3. *C. bijugum** K.H. Rech.
4. *C. pinnatifidum** Jaub. and Sp.

5. *C. chorassanicum** (Bge.) M. Pop.
6. *C. reticulatum** Ladiz.
7. *C. cuneatum** Hochst. ex Rich.
8. *C. yamashitae** Kitamura.
9. *C. echinospermum** P.H. Davis.

20.2.2 Perennial Species

1. *C. acanthophyllum* Boriss.
2. *C. macracanthum* M. Pop.
3. *C. anatolicum** Alef.
4. *C. microphyllum** Benth.
5. *C. atlanticum* Coss. ex Maire.
6. *C. mogoltavicum* (M. Pop.) Koroleva.
7. *C. balcaricum* Galushko.
8. *C. montbretii** Jaub. and Sp.
9. *C. baldshuanicum* (M. Pop.) Lincz.
10. *C. multijugum* van der Maesen.
11. *C. canariense** Santos Guerra and Lewis.
12. *C. nuristanicum* Kitamura.
13. *C. fedtschenkoi* Lincz.
14. *C. oxyodon* Boiss. and Hoh.
15. *C. flexuosum* Lipsky.
16. *C. paucijugum* (M. Pop.) Nevski.
17. *C. floribundum** Fenzl., *C. pungens** Boiss.
18. *C. graecum* Orph.
19. *C. rassuloviae* Lincz.
20. *C. grande* (M. Pop.) Korotk.
21. *C. rechingeri* Podlech.
22. *C. heterophyllum** Contandr et al.
23. *C. songaricum** Steph. ex. DC.
24. *C. incanum* Korotk.
25. *C. spiroceras* Jaub. and Sp.
26. *C. incisum** (Willd.) K. Maly.
27. *C. stapfianum* K.H. Rech.
28. *C. isauricum** P.H. Davis.
29. *C. subaphyllum* Boiss.
30. *C. kermanense* Bornm.
31. *C. tragacanthoides* Jaub. and Sp.
32. *C. korshinskyi* Lincz.

20.2.3 Unspecified

C. laetum Rassulova and Sharipova.

*Species with confirmed somatic chromosome number of $2n = 16$.

Source: The CGIAR System-Wide Information Network for Genetic Resources (SINGER; http://singer.cgiar.org/Search/SINGER/search.htm and van der Maesen 1987).

The wild relatives of chickpea have a very narrow geographical and ecological range (Abbo et al. 2003). *C. reticulatum* and *C. echinospermum* are limited to a few provinces of southeastern Turkey (Berger et al. 2003). It is possible that they also occur in similar habitats in Iran or Iraq, although verification of this is not currently possible. *C. reticulatum* and *C. echinospermum* rarely co-occur, except for a few likely hybrid populations in the Euphrates valley north of Cermik, but do have adjacent distributions. *C. echinospermum* typically occurs on more basaltic substrates at lower elevations in open pastures and disturbed meadows with lower tree cover than for *C. reticulatum*, which occurs more frequently on sand-stone or granitic substrates in mixed pastures and some disturbed habitats (von Wettberg et al. 2018). Taxa in the tertiary gene pool have somewhat ecologically and geographically broader distributions. *C. pinnatifidum*, in particular, occurs in drier habitats in southeastern Turkey.

The cultivated chickpea (*Cicer arietinum* L.) and *C. reticulatum* are interfertile, with similar seed proteins, and are morphologically similar, but some domestic accessions may differ from *C. reticulatum* by a reciprocal inversion, by a paracentric inversion or by location of chromosomal satellites, whereas *C. echinospernum* differ from the *Cicer arietinum* L. by a single reciprocal translocation, and hybrids between these tend to be sterile (Ladizinsky 1998). All other annual and perennial *Cicer* spp. are genetically isolated in the tertiary gene pool and equidistant from the domestic species as per amplified fragment length polymorphism (AFLP) diversity analyses (Nguyen et al. 2004).

20.3 Plant Genetic Resources

Chickpea germplasm accessions are conserved worldwide in more than 30 gene banks. Most of these accessions originated in India, Iran, Syria and Turkey. Out of 97,400 accessions available worldwide, ICRISAT holds the largest collection of chickpea, i.e. 20,764 accessions, which comprises 20,456 of cultivated types and 308 accessions of 10 wild Cicer species collected from 60 countries (Chandora et al. 2020). Of the entire cultivated chickpea accessions, 75.2% are of desi small seed type, 23.1% are of kabuli large seed type and the rest are of intermediate type (Upadhyaya 2003). ICARDA gene bank has collected and conserved 15,734 accessions including 540 accessions of wild *Cicer* species in its global germplasm repository from 61 countries across six continents (Asia, Africa, the Americas, Europe and Australia). The Indian National Gene Bank at NBPGR, New Delhi, conserves 14,704 chickpea accessions which represent a large proportion of the

Table 20.1 Ex situ *Cicer* collections in gene banks

Institute	Wild	Cultivated	Total
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India	308	20,456	20,764
International Centre for Agricultural Research in the Dry Areas (ICARDA), Beirut, Lebanon	540	15,194	15,734
National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India	69	14,635	14,704
Australian Temperate Field Crops Collection (ATFCC), Australia	246	8409	8655
Western Regional Plant Introduction Station, USDA- ARS, USA	194	7844	8038
National Plant Gene Bank of Iran, Seed and Plant Improvement Institute (NPGBI-SPII), Iran	–	5700	5700
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (MR), Russian Federation	–	2767	2767
Plant Genetic Resources Program (PGRP), Pakistan	89	2057	2146
Plant Genetic Resources Department, Aegean Agricultural Research Institute (AARI), Turkey	21	2054	2075
Institute of Plant Production n.d. a. V.Ya. Yuryev of NAAS, Ukraine	–	1760	1760
Estación de Iguala, Instituto Nacional de Investigaciones Agricolas, Iguala, Mexico	–	1600	1600
Institute of Biodiversity Conservation (IBC), Ethiopia	–	1173	1173
Research Centre for Agrobotany (RCA), Hungary	9	1161	1170
Uzbek Research Institute of Plant Industry (UZRIPI), Uzbekistan	–	1055	1055
Total	1476	85,865	87,341

Source: http://www.fao.org/wiews-archive/germplasm_query.htm?i_I=EN

cultivated diversity found in India and some genotypes introduced from other countries.

Other gene banks like Australian Temperate Field Crops Collection (ATFCC), Victoria, Australia, and Western Regional Plant Introduction Station, USDA-ARS, USA, conserve 8655 and 8038 accessions, respectively. All these collections also include duplicates which generally happen in the process of collection, conservation and exchange. Wild species of *Cicer* represents only less than 1% of the total accessions (conserved in about 10 gene banks worldwide). Due to the narrowed gene pool of cultivated chickpea, recent utilization of wild species through pre-breeding programmes has significantly increased. Therefore, now priority is being given to the collection and conservation of wild species. Chickpea germplasm collection held in various major gene banks (ex situ) across the world is presented in Table 20.1.

Systematic characterization and evaluation is necessary to facilitate effective utilization of available genetic resources in crop breeding programmes and for efficient management of germplasm. Since the 1970s, a large number of chickpea

germplasm accessions have been characterized and evaluated, in batches, for morphological and agronomic traits (Singh and Pratap 2016). About 99% of germplasm accessions of chickpea at ICRISAT have been characterized and evaluated for agro-morphological traits (Sharma et al. 2005; Upadhyaya et al. 2006). The first large-scale evaluation of 5477 accessions was carried out by Narayan and Macefield (1976), who observed substantial amount of genetic variability for plant type, flower colour, days to flowering, days to seed maturity, pod size, number of seeds per pod, weight of 100 seeds and seed colour from different geographical locations. This was followed by various joint evaluations by collaborative research at several national and international organizations (Muehlbauer and Sarker 2017). In India, systematic research in chickpea started with the inception of All India Coordinated Pulse Improvement Program (AICPIP) during 1967 (http://www.aicrpchickpea.res.in) followed by the establishment of a separate All India Coordinated Research Project (AICRP) on chickpea during 1993. However, characterization of Indian chickpea collections was mainly taken care by NBPGR, which is also the host institute for the Indian National Gene Bank. Along with characterization, NBPGR is also actively carrying out evaluation of conserved chickpea germplasm.

20.4 Floral Biology, Emasculation and Pollination Techniques

Chickpea belongs to the family Fabaceae (Leguminosae), subfamily Faboideae (Papilionaceae), tribe Cicereae and the genus *Cicer* (http://plants.usda.gov/core/profile?symbol=CIAR5).

20.4.1 Floral Biology

The inflorescence is an axillary raceme with generally a single papilionaceous flower. Sometimes two to three flowers were also reported to occur rarely at the same node. Peduncle is 6–30 mm long, while the pedicel is 6–13 mm long. Both the peduncle and pedicel look like a single part because they are straight in line up to fertilization, and then the pedicel bends down. Size of flower may vary from 8 to 10 mm. Flowers are zygomorphic with papilionaceous corolla. Each flower has five petals: a large standard petal, two lateral wing petals and two fused keel petals. The flower contains diadelphous stamens (nine fused and one free) of 6–8 mm and a style (3–4 mm) with a slightly broadened stigma (Auckland and van der Maesen 1980). Colour of the petals may be pink or purple in desi chickpeas, while kabuli flowers are white to cream in colour.

20.4.2 Anthesis

The chickpea flower goes through five stages of development: closed bud, hooded bud, half-open flower, fully open flower and fading flower. The stigma is receptive to

pollen over a 3-day period from the hooded bud to the fully open flower stage. Pollen matures and anthers dehisce at the half-open flower stage, resulting in self-pollination. The keel petal remains closed at this stage, preventing the entry of foreign pollen. Mature pollen is yellow in colour and slightly sticky. Flowering in chickpea is indeterminate, starting at the lower nodes and continuing to the upper nodes until the whole plant matures (Loss et al. 1998). Anthesis continues throughout the day.

Plants produce a large number of flowers, but only around 20–50% of flowers will develop into pods (Loss et al. 1998). Pods start appearing about 6 days after fertilization and may take up to 4 weeks for completing seed development. Outcrossing rates in chickpea are very low. When outcrossing is observed in close plantings, rates are in the range of 0–1.9%. Outcrossing rate up to 5.9% has been seen in open-flower mutation (Srinivasan and Gaur 2012).

20.4.3 Technique of Emasculation and Pollination

Due to cleistogamous nature of the flower and due to its small flowers, crossing is difficult and tedious. Artificial hybridization with and without emasculation has been followed in chickpea. The success of the artificial hybridization ranges from 10% to 50% depending upon the weather, particularly temperature and humidity, besides the genotypes involved. Artificial hybridization techniques have been reviewed by Argikar (1970), van der Maesen (1972), Smartt (1976) and Auckland and van der Maesen (1980). For emasculation, the flower bud that is due to open the following day is selected. The selected flower bud is held at the base between the thumb and forefinger. The front sepal is stripped off with the help of forceps, and the keel is pushed downwards. The exposed anthers are then gently removed. After that, a coloured cotton thread or a tag is tied around the internodes below the emasculated bud. Kalve and Tadege (2017) have shown that the crossing by keel petal incision or petal removal is an effective approach which significantly increases the crossing success rate.

Pollination is generally done in the morning on the following day. Half-opened flowers are the best source of matured pollen. Such flower buds are collected from the intended male parent in a Petri dish or a glass tube. At the time of pollination, the standard and wings of the selected buds are removed, and the upper portion of the keel is lightly pressed between the thumb and the forefinger to shed enough pollen on the stigma of the emasculated flower. Pollen grains germinate in nearly 30 min after pollination. The germinated pollen tube takes 4 h to reach the base of the ovary (Malti and Shivanna 1983). However, it takes 24 h for fertilization after pollination. In order to increase the success rate of artificial hybridization, the following points are taken care of:

1. Selection of large flower buds.
2. Selection of lateral buds rather than the terminal ones since the success is reported to be better on the lateral buds (Sindhu et al. 1981).

3. Avoiding mechanical injury to the floral parts at the time of emasculation and pollination.
4. Attempting hybridization after the formation of the first pod (Bahl and Gowda 1975).

Timing of pollination and fertilization is also important in deciding the success rate. Under the conditions of low temperature, emasculation in the afternoon followed by pollination in the following morning gives better results. However, when the temperatures are high, morning emasculation followed by immediate pollination or pollination in the same afternoon is reported to be successful (Khosh-Khui and Niknejad 1972; Singh and Auckland 1975; Bejiga and Tessema 1981; Pundir and Reddy 1984).

Tullu and van Rheenen (1989) showed that field environment is more favourable for crossing chickpea than green house. They have reported that the time of emasculation and pollination has no significant effect on crossing success. In contrast, emasculation followed by pollination was found to be more effective in some parts of the world, while evening emasculation and next-day pollination were found to have better results in other parts. Selection of parents for crossing has also been found crucial for successful hybridization. It has been reported that crossing success may be influenced by the parental identity and the environment in which plants are growing (Pittman and Levin 1989). The female parent plays a crucial role in determining the crossing success between both the parents. It was reported that for better crossing in chickpea, parent with small seed size should be used as female parent (Anbessa and Warkentin 2005). Moreover, female flower with anthocyanin pigmentation is better than the one without pigmentation which often scheduled for natural flower drop.

Crossing devoid of emasculation was found as a second option for chickpea crossing (Arora and Jeena 2000; Dahiya 1974 ; Retig 1971; Arora and Singh 1990). For this method to succeed, identification of flower stage is very important so that the artificial pollination can be done before its pollen grains are shed naturally. When hybridization without emasculation is attempted, there is always some chance of self- pollination. Therefore, this technique should only be used when parents are chosen on the basis of marker genes which can be used to eliminate self-pollinated progenies in the F_1 generation. In chickpea, sufficient information is available on inheritance of various traits (Dahiya 1974; Retig 1971). Therefore, monogenic traits with complete or incomplete dominance can be used as ‘markers’ to identify selfs. The reduced damage to flowers and the reduced time taken when flowers are not emasculated while making crosses can result in more flowers setting seed for the same amount of effort even when some selfed plants are discovered (Singh 2001).

20.5 Molecular Cytogenetics and Breeding

All chickpea cultivars and their wild relatives are self-fertilizing diploids ($2n = 2x = 16$ chromosomes) (Ahmad and Godward 1980; Mercy et al. 1974; Singh and Singh 1997) with a genome size of 740 Mbp (Varshney et al. 2013c). A few chickpea species have $2n = 14$ chromosome number. An attempt to cytogenetically characterize the perennial *Cicer* species was carried out by many researchers. In 1972, van der Maesen estimated $2n = 14$ or $2n = 16$ as the chromosome number in the perennial *Cicer* species. The initial description of the karyotype of the perennial *C. anatolicum* (Ahmad 1989; Hejazi 2011) established $2n = 16$ as the chromosome number, as is the case for the annuals. Ensuring analysis revealed much similarity in the karyotype of *C. songaricum* with that of *C. arietinum*, *C. reticulatum* and *C. echinospermum*.

Abbo et al. (1994) and Staginnus et al. (1999) reported first sequences localized through fluorescence in situ hybridization (FISH) technique as the ribosomal RNA genes. While only one chromosome pair carries a visible satellite, two sites hybridize with a 45S rDNA sequence, which was interesting in light of the presence of two satellited chromosome pairs in *C. reticulatum* (Abbo et al. 1994; Ohri and Pal 1991). Additionally, two sites with 5S rRNA and 45S rDNA sequences have been recognized on chromosome B (Vlacilova et al. 2002). About 50% of the chickpea genome is composed of repetitive DNA (Jain et al. 2013; Varshney et al. 2013c). These repetitive sequences are very informative and hence serve as cytogenetic markers, particularly where the chromosomal distribution is non-random (Jiang and Bikram 2006; Schwarzacher 2003). Five microsatellite motifs {(A)16, (CA)8, (TA)9, (AAC)5, (GATA)4} were selected (Sharma et al. 1995) using FISH technique, but did not produce any chromosome-specific karyotypes. All these five microsatellite motifs are localized within each chromosome but show a varied distribution and intensity from repeat motif to repeat motif (Gortner et al. 1998).

Zatloukalova et al. (2011) and Staginnus et al. (1999) reported a site in the pericentromeric region of chromosome A and a major cluster on the short arm of chromosome B. Nonetheless, the potential of repetitive DNA sequences was demonstrated in several studies. For instance, Staginnus et al. (1999) isolated the two tandemly organized chickpea-specific repeats from a genomic library (CaSat 1, CaSat 2) which were very informative. CaSat 1 defined a large cluster of sites in the sub-telomeric region of both chromosomes A and B, while CaSat 2 proved to be present at each of the eight centromeres.

Individual chickpea chromosomes have been successfully sorted by flow cytometry (Vlacilova et al. 2002) and utilized for mapping specific DNA sequences and genes to individual chromosomes. Thus, specific genes (coding for various rRNA loci), major random repetitive DNA sequences, sequence-tagged microsatellite site (STMS) markers, En/Spm-like transposon sequences, simple sequence repeats and *Arabidopsis*-type telomeric sequences have been successfully hybridized to, and localized on, the chickpea chromosomes by fluorescence in situ hybridization (FISH) (Abbo et al. 1994; Galasso et al. 1996; Gortner et al. 1998; Staginnus et al. 1999; Vlacilova et al. 2002; Valarik et al. 2004). Using polymerase chain reaction

(PCR) and FISH, Vlacilova et al. (2002) have successfully associated two STMS markers belonging to linkage group 8 of Winter et al. (2000) to the shortest chromosome of the chickpea genome. Recently, FISH analysis on super-stretched flow-sorted chickpea chromosomes has revealed spatial resolution of neighbouring loci that has not been obtained by any other method (Valarik et al. 2004).

Owing to early operating and strong post-fertilization crossability barriers (Bassiri et al. 1987; Ahmad et al. 1988; Stamigna et al. 2000; Ahmad and Slinkard 2003, 2004), only a few authentic interspecific hybrids are known in the genus *Cicer*. Ladizinsky and Adler (1976a, b) studied meiotic chromosome associations in six interspecific hybrids of *Cicer*. The interspecific hybrid *C. arietinum* × *C. reticulatum* is easy to make, develops normally, has regular meiosis with eight bivalents and is fully fertile (Ladizinsky and Adler 1976a, b). In contrast, the hybrid *C. arietinum* × *C. echinospermum* is characterized by six bivalents plus a quadrivalent (Ladizinsky and Adler 1976a, b), which along with other cryptic structural hybridities renders the F_1 or F_2 plants highly sterile. A reciprocal translocation also differentiates the genomes of *C. reticulatum* and *C. echinospermum*, which results in complete sterility (Ladizinsky and Adler 1976a, b). Chromosome association data indicate a close chromosome homology between *C. bijugum*, *C. judaicum* and *C. pinnatifidum* (Ladizinsky and Adler 1976a, b; Ahmad 2005). Univalent formation was lowest in *C. pinnatifidum* × *C. bijugum* and highest in *C. judaicum* × *C. pinnatifidum*. The only hybrid between *C. judaicum* and *C. chorassanicum* has been reported by Ahmad et al. (1987) and is characterized by a high number of univalents and very low pollen fertility. Authentic interspecific hybrids of *C. arietinum* × *C. pinnatifidum* (Badami et al. 1997) and putative hybrids, *C. chorassanicum* × *C. pinnatifidum* and *C. chorassanicum* × *C. yamashitae* (Ahmad et al. 2005), have been produced, but no chromosome pairing data are available due to the albino nature of the hybrids. Genetic studies among interspecific hybrids between cultivated chickpea and accessions from six recently identified wild *C. echinospermum* sites in southeastern Turkey indicated that both hybrid sterility and hybrid breakdown distinct subgroups of *C. echinospermum* are conditioned by one to few genetic loci (Kahraman et al. 2017).

Despite their narrow distribution, the annual wild *Cicer* species have a great potential for chickpea improvement through base broadening (von Wettberg et al. 2018) and by providing adaptive traits lost in the cultigen. Because wild and domestic *Cicer* have very contrasting evolutionary trajectories, there are good reasons to expect different adaptive traits among the wild species. There is no robust reproductive chilling (Berger and Turner 2007; Berger et al. 2012) or vegetative cold tolerance in domestic chickpea relative to wild *Cicer*, whereas heat tolerance is relatively common (Devasirvatham et al. 2012a). Wild versus domestic differences are also evident in phenology. These responses differ in wild *Cicer*, where vernalization and photoperiod responses become much more important (Berger et al. 2005; Sharma and Upadhyaya 2015). These differing behaviours suggest that wild versus domesticate differences are likely to emerge in responses to both biotic and abiotic stresses, as the current round of phenotyping attests (Kozlov et al. 2019).

The annual wild *Cicer* species have long been recognized as a promising source of resistance or tolerance to a range of important biotic stresses (*Fusarium* wilt, leaf miner, bruchids and nematodes) (Singh et al. 1998). However, the narrowness of the world's wild *Cicer* collection at that time made it impossible to evaluate whether this resistance (Singh et al. 1998) was prescriptive of the species as a whole or merely a symptom of a limited collection (Berger et al. 2003). For example, *C. reticulatum* was rated as highly susceptible to *Ascochyta* blight and *C. echinospermum* as moderately susceptible to susceptible (Singh et al. 1998), but these scores were based solely on the evaluation of material derived from 18 and 10 independent accessions, respectively. Making matters worse, 5 of these 18 independent *C. reticulatum* accessions were collected from the Savur region, recently identified as a single megapopulation (von Wettberg et al. 2018). To address this constraint, the newly collected germplasm is currently being evaluated for a wide range of biotic resistance in Australia (*Ascochyta* blight, *Phytophthora*, *Pratylenchus thornei* and *Pratylenchus neglectus* tolerance; Reen et al. 2019) and Turkey (*Fusarium*, *P. thornei* and *P. neglectus* tolerance). Although many of the activities are ongoing, there is a history of wild *Cicer* exploitation in chickpea improvement (Singh and Ocampo 1997). *C. echinospermum*, in particular, has been used as a source for *Ascochyta* resistance.

An evaluation of global chickpea genetic resources from contrasting reproductive phase temperature habitats showed no reproductive chilling tolerance in the cultigen but promising tolerance among wild *Cicer* (Berger et al. 2012; Berger and Turner 2007). However, this evaluation was subject to the same constraints as the earlier ICARDA work and was equally unbalanced. Recent evaluation of the new, much wider *Cicer* collection in Turkey and southern Australia has identified a wide range of *C. echinospermum* and *C. reticulatum* accessions that can set pods earlier and at lower temperatures than the domestic checks. This material is also being evaluated for short- and long-term water use and water-deficit response using mini-lysimeters and is showing markedly different behaviours than domestic chickpea. The same applies to regulation of phenology, where variation in flowering response (Kozlov et al. 2019) may be useful for adapting chickpea to new systems niches, such as the development of a vernalization response of winter chickpea for use in cold areas. Spatially accurate GPS data exist for the recently collected *C. echinospermum* and *C. reticulatum* accessions (von Wettberg et al. 2018), plus from the expanded collection. This would enable identification of key climatic variables associated with these sites and prioritization of accessions as potential sources of heat, cold and drought stresses, in both the vegetative and reproductive growth phases (Li et al. 2018). It also allows natural sites to be prioritized for in situ preservation, such as the lowest and highest elevation sites, or those on particular substrates, or those with unique rhizobial associates (Greenlon et al. 2019).

20.6 Genetic Studies on Qualitative and Quantitative Traits

Web-searchable International Crop Information System (ICIS) database is being developed in various crops to incorporate information on genetic studies pertaining to qualitative and quantitative traits (Balachandra 2005). Collective responsibility is being given to International Center for Agricultural Research in the Dry Areas (ICARDA), Morocco; International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India; United States Department of Agriculture (USDA); and the Australian Temperate Field Crops Collection (ATFCC), Australia in conjunction with the International Rice Research Institute (IRRI), Philippines.

20.7 Qualitative Traits

Qualitative traits are characterized by distinct phenotype groups, and Mendelian genetics are used to study inheritance patterns of these traits. A comprehensive list of qualitative traits in chickpea and their gene nomenclature is given by Muehlbauer and Singh (1987) and Pundir et al. (1985). They were further updated as a descriptor list by the International Board for Plant Genetic Resources (IBPGR) in 1993. Examples of qualitative traits include leaf shape and arrangement, plant habit, stem and foliage characteristics, flower colour, seed and cotyledon characteristics, podding, nodulation and disease resistance. Rubio et al. (2004) identified useful qualitative genes in chickpea, viz., erect or bushy, single or double and early or late qualitative genes across a range of environments. Resistance to iron chlorosis (Toker et al. 2010), seed coat thickness (Gil and Cubero 1993), vigour (Sabaghpour and Kumar 2003) and speed of germination (Dahiya et al. 1994) are other examples. Numerous potentially beneficial phenotypes, which may be qualitatively inherited, have been identified in species closely related to chickpea (Croser et al. 2003). However, to date few have been confirmed as being qualitatively inherited as phenotypes once incorporated into chickpea backgrounds. Examples of genes of interest include virus, insect, disease and nematode resistances. Dehydrin has been identified in *Cicer pinnatifidum* (Bhattarai and Fettig 2005), which may confer drought resistance. Purple flower colour, pigmentation and single-podded traits in *C. reticulatum* were governed by a dominant single gene (Adak et al. 2017). Efforts are also under way to use GM methods to confer *Helicoverpa* resistance (Gupta et al. 2020; Khatodia et al. 2017; Sanyal et al. 2005) in chickpea.

20.8 Quantitative Traits

In quantitative traits which are governed by many genes, phenotypic variation expressed as phenotypic continuum. Biometrical tools are employed in quantifying the genetic components of quantitative traits. In the two botanical cultivar groups, microsperma (desi) and macrosperma (kabuli), dominance of lower magnitude was

observed for leaflet length, width and shape index, whereas dominance of higher values was recorded for seed per pod. Overdominance was recorded for pod number, seed number and seed yield per plant (Martinez et al. 1979; Moreno and Cubero 1978). The most comprehensive documentation of quantitative traits in chickpea was given in the ICRISAT chickpea germplasm catalogue (Pundir et al. 1988). After screening thousands of entries, Pundir et al. (1988) obtained bell-shaped frequency distribution curves (or nearly so) for the following traits: days to flowering, duration of flowering, plant height, canopy width, seed weight, days to physiological maturity, number of primary and secondary branches, number of pods per plant, seed yield and seed protein concentration. Analysed 28 diallel trials carried out over 8 years and estimated the genetic variance for several agronomic traits. Although days to flowering, plant height and seed size were predominantly affected by additive gene action, both additive and non-additive effects were important for seed yield. This observation is partly in line with the earlier work of Jaiswal and Singh (1989), who reported preponderance of non-additive gene effects on yield and yield components among *C. arietinum* × *C. reticulatum* hybrid progeny.

Consequently, Jaiswal and Singh (1989) anticipated poor yield gain under selection in such crosses. However, impressive yield improvement following introgression in such interspecific crosses was reported by Singh and Ocampo (1997) pointing to the importance of additive effects on yield in chickpea. The genetic basis of freezing tolerance at the seedling stage was investigated, and additive, non-additive as well as their respective interaction gene effects were identified (Malhotra and Singh 1991). Root traits confer advantage in drought-prone environments, and a polygenic control of root length density and root mass with broad-sense heritability values of 0.23 and 0.27, respectively, was demonstrated in recombinant inbred lines (Kashiwagi et al. 2006; Serraj et al. 2004). Additive inheritance of root length and speed of radical emergence, two important adaptive traits for rainfed farming, were reported by Waldia et al. (1993). The ability to maintain dry matter accumulation in the seeds is a major adaptive trait under terminal-drought conditions (Palta et al. 2004). Leport et al. (1999) studied the yield physiology of a number of Australian chickpea cultivars in water-limited conditions with and without irrigation after flowering. They reported multifactorial inheritance of dry matter distribution after flowering.

Seed coat thickness is an important parameter for processing, with desi seeds having thicker coats compared with those of kabuli type. A number of genes appear to control this trait, with thick seed coat partly dominant to thin seed coat. Farmers (producers) prefer to produce large-seeded chickpeas due to consumer preference, since a larger seed size commands a higher price in regional and international markets. The inheritance of seed size was determined as monogenic, digenic and polygenic (Upadhyaya et al. 2006; Sundaram et al. 2019; Kumar and Singh 1995; Malhotra et al. 1997; Hovav et al. 2003; Hossain et al. 2010; Sharma et al. 2013). The inheritance pattern of the extra-large-seeded trait was polygenically controlled by partial dominant alleles (Kivrak et al. 2020). Several other consumer quality traits like seed mass, seed volume before and after soaking as well as swelling and hydration capacity show considerable phenotypic and genotypic variation Yadav

et al. 2003; Sharma et al. 2013). The inheritance of seed colour, shape and size as well as a range of nutraceutical characteristics such as cotyledon and seed coat flavonoids has been studied and carotenoids (Abbo et al. 2005) and seed Ca concentration (Abbo et al. 2000). Narrow-sense heritability values of 0.5–0.9 were reported with negative correlation with seed weight (Abbo et al. 2005). The protein content showed continuous distribution suggesting that it is a quantitative trait controlled by multiple genes (Gaur et al. 2016). A number of QTLs for pro-vitamin A carotenoids were identified on the chickpea genetic map, with one linked to a QTL for seed weight as expected from the correlation analysis (Rezaei et al. 2019).

Molecular mapping of the quantitative trait loci (QTLs) and development of linked and/or perfect DNA markers have blurred the distinction between qualitative and quantitative traits. *Ascochyta* resistance gene with a qualitative effect as part of the overall quantitative gene management has been provided by Cho et al. (2005). Complex genetic linkage maps have been developed (Cho et al. 2002; Flandez-Galvez et al. 2003) that include qualitative information on DNA sequences representing known chickpea morphological or phenotype genes, biochemical genes (e.g. isozymes), analogues to genes in other species (e.g. lentils and pea) and DNA sequences without known function. Tekeoglu et al. (2000) and Santra et al. (2000) identified several QTLs for *Ascochyta* blight response in several chickpea crosses. Flandez-Galvez et al. (2003) and Collard et al. (2003a, b) identified QTLs for *Ascochyta* blight resistance in interspecific and intraspecific chickpea crosses, thereby corroborating previous reports on the oligogenic nature of this trait (Santra et al. 2000; Tekeoglu et al. 2000). *Fusarium* wilt resistance in chickpea was also studied using both Mendelian (Upadhyaya et al. 1983; Kumar 1998) and quantitative approaches (Winter et al. 2000; Jana et al. 2003; Cobos et al. 2005).

20.9 Breeding Objectives

Chickpea is grown in more than 50 countries of the world, and the productivity of chickpea is usually low in important chickpea-growing countries, with the average yield ranging from 1041 kg/ha in India to 1730 kg/ha in the USA. Genetic enhancement through breeding is essential to achieve increased yield coupled with stable productivity. Chickpea breeding objectives generally differ in different regions depending on the problems and priorities of farmers and consumer preferences of the province. The major concern for any chickpea breeding programme is its narrow genetic base which imposes a lesser degree of genetic variability and potential genetic gain. To achieve the desired level of genetic improvement, broadening of the genetic base of chickpea is very much required.

The major objectives of breeding in chickpea are:

20.9.1 Breeding for Higher Yield

Chickpea is indeterminate in its growth habit, photo-sensitive as well as thermo-sensitive (Bahl et al. 1979), and is characterized by poor partitioning of the photosynthates resulting in very low harvest index values (Jain 1975). High yield potential can be achieved by improving biomass and its favourable partitioning. Another way of enhancing yield potential is by breeding cultivars responsive to fertilizer and irrigation. This is a long-term objective since there is no variability for this trait in the existing germplasm, and therefore some novel techniques including the modern biotechnological tools need to be used for realizing this objective.

20.9.2 Breeding for Quality Characters Including Biofortification

Breeding programmes have given greater emphasis to market-preferred seed traits in recent years. In desi chickpeas, most markets prefer small- to medium-size seeds (100-seed weight 16–22 g) and pay only modest premiums for the large grades. There is preference for yellow to light brown seed coat colour, and small niche markets exist for green- and black-seeded types. More than 70% of desi chickpea is used for making dhal, and a portion is further processed into flour (besan). High milling efficiency (dhal recovery) is therefore an important trait. In kabuli chickpeas, larger seeds get a high price premium. There is generally a preference for white or beige seed coat colour and ram's head seed shape. As the bulk of kabuli chickpea is cooked as whole grain, cooking time and seed volume expansion (on soaking) are considered important quality traits. Most kabuli-breeding programmes have shifted an emphasis from medium (around 25 g/100 seed) to large (>30 g/100 seed) seed size; there is also an increasing demand for extra-large kabuli (>50 g/100 seed) chickpea, as this variety commands very high premium. Earlier the demand for extra-large kabulis in the Indian subcontinent was met through import, mainly from Mexico and Turkey. The extra-large kabuli germplasm introduced in India from other countries was found poorly adapted (Yadav et al. 2004). The Indian National Programme, in partnership with ICRISAT, has ongoing breeding programmes for development of fusarium wilt-resistant, extra-large-seeded kabuli varieties adapted to local environments. A few varieties like Phule G 0517, PKV 4-1 and MNK-1 have been developed with seed size more than 50 g/100 seeds (Dixit et al. 2019).

There has been negligible input into the improvement of nutritional quality of chickpea. Protein content of existing cultivars is generally in the range of 18–22%, but much larger variability (12.4–32.5%) exists in the cultivated and wild species, and this could be exploited to breed higher protein (up to 25%) varieties. The nutritional value of off-grade chickpeas was found to be acceptable in ruminant and pig diets (Mustafa et al. 2000). The sulphur containing-amino acids methionine and cystine are the limiting amino acids. Transgenic technology is being used to enhance the level of sulphur-containing amino acids because the required variation is absent from the primary gene pool. Transgenics developed by introducing a seed-specific chimeric gene encoding sunflower seed albumin (SSA) produced

24–94% higher methionine but 10–15% lower cysteine than comparable non-transgenic chickpea (Higgins et al. 2004).

There is a need to assess genetic variability for various nutritional and antinutritional traits in the germplasm of cultivated and wild species. Studies are also needed on G × E interactions and genetics of these traits. Identification of contrasting parents for the content of each nutritional and antinutritional trait would be required for development of genetic populations needed for mapping of genes controlling these traits. Availability of such basic information on genetic variability and genetics of nutritional and antinutritional traits would help in development of breeding strategies (both conventional and biotechnological approaches) for improvement of these traits in chickpea.

20.9.3 Breeding for Tolerance to Biotic Stresses

The serious damage caused by disease and pest results in yield instability. *Ascochyta* blight, wilt and root rot are the major diseases; pod borer and leaf minor are the major pests; and cyst, root knot and root lesion nematodes are the major soil microorganisms that cause considerable yield losses. Obviously, breeding for resistance to these stresses will help stabilize chickpea production.

20.9.4 Breeding for Abiotic Stress Resistance (Climate Change)

Considerable yield losses occur due to abiotic stresses like drought, salinity, cold and frost. Resistance or tolerance to these stresses is more complex. However, resistant germplasm against these stresses has been identified. Therefore, breeding for resistance or tolerance to abiotic stresses is also an important objective. Chickpea production faces many challenges due to unpredictable climate change as it increases the frequency of drought and temperature extremes (Gaur et al. 2013a, b; Kadiyala et al. 2016). Therefore, breeding chickpeas for such stress conditions is essential for development of high- and stable-yielding varieties of chickpea (Devasirvatham and Tan 2018).

20.9.5 Exploitation of Heterosis and Prospects of Hybrid Development

Although exploitation of heterosis is difficult in chickpea due to many problems including difficulty in producing large quantity of hybrid seeds coupled with very high seed rate. Male sterility will be useful to employ in population improvement schemes. In the absence of stable male sterility, development of stable and useful male sterile genotypes becomes an important objective in chickpea. This may however be considered as a long-term objective.

20.10 Breeding Approaches: Conventional and Non-Conventional Including the Use of Genomic Tools

Significant improvement has been made in chickpea by following various conventional breeding approaches. Selection from indigenous and exotic landraces led to the development of most of the chickpea varieties during the early phases of chickpea breeding. This invariably led to the further narrowing down of the genetic base, thereby diverting the focus to hybridization-based breeding programmes with an aim to broaden the genetic base. One of the commonly used hybridization-based methods is pedigree method—not practised in original form as it is cumbersome and only limited number of crosses could be handled. It was found that 120 *desi* and 53 *kabuli* parents were used to develop 138 varieties (105 *desi* and 33 *kabuli*). The pedigree analysis revealed that these varieties were developed through hybridization using IP 58 (27), C 1234 (26), JG 62 (18), S 26 (18) and Chaffa (15) in *desi*, while Rabat (26), Pb 7 (24), Banda Local (14), Etah Bold (14), Guamchil 2 (14), P 458 (14) and GW 5/6 (14) as the frequently used parents for the development of *kabuli* varieties, thereby evidently specifying that very few genotypes were utilised for the development of chickpea varieties released in India. Instead of pedigree method, bulk method and its modifications are commonly used by following hybridization. It is well recognized that individual plant selection is not effective for yield in early segregating generations in chickpea. Thus, the selection is preferred for simple traits such as seed traits, maturity and resistance to diseases in early generation (F2, F3) while yield, etc., based selection is done in later generations. A recombinant-derived family method that uses early-generation selection for yield in F_2-derived F_4 or later-generation families to eliminate inferior crosses and inferior F_2-derived families has also been suggested (Slinkard et al. 2000) and is used at the University of Saskatchewan, Canada, thereby necessitating to involve more and diverse germplasm lines, primitive landraces and wild *Cicer* species in the hybridization programme for the cultivar development (Verma et al. 1990; van Rheenen et al. 1993; Nadarajan and Chaturvedi 2010; Mishra et al. 2013a, b; Singh et al. 2014).

Most of the chickpea breeding programmes were earlier confined to intraspecific hybridization by involving *desi* × *desi*, *kabuli* × *kabuli* or *desi* × *kabuli* crosses. Crosses between *desi* and *kabuli* parents are extensively used for transferring genes for *Fusarium* wilt, *Ascochyta* resistance and drought tolerance to *kabuli* from *desi* and genes for improved seed quality, especially large seed size from *kabuli* to *desi* chickpea. *Desi* × *kabuli* hybridization programmes have consistently led to high-yielding progenies and have served as the source for many new cultivars (Yadav et al. 2004). Efforts have been focussed to utilize interspecific crosses for enhancing the genetic variability by introgressing useful genes into the cultigen(s) from wild *Cicer* species which have been inadvertently being lost at the time of domestication such as for stable disease resistance for *Ascochyta* blight, *Fusarium* wilt, root rot, botrytis grey mould, cold tolerance, heat tolerance, drought tolerance, nodulation, etc.

Only two annual wild species, *Cicer reticulatum* and *C. echinospermum*, have so far been exploited in the breeding programmes, as the crossing of the cultigen with other species has remained a challenge even with embryo rescue techniques. Interspecific derivative lines have been developed from a successful *Cicer arietinum* × *C. pinnatifidum* cross at PAU, Ludhiana, expressing significant variability for yield traits and disease resistance (Kaur et al. 2013; Salaria 2020). Continuous efforts are required for exploiting wild *Cicer* species. There is a need to exploit species belonging to the tertiary gene pool, as they contain many useful genes, particularly resistant to biotic and abiotic stresses (Ahmad et al. 2005). A *desi* chickpea variety, Pusa 1103, developed by the ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India, from an interspecific cross of *C. arietinum* with *C. reticulatum*, has been released for commercial cultivation in north India, and recently the Punjab Agricultural University, Ludhiana, developed a high-yielding variety PBG 8 from an interspecific cross of *C. arietinum* with *C. judaicum* for commercial cultivation in Punjab state. PBG 8 is moderately resistant to *Ascochyta* blight and botrytis grey mould and higher level of tolerance to pod borer.

As conventional breeding takes 6 to 7 years to develop homozygous lines after hybridization, therefore breeding programmes have been focussed to reduce the number of years required to reach homozygosity by taking more than one generation per year for the development of a variety. One of the methods to have more than one generation in a year is by taking the advantage of off-season nurseries or greenhouse facilities. Another method for generation advancement is rapid generation advancement (RGA) technology for accelerating the breeding cycle by involving the use of immature seeds to produce miniature plants in artificial medium under controlled conditions and allowing them to produce few flowers bearing seeds which are harvested before normal seed maturity (Samineni et al. 2020).

Whole of this process is based on providing extended photoperiod (15–16 h) through artificial lighting as long days induce early flowering in chickpea provided until flower initiation (Sethi et al. 1981). But only single seed descent (SSD) method could be followed using RGA whereby one or more early generations are advanced without selection in the greenhouse. As by following SSD, large populations could be accommodated depending on the greenhouse facility and other resources. Results obtained by Samineni et al. (2020) have implicated the utility of RGA in breeding programmes such as rapid progression towards homozygosity, development of mapping populations, reduction in time, space and resources for the cultivar development (speed breeding).

Besides above-mentioned breeding methods, mutation breeding has also been used in chickpea improvement for enhancing genetic variability. Sufficient efforts have been put forth in chickpea to study the effect of various mutagen treatments (physical and chemical mutagens) on yield potential, resistance/tolerance to biotic and abiotic stresses, effect on chlorophyll, tolerance to herbicides and quality parameters along with elucidation of its origin. Sarma et al. (1991), Charumathi et al. (1992) and Khan et al. (2005) reported that a significant amount of variability is produced particularly with the increase in grain yield across the different mutagenic treatments of gamma radiations (50 kR) in combination with gibberellic acid (John

1995). However, Rao (1988) found that gamma radiations have an adverse effect on the grain yield in chickpea. Gamma rays have also been found effective in the development of disease resistance (CM 98 against *Ascochyta* blight and wilt (Haq et al. 1999)). As far as quality is concerned, gamma rays brought a significant increase in degree of softness of seed, thereby improving the cooking quality (Graham et al. 2002). Some mutants have been directly released as varieties, whereas many other mutants have been used as parents in the crossing programmes. At least 12 varieties have been developed through mutation breeding. These include seven varieties (Pusa 408, Pusa 413, Pusa 417, Pusa 547, RS 11, RSG 2 and WCG 2) developed by IARI and State Agricultural Universities in India; four varieties (CM 72, CM 88, CM 98 and CM 2000) developed by the Nuclear Institute of Agriculture and Biology, Faisalabad, Pakistan; and one variety (Hyprosola) developed by the Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.

Recent advances in the in vitro culture, transformation and plant regeneration protocols for chickpea offer unique opportunities to realize the full potential of chickpea production. Transgenic technology can be utilized to improve those traits for which adequate variability is not available in the gene pool. These include resistance to pod borer and other biotic and abiotic stresses, as well as sulphur-containing amino acids. However, as of date, no transgenic chickpea variety has been approved for cultivation anywhere in the world.

With the advent of the molecular markers, marker-assisted selection (MAS) is being considered for improving the precision and efficiency of conventional plant-breeding methods by understanding the genetic basis of the traits. MAS is considered to be useful for improving traits wherein direct selection is difficult or is convenient (e.g. root traits for drought avoidance, antinutritional factors, quality traits). MAS finds a significant role in pyramiding genes for resistance from different sources particularly when the resistance is controlled polygenically (e.g. resistance to *Ascochyta* blight), combining genes conferring different resistance mechanisms (e.g. antixenosis, antibiosis and tolerance for pod borer) and resistance against two or more stresses (e.g. fusarium wilt and pod borer). Besides this MAS is exploited for the introgression of genes from wild species into cultivated germplasm with minimum linkage drag and mapping of quantitative trait loci (QTLs) governing economically important traits. The molecular tools hasten the conventional breeding and are a rapid precise alternative for the improvement of quantitative traits like yield and resistance/tolerance to various biotic and abiotic stresses. During the last decade, significant improvements have been made in the generation of plenty of genomic resources. It has been even made possible to locate genomic regions of various quantitative traits for use in MAS by various molecular marker technologies, thereby encouraging the usage of molecular breeding approaches in chickpea breeding programmes such as marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), advanced backcross quantitative trait loci (AB-QTL) analysis and genomics-assisted breeding (GAB).

Development of molecular markers in chickpea on a large-scale have been made possible through next-generation sequencing technologies leading to the construction of dense linkage maps and identification of several molecular markers

associated with agronomically important traits. The application of a holistic approach combining genomics with breeding and physiology, termed as genomics-assisted breeding (GAB) (Varshney et al. 2005), provides strategies for improving component traits of biotic and abiotic stress tolerance that should prove more effective and efficient than the conventional methods. Various functional genomic approaches like suppression subtractive hybridization (SSH), microarray and EST sequencing, serial analysis of gene expression (SAGE) and their modifications such as super serial analysis of gene expression (SuperSAGE) and deep SuperSAGE have been utilised for the identification of transcripts for different abiotic stress-responsive genes in chickpea for quantification of global gene expression (Matsumura et al. 2005; Buhariwalla et al. 2005; Molina et al. 2008).

Considerable reduction in sequencing costs has been made on utilization of advances in next-generation sequencing (NGS) technology (Varshney et al. 2009) and has led to the evolution of genotyping methods from individual marker- to whole-genome sequence-based genotyping. This brought about the development of large-scale genomic resources, including genome sequence assemblies, resequencing of few thousand lines, high-resolution genetic maps and a range of low- to high-density genotyping platforms. Even the identification of alleles and haplotypes associated with agronomic traits in chickpea has been made possible on utilization of such genomic resources (Varshney et al. 2019b). Fine mapping of the *QTL-hotspot* region from ~ 7.74 Mb to ~ 300 kb, for drought tolerance-related traits (Jaganathan et al. 2015; Kale et al. 2015), is done by genotyping-by-sequencing and skim sequencing-based bin mapping. 'Axiom®*CicerSNP* Array', a high-throughput single-nucleotide polymorphism (SNP) genotyping platform, has facilitated the construction of dense genetic maps for the advancement of genetics and breeding efforts in chickpea (Roorkiwal et al. 2018). Whole-genome resequencing (WGRS) has led to the dissection of genetic diversity, population structure, domestication patterns, linkage disequilibrium and the unexploited genetic potential for chickpea improvement (Varshney et al. 2019a). Modern genomics technologies have the potential to hasten the process for trait mapping, gene discovery, marker development and molecular breeding in addition to the enhancement of the rate of productivity gains in chickpea. Integration of genome-wide sequence information with precise phenotypic variation permits to capture accessions with low-frequency alternatives responsible for essential phenotypes such as yield components, abiotic stress tolerance or disease resistance. Chickpea breeding programmes are focussed on improving yield and its component traits under biotic and abiotic stress conditions. But, the changes in climatic patterns have levied challenges to enhance yield levels of chickpea, in addition to meet the growing demands w.r.t. health benefits of consumers. Therefore, breeding efforts need to be focussed for the development of superior climate-resilient chickpea varieties to meet out the nutritional issues of developing countries.

20.11 Emerging Challenges at National and International Levels

Climate change has played a key role in the evolution. Under present context, effects of global warming are experienced in the form of climate change like erratic rainfall patterns (reduction in the number of rainy days, more drought and flood incidence, etc.), abrupt rise or drop in temperature and humidity, longer consecutive foggy days leading to low intensity of sunlight during crop growth, etc. Under such adverse climatic conditions and selection pressure leading to the newer diseases (*Alternaria* blight, stem rot, collar rot, rust, etc.) along with the incidence of prevalent diseases (*Ascochyta* blight, botrytis grey mould, wilt, dry root rot, etc.), increased infestation of the insect pests (gram pod borer, cut worm, etc.) under high humidity or foggy climatic conditions is becoming a consistent phenomenon of climate change not only in India but also in other chickpea-growing countries worldwide. Even the availability and utilization of macro- and micro-nutrients in soil or nutrients applied to the crop is hampered due to low intensity of sunlight (due to fog) during chickpea-growing season. Under harsh environmental conditions, even the nodulation may also be affected. Chickpea being a sensitive crop is susceptible to a large number of biotic (diseases, insect pests, nematodes, weeds) and abiotic (drought, heat, cold, salinity, alkalinity, etc.) stresses. Low productivity levels of chickpea are due to abrupt rise (terminal heat stress) or drop in temperature (cold or frost stress), terminal soil moisture stress or excess rainfall during crop growth. The emerging challenges in chickpea have been discussed as biotic and abiotic constraints, ideal plant type and hastening breeding cycle.

In chickpea a large number of biotic stresses (fusarium wilt, *Ascochyta* blight, botrytis grey mould, pod borer, bruchids) and abiotic stresses (drought, heat, cold) limit the realization of yield potential at farmers' fields. To curtail losses due to these stresses, several strategies including genetic options have been recommended and adopted (Nadarajan and Chaturvedi 2010; Kaur et al. 2013) to enhance chickpea productivity and production.

20.11.1 Biotic Stress

20.11.1.1 *Fusarium* Wilt

It is a major problem reported from 32 countries across 6 continents in the world causing 10 to 90% losses (Jimenez-Diaz et al. 1989; Singh and Reddy 1991). Initially symptoms of wilt vary among different chickpea genotypes. Resistance to wilt is controlled by a few major genes that are part of oligogenic resistance mechanism, thereby delaying the onset of disease symptoms. Resistance against *Fusarium* wilt has been reported in the indigenous chickpea germplasm (Singh et al. 2012). Around eight pathogenic races of *Foc* (races 0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been reported worldwide (Jendoubi et al. 2017). For the management of *Fusarium* wilt, the most effective and efficient method is the development of resistant cultivars. Reliable and efficient screening methods have been established by developing sick-plots for evaluating a large number of genotypes under field

conditions at several AICRP centres. Till date conventional breeding methods are followed for the development of *Fusarium* wilt-resistant varieties, but it is time-consuming and depends on inoculum load and specific environmental factors that influence disease development. The utilization of molecular markers closely linked to genes/QTLs controlling *Fusarium* wilt offers a great potential for the identification of resistant genotypes.

20.11.1.2 *Ascochyta* Blight

It is one of the most important foliar diseases of chickpea leading to yield losses as high as 100% (Nene and Reddy 1987; Singh 1990) and prevalent in many parts of the world including India. *Ascochyta rabiei* (causal organism) isolates have been classified based on their levels of virulence (Udupa et al. 1998; Chen et al. 2004; Jayakumar et al. 2005) into either a two- or three-pathotype system (I, II and III). It is speculated that the disease might have spread from its site of origin to distant continents through chickpea germplasm exchanges. Two most damaging symptoms of this disease are stem breakage along with girdling and collapse of twigs. The frequency of resistant and moderately resistant-type accessions is observed to be comparatively higher in accessions originated from Southwestern Asian countries particularly Iran and Syria than the accessions originated from Indian subcontinent (Gayacharan et al. 2020). Presently, chickpea breeders have shifted to gene pyramiding in elite genotypes instead of incorporating vertical resistance for the incorporation of stable resistance. Besides this, deployment of different lines having resistance to different races of the pathogen prevalent in different regions can be effective in minimizing yield losses caused by *Ascochyta* blight.

20.11.1.3 *Botrytis* Grey Mould

Botrytis grey mould (BGM) is the second most important foliar disease of chickpea. BGM is predominant in 15 countries including India, Bangladesh, Nepal, Pakistan, Australia, Argentina, Myanmar, Canada, Columbia, Hungary, Mexico, Spain, Turkey, the USA and Vietnam. Maximum crop losses are observed in seasons with wet spring, particularly when crops develop dense canopies. Earlier it was reported that there is no reliable source known to have resistance to BGM (Singh and Reddy 1991) in cultivated germplasm. Interspecific derivative lines of *C. arietinum* × *C. pinnatifidum* crosses, developed at PAU, Ludhiana, have exhibited a moderate to high level of genetic resistance against BGM (Kaur et al. 2013; Salaria 2020) and can be utilized to incorporate such durable resistance into elite genotypes for the development of high-yielding chickpea cultivars.

20.11.1.4 Pod Borer

One of the major insect pests infesting chickpea crop is pod borer (*Helicoverpa armigera*). It predominantly causes damages across Asia, Africa, Australia and some other chickpea-growing regions. Pod borer is a polyphagous insect and known to cause damage to more than 182 plant species. The breeding approach followed for conferring pod borer resistance in chickpea is an integrated one involving both the antixenosis/antibiosis and avoidance mechanisms (Clement et al. 1992). The

development of cultivars resistant or tolerant to *H. armigera* could be integrated in the pest management strategy by identifying, characterizing and utilizing the genetic mechanisms conferring durable resistance to pod borer (Dua et al. 2002). More than 14,000 chickpea germplasm accessions screened under field conditions at ICRISAT for resistance against *H. armigera* (Lateef and Sachan 1990) led to the identification and release of moderately resistant/tolerant chickpea cultivars (Lateef 1985; Lateef and Pimbert 1990). Still complete resistance against pod borer is far from reach. Different chickpea cultivars express differential inhibition activity of gut proteinases of *H. armigera*, indicating that *H. armigera* is adapted to a wide range of host protein inhibitors (Singh et al. 2008). A high-throughput Axiom*CicerSNP* array led to the construction of a dense linkage map comprising of 3873 loci spanning a distance of 949.27 cM. This genomic region, after validation, could be useful to improve *H. armigera* resistance component traits in elite chickpea cultivars (Barmukh et al. 2020).

20.11.1.5 Bruchids

Significant losses by storage pests occur in the Mediterranean region and in India. Bruchid (*Callosobruchus chinensis*) infestation levels can cause losses ranging from 13% (Dias and Yadav 1988; Mookherjee et al. 1970) to total loss (Weigand and Tahhan 1990) in storage. Talking about resistance to storage pests like bruchids, there is no resistant genotype available in the cultivated chickpea germplasm. Chickpea grains with higher sugar and lower phenol contents were found to be more susceptible to bruchids (Swamy et al. 2020), whereas wild chickpea accessions have exhibited variable resistance to bruchids (Singh et al. 1994, 1998). Owing to crossing barrier, it has not been possible to transfer this trait to the cultivated background. Thus, it is advised to go for chemical control measures (Duke 1981). Recent studies in legume crops indicated that seed storage in three-layered polythene bag resulted in effective control of bruchids and their further spread (Vales et al. 2014; Sudini et al. 2015).

20.11.1.6 Weeds

Besides above-mentioned biotic factors, seasonal weeds associated with chickpea crop such as *Phalaris minor* (L-Retz), *Avena fatua*, *Lolium temulentum* (L), *Trifolium* spp., *Chenopodium album* (L), *Melilotus* spp., *Lathyrus tuberosus* (L), *Convolvulus arvensis* (L), *Anagallis arvensis* (L), *Asphodelus tenuifolius* (Cavan), *Medicago denticulata* (L. wild), *Rumex dentatus* (L), *Fumaria parviflora* (Lamk), *Circium arvense* (L. Scop), *Cyperus rotundus* (L) and *Cynodon dactylon* (L. Pers) pose a serious threat to chickpea productivity by causing a smothering effect to the crop. It is specifically observed to be a major problem during winter rains when the weeds become a major yield-limiting factor. Under present situation when the farm labour days are becoming expensive, there is a need to have varieties tolerant to herbicides (Sandhu et al. 2010; Gaur et al. 2012). Significant levels of genetic variations against post-emergence herbicide (imazethapyr) have been observed to be present on screening reference set and elite breeding lines in chickpea (Gaur et al.

2013a; Gupta et al. 2018). These have paved the way for the development of post-emergence herbicide-tolerant chickpea varieties.

20.11.2 Abiotic Stress

20.11.2.1 Drought

Globally one of the effects of climate change is drought responsible for high-yield losses in chickpea. Usually, terminal drought has adverse effects on the crop productivity (Khanna-Chopra and Sinha 1987). Cultivation of early maturing cultivars is suggested for areas frequently affected by drought as it helps in judicious utilization of the available soil moisture and escape of the crop from drought. Recently, studies on root traits have gained much importance as genotypes with longer root systems have better potential for the extraction of moisture from deeper layers of soil, thereby exhibiting better drought tolerance. Among wild *Cicer* species screened, a few accessions of *C. pinnatifidum* and *C. reticulatum* were found to be resistant/tolerant to drought (Toker et al. 2007). The ICC 4958 genotype from cultivated germplasm is a potential donor extensively utilized for the incorporation of drought tolerance. Chickpea introgression lines with improved drought tolerance (ICC 4958, used as donor) gave promising results in India and Kenya (Gaur et al. 2012). The introgression lines with improved root traits screened at several locations in central and southern India exhibited high G × E interaction.

20.11.2.2 Heat Stress

Chickpea is a cool season crop. In the era of climate change and varying cropping pattern, the crop is being exposed to high temperature (>35 °C) during the reproductive phase, leading to severe yield penalties. Reproductive period is most sensitive to heat stress conditions, if temperature rises above the threshold level, the pod formation and seed set is affected adversely, thereby, in turn, leading to reduced grain yield (Summerfield et al. 1984; Wang et al. 2006; Basu et al. 2009; Kumar et al. 2013). Additionally, adverse effects of high temperature are experienced during seed germination, respiration, membrane stability, photosynthesis, hormone level, nutrient absorption, protoplasmic movement, fruit maturation and materials transport leading to withering, burning of lower leaves, desiccation of poorly developed plants, stunting flower and pod abortion, reduced root nodulation and nitrogen fixation affecting quality of seeds and seed yield (Saxena et al. 1988; Kurdali 1996; Chen et al. 1982; Wahid and Close 2007). Even though chickpea is comparatively more tolerant to heat stress compared to other cool season legume crops (Summerfield et al. 1984; Erskine et al. 1994; McDonald and Paulsen 1997; Patrick and Stoddard 2010), still severe heat stress leads to high-yield losses and crop failure (Devasirvatham et al. 2012b). Large genetic variations for heat tolerance in chickpea-cultivated germplasm have been observed as revealed from multi-location screening of reference set against heat stress in India (Krishnamurthy et al. 2010). A field screening technique standardized by Gaur et al. (2014) for heat tolerance led to the identification of several sources of heat tolerance. The JG 14, a heat tolerant

variety, released in India is found to be promising for normal as well as late planting situations in central and southern states.

20.11.2.3 Cold Stress

Chickpea being a winter season crop is more productive than the traditionally grown spring season crops in the Mediterranean region (Singh and Hawtin 1979). This is mainly due to the long cropping season and better moisture availability. Chickpea crop grown during winter season experiences difficulties such as flower drop and pod abortion, thereby leading to severe yield losses particularly when the mean day temperature falls below 15 °C (Savithri et al. 1980; Srinivasan et al. 1999; Clarke and Siddique 2004; Nayyar et al. 2005). Studies have revealed lack of cold/chilling tolerance in the domesticated gene pool, while significant tolerance potential has been observed in the annual wild relatives of chickpea (Berger and Turner 2007; Berger et al. 2003, 2012). Preliminary studies in Australia demonstrated that the wild relatives that readily crossable with chickpea (*C. reticulatum*, *C. echinospermum*) have considerably more cold tolerance at vegetative stage and chilling tolerance at reproductive stage compared to domestic chickpea. Therefore, significant efforts are required for the identification of novel sources for cold tolerance followed by the development of the breeding population(s) meant for the identification of cold-tolerant genotypes.

20.11.3 Ideal Plant Type

Today when the effects of global warming experienced in the form of climate change are leading to the changes in the cropping pattern, there is an urgent need to restructure existing bushy/semi-spreading chickpea plant types for the enhancement of photosynthetic and input use efficiency, reduction in the cost of cultivation, minimizing foliar diseases, varieties amenable to intercropping, mechanical harvesting and to ease the intercultural operations. Two different ideotypes were identified to be suitable for winter and spring sown crops under Italian conditions. It was suggested that winter types should be resistant to *Ascochyta* blight, cold tolerant, longer vegetative cycle, erect growth habit and bearing of pods on upper parts of the branches to facilitate mechanical harvesting, whereas, for spring sown crop, short vegetative cycle, good adaptability to southern and central environments, high yield and good grain quality were suggested by Saccardo and Calcagno (1990). Several mutants with desirable traits like cymose inflorescence having more than three flowers per node (Gaur and Gour 2002), brachytic growth habit (Gaur et al. 2008a, b) and determinate growth habit (Hegde 2011) and mutant with short internode (E100Y) and erect growth (Chaturvedi et al. 2010) offer ample scope to alter the ideotype of chickpea varieties for their suitability to mechanized harvesting (Sandhu et al. 2010; Gaur et al. 2012).

20.11.4 Reduction in Breeding Period

Significant efforts have been put forth at national and international levels for hastening the chickpea breeding programmes to tackle the repercussions of climate change. Considering the importance of rapid generation advancement for shortening the breeding cycle, Punjab Agricultural University, Ludhiana, has off-season nursery facility at Keylong (H.P), and few years back, ICAR-Indian Institute of Pulses Research has also established off-season nursery centre at Dharwad (Karnataka). The Dharwad centre helps other chickpea breeding centres for rapid generation turnover. More than 200 high-yielding varieties with varying levels of tolerance to biotic and abiotic stresses have been developed with significant gains in production and productivity with more than 51% chickpea's share in total pulse production (19.27 mt) in India during 2013–2014.

Still, to improve the complex traits wherein phenotyping/selection in the segregating generations is difficult, deployment of molecular markers linked with targeted genes/QTLs is suggested to hasten the breeding cycle. Recent advances in the development of hefty numeral of molecular markers linked with useful genes/QTLs governing traits of breeders' interest have encouraged applications of marker-assisted breeding in chickpea improvement. One of the successful examples is deployment of MABC (marker-assisted backcrossing) wherein a 'QTL-hotspot' containing QTLs for several root and drought tolerance traits was transferred from the drought-tolerant line ICC4958 to a popular *desi* chickpea variety JG 11 (Varshney et al. 2013c). They have suggested ways to go for marker-assisted breeding for the development of future varieties in chickpea on reviewing the status of genomic resources available for chickpea improvement. Further, Gaur et al. (2013b) reported identification of improved lines with significantly higher yield.

Recently, besides utilising off-season nurseries along with molecular breeding, RGA (rapid generation advancement) technology is gaining impetus for accelerating the breeding cycle. This technique offers the flexibility to take about six generations in a year compared to conventional breeding with two generations per year using off-season nurseries. This technology involves the usage of immature seeds for the production of miniature plants in artificial medium under controlled conditions, thereby allowing them to produce few flowers, bearing seeds, which are harvested before normal seed maturity. The whole process is based on providing long days of conditions with 15–16 h photoperiod through artificial lighting for the induction of early flowering in chickpea (Sethi et al. 1981). The only drawback perceived is that out of all the chickpea breeding methods only single seed descent (SSD) method could be followed for the RGA whereby one or more early generations are advanced without selection in the greenhouse. As by following SSD, large populations could be accommodated depending on the greenhouse facility and other resources. Samineni et al. (2020) suggested the utility of RGA in breeding programmes such as rapid progression towards homozygosity, development of mapping populations, reduction in time, space and resources for the cultivar development (speed breeding).

20.12 Precise and High-Throughput Phenotyping Protocols for Key Traits

The term 'phenotype', derived from the Greek words 'phainein' and 'typos' (meaning show and type, respectively), was explained as 'all types of organisms distinguished by direct inspection or with finer methods of measurement or description' by Wilhelm Johannsen in 1911 (Johannsen 1911). Fiorani and Schurr (2013) defined plant phenotyping as the set of methodologies and protocols used to accurately measure plant growth, architecture and composition at different scales. Traditionally phenotyping methods deal with either one or few specific plant characteristics at a given time without thorough functional analysis of constituent traits linking genotype with the phenotype. One of the key difficulties with conventional (direct) measurements of basic plant traits is that they are laborious and often destructive. This is mainly evident while working with larger plants and plant species with many small leaves. Additionally, measurement of many traits in segregating generations becomes difficult as they are invasive, labour-dependent and time-consuming, thereby decreasing the breeding efficiency as selection is delayed to later generation(s). Besides this, often the environmental and soil variables are not taken into consideration while monitoring, aggravating the phenotypic bottleneck(s). Many times, depending upon the study conducted, other important factors (other than those under consideration) are overlooked. Therefore, phenotypic prediction based on the genetic composition of lines or cultivars must be considered to address all the above-mentioned issues (White et al. 2012). In plant breeding, field experiments conducted at multiple locations are indispensable for evaluating the adaptability of new candidate genotypes in order to examine their pattern of genotype × environment interaction (Chapman et al. 2014). Plant phenotyping techniques have impressively evolved over the last two decades. In today's era of genomics, data generated by plant phenotyping needs to be high-quality quantitative data to adapt to the needs of modern breeding techniques. Modern plant phenotyping is all about increased accuracy, precision, throughput at all levels with reduction in costs and automation to minimize labour demand.

On realizing the need for rapid and precise phenotyping of multiple traits, many next-generation and high-throughput plant phenotyping platforms (HTPPs) have been developed (Hartmann et al. 2011) to measure trait values accurately for the assessment of variation among individuals, thereby enabling better HTPPs approaches to address the relationship between traits, plant development, growth and reproduction under various conditions. Now, high-throughput phenotyping strides for non-invasive technologies and is one of the rapidly advancing fields (Berger et al. 2010; Furbank et al. 2009). These are based on various imaging techniques to record plant structure, estimation of biomass, analysis of phenology, plant health, tissue-water relations, transpiration, photosynthetic activity, etc. Such phenotyping systems can work well under field setting or controlled environment where individual plants are automatically weighed and watered. Owing to the high cost involved in HTPPs, cost-effective automated and semi-automated methods for data acquisition and analysis are now being developed (Gehan and Kellogg 2017).

These HTPPs provides physiological and morphological data along with simultaneous analysis of the massive data generated and can give better understanding of the whole phenome of the plant under a wide range of environmental and growth conditions.

Mainly HTPPs are categorized into two types, viz., ground-based HTPPs (enabling the data to be captured at a plot level) and aerial HTPPs (involving a very high level of automation and precision to cover larger plots and even the entire fields). Ground-level HTPPs involve the usage of carts, tractors or gantry-mounted sensors, while aerial HTTPs generally involves small airplanes, helicopters and unmanned aerial platforms (UAPs) such as polycopters and drones. The recent alternatives to airplanes in aerial HTPPs include 'phenotowers' (Rascher et al. 2011) and 'blimps' (Losos et al. 2013). A few HTPPs deployed for phenotyping several crop plants are available for *Arabidopsis* (Granier et al. 2006; De Diego et al. 2017), cotton (Andrade-Sanchez et al. 2013), barley (Hartmann et al. 2011), maize (Trachsel et al. 2011), wheat (Bai et al. 2016; Zhang et al. 2017), rice (Yang 2012), sorghum (Hartmann et al. 2011; Golzarian et al. 2011), etc. mostly run by large seed companies and advanced crop research institutes around the world. Some of the popular HTPPs are LemnaTec; Digital Phenotyping-KeyGene; International Plant Phenotyping Network; Julich Plant Phenotyping Centre; LEPSEMontpellier Plant Phenotyping Platform; PPHD-INRA; Dijon; Phenopsis; Arabidopsis Platform, INRA; PhenoFab, Wageningen; The Biotron, Canada (KeyGene + LemnaTec); and Australian Plant Phenomics Facility.

20.12.1 Phenotyping for Abiotic Stresses

High-yield losses have been recorded due to the abiotic stresses, especially owing to adverse agro-climatic conditions experienced at the time of reproductive phase, leading to instability in chickpea production worldwide. Breeding for tolerant chickpea types with wider adaptation to different and diverse growth conditions and regions is the best strategy. But this requires a fine-tuned amalgamation of advanced phenotyping and genotyping methods. It is suggested that for abiotic stresses due consideration should be given for the phenotyping of plant phenology, early vigour, root traits, stomatal conductance, canopy temperature and stay-green trait, pollen viability, biomass, harvest index and grain yield. Chickpea breeders generally focus on selecting lines with adaptation to wider agro-climatic conditions. However, adaptation is dependent on the season, sowing date and water regime combinations. These combinations often affect phenological development of thermal time to flowering, duration of flowering, end of flowering and pod set, with accelerated development under late-sown and dry conditions (Sadras et al. 2016).

In chickpea, flowering and podding (reproductive and grain filling stages, respectively) are generally the most critical stages affected by adverse conditions. Conventionally, flowering is recorded visually based on the percentage of plants per plot (Mallikarjuna et al. 2017), which is subjective and prone to human errors. For this, an image-based phenotyping method can be opted to measure such qualitative traits

effectively as a replacement of the visual method. There are successful examples of HTP technology utilized to phenotype heading and flowering (Sadeghi-Tehran et al. 2017) of various crop species (wheat, maize, barley, rice, *Brassica*), thereby suggesting that the assessment of flowering time in chickpea by HTPP is very feasible and should be explored to avoid errors between scorers and days.

Early vigour is an adaptive trait for drought and chilling stress in chickpea (Croser et al. 2003). Several conventional methods to assess early vigour involve visual scores based on a pre-determined scale (Sivasakthi et al. 2017, 2018) or vegetative biomass harvest (Berger et al. 2004). Although effective, these methods are highly subjective and/or labour intensive and, thus, not suitable on large-scale field trials. A robust and rapid assessment of early vigour is offered by HTP technology using sensors or multispectral imagery in various grain crop species such as wheat (Kipp et al. 2014), barley (Di Gennaro et al. 2018) and field pea (Nguyen et al. 2018) and can also be utilised for boosting genetic gains in chickpea.

Talking about chickpea root traits, root length density, root volume, root depth and root mass play a critical role in drought and heat adaptation (Kashiwagi et al. 2015; Ramamoorthy et al. 2017). Several QTLs controlling root traits have been reported (Gaur et al. 2008a, b; Varshney et al. 2013a, b, c; Kale et al. 2015; Samineni et al. 2016). So far, the commonly utilized methods for characterizing root traits in chickpea such as polyvinyl chloride cylinder (PVC) growth systems (Varshney et al. 2013a, b, c), soil cores (Purushothaman et al. 2017), semi-hydroponic systems (Chen et al. 2017) and shovelomics (Burridge et al. 2016) with subsequent WinRHIZO imagery analysis are time-consuming and labour intensive. Advanced image-based root phenotyping methods such as X-ray computer tomography, magnetic resonance imaging, positron emission tomography and GROWSCREEN-Rhizo have shown a promise in chickpea germplasm improvement against drought and heat stresses as they simultaneously combine phenotyping of shoot and root (Tracy et al. 2020).

Stomatal conductance and canopy temperature (CT) are well recognized adaptive traits for terminal drought and heat tolerance in chickpea. Canopy temperature can be measured by handheld (Sivasakthi et al. 2017; Biju et al. 2018) or airborne (Rutkoski et al. 2016; Bian et al. 2019) thermal and hyperspectral imagery to screen crop genotypes for drought and heat adaptation. Stay-green trait is termed as the plant's ability to retain green leaves and photosynthetic activities for an extended period. Functional stay-green has shown positive association with deeper roots and cooler CT (the adaptive traits for heat and drought). Proximal and remote sensing technology using sensors and cameras can be a method of choice for HTP screening of stay-green phenotypes for different crop species (Blancon et al. 2019; Sadras et al. 2019) along with chickpea (Cai et al. 2016).

To assess the effect of heat, cold and drought stresses during reproductive growth, pollen viability is a key adaptive trait. Screening of pollen traits using standard microscopy is cumbersome, tedious and labour intensive. Nowadays with the help of advanced image-based phenotyping methods, automated quantitative analysis of pollen fertility has been made possible. Costa and Yang (2009) developed an image processing pipeline to effectively count the number of stained viable pollens from digital microscopy RGB images. A novel method utilizing Pollen Counter

software has been introduced by Tello et al. (2018) to successfully quantify fertile pollen grains within stained aliquots of pollen suspension under a microscope.

Any kind of stress has a direct effect on grain yield which is a function of biomass and harvest index. Therefore, they are most important targeted traits for phenotyping in any breeding programmes as it is an outcome of G × E interactions. High-throughput estimation for biomass is a typical approach in various crop species and can be conducted fairly straightforward by proximal and remote sensing tools (Araus and Cairns 2014). Normalized difference vegetation index (NDVI) is an inexpensive screening tool to capture physiological characteristics such as yield and crop growth rate in chickpea (Lake and Sadras 2016). Recently, airborne multispectral imagery has been deployed to evaluate yield potential in chickpea, where the mean NDVI was found to be consistently correlated to dry seed yield (Quirós et al. 2019).

Phenotyping for salinity by Vadez et al. (2015) utilized a high-throughput, 3D scanning technique to monitor leaf area development in relation to plant water use in cowpea and peanut. Several studies in cereals utilize high-throughput phenotyping technology under controlled environmental conditions to gain a better understanding of the physiological processes associated with salinity stress (Hairmansis et al. 2014; Pound et al. 2016; Rajendran et al. 2009). In contrast, similar studies examining salinity response in legume species have not been reported. Similarly, salinity response, measured as the effect of salt on growth rate at different developmental times, possibly could explain genotypic variation for salinity tolerance in chickpea. Atieno et al. (2017) utilized an image-based phenotyping platform (Fig. 20.1) to enable quantitative, non-destructive assessment of temporal responses of chickpea to salinity and related the responses to seed yield under saline conditions and proposed seed number as a selection trait in breeding salt-tolerant chickpea cultivars.

20.12.2 Phenotyping for Biotic Stresses

20.12.2.1 *Ascochyta* Blight

Chickpea production is limited by several biotic factors. One such biotic factor is *Ascochyta* blight (*Ascochyta rabiei*) disease in chickpea. To minimize the impact of *Ascochyta* blight, timely information on disease outbreak and epidemics is essential for the implementation of disease control methods. One such example for the utilization of HTPPs for AB screening is by Zhang et al. (2019). They studied the feasibility of monitoring *Ascochyta* blight disease severity in chickpea using remote sensing techniques. Disease severity was monitored using an unmanned aircraft system integrated with different types of sensors (three-band multispectral, five-band multispectral and thermal cameras). It was observed that different flight altitudes (60 m and 90 m above ground level) that lead to different image resolutions did not influence the disease detection efficiency, especially with the three-band camera. Hyperspectral sensing was found to be useful in predicting disease severity demonstrating that the disease severity of *Ascochyta* blight in chickpea can be monitored using remote sensing methods under active field conditions. With timely

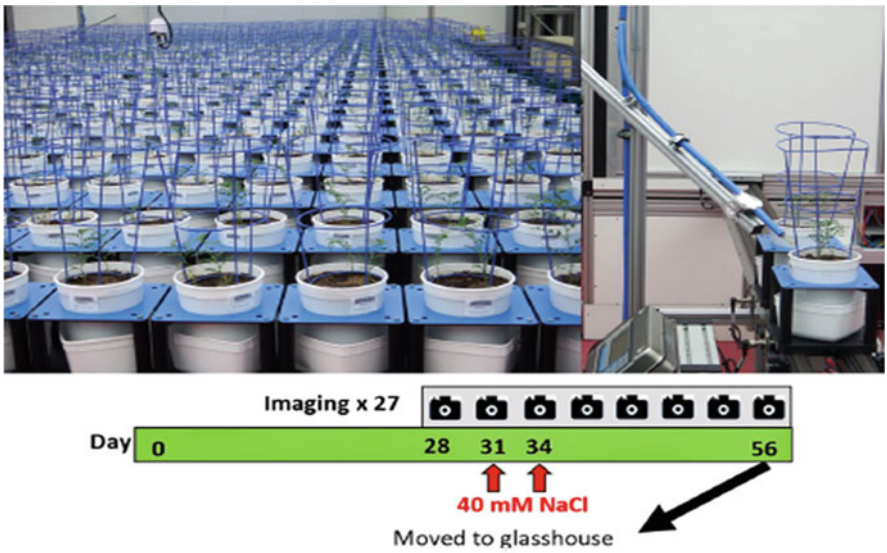

Fig. 20.1 Salinity tolerance phenotyping in The Plant Accelerator. Plants were imaged at 28 DAS for 3 consecutive days prior to 40 mM NaCl application in two increments over 2 days. Plants were daily imaged until 56 DAS. Right pane shows 6-week-old chickpea plants on conveyor belts leaving the imaging hall proceeding to an automatic weighing and watering station. (Adapted from Atieno et al. 2017)

and accurate disease severity information from high-throughput phenotyping technologies, the effects of *Ascochyta* blight on chickpea yield and quality can be minimized with timely application of appropriate management techniques.

20.12.2.2 *Phytophthora* Root Rot

Chickpea is grown as a rotational crop for its ability to fix atmospheric nitrogen through symbiotic fixation (Yadav and Chen 2007). Susceptibility of chickpea to soil-borne pathogens is a major limitation for the expansion of chickpea production area particularly owing to the depleting soil health. An *in planta* infection method to screen chickpea for PRR resistance was developed by (Amalraj et al. 2019) using hydroponically grown seedlings inoculated with *P. medicaginis* zoospore suspension culture. They utilized three chickpea genotypes for the initial pilot scale study, viz., Rupali (susceptible), Genesis 114 (moderately susceptible) and highly PRR-resistant breeding line 04067-81-2-1-1 (a backcross derivative from *C. echinospermum*). They conducted the experiment in a temperature-controlled growth room at the University of Adelaide, Waite Campus, South Australia, Australia, at 20/14 ± 2 °C day/night temperatures with a 16 h photoperiod. Covered plastic pots (4.5 L) were utilized to grow plants in continuously aerated nutrient solution.

The composition of the full-strength nutrient solution was as per buffered with 1.0 mM MES {2-(N-morpholino)ethanesulfonic acid} and adjusted to 6.5 pH using

Fig. 20.2 Phenotypic variation for PRR in chickpea grown in hydropnics at 9 days after inoculation with *P. medicaginis* zoospores. (**a**) Wilting symptoms (04067-81-2-1-1 on the left, Rupali on the right) of chickpea genotypes grown in hydroponics at 9 days after inoculation with *P. medicaginis* zoospores. (**b**) Root symptoms (04067-81-2-1-1 on the left, Rupali on the right). (**c**) Lateral and tap root death in Rupali. (Adapted from Amalraj et al. 2019).

KOH. Seeds were washed with commercial bleach (0.042% (w/v) sodium hypochlorite) added to de-ionized water for 5 min, rinsed twice in tap water and imbibed at 4 °C for 48 h. Imbibed seeds were germinated on mesh in 10% aerated nutrient solution in the dark for 3 days, and seedlings were then transferred to continuously aerated 25% nutrient solution and exposed to light. Each pot had one healthy individual from each genotype, and the pots and position of each genotype were set up in a completely randomized block design with six replicates in control (no inoculation) and in treatment (with inoculation) pots. *P. medicaginis* zoospore suspension culture was added to the treatment pots at a concentration of 1.5×105 spores/mL. Plants were examined daily after inoculation for PRR symptoms including canker development, chlorosis and wilting/death (Fig. 20.2). The experiment was terminated at 16 days after inoculation and repeated three times.

To visualize the progression of PRR disease over the duration of the experiments, the KME-survival was plotted, based on the initial observation of PRR symptoms after inoculation until the termination of the experiment. The experiment completion was based on the death of the PRR-susceptible parental genotype included in each of the RIL mapping population. To visualize the spatial progression of PRR disease across the experiment, heat maps were plotted for each RIL population to graphically display the KME-survival and canker length at the termination of the experiment.

20.13 Breeding Progress/Varietal Development

20.13.1 Conventional Breeding

Chickpea breeding in its nascence started with selection from either indigenous or exotic landraces. After selection, chickpea breeding grew to hybridization-based programmes with an aim to have an ideal genotype by combining useful traits from superior parents that invariably led to the narrowing of the genetic base. The breeding programmes consequently started aiming towards increasing the genetic variability by utilising diverse germplasm be it from primary, secondary or tertiary gene pool or by creation of new variation by mutation breeding. The detailed account of how chickpea breeding kick started in India is given below.

Systematic chickpea breeding started in 1905 at Imperial Agricultural Research Institute, Pusa (Bihar), followed by other centres focussing mainly on the collection of landraces. In the early 1970s, varietal development majorly emphasised on increasing yield potential over superior landraces. Henceforth most of the varieties like Dahod Yellow, Chaffa, Annegri-1, Ujjain 21, BR 78 and Gwalior 2 were developed directly by selection and purification of local germplasm or existing landraces. In the early 1980s, significant crop losses in northern states like Punjab, Haryana, North West Rajasthan and Jammu region were caused by the outbreak of *Ascochyta* blight. Focussed breeding efforts to develop Ascochyta blight-resistant/tolerant varieties for such areas subsequently brought about the release of high-yielding *Ascochyta*-resistant varieties like PBG 1, PBG 5, GNG 469, Gaurav, PBG 7 and GNG 2171. Therefore in the 1980s, breeding was mainly aimed towards disease resistance to cater AB problem in northern states and wilt problem in central and southern regions of the country. This led to the identification and development of disease-resistant/disease-tolerant donors/varieties with *Fusarium* wilt and *Ascochyta* blight resistance by following systematic breeding programme(s). This initiative led to the development of varieties with the potential to minimize wilt incidence such as KWR 108, H 82–2, GPF 2, Vijay, JG 11, Vishal, Gujarat Gram 1, Gujarat Gram 2, GNG 663, JG-16, KPG 59, Digvijay, Rajas, BGM 547, BGD 128, GNG 1581, etc.

In 1981–1982, two separate trials were initiated under All India Coordinated Pulses Improvement Project (AICPIP) for the evaluation of *kabuli* and *desi* genotypes. Then in 1982–1983, trials for *desi* chickpea were split into two categories, viz., normal and late-sown. This initiative led to the identification of JG 74 for central and northern Indian conditions, and simultaneously a special trial for screening against *Ascochyta* blight (AB) was also started. To identify and release high-yielding and large-seeded *desi* chickpea varieties, a new trial for 'bold seeded' types was constituted in 1983–1984 to fit pulse crop(s) in wheat rice cropping system and to gain back the chickpea area lost in the northern states due to the incidence of AB. A key drive was initiated to breed for short duration, multiple resistance, drought tolerance and high input responsive varieties in the 1990s. This was chiefly instituted to escape from terminal drought and heat for successfully raising a crop particularly for the environment(s) with short growing season. The drive for

breeding short-duration (90–110 days) genotypes directed the development of varieties like JG 16, JG 11, Vijay, Vikas, Vishal, JGK 1, KAK 2, ICCV 2, ICCV 10, etc., thereby expanding unexplored chickpea area in southern and central parts of the country.

In spite of the reduction in maturity duration, the yield potential remained almost similar to the long-duration varieties. Correspondingly, early maturing varieties amenable to late planting like Pusa 372, Udai, RSG 963, BGM 547 and Rajas were developed for the states like Uttar Pradesh, Bihar, parts of Chhattisgarh, Jharkhand, Haryana and Punjab after the harvest of rice or cotton, as fields are vacated quite late. Owing to the depleting soil health, two special trials for the evaluation of genotypes under high input conditions and for salinity tolerance were instituted in 1991–1992. Later in 1995–1996, a trial to evaluate breeding lines for drought was started with an aim to cater effects of global warming. Large-seeded *desi*-type varieties, Pusa 256, JG 11, Samrat, Phule G 5, Vishal and BGM 547, were developed and identified through coordinated testing system for large-seeded *desi* (>20 g/100 seeds) and *kabuli* (>25 g/100 seeds) varieties, implemented in 1983–1984 and 1995–1996. Likewise, large-seeded *kabuli*-type varieties, BG 1003, BG 1053, Haryana Kabuli Chana 1, Haryana Kabuli Chana 2, KAK 2, JGK 1, Vihar and Virat, were also developed.

Similarly, to address the location-specific problems, DCP 92–3, a wilt-resistant variety was released for the areas receiving frequent winter rains with high soil moisture and/or high fertility responsible for more vegetative growth followed by subsequently lodging of the crop. Later for the poor soils with moderate salinity levels, CSG 8962 variety for mild salinity conditions was identified for North-Western Plains Zone; for heat stress, JG 14 in central India and RSG 888 for rainfed conditions of Rajasthan, Haryana and Punjab were released to address moisture stress. Recently, new *kabuli* varieties, viz., HK 05–169, L 555 (GLK 26155), GNG 1969 and L 556 (GLK 28127), were released for northern Indian conditions, whereas cold-tolerant *kabuli* varieties such as CSJK 6 and Phule G 0027 were released for northern hilly regions and JSC 55 and JSC 56 for central India (late-sown conditions). Presently, due importance is being given for the development of extra-large-seeded *kabuli* chickpea (>40/100 seeds) varieties. Besides this, work is in progress for the development of varieties with more than 50 g 100-seed weight. Several promising lines are in advanced varietal trails, while a few varieties such as Phule G 0517, PKV 4-1 and MNK-1 have been developed with more than 50 g/100-seed weight to capture market for premium prices. The promotion of varieties among farmers is done through FLD's by SAUs and State Agriculture Department.

Currently with the hike in the labour wages along with labour shortage, the Indian farming community is gradually on the way of opting mechanization of field operations as it improves efficiency along with the reduction in the cost of cultivation. Besides this, there is an increase in the demand of machine-harvestable chickpea cultivars also. The chickpea cultivars available that are of semi-spreading and semi-erect growth habit with short to medium stature are not well suited for machine harvesting. The minimum standard requirement for the development of machine-harvestable chickpea cultivars is that the genotypes must have >70 cm

height with erect growth habit. NBeG 47, Phule Vikram, RVG 204 and BG 3062 are the few machine-harvestable varieties released recently for southern and central Indian conditions. Since the inception of AICRP (All India Coordinated Research Project) on chickpea, more than 210 chickpea varieties have been developed suitable for cultivation under different agro-climatic conditions of the country. The milestones in chickpea varietal development during the past 100 years as adopted from Kushwah et al. (2020) are given in Table 20.2.

AICRP on chickpea is currently emphasising on the collection, evaluation, characterization and utilization of germplasm for the development of the improved varieties. Partnerships with national and international institutions have been made to take the advantage of innovative knowledge in the frontiers like biotechnology, information technology, etc. Overall, there is a need to have dedicated research efforts for the development of irrigation and high-fertility responsive cultivars to regain back chickpea area in northern Indian states. With the alarming climate change-related issues, drought tolerance would be the most important trait for two-thirds of the rainfed chickpea area. Besides this, precise and efficient breeding efforts are required for the enhancement of resistance/tolerance to abiotic and biotic stresses to achieve yield stability. This would encompass novel methods to widen the genetic base of the breeding populations, genomics-assisted breeding, precision phenotyping, rapid generation advancement and efficient breeding data management system.

20.13.2 Genomics-Assisted Breeding

Amalgamation and usage of genomic tools in breeding practices for the development of superior lines with enhanced biotic or abiotic stress tolerance along with higher yield levels is termed as genomics-assisted breeding (GAB). Conventional breeding approaches were able to improve yield but still could not break the yield plateau and address the issue of narrow genetic base significantly. Efforts have been put forth at various international platforms to generate genomic resources. GAB tends to establish and utilize relationship between genotype and phenotype for the crop improvement including range of approaches, viz., genomics, transcriptomics and proteomics, for the identification of the molecular markers associated with traits of interest. This acts as a tool for breeders to predict phenotype from the genotype. ICRISAT along with its partners fast-tracked the development of genomic resources during the past few years. The genomic resources have been integrated with breeding via GAB and are making an impact on chickpea improvement (Pandey et al. 2016).

For the development of biotic or abiotic stress-tolerant genotypes with improved yield levels, GAB holds a promise, as it is one of the unconventional breeding approaches utilizing several genomic tools. As mentioned earlier GAB encompasses genomics, proteomics and transcriptomics for the detection of tightly linked molecular markers associated with economically important traits for the prediction of phenotype from the genotype. With the advent of NGS technologies, high-throughput genotyping has made possible to develop large-scale genome-wide

Table 20.2 Achievements made during the past 100 years in chickpea improvement

Year	Variety	Technique used/first of its kind/ adaptation
1926	NP 17, NP 25, NP 28 and NP 58	Direct selection
1940s	C12/C34 and type 87	Hybridization
1948	Chaffa	Wide adaptability
1960s	Annigeri 1	First variety for southern India
1960	C 104 C 235	First wilt-resistant Widely adaptable in northern India states
1969	GNG 114	First release through All India Coordinated Pulse Improvement Project (AICPIP)
1970	Radhey	Bold (large)-seeded variety for central India
1970	RS 11	Spontaneous mutation from RS 10
1976	L 144	First kabuli variety
1979	Hare Chhole	First green-seeded variety
1982	GL 769	First *Ascochyta* blight-resistant variety
1984	Pusa 256	First variety developed by *desi* × *kabuli* introgression
1985	Pusa 408, Pusa 413, Pusa 417	Mutation breeding
1985	Pusa 261	Tall variety from Russian tall donors
1992	KPG 59 (Udai)	First variety for late-sown condition by AICRP
1993	ICCV 2 (Swetha)	First short-duration *kabuli* variety
1994	Vijay	First drought-tolerant variety for rainfed condition
1998	DCP 92-3	First lodging-resistant variety for high input conditions
1998	CSG 8962	First salinity-tolerant variety
1999	JGG 1	First officially released Gulabi gram
1999	JG 11	First variety developed through polygon breeding
1999	KAK 2	First large-seeded *kabuli* variety
2002	RSG 888	First drought-tolerant variety
2003	Vihar	First large-seeded *kabuli* variety for South India
2005	Pusa 1088	First variety through interspecific hybridization
2008	IPCK 2002-29	Large-seeded kabuli variety for Central India
2009	MNK 1, Phule G 0517, IPCK02, PKV 4–1	Extra-large-seeded (>50 g/100-seed wt.) kabuli varieties
2011	JG 14	Heat-tolerant variety
2017	Andhra Pradesh (NBeG 47), Karnataka (GBM 2) and Maharashtra (Phule Vikram)	Machine harvestable
2019	Phule G 08108, JG 20016-24, BG 3062	Machine harvestable for Central India

(continued)

Table 20.2 (continued)

Year	Variety	Technique used/first of its kind/ adaptation
2019	BGM 10216 (drought tolerance) and MABC WR SA 1 (*Fusarium* wilt resistance)	Marker-assisted backcrossing (MABC)
2020	PBG8	First variety from *C. arietinum* × *C. judaicum*-interspecific hybridization
2020	Manav (*Fusarium* wilt resistance)	Marker-assisted backcrossing (MABC)

(Adapted from Kushwah et al. 2020)

markers. To pyramid traits of interest governed by several major genes/QTLs together in a specific genetic background, marker-assisted backcrossing (MABC) is the most preferred approach. Though the most preferred method of choice, MABC approach becomes less efficient in cases where characters are polygenically controlled. Consequently marker-assisted recurrent selection (MARS) has been considered as an alternate option for improving polygenic traits. Simultaneously, genomic selection (GS) or genome-wide selection approach adopted from human and veterinary sciences is emerging as a powerful approach for the selection of desirable progenies obtained from the desirable crosses (Jannink et al. 2010). To simultaneously identify as well as transfer desirable alleles from wild species or wild relatives into elite ones, advanced backcross QTL (AB-QTL) approach has been exploited to accumulate several superior alleles for tolerance to biotic and abiotic stresses from wild species. Successful utilization of AB-QTL approach has been used by Singh (2005) for the introgression of productivity and disease resistance traits from *C. reticulatum* into cultivated chickpea.

Genetic resistance in chickpea to *Ascochyta* blight is recessive, exhibiting complex inheritance patterns, and MABC approach has made it possible to unravel QTLs for resistance to AB. The successful example of MABC is from Tar'an et al. (2013) where in introgression QTLs for double podding and for resistance to AB have been simultaneously transferred in elite chickpea cultivars via continuous backcrossing of moderately resistant donors to susceptible but adapted cultivars. A stepwise MABC approach by Varshney et al. (2014a, b) is given for the development of *Fusarium* wilt (FW) and AB-resistant lines wherein two QTLs for AB and *foc* 1 locus for FW are incorporated into C 214 (elite cultivar). On undergoing three rounds of backcrosses and three rounds of selfing, 22 FW-resistant lines and 14 AB-resistant lines were generated (Varshney et al. 2014b), thereby resulting into the development of three FW-resistant lines and seven AB-resistant lines. This method has also been employed to introgress resistance against two races (*foc*2 and *foc*4) discretely along with the gene pyramiding for FW resistance to two races (*foc*1 and *foc*3) and two different QTLs conferring AB resistance in chickpea (Varshney et al. 2014b). Genes from five germplasm lines displaying FW resistance against *foc 2* race have been introgressed by SSR markers into the genetic background of an elite cultivar Pusa 256 (Pratap et al. 2017).

Similar efforts by various national institutes like ICAR-Indian Agricultural Research Institute (New Delhi), Punjab Agricultural University (Ludhiana) and ICAR-Indian Institute of Pulses Research (Kanpur) are underway for the transfer of FW and AB resistance into promising high-yielding chickpea cultivars. In addition, introgression of genomic regions has also been performed for drought- and yield-related traits. The genomic region responsible for drought tolerance, located on LG 4 and labelled as *QTL-hotspot*, is introgressed into an elite cultivar of chickpea, JG 11, using MABC approach (Varshney et al. 2014a). The introgressed lines have shown yield advantage of 24% under irrigated conditions and 12% under rainfed conditions. Furthermore, drought tolerance-related traits controlled by the LG 4 *QTL-hotspot* harbouring several drought tolerance QTLs have been transferred into elite chickpea cultivars via MABC approach (Thudi et al. 2014).

Coming to MARS, its competence depends on the total genetic gain attained following selection accuracy, selection efficiency and marker-trait associations along with the distribution of desirable alleles across the parents. In chickpea, for the accumulation of the required set of alleles against drought stress, MARS has been exploited on utilizing crosses, viz., ICCV 04112 × ICCV 93954 and ICCV05107 × ICCV 94954 (Thudi et al. 2014). Two crosses, JG 11 × ICCV 04112 and JG 130 × ICCV 05107, were attempted in chickpea with an aim to combine the desirable yield QTLs through MARS approach (Thudi et al. 2014). For this, overall 188 F_3 plants each from two crosses (mentioned above) were genotyped by SSR markers, while for phenotyping $F_{3:5}$ progenies were assessed at multi-locations. Association of genotypic with phenotypic data led to the identification of a few major and several minor QTLs related to yield and yield component traits. Based on the QTL information for several yield and yield-related parameters in F_5 progenies, four lines and three lines from JG 11 × ICCV 04112 and JG 130 × ICCV 05107 crosses, respectively, were selected for recombination cycle for several combinations of favourable alleles. The shortlisted lines were further subjected to two recombination cycles, and F_1 plants from both the crosses with favourable homozygous alleles for yield and yield-related traits were recognized and grown. Further, the selected F_1 plants were advanced to F_4 generation for further evaluation, thereby increasing the frequency and accumulation of favourable alleles for economically important traits following the number of MARS-based recombination cycles.

To utilise genomic selection (GS) approach, efforts are underway for chickpea yield improvement in the near future. This has been made possible by the availability of precise phenotyping techniques and large linkage disequilibrium (LD) blocks in chickpea breeding populations along with the availability high-throughput genotyping systems like DArT and SNP markers. ICRISAT has made pioneer efforts for the exploitation of GS approach in chickpea breeding by utilizing a set of 320 elite chickpea lines genotyped by DArT markers. Phenotyping was carried out at Patancheru and New Delhi for yield and yield-related traits. Six different statistical GS models were employed, utilizing phenotypic and genotypic data giving results with higher prediction accuracies (up to 0.91) for yield and yield-related traits (Roorkiwal et al. 2016). Higher prediction accuracies could be expected on inclusion

of G × E effects by GS approach by considering multiple variables simultaneously in chickpea breeding programmes (Roorkiwal et al. 2018). Additionally, an alternate way to increase the prediction accuracies is by incorporating information regarding the large-scale genome-wide significant markers obtained from the results of GWAS to diverse GS models (Li et al. 2018). GS models hold a promise in pre-breeding programmes as it will help in screening for the identification of introgressions (Crossa et al. 2017). Varshney et al. (2018) have outlined sequence-based breeding based on GS approach wherein sequencing at higher depth is suggested for all possible parental lines of a specific breeding programme. These founder genotypes can be sequenced for the development of GWAS approach or HapMap that could be further utilized for the selection of desirable superior parental combinations with higher frequency of favourable alleles.

If a greater number of lines are to be selected, then a large number of crosses have to be attempted followed by early-generation selection. GS can now be done on selected lines srepresenting the lines from such crosses by means of the training model developed from the germplasm set. Although not feasible for large-scale breeding programmes, the best genotyping platform for GS approach could be a fixed SNP array. Consequently, segregating F_6/F_7 populations could be sequenced at lower coverage using skim sequencing or 384-plex-based genotyping platform. Practical haplotype graph (PHG) could be developed via high-throughput genotypic data generated for parental lines and other available germplasm lines as PHG is helpful in the identification of SNP markers. By means of sequence-based approaches, such SNP markers can be evaluated using rhAmp-SNP genotyping technology or DArT-seq SNP genotyping technology. Thereby exploiting GS approach-based breeding programme(s) utilizing segregating populations and elite lines for the selection of genotypes with higher genomic estimated breeding values (GEBVs) will be quite useful.

20.13.3 Development of Transgenics

Genetic improvement, either by traditional or molecular methods, has been hampered by the limited genomic resources coupled with narrow genetic diversity in the elite gene pool. However, most intractable stresses like insect pest (gram pod borer, aphids, bruchids), weeds, drought, salinity and low methionine content in the seeds are challenging because breeding efforts for these traits are limited, due to cross-incompatibility and lack of resistant sources in the available germplasm (Acharjee and Sarmah 2013). Genetic engineering can provide a potential alternative to address the issue by employing genes available across kingdoms. Recent advances in the transformation and plant regeneration protocols for chickpea now mean that transgenic technology can be used to improve those traits for which adequate variability is not available in the primary gene pool. These include resistance to pod borer and other biotic and abiotic stresses, as well as sulphur-containing amino acids. In chickpea, the combination of recombinant DNA technology and plant tissue culture has paved the way for the creation of novel options for biotic stress management,

especially insect pests. Special attention has been focussed on insecticidal *Bt* expressing transgenic plants that provide tolerance to herbicides (Shelton et al. 2002). The development of transgenic chickpea lines showing resistance to *H. armigera* is considered as one of the best approaches to counter yield loss (Asharani 2009). The genes utilized for development of resistance against pod borer, bruchid, aphid, drought and salt tolerance, namely, Bt, alpha amylase inhibitor, ASAL, P5CSF129A and P5CS, respectively, are discussed by several researchers (Das et al. 2017; Kumar et al. 2018; Aggarwal et al. 2018; Bhowmik et al. 2019). Till date, confined field trials of transgenic chickpea for insect-resistant trait are reported; however, the efforts require constant support to bring forth viable transgenics, addressing all regulatory issues. Efforts should be directed towards development of a large number of transgenic lines, evaluate them in open-field conditions and identify elite event(s) with high trait efficacy. Efforts should also be initiated for targeted integration of transgene in genome using genome editing techniques (CRISPR/Cas9systems).

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Pigeonpea Breeding

21

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Abstract

In recent years, pigeonpea has witnessed a number of advances in genetic research and breeding. The availability of the modern genomic resources such as whole genome sequence provides the basis and validation of plant responses for future crop improvement. Genome maps and quantitative trait loci (QTLs) have provided the opportunity to relive the bottlenecks that previously hampered the breeding progress. Next-generation sequencing (NGS)-based trait mapping methods allow rapid identification of candidate gene(s) associated with the traits of interest. Growing sequencing data on multiple genomes and pangenome analysis unleashes opportunity for the discovery of large-scale structural variations (SVs) and the mining of superior haplotypes to accelerate breeding of climate-smart varieties. Refinements in our understanding of male sterility phenomenon in combination with molecular basis of heterosis hold promises to enhance gains from hybrid breeding. In this chapter, we discuss the advances in genomics and breeding of pigeonpea crop. Concerted efforts backed by the multidisciplinary research would lead to the development of superior varieties and identification of potential genetic resources for future use by the research community.

Keywords

Pigeonpea · Gene · Cytoplasmic male sterility · Genetic gain · Breeding

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_21

21.1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] also known as tur, arhar, and redgram is a protein-rich food legume. It is an integral part of food diet especially in Asia due to its affordability and protein-enriched seeds. It is used not only in human diets but also as fodder and feed for animals and fuel wood for the tribes and rural living, and it prevents soil erosion too. Pigeonpea is utilized in intercropping systems to avoid the need of nitrogen fertilizers and boost the nitrogen usage efficiency of the crop field system, as is the case with all members of the *Leguminosae* family.

From the last two decades of the twentieth century, the crop witnessed the major genomic initiatives and related research in pigeonpea. After these advances, understanding of these structural and functional aspects of the genomic regions in crop has helped breeders to deal with many constraints in faster and efficient manner. After the first decade of this century, development of various molecular markers helped breeder to identify the relatable traits, and marker-assisted breeding technologies could be employed since then. Identification of germplasm and resequencing of the many genotypes have led to the development of many elite varieties, which are used in backcrossing programs and development of mapping population. Not only cultivars but also wild breeding lines are identified with resistant genomic loci for many resistance traits against biotic and abiotic constraints. After the genome sequencing initiatives, the finding of candidate genes has been made easy by using functional and system biology approaches that further accelerated the genetic gains. Not only cultivars and landraces but also wild relatives are exploited under pangenomic studies to identify more resistant traits and find the more diverse traits available.

Till date, over 150 varieties are released in India (Naik et al. 2020). Despite many efforts, the expected yield gain from pigeonpea is not yet exploited (Varshney et al. 2012). Therefore, it is concluded that the efficiency of breeding system is required to be improved, and moreover a variety of systems suitable for different agroecologies within India need to be developed (Singh et al. 2017). Also, cytoplasmic male sterility (CMS) technology is used to produce CMS-based hybrid for exploiting grain yields in pigeonpea (Bohra et al. 2020a). Many recent advanced protocols are now being used to increase the genetic gain through genomic selection (GS), speed breeding (SB) or rapid genetic advancement protocols (RGA), and haplotype-based breeding (HBB) (Bohra et al. 2020b). All these methodologies are recently under observation and have been reported to deliver more yield gain in the farmer's field, and breeders are also focusing more towards the strengthening of the seed delivery system and agronomic practices. This chapter highlights about the pigeonpea origin, morphological characters of flowers, wild relatives, germplasm availability, varietal development, and molecular and genomic tools with efficient traditional and conventional breeding practices including high-throughput phenotyping platforms.

21.2 Origin, Domestication, and Its Wild Relatives

Pigeonpea belongs to the genus *Cajanus,* which is comprised of 32 species (Mallikarjuna et al. 2012). Among all the species, the genetic diversity studied by Kassa et al. (2012) has suggested that the *Cajanus cajanifolius* is the most probable progenitor of cultivated pigeonpea found in eastern India, including eastern state Odisha (Van der Maesen 1986). These two studies complemented each other and confirm that India is the country where pigeonpea has originated. Based on the Van der Masen's wild distribution map, it suggested that, apart from these southern origins, some eastern coast states such as Andhra Pradesh and Madhya Pradesh have some landraces and wild relatives exhibiting high level of genetic diversity, suggesting the domestication (Saxena et al. 2014). The processes of domestication strongly show severe bottleneck effect by representing 75% less allelic diversity than the wild species in the clade (Saxena et al. 2014). Pigeonpea archae-botanical findings are most abundant in India, with a few others coming from Southeast Asia. The Neolithic sites in Kalaburagi, Karnataka (Sanganakallu), and its border areas (Tuljapur Garhi in Maharashtra and Gopalpur in Orissa) show the abundance of pigeonpea and from there it is encountered with Europeans and dispersed in Southern African states prior to 1650 BC (Fuller et al. 2019). Within these domesticated processes, the species *C. kerstingii*, with haploid chromosome ($n = 16$), is found to be originated in Africa (Bohra et al. 2010).

The concept of the gene flow highlighting various gene pools was established by Harlan and de Wet in 1971. On the basis of the crossability barriers, primary gene pool (GP1), which produces fertile F_1 offspring, is comprised of all cultivars and landraces of *C. cajan.* Many early taxonomic studies suggested that the *Atylosia and Cajanus* are two different species, but Van der Masen later merged them under *Cajanus* (1986). The secondary gene pool (GP2) has wild relatives with many beneficial traits and may produce partially fertile hybrid in interspecific cross. GP2 has many species such as *C. scarabaeoides, C. reticulum, C. trinervirus, C. lineatus, C. latisepalus, C. cajanifolus, C. lanceolatus, C. sericeus, C. albicans, C. acutifolius,* etc. The tertiary gene pool (GP3) has species which are either not crossable with cultivated species or crossable with the aid of embryo rescue or tissue culture techniques. Some of the species from GP3 are *C. goensis, C. crassus, C. rugosus, C. cinereus, C. volubilis, C. platycarpus, C. mollis*, and *C. confertiflorus.* Some other related generas are *Rhynchosia, Dunbaria, Flemingia, Paracalyx, Eriosema, Adenodolichos, Bolusafra, Carissoa, Chrysoscias,* and *Baukea*, which are from the far reaches of the gene pool (GP4) but anyhow are connected during domestication process (Bohra et al. 2010).

The importance of the wild relatives cannot be ignored as the genetic base of the released varieties is mainly contributed by 48% of the top 10 ancestors of pigeonpea (Kumar et al. 2003). Many high yield traits and resistant genes against various constraints from biotic and abiotic category have been introgressed into the cultivars from these potential wild relatives in several other crops (Bohra et al. 2021a), which are further discussed under trait dissection. The undeniable fact is that domestication, allopatric and, sympatric speciation, polyploidization and inbreeding have all had

severe genetic bottleneck impacts on diversity during the course of evolution. In order to harness the beneficial genetic traits, breeders realized the importance of wild crops and targeted their germplasm conservation in gene banks (Varshney et al. 2021a).

21.3 Genetic Resources Available

In pigeonpea, the development of genetic resources started by conservation of the germplasm accessions, development of the reference genetic maps, transcripts assemblies, experimental population, genotyping platform, and markers associated with trait of interest.

21.3.1 Collection of Germplasm

Exploration is the process of gathering the diverse genetic germplasm accessions and preventing them for use in breeding programs in the form of core, mini core, and composite collections. According to the concept given by Brown in 1989, core collection is 10% of the entire collection and represents the maximum genetic diversity ~70% of the total alleles. Similarly, mini core collection reduces it to 10% of the total core collections. Due to its reduced size, mini core collection has provided an easy access to scientists, which further helped them to evaluate it across multiple locations for target traits, finally helping them to identify promising germplasm for future use. The core and mini core collections of pigeonpea are composed of 1290 and 146 accessions, respectively, developed by ICRISAT. Similarly, to explore diversity, a global composite collection was put forward which is comprised of 1000 accessions, from the mini core collection (146), mini core comparator (146), additional representative accessions from 79 clusters of core collection (236), control cultivars (4), 63 accessions of 7 wild species, diverse sources for biotic (77) and abiotic (16) stresses, promising germplasm accessions (59), released cultivars (16), and accessions with distinct morpho-agronomic traits (237) (Upadhyaya et al. 2008). In total, 94% were cultivated and 6% were wild relatives in composite collections and contain accessions that were collected in ICRISAT from 54 countries. Among all the collected accessions, 58.8% accessions were collected from Asia and 12.9% from Africa (Upadhyaya et al. 2011). In total, 38,735 accessions of pigeonpea have been collected at various institutes such as ICRISAT, NBPGR, etc. (Saxena et al. 2016).

21.3.2 Genome Assemblies

A draft map of genome was presented by Singh et al. (2012) using 454 GS FLX platform with mean read length of >550 bp with >tenfold coverage which successfully assembled ~511 mb of the genome. The genotype used was "Asha" (ICPL

87119), and 20 kb long fragments were generated by GS-FLX. In these studies, 237.2 Gb and 10.1 Gb sequencing data were generated on Illumina sequencing and GS-FLX sequencing platform, respectively, which was later on assembled on the Newbler GS de novo assembler. Further gene annotation was done using *Arabidopsis thaliana* gene models as a reference which identified 1213 disease resistance/defense response genes and 152 abiotic stress tolerance genes in pigeonpea. Using homology-based approaches, they identified a total of 1,127,729 REs in the pigeonpea genome covering a total of 326,671,068 bp of sequence. These approaches identified REs belonging to six major categories, namely, (a) LINEs including L1, R1, and RTE-BovB; (b) LTR-retrotransposons including LTR, *Caulimovirus*, Copia, and Gypsy; (c) DNA transposons including En-spm, Harbinger, hat-AC, hat-Tag1, hat-Tip100, Rc/Hiltron, MuDr, TcMAR-Pogo, and RC/Hiltron; (d) unclassified interspersed repeats; (e) simple tandem repeats; and (f) low complexity repeats.

Varshney et al. (2012) developed the genome assembly of "Asha" variety using next-generation sequencing (NGS) and Sanger-sequenced BAC (bacterial artificial chromosome) clones. Using platforms Illumina GA and HiSeq 2000 sequencing system, two libraries were formed: 11 small insert libraries (180–800 bp) and 11 large insert libraries (2–20 kb), with a set of 88,860 BAC end sequences generated by using Sanger sequencing platform. On getting the long reads, data was assembled using the assembler SOAP de novo with a contig N50 of 21.95 Kb, and the longest contig length was 185.39 kb. Total assembled genome represents 72.7% of the total genome, i.e., 605.78 M of the total 833.07 Mb of pigeonpea genome. Further, gene annotation was done using gene models, which resulted into the identification of 48,680 genes with 51.67% of the repeat elements in total genome.

In general, both studies have taken many steps to sequence the raw sequencing reads that was further aligned to form contigs and then calculated the amount of shared PE relationships between each pair of contigs, and then those scaffolds were assembled into the genome. The BAC end sequences used for mapping the sequence obtained a final super scaffold, and a genetic map of *Cajanus cajan* ICP 28 × *C. scaraboides* (ICPW 94) was created (Varshney et al. 2012). Varshney et al. (2010) utilized a scaffold of 51,606 kb, while Singh et al. (2012) used 4522 bp of the contigs in their N50 assembly. The completion of the genome has a significant contribution in various genetic resources, with a total of 309,052 SSRs markers and 28,104 SNPs in the study done by Varshney et al. (2012), whereas the study by Singh et al. (2012) has given 1,89,895 SSRs including di-peptide and hexa-peptide repeats. These genome studies will contribute in advancing the development of many other genetic resources and help breeders to develop elite varieties.

21.3.3 Transcriptomic Resources

As the second-generation sequencing technologies start developing, trait-specific genes were targeted in breeding programs. The importance of gene, their regulatory

mechanisms, and their participation in various biological processes came into light. Based on Sanger's sequencing, the first set of high-quality ESTs (9468) was developed that further served the identification of 19 and 20 genes responsible for fusarium wilt (FW) and sterility mosaic disease (SMD), respectively. Using Illumina and FLX/454, many transcript assemblies are being submitted with 21, 434 transcript assembly contigs (TACs) (Kudapa et al. 2012), 48, 726 TACs (Dubey et al. 2011), and 43, 324 TACs (Dutta et al. 2011). Another study by Sinha et al. (2015b, c) evaluated the genes expressed by crop under stress and validated the set of reference gene expressed under heat, stress, and drought condition.

Another study by Pazhamala et al. (2017) represented the global expression analysis of the gene in pigeonpea during its all developmental and reproductive phases. This study may have worked as the bridge to fill the gap between whole genomic sequence information and crops phenotype. *Cajanus cajan* gene expression atlas (CcGEA) is comprised of 28,793 genes developed by sing RNA sequencing that highlights all the genes expressed during developmental growth phases, seed formation, pollen pistil interaction, and fertilizing conditions. The network analysis of these genes identified 28 hub genes and 3 highly connected genes for flower-related trait in pigeonpea. Exploring genome sequences in combination with pangenomic studies will refine and upgrade the expression atlas for future high-throughput breeding programs (Bohra et al. 2020a).

21.3.4 Experimental Populations

Previously, researchers found that using a biparental population resulting from the crosses between two inbred lines had a high power to detect quantitative trait loci (QTLs) as all allelic frequencies are typically close to 50%. Apart from that fact that many studies rely upon mapping QTLs in biparental population, they lack mapping precision due to limited effective recombination, low genetic diversity and genetic bottlenecks effect caused by choosing only two founder parents. This may limit the number of QTL captured as no more than two alleles segregate at any locus. Recently, the two most popular designs are employed in plants NAM (Nested association mapping) and MAGIC (multi-parent advanced generation inter-cross) populations. NAM is a set of biparental populations all linked by a common parent, whereas MAGIC is descended from 4, 8, or 16 parents consistent with a simple funnel breeding design. MAGIC population usually inherits alleles from all parents and arranged in random mosaic. By increasing genetic recombination frequency and variations, these designed populations can overcome limitation of biparental mapping populations (Scott et al. 2020). MAGIC population available in pigeonpea involves eight founder parents with seven funnels for mapping various genes.

21.3.5 Whole Genome Resequencing Approaches

From whole genome resequencing (WGRS), Varshney et al. (2017) resequenced 292 accessions including landraces, breeding lines, and wild relatives, and the obtained data were mapped into the reference genome of "Asha" that helped breeders find many structural variations (SVs). The SVs help breeder to understand the diversity in the species and understand how many important agronomic traits are being ignored leading to inbreeding depression in the population. In the study, the researchers comprehended that the extent of genetic bottleneck during domestication was more intense than the moderate level observed from landraces to breeding lines. Similarly, the first-generation haplotype map of pigeonpea is based on WGRS data of 20 *Cajanus* accessions which revealed 5.5 million of variants including 4.6 million of the SNPs and 0.7 million of InDels along the structural variations such as CNVs and PAVs (Kumar et al. 2016).

21.4 Floral Morphology and Pollination

Pigeonpea crops have found to be successfully grown between 30°N and 30°S latitude, with a temperature range from 29°C to 36°C. The growth seems to be slow during initial days, depending upon the different maturity groups, and then it becomes large bushy by its flowering time. Depending upon the days to flowering, the different maturity groups are divided at 17°N at ICRISAT which is categorized into super early (<50 days to flowering), extra early (51–60 days), early (61–100 days), medium (101–140 days), and late (141–160 days). Wallis et al. (1981) suggested that the trait of earliness is linked to photoperiod insensitivity which is found to be true.

Pigeonpea forms two types of plant types, viz., determinate (DT) and indeterminate type (NDT). The determinate inflorescence forms somewhat corymb shape bunch, whereas the indeterminate forms a terminal panicle. The peduncle in DT is almost 1–8 cm long and flowers are clustered at the top end. An individual flower is comprised of a calyx with five sepals, and a corolla with a standard, two wings, and two keel petals. In total, flower has 10 stamens in diadelphous condition, 9 fused in a column, and 1 free, and the ovary consists of 2–9 ovules. Flowers are normally yellow but could be in shades of yellow such as ivory, light yellow, yellow, and orange yellow. Streaks found in petals could be red or purple and ranges from no/sparse streaks to dense/uniform coverage of streaks (Sameer et al. 2017). Pigeonpea is a daylight plant; therefore its inflorescence development and pod setting are greatly influenced by the availability of light. No pods are set under the dense crop canopy. Dry, bright days are favorable for fertilization, while cloudy, damp weather results in excessive flower drop (Howard et al. 1919; Mahta and Dave 1931). Precise information on the quality, level, and the duration of light required for fertilization and seed setting is lacking.

The flower found in pigeonpea is cleistogamous which is further supported by the evidences provided by Saxena et al. (1992a, b). The study stated that the keel part

usually surrounds the part of standard wing petal which causes a considerable delay in opening of buds. Therefore, even in an open flower the standard unwraps itself but the wings still remain enclosed within the keel resulting in partial cleistogamy (Sultana and Saxena 2019). Cleistogamy promotes self-pollination, whereas in pigeonpea this statement is ruled out as a considerable amount of out-crossing is found. Hence, pigeonpea is a well-known example of often-cross-pollinated crop which ranges from 3% to 40% of out-crossing.

From the report submitted by Onim (1981), pollen in pigeonpea is shed when the flower is intact in bud and germination is not seen until the flower starts to wither, i.e., 24–48 h after their anther dehiscence. Dutta and Deb (1970) studied the pollen tube growth in a style pollinated with pollen from the same flower and observed it to be very slow, taking 54 h to reach the base of the ovary. Those two mechanisms provide a sufficient time gap for the foreign pollen to be introduced on to the stigma by insect pollinators before self-fertilization takes place. The study by Saxena et al. (1990) and Onim (1981) observed the entomophily in pigeonpea to support many previous studies such as at ICRISAT Center by Williams (1977) which counted between 5500 and 107,333 pollen grains on single *Xylocopa spp.* and *Megachile spp.* of insect pollinators, of which pigeonpea pollen accounted for 98–100%. On this basis, it is found to be beneficial for broadening the genetic base of the segregating pigeonpea population.

To study pollination, it was observed that anthers dehisce a day before flower opens. Anthesis starts from 06.00 h. and continues till 16.00 h, and the peak was observed between 09.00 and 10.00 h and varies from 6 to 36 h. Pollination takes place with the help of pollen of the same flower, and it requires 54 h to reach to the ovary, and also the pollen tube is grown within the style (Dutta and Deb 1970). As pigeonpea is a daylight plant, full-day sunlight works in favor of the anthesis (Sharma and Green 1977). Temperature also plays a major role in controlling the fertility as higher temperature can cause sterility (Saxena et al. 2014); therefore environmental conditions ought to play a major role in fertilization.

21.5 Cytological Studies

The nuclear variation in *C. cajan* was studied through cytological research and other related wild species (20). The study supported that all the species have $2n = 22$ as stated in the earlier reports too (Dundas 1990). According to Ohri and Singh's (2002) research, the *C. cajan* and *C. cajanifolius* are remarkably similar; their origin stories supported the fact. The similarity found in respect to the number and morphology of satellite chromosome in both species. This study further helped breeders to understand the chromosomal abnormalities and possible cross-abilities among these related wild species. Under such observation, hybrid of *C. cajan* with *C. cajanifolius* and *C. lineatus* shows a normal meiosis, and *C. cajan* crossed with *C. lineatus* shows reduction of pairing in interstitial and terminal regions and two heteromorphic bivalents (Dundas 1990). Therefore, breeders could find the success

of breeding programs between interspecific cross of *C. cajan* and its related wild species for some beneficial traits.

Another cytological study attempted to understand cytoplasmic male sterility (CMS). Among all the CMS systems, only A_4 derived by crossing *C. cajanifolius* has been further used for hybrid production. To understand this sterility and fertility transition in near isogenic lines, cytological studies were performed which helped researchers to understand the sterility in pollen which is caused by the restricted supply of nutrients and energy (Dalvi et al. 2008; Pazhamala et al. 2020; Bohra et al. 2021b, c, d).

21.6 Quantitative and Qualitative Traits in Germplasm Collection

The germplasm collection of pigeonpea with 11,402 accessions was analyzed to study the diversity pattern in 14 qualitative and 12 quantitative traits (Upadhyaya et al. 2005). Semi-spreading growth habit, green stem color, indeterminate flowering pattern, and yellow flower color were predominant among qualitative traits. Other qualitative trait is growth habitat having three categories being compact, spreading, and semi-spreading, with semi-spreading being the most heterozygous characteristic (84.22%) followed by the compact (13.22%) type. Plant pigmentation, flowering pattern, color, streaks, streak pattern, seed color, pod shape, and seed eye color were all categorized under qualitative characteristics with their diversity explored in 56 diverse countries (Upadhyaya et al. 2005). The rest of the characteristics such as 50% days to flowering, number of primary and secondary branches, days to 75% maturity, plant height, seed per pod, seed weight, protein content, shelling, and harvest index were accountable under quantitative traits.

21.7 Breeding Efforts for High-Yielding and Disease-Resistant Cultivars

Since the pigeonpea breeders realized the importance of systemic breeding programs for developing varieties/hybrids that are more resistant to biotic and abiotic stresses, various varieties/hybrids have been released for farmer's cultivation. Previously, when constraints were found while increasing the yield in pigeonpea, breeders adopted various methods to increase the yield such as pure line selection from germplasm followed by pedigree breeding and mutational breeding. Using the same approaches, 150 varieties were released since 1960–2018, 89 were developed by pedigree selection, 57 by pureline selection, and 3 by mutation and population improvement breeding programs (Naik et al. 2020). Earlier approaches involved the collection and selection of landraces from the farmer's field. Later on, these landraces were advanced by using pedigree method, and some of the varieties produced by the same approaches are well known as C11, T7, BDN1, Bahar, NA1, ICP8863 (Maruti), ICP7035, etc. (Bohra et al. 2017a, b). Many other varieties

were maintained for their resistance genes such as, IPA8F, IPA9F, IPA16F, and ICP7035 for both fusarium wilt and sterility mosaic disease, KPL 44 for fusarium wilt, ICPL366 for sterility mosaic disease, ICP7105 for *Alternaria* blight, PB 9 for *Phytophthora* blight, IPAC79 for water logging tolerant, and many more.

Many varieties were developed by mutational breeding approach using chemicals such as ethyl methane sulfonate ($C_3H_8SO_3$), fast neutrons, and gamma rays, which were found successful in creating useful variability. Mutational breeding has given some remarkable varieties against traits such as yield, seed size, earliness, and some biotic constraints. These varieties (viz., Co 3, Co 5, TT5, TT 6, and TAT 10) have been bred through mutagenesis. Among these varieties, Co3 is produced by EMS, and Co 5 by gamma rays, and TAT 5 and TAT 10 were produced by fast neutrons. One of the desirable traits in all geographical regions is earliness, plant type (determinate and indeterminate), and protein content. Breeding of early maturating lines was identified first when the late maturating group landraces were grown in Gorakhpur, Uttar Pradesh, and spontaneously identified an early maturating named as T-1 which leads to development of T-21 by crossing T-1 with T-190 in Kanpur. Subsequently, a few more extra early maturating cultivars including Co 2 from PB 4278, Hy 2 from PI 4628, Hy 4 from PI 4839, Hy 5 from PI 3701, Co 4 from S 80, AL 15 from 809, Pusa 855 from T 21, Pusa 992 from ICPL 90306, AKP 1 from ICPL 87101, and CORG (RG) 7 from PB 9825 were also developed through the selection of spontaneous mutants. Recently, transgressive segregation selection is also used to produce such high-yielding varieties.

21.8 Pigeonpea Constraints and Breeding Approaches

21.8.1 Biotic Stresses

Major biotic diseases in pigeonpea are fusarium wilt (FW), sterility mosaic disease (SMD), and phytophthora blight.

21.8.1.1 Fusarium Wilt

It is a major biotic constraint governed by single dominant gene which results in yield loss from 30% to 100%. First completely resistant variety against wilt is (Maruti) ICP 8863 and then ICPL 87119 (Asha), which were produced in India. Wilt can be diagnosed by symptoms such as loss of turgidity, slight interveinal chlorosis, browning of xylem vessels, and purple band on the stem. The pathogen which causes fusarium wilt is *Fusarium udum.* One of the greatest approaches targeted by breeders to overcome this problem was to identify the potential resistant germplasm, including its wild relatives. Many other varieties were identified in ICRISAT with potential germplasm such as ICPL 20109, ICPL 20096, ICPL 20115, ICPL 20116, ICPL 20102, and ICPL 20094 (Sharma et al. 2016). This approach was limited till conventional breeding approaches. Later, using the modern NGS-based technologies, genotyping by sequencing (GBS), leads to the discovery of 3 QTLs, qFW11.1, qFW11.2, and qFW11.3, from three populations, namely,

PRIL_B, C, and F_2. Similarly, by using another approach Seq-BSA (sequencing-based bulked segregant analysis), resistant (R) and susceptible (S) bulks from RIL population derived from crossing ICPL 20096 × ICPL 332 were sequenced. SNP index was calculated between R and S-bulks by using draft genome of a resistant parent ICPL 20096. The result provided with seven candidate genes for FW and SMD. Further, on the same study, in silico protein analysis has revealed two promising candidate genes *C. cajan*_03203 for FW resistance that could be further used in genomic-assisted breeding programs (Singh et al. 2016a).

21.8.1.2 Pigeonpea Sterility Mosaic Virus (PPSMV)

A species of the genus *Emaravirus* also known as "green plague" of pigeonpea is characterized by sterility of flower and mosaic-like appearances over the leaf. *Sterility mosaic disease* (SMD) induces symptoms like stunted and bushy plants, leaves of reduced size with chlorotic rings or mosaic symptoms, and partial or complete cessation of flower production (i.e., sterility). The causal agent of the disease is PPSMV, a virus with a segmented, negative-sense, single-stranded RNA genome, transmitted in a semi-persistent manner by an eriophyid mite *Aceria cajani* Channabasavanna (Acari: Arthropoda). Both the virus and the vector are highly specific to pigeonpea and a few of its wild relatives, such as *C. scarabaeoides* and *C. cajanifolius*. SMD is controlled by four independent loci, two duplicate dominant gene (*Sv1 and Sv2*), and two recessive (sv3 and sv4) genes. Expression of SMD is noticed only when one dominant allele at loci 1 and 2 and homozygous recessive genes at loci 3 and 4 are present.

As explained, SMD virus is transmitted by a mite; therefore, application of spray against the mite has controlled mite population thus limiting the spread of the disease. Apart from these conventional methods, introgression breeding through genomic-assisted breeding programs would be an important strategy for the development of disease-resistant varieties. Genotyping by sequencing approach was used for simultaneous identification and genotyping of SNPs, and the candidate genomic region identified on CcLG11 was the promising QTL for molecular breeding in developing superior lines with enhanced resistance to SMD (Saxena et al. 2017a). Six QTLs explaining phenotypic variation were identified on LG7 and LG9 after extensive phenotyping for SMD resistance (Gnanesh et al. 2011). An approach discussed above for *Fusarium* wilt by Singh et al. (2016a) also identified seven SNP related to both FW and SMD, and protein expression profiling identified another candidate gene, namely, *C. cajan*_01839 for SMD resistance.

21.8.1.3 *Phytophthora Blight* (PB)

Another major constraint in pigeonpea which results in 100% yield loss even under a favorable environment is PB. The causative agent, *Phytophthora cajani*, was first isolated from India from infected pigeonpea plants with stem canker symptom. The disease is easily identified with characteristics such as lesion on stem (dark brown or black) and leaf, stem broken at the point of infection initiation, stem cankers and galls, and eventually mortality. It is a soilborne fungi which is mostly found as dormant mycelium in soil and infects plant debris. It is controlled by a dominant

gene known as Pd1. This fungus holds sporadic in nature, but in the places of heavy rainfall, it becomes epidemic in proportions. Only conventional method of selecting resistance germplasm screened in sick plots is the best way for identifying resistance source for PB (Singh and Chauhan 1992).

21.8.1.4 *Helicoverpa armigera* and *Bruchids*

They are the most devastating insects' pest for pigeonpea since ages. Conventional breeding alone cannot be the solution of this problem, as the resistance source is not yet found in any germplasm. Gene pyramiding with two different insecticidal gene and tissue-specific expression of a chimeric cry1AcF (encoding cry1Ac and cry1F domains) gene in transgenic pigeonpea has been demonstrated towards resistance to *H. armigera*. Apart from this, an advanced generation population from the cross utilizing "*Cajanus acutifolius*," a wild relative from the secondary gene pool as the pollen parent, has shown considerable resistance *for pod borer damage* (Mallikarjuna et al. 2012). Therefore, many insect resistance genes are exploited from the wild relatives mainly from secondary gene pool. Some of the lines produced by crossing *C. acutifolius* showed high level of resistance to *pod borers, pod fly, and bruchid* under unprotected field conditions. Bruchid resistance is an important trait for pigeonpea seeds under storage as resistance to the pest has not been observed in cultivated pigeonpea. Another species from the secondary gene pool, *C. lanceolatus*, showed resistance against bruchids. Bruchid growth and survival was inhibited in the lines derived from *C. lanceolatus*. Some of the lines showed delayed bruchid growth and delayed life cycle, thus showing antibiosis mechanism of resistance to bruchids. Lines were screened for protein content, and some of the lines showed higher protein content than both their parents. Further, biochemical analysis showed higher content of proteinase inhibitor activity in some of the lines.

21.8.2 Abiotic Stresses

Major abiotic stresses in pigeonpea are water logging, drought, and salinity. As researchers are targeting the enhanced yield of pigeonpea, the prevailing abrupt climatic changes are putting checks to the progress towards self-sufficiency.

21.8.2.1 Drought

It is the water limiting condition where the plants experience the shortage of moisture to complete its life cycle. Drought can occur at any stage of crop development, and it mostly depends on onset time, intensity, and duration of water scarcity. Despite pigeonpea having deeper root system to withstand considerable level of moisture stress, it poses penalty on grain yield. Long-duration pigeonpea needs adequate water, but even in short-duration varieties, yield is affected due to water stress. Stress leads late flowering and hinders early pod development. Since drought is a complex trait, a candidate gene could be responsive under such stress. The study by Sinha et al. (2016) showed upregulated gene under drought stress condition.

Three genotypes ICPL 151, ICPL 8755, and ICPL 227 showing different responses under drought condition were analyzed by expression profile of universal stress protein domain and later validated by q-RT PCR which revealed 6, 8, and 18 to be >wo-fold differentially expressed in CPL 151, ICPL 8755, and ICPL 227, respectively. From the total 10 differentially expressed genes including plant U-box protein (four genes), universal stress protein A-like protein (four genes), cation/H(+) antiporter protein (one gene), and an uncharacterized protein (one gene) were abundantly expressed under stress condition.

Genes *C. cajan_29830* and *C. cajan_33874* belonging to *uspA* were found significantly expressed in all the three genotypes with ≥two-fold expression variations. These two candidate genes were specific to this crop only; thus they confer drought tolerance specifically to pigeonpea. Drought is the only trait whose haplotype has been investigated (set of gene inherited together by non-random segregation), and haplotype-based breeding is the ultimate option when using a whole genome resequencing approach. By using WGRS data of 292 genotypes from pigeonpea, 10 drought responsive candidate genes were identified. Further on, using marker trait association and haplo-pheno analysis, three genes were identified regulating five component drought traits. The haplotype *C. cajan*_23080-H2 for plant weight (PW), fresh weight (FW) and turgid weight (TW); the haplotype *C. cajan*_30211-H6 for PW, FW, TW, and dry weight (DW); the haplotype *C. cajan*_26230-H11 for FW and DW; and the haplotype *C. cajan*_26230-H5 for relative water content (RWC) were identified as superior haplotypes under drought stress condition.

21.8.2.2 Water Logging

Pigeonpea often encounters overflooding at seedling stage, especially in low-lying areas. Upon exposure to submergence for 3–4 days, the crop experiences hypoxia with considerable injury to roots, eventually causing death of the plant. Further, the weather conditions during seedling stage, such as intermittent rains and moderate temperatures of 25 ± 1°C, favor the occurrence of phytophthora stem blight (PSB) and the ensuing diseases causing significant loss in crop yield. Recently, ICAR-IIPR, Kanpur, have identified a pigeonpea genotype, namely, "IPAC 79," showing remarkable tolerance against both water logging and PSB based on long-term observations. The genotype IPAC 79 is an advance line derived from the cross "Bennur local/BRG 1." It was found to be tolerant up to 96 h of waterlogging, when the screening conducted after 20 days of seedling stage. Under waterlogged conditions, the genotype showed 53.8% survival as compared to only 0.6% survival in sensitive line (ICPL 7035), and 11.2% and 18.6% survival in national check varieties, viz., Bahar and NDA 1, respectively. It is important to note that IPAC 79 was also resistant to PSB. Scientists at ICRISAT have developed the advanced generation derived out by crossing with the wild *C. acutifolius* lines showed growth in stress condition. Formation of lenticels marked waterlogging situation, and this region gives rise to roots which enter the soil through the water surface.

21.8.2.3 Salinity

Salinity hinders the growth due to increased salt concentration and accumulation of those in soil (Jha et al. 2019). Higher $NaCl/Na_2SO_4$ content in the soil affects crop yield physiologically. Salinity delays days to 50% flowering by 1–2 weeks and prolongs the peak period of flowering and reduces the number and weight of the seeds. The wild relatives of pigeonpea, *C. scarabaeoides, C. albicans,* and *C. platycarpus,* showed a wide range of variation in their salinity tolerance. The transfer of salinity tolerance from *C. albicans* to *C. cajan* would be feasible as the high level of salinity tolerance in this wild species is expressed as a dominant genetic trait (Choudhary et al. 2011).

21.8.3 Yield

To understand the yield, many agronomic traits such as days to flowering, maturity, relative water content, electrical conductivity, seed protein content, seed weight, high biomass, and water uptake traits were targeted for the QTL identification (Bohra et al. 2020a; Obala et al. 2020). The study uses the interspecific cross between *Cajanus cajan* (L.) Millspaugh acc. ICPL 20340 and *Cajanus scarabaeoides* (L.) Thouars acc. ICP 15739 resulting in higher phenotypic variance which suggested the major contribution made by wild parent.

Further, using SSR marker partial linkage map with 83 loci, 15 maker trait associations of 4 different linkage groups were identified, i.e., LG01, LG02, LG04, and LG05. Their study reported genetic variation for six seed traits including seed length (SL), seed width (SW), seed thickness (ST), seed weight (SWT), electrical conductivity (EC), and water uptake (WU), which resulted in the identification of 2, 3, 3, 2, 1, and 4 QTLs, respectively (Bohra et al. 2020a). Similar approach used by Obala et al. (2020) with SNP genotyping identified 192 main effect QTLs and 573 epistatic QTLs, and phenotypic variance from 0.7% to 91.3% and 6.3% to 99.4%, respectively, were detected. Major effect (PVE $\geq$ 10%) of M-QTLs included 14 M-QTLs for seed protein content, 16 M-QTLs for seed weight, 17 M-QTLs for seed yield, 19 M-QTLs for growth habit, and 24 M-QTLs for days to 50% flowering, and these QTLs were mapped and genotyped using GBS approach in 5 different populations between CP11605 $\times$ ICP14209, ICP8863 $\times$ ICP11605, HPL24 $\times$ ICP11605, ICP8863 $\times$ ICPL87119, and ICP5529 $\times$ ICP11605.

21.9 Hybrid Development and Heterosis Vigor

The constantly increasing gap in the demand to supply for pigeonpea had been the matter of concern to the pigeonpea researchers in India. The cross-pollination behavior of pigeonpea is a desirable to develop and establish the hybrid system to exploit the commercial heterosis. Keeping this in view, pigeonpea research was directed towards a new initiative on hybrid pigeonpea breeding at ICRISAT, Hyderabad, immediately after the identification of male sterile line in 1974, which

in turn led to the development of new GMS hybrid called ICPH 8 in 1991 which resulted in 31–40% superiority in farmer's field in the central zone (Saxena et al. 1992a, b). Then after five GMS hybrids (PPH4, CoPH1, CoPH2, AKPH4101, and AKPH2022), the early maturing groups were released by the state and central varietal release committee. Nevertheless, the GMS-based hybrids did not yield much success due to difficulty in the production of commercial F_1 seed.

The bottlenecks of GMS system led to the development of stable and economic CGMS system in pigeonpea. The first CMS line in pigeonpea was made by Reddy and Faris (1981) using wild relative of pigeonpea, *C. scarabaeoides*. Previously, pigeonpea was believed to be a self-pollinated crop, and natural out-crossing was treated as a constraint. The first CGMS line GT 288A and its maintainer B line were registered by Pulse Research Station, SDAU, GAU, SK Nagar, Gujarat, in 2000. Consequently in 2006 the first CMS hybrid GTH 1 was developed by SDAU, Gujarat, and released by CVRC for cultivation in the central zone. Subsequently, ICRISAT released its first CMS hybrid ICPH 2671 in 2010 (Saxena et al. 2013). There are 39 CGMS lines that have been registered with ICAR-NBPGR and five CMS-based hybrids (ICPH 2671, ICPH 2740, ICPH 3762, IPH 15-03, and IPH 09-5) that have been released for cultivation as of today.

After the failure of the GMS, CMS came into light with the first concept of hybrid seed production technology that harnesses the non-functionality of pollen resulting from the impaired harmony between the nuclear and cytoplasmic genomes (Bohra et al. 2016; Mishra and Bohra 2018). Mitochondrial genes have been reported in different crops that confer CMS trait. The fertility in CMS system is rescued because of fertility-restoring elements located in the nucleus. Therefore, this system could also be known as cytoplasmic-genetic male sterility (CGMS). In pigeonpea and many other crop plants, Bohra et al. (2016) reviewed the progress of mapping sterilizing cytoplasmic elements and fertility restoration loci by the use of NGS-based technologies. Many other crops tend to be discovered by the molecular mechanism using the omics-based approaches in sterilizing cytoplasm. The three-line system used in pigeonpea has three components and the genotype carrying sterilizing (mitochondria derived) and *Rf* (nucleus encoded) factors that are referred to as sterile (A), restorer (R) line, and third line "maintainer" B line. The maintainer (B) line is essentially required to retain the sterility status in pollen; both A and B line are isogenic which differ only in sterility-inducing cytoplasm. In simple words, A × B cross regenerates the genetic constituent of A line, but B line is devoid of any *Rf* gene. Now, A × R cross is used to restore fertility; thus, this explains improved heterosis in the hybrid.

It has long been assumed that wild germplasm was used to introduce sterility system (Bohra et al. 2021b, c). The very first hybrid was also produced by crossing with *C. scarabaeoides*. So far, nine such systems, namely, *C. sericeus* (A1), *C. scarabaeoides* (A2), *C. volubilis* (A3), *C. cajanifolius* (A4), *C. cajan* (A5), *C. lineatus* (A6), *C. platycarpus* (A7), *C. reticulatus* (A8), and *C. lanceolatus* (A9), have been reported in pigeonpea with varying degree of success (Singh et al. 2017; Saxena et al. 2017a; Varshney et al. 2017; Sameer et al. 2017). Out of these, A4 cytoplasm with gene *nad7a* (Sinha et al. 2015a; Bohra et al. 2021d) has been

promising because of its stability under various agro-climate zones and availability of good maintainers and restorers.

Another form of sterility is conferred in pigeonpea which can be reversed to fertility or vice versa. Various environmental factors such as photoperiod (PGMS) and temperature (TGMS) alter the gene expression causing sterility and fertility transition. This is also known as two-line system where the temperature above critical temperature (25°C) causes sterility. This system is known as temperature genetic male sterility (TGMS) in which temperature above 25°C is considered a restrictive condition (RC) and causes sterility and below 25°C is known as permissive condition (PC). The temperature-sensitive selections were made in advanced generations of the populations derived between *C. cajan* (ICPA 85010) as female parent and *C. sericeus* as the pollen donor.

The concept of hybrid vigor or *heterosis*, first introduced by Shull in 1914, explains the tendency for the progeny of parents to exceed the mid-parent (average of the two); in simple words F1 hybrid is superior to its parents. Heterosis can be estimated in three ways: mid-parent heterosis (MPH), better parent heterosis, and standard heterosis. Mid-parent heterosis (MPH) can be explained by the formula (F1-MP) ÷ 100 × MP, where MP is the mean of two parent and F1 is the mean of F1. Therefore, heterosis is measured over mid-parent which is the average value of two parents. Superiority over parental lines in the reference of the many yield-related quantitative traits such as seed weight, days to 50% flowering, days to maturity, plant height, etc. was used to identify hybrid superiority. Molecular mechanism to understand this phenomenon of hybrid vigor was not yet known which is already known in many other crops such as rice, maize, and wheat. Using whole genome resequencing approach (WGRS), multiyear and multilocation phenotyping data from 104 parental lines and 435 of their single-cross hybrid progenies, combined with 292 lines from a previous study (Varshney et al. 2017), a total of 396 inbred lines, were used for identifying hybrid vigor. After the assessment of yield-related traits in different locations, an analysis for MPH came out to be 78.6% positive MPH values in the hybrids. Further by using genome wide prediction and association mapping, the authors identified 129 SNPs and 52 CNVs effectively for studying heterotic effect.

21.10 Breeding Approaches of Post NGS Era

The latest development of the genomic resources led the breeding of pigeonpea to a new platform for the genetic enhancement.

21.10.1 Marker Technologies

After the availability of marker technologies, trait mapping and diversity analysis became easier for the breeders. The development of SSR markers catalyzed the molecular analysis in a population. Initially using Sanger's platform, SSR markers were developed by using genomic libraries or mining ESTs from the transcriptome.

The same approach was used by Bohra et al. (2011) to discover the first and large set of SSR marker (3072) in pigeonpea by using BAC end sequences. After the high-throughput, automated, and cost-effective technologies came into light, the discoveries of marker have become easier. The trait-associated mapping also became easy with the genome sequencing and whole genome resequencing approaches. Diversity array technologies (DArT), hybridization-based genotyping technologies, generated thousands of polymorphic loci that were further used for designing linkage map and to study diversity of analysis across the genotypes. Next-generation sequencing technologies have made SNP marker development very easy. Single-nucleotide polymorphism (SNP) marker is nowadays a marker of choice; therefore, development of cost-effective KASP technologies has given 1616 SNPs (Saxena et al. 2011) for genotyping assay in pigeonpea.

Using Illumina platform, SNP discovery using NGS, RNA-Seq, complexity reduction of polymorphic sequences (CRoPS), restriction-site-associated DNA sequencing (RAD-Seq), and genotyping by sequencing (GBS) have simplified the way enough. GBS offered a way to sequence and genotype polymorphism among two genotypes simultaneously. Recently a GBS approach was used in pigeonpea for the trait mapping and identification of candidate gene for FW, SMD, and determinacy (Saxena et al. 2017b, c, d). The molecular map was designed using SNP marker, GBS approach in the F2 population of CMS line its isogenic line (Saxena et al. 2018). Using microarray technology, a 62 k genic-SNP array (Singh et al. 2020) "CcSNPnks" and 56k SNP- array (Saxena et al. 2018) is widely used high-density genotyping platform for pigeonpea to demonstrate population structure analysis, diversity analysis, phylogenetic study, high-density linkage mapping, QTL mapping, and screening of haplotypes.

In the field of hybrid breeding programs, the molecular marker technologies lead researchers to find the affected molecular pathways and candidate gene confer against CMS trait. Based on mitochondrial genome sequencing, the open reading frame (ORFs) and gene in the ICPB 2039 and ICPA 2039 have been identified. Further, on the comparative analysis between ICPA 2039 and ICPB 2039 of 34 mitochondrial genes, the deletion in *nad7A* gene in CMS line has been provided. Thus, InDel-based marker *(nad7a_del)* is used for testing the hybrid purity (Sinha et al. 2015a). Later on, to test the hybrid purity, many hybrid purity testing kits by using SSR marker system were used (Bohra et al. 2015, 2017a, b), but due to the expensive way of testing, it was later replaced by SNP assays (Bohra et al. 2020a). To differentiate restorer line from non-restorer lines in A4 hybrid system, two markers, namely, "CcLG08_RFQI1" and "CcLG08_ RFQI4," have been developed. Therefore, after the development of the marker system, the phenotyping activities could be avoided in order to be time effective.

21.10.2 Candidate Gene Mapping and Breeding Using Transcriptomics Resources

Based on Sanger technologies, the first set of transcriptomics resources include 9468 ESTs that served for the candidate gene for fusarium wilt and SMD with 19 and 20 genes, respectively, and set of 3583 SSRs. Sinha et al. (2015a, c) discovered and validated stress-responsive genes for the candidate gene to be responsive to salinity, drought, and heat,. A major asset to pigeonpea is its expression atlas, a global view on the gene expression that helps in bridging the gap between whole genome sequence information and plant phenotypes. From its developmental stages to its reproductive and later followed by senescence, expression atlases "CcGEA" highlight the impact of each gene. It basically covers the whole life cycle of pigeonpea, which is developed using RNA-sequencing technologies. The "*C. cajan gene expression atlas" CcGEA* catalogue has a total of 28,793 genes expressed during different variable stages especially during flower development and fertilization. This catalogue also suggested the posttranscriptional and epigenetic modification in seed and embryo development of pigeonpea.

Co-expressional analysis also highlighted the identification of 28 genes and three highly connected genes known as "Hub genes" (Pazhamala et al. 2017). Multiomics approach was used to reveal fertility transition of candidate genes contributing towards hybrid breeding program in two-line system (TGMS); however transcriptomics by RNA-Seq was largely relied on. The male and female anther were studied at their developmental stages, and by integrating omics approaches, transcriptome and proteome revealed 17 DEGs in sterile anther. The study revealed that this transition of fertility to sterility is mainly due to auxin homeostasis, impaired cell wall, and sugar transporters. Similarly, Bohra et al. (2020a, b) revealed a set of 505 genes that showed altered expression in response to CMS. From this set, 412 genes were upregulated, while 93 were downregulated in the fertile maintainer line as compared to the CMS line. The study revealed gene showing impaired pathways related to glucose and lipid metabolism, ATP production, pollen development/pollen tube growth, and reactive oxygen species (ROS) scavenging.

For candidate trait mapping and QTL, discovery methods are still conventional, and these mapping can be done by using SSR and SNP marker. SNP array and linkage maps based on GBS approach have helped researchers in trait dissection. Other similar approaches like WGRS/skim sequencing with the availability of the reference genome facilitated the efforts. The first generation Hap-map in pigeonpea-based WGRs data from 20 accessions revealed 5.5 million genome-wide variants including 4.6 million SNPs and 0.7 InDels along with the structural variations including copy number variable (CNVs) and presence and absence variable (PAVs) (Kumar et al. 2016). Similarly, with WGRS data obtained by using 292 pigeonpea accessions revealed large structural variations (SVs) of greater than or equal to 1000 bp in the breeding lines, viz. 282 CNVs; 35 PAVs, wild has 173 CNVs; 77 PAVs and landraces have 228 CNVs and 37 PAVs.

21.10.3 High-Density Linkage Map and QTL Mapping

Prior to the large set of SSR markers discovered by Bohra et al. 2011, there were inadequate diversity analyses which impaired the development of high-density linkage map. Later, the team discovered the first map with 239 loci for an interspecific cross between *C. cajan* (ICP 28) × *C. scarabaeoides* (ICPW 94). After this pigeonpea got more linkage map, none of them were of high density. Over the discovery of high-throughput technologies, high-density map in pigeonpea became an easy shot. The first high-density linkage map was discovered by SNP marker for interspecific cross using KASP assay platform with 875 loci (Saxena et al. 2012a, b). After that, more population maps were made covering 1101 loci (Saxena et al. 2017b), 964 loci (Saxena et al. 2017c), and 787 loci (Saxena et al. 2017d). To date, the most comprehensive genetic map of pigeonpea harbors 6818 SNPs loci that span 974 cM of the genome (Yadav et al. 2019). Availability of such density genetic maps is the key to understand the genomic importance of essential agronomic traits, for the trait mapping. In the future, this kind of trait mapping has several advantages over traditional marker-based mapping. In addition to taking much less time, these approaches identify genes or QTLs for a given trait which can be used as diagnostic marker(s).

21.10.4 NGS-Based Trait Dissection

As the popularity of NGS-based platform raised, WGRS approaches and next-generation trait mapping particularly Seq-BSA were employed for trait discovery. An approach used by Singh et al. (2016a) revealed the candidate gene responsible for the major biotic stress in pigeonpea recombinant inbred population (RIL) population ICPL20096 × ICPL332. Four SNPs showed the associated with FW, while the remaining four showed correlation with SMD. A similar InDel approach used to identify the same traits by Singh et al. (2017) revealed 16 InDels from which five were validated by WGRS data. Table 21.1 has highlighted the major candidate genes and high-density QTLs region with their respective traits and approach used for their validation and identification.

21.10.5 Adopting Speed Breeding Technology and RGT Technologies

Speed breeding is recently in spotlight for cool season legume; nevertheless the extra early and early maturing pigeonpea holds immense potential. The concept of speed breeding was first introduced by Watson (2019). The speed breeding (SB)/rapid generation advancement (RGA) concept describes the breeding techniques which focused on enhancing the genetic gain by reducing the length of breeding cycle which is inversely related to genetic gains (Moose and Mumm 2008). In pigeonpea as described earlier, photoperiod plays an important role in the completion of its life

Table 21.1 Major candidate genes and high-density QTLs regions identified in pigeonpea using WGRS data

Trait	Number of QTLs/candidate gene	Population	Method/ approach	References
Fusarium wilt (FW)	3 QTLs *(qFW11.1, qFW11.2, and qFW11.3)*	ICPB2049 × ICPL99050	Genotyping by sequencing (GBS)	Saxena et al. (2017b)
		ICPL20096 × ICPL332		
		F2 (ICPL85063 × ICPL 87119)		
	Two gene (*C. cajan_03691* and *C. cajan_18888*)	BDN 711 × ICPL 20096	GBS	Saxena et al. (2021)
	C.cajan_03203	ICPL 20096 × ICPL 332	Bulk segregant assay (BSA-Seq)	Singh et al. (2016b)
	3 InDels	ICPL 20096 × ICPL 332	BSA-Seq and WGRS	
Sterility mosaic disease (SMD)	CcLG11	ICPB 2049 × ICPL 99050	GBS	Saxena et al. (2017c)
		ICPL 20096 × ICPL 332		
		F2 (ICPL 85063 × ICPL 87119)		
	Four gene (*C. cajan_07858, C. cajan_20995, C. cajan_21801, and C. cajan_17341*)	BDN 711 × ICPL 20096	GBS	Saxena et al. (2021)
	C.cajan_01839	ICPL 20096 × ICPL 332	BSA-Seq	Singh et al. (2016b)
	3 InDels	ICPL 20096 × ICPL 332	BSA-Seq and WGRS	
	qSMD4	ICP 8863 × ICPL 20097	Biparental	Gnanesh et al. (2011)
		TTB 7 × ICP 7035		
Drought tolerance	*C. cajan_23080*-H2	_	WGRS (haplotypes)	Sinha et al. (2020)
	C. cajan_30211-H6			
	C. cajan_26230-H11			
	C. cajan_26230-H5			
Cleistogamous flower	*qCl3.2*	ICPL 99010 and ICP 5529	Axiom *Cajanus* SNP Array	Yadav et al. (2019)

(continued)

Table 21.1 (continued)

Trait	Number of QTLs/candidate gene	Population	Method/ approach	References
Determinacy	*CcTFL1*	ICPA 2039 × ICPR 2447	SNP genotyping and marker trait association (MTA)	Mir et al. (2014)
		C. cajan(ICPL 85010) × *C. volubilis Blanco* (ICP 15774)		
Seed protein content (SPC) and seed weight (SW), seed yield (SY), growth habit (GH) and days to first flowering (DFF)	192 main effect QTLs and 573 epistatic QTLs	ICP 11605 (P1) × ICP 14209 (P2); ICP 8863 (P1) × ICP 11605 (P2); HPL 24 (P1) × ICP 11605 (P2); ICP 8863 (P1) × ICPL 87119 (P2); ICP 5529 (P1) × ICP 11605 (P2)	GBS	Obala et al. (2020)

cycle, and hence, SB protocol may work on it. A 12-h day length has been established as the optimum photoperiod in pigeonpea (McPherson et al. 1985). In cool season, rapid generation from 5 and 3 generations in legumes such as field pea, chickpea, respectively, can be achieved by regulation of growth condition and photoperiod exposure (Gaur et al. 2007; Mobini and Warkentin 2016). Mobini et al. (2015) applied plant hormone in *Faba* bean and lentils to gain seven and eight generations, respectively; they used cytokinin and auxin to induce early flowering and harvested immature seed for generation advancements. In pigeonpea, Saxena et al. (2019) achieved four generations per year in the early maturing genotypes, namely, ICPL 85024, ICPL 87093, ICPL 00004, and ICPL 00151, as they were photo-insensitive lines. Therefore, SB protocol with single seed descent is extremely useful for preserving genetic variability, and combination of SB recipe with genomic selection or marker-assisted selection will promote the targeted breeding (Bohra et al. 2020b).

21.10.6 Genomic Selection (GS) Using Marker Technologies

From conventional breeding approaches, research programs drastically shifted towards marker-assisted breeding. From the use of effective marker technologies, pigeonpea has been derived out of many linkage maps and revealed trait-responsive QTLs. Deploying trait-associated markers for the introgression or selection of QTL (s) in the elite genetic background for the development of elite pigeonpea varieties is currently imparted by the national and international breeding programs. Genomics

assisted breeding efforts have been successful in legume crops (Varshney et al. 2019, 2021a). In general, the transfer of QTLs using the MABC technology is limited with the major effect QTL instead of minor QTLs responsible for complex traits of plant phenotype (Varshney et al. 2021b). Due to this inability of MABC program, genomic selection (GS) can be used to locate minor effect loci by calculating the estimated breeding values of the model prepared. Prior information of the phenotype is not required; genotyping works for controlling the polygenic inheritance. In simple words, the concept of GS was first proposed by Meuwissen et al. (2001) in animals for the estimation of breeding values of unobserved phenotypes based on genome-wide marker data. GS needs the genome wide associated markers, and genome estimated breeding value (GEBV) is the basis for the selection of the genotypes from the respective breeding programs. In pigeonpea, no GS model yet has been reported, but in other legume crops such as field pea, soybean, etc., the result revealed to conclude superiority of GS over phenotypic selection especially in the case of field pea with respect to yield response (Annicchiarico et al. 2019).

21.11 Challenges for Pigeonpea as International Crop

Pigeonpea being the rainfed crop harbors several challenges starting from evolutionary to adoptability to agronomic and crop husbandry.

21.11.1 Limitation Due to Photoperiod Response and Temperature across the World

Apart from India, pigeonpea is widely cultivated in South Africa, Asia, and Latin America. These regions have not yet able to produce the potential yield. Traditional cultivars of pigeonpea cannot fit as preceding or succeeding crop in situations of rainfed and irrigated ecologies due to high sensitivity to photothermal regimes. Therefore, photothermal insensitive lines are of particular interest to pigeonpea breeders to develop cultivars for niches and also to ensure synchrony in flowering. Short-duration insensitive accessions are very useful in sequential, double or multiple cropping systems. The photothermal insensitivity trait is well distributed across the classes of flowering pattern and growth habit indicating that the photothermal insensitivity can be easily combined with other traits in efforts to maximize the pigeonpea yields (Upadhyaya et al. 2007). However, the biggest challenge is the inheritance of insensitivity in pigeonpea. But, several high-yielding varieties have been released for cultivation due to their photothermal sensitivity which is restricted to their own geographical regions. Pigeonpea needs shorter days and longer hour of darkness to promote flower induction (Vales et al. 2012). Perfect combination induces flower induction because this trait restricts pigeonpea to adaptation beyond 30° northern and southern latitudes. Medium maturating and late varieties are highly connected to photoperiod response; in other words, inverse correlation between earliness and photo-insensitivity in pigeonpea is recorded (Saxena et al. 1981).

Therefore, photoperiod and low-temperature sensitivity have surely restricted the expansion of pigeonpea across higher latitudes. Not only environmental factors but also biotic and abiotic factors still block the way out for pigeonpea to a diversely cultivated crop all around the world.

Drought is the major abiotic constraints in many areas; therefore through multi-location and multiyear evaluations, medium-duration genotypes such as ICEAP 00673, ICEAP 01170, and ICEAP 01179, as well as long-duration genotypes such as ICEAP 01423 and ICEAP 01202 possessing, drought tolerance coupled with high yield has been identified.

21.11.2 Natural Cross-Pollination

It is one of the major constraints to maintain genetic purity of varieties and breeding material (Howard et al. 1919). To maintain pure breeding lines for a particular trait, this turns out to be a serious issue due to poor seed production (Sultana and Saxena 2019).

21.11.3 Limited Genetic Diversity in Various Geographical Areas

Focusing on to primary gene pool, we have processed an inbreeding depression of the *Cajanus* which has limited diversity and limited access to beneficially agronomic traits. More breeding programs crossing the elite varieties are leading more towards limited collections that add on to the lagging genetic enhancements with interventions of wild relatives (Saxena et al. 2014). To access more in primary pool, breeders started exploring the secondary and tertiary pools. The utilization of the wild relatives will provide resistance, quality, and breeding efficiency, which is highlighted by Khoury et al. (2015). Further, their study highlight the extent of diversity in pigeonpea, which cultivars lack as compared to the wild pigeonpea.

21.12 Approaches Used for Varietal Development

In the view from geographical regions and different maturating groups, distinct maturity groups pertaining to the zone of cultivation of pigeonpea have been explored (AICRP on Pigeonpea Coordinator Report 2018–2019). Developing any of the cultivars, breeders focused on increasing the seed yield. In total, all 150 varieties have been released till 2018 since 1960. Most of the varieties were developed following pedigree (89), pure-line germplasm selection (57), and mutational breeding (3). The data presented by IVT-AICRP suggested that hybridization coupled with pedigree method was observed in 455 varieties. Of the five maturity groups, medium and early represent large groups comprised of 48 released and 182 IVT entries and 33 released and 142 IVT varieties, respectively (Naik et al. 2020). Further their study suggested that the landmark pigeonpea varieties released

for cultivation were having the genetic base contributed by T190, C11, UPAS 120, ICP 8863 and T 21 genotypes. The variety UPAS 120 is pureline selected from P4768 in 1976 for its superior trait, early maturating, and tolerance to pod borer, initially cultivated in NEPZ. This variety is the most used of genetic bases in all varieties, as it's been used as parent 25 times which is further followed up by Asha. Asha (ICP 87119) has been widely used as parent 44 times, even exploited in the NAM population as founder parent due to its resistance against FW and SMD. It is released in 1986, derived out by hybridizing C11 as cytoplasmic donor and ICP 6 male parent. UPAS 120 is used as donor for most of early and medium maturing groups, whereas Asha is used as parent for late maturity group.

ICP 8863 well known as Maruti is also achieved by a pureline selection from Hyderabad landrace and now suitable for South zones, and it shows resistance against wilt. Many more remarkable varieties are released such as BMSR 736, TJT-501, IPA203, IPA 206, Bahar, etc. (Naik et al. 2020) to understand the contribution of varieties to form the genetic base of the cultivated pigeonpea and how the development of these varieties has supported the invention of more elite cultivars. A new landrace "*ICP 7035*" has been identified in the state of Madhya Pradesh in Jabalpur (Bedaghat) which offers multiple resistance against all the three strains of SMD and has sweet immature seeds. It is considered as the "jewel" among all the germplasms due to its multiple benefits in soil conservation and resistance against pest and multiple diseases. Its performance has been reported well in SMD-endemic areas of India, Myanmar, and Nepal (Sharma et al. 2021).

21.13 Hybrid Released in India

21.13.1 GMS-Based Pigeonpea Hybrids

ICRISAT developed the first GMS-based pigeonpea hybrid ICPH 8 and released for cultivation in India (Saxena et al. 1992a, b). In farmers' fields, this hybrid recorded 31–40% yield advantage over the best control. This was followed by the release of five more GMS-based hybrids that exhibited high levels of standard heterosis (Table 21.2). But, none of these could reach farmers' fields at commercial level. As the male sterility is controlled by a pair of recessive gene (msms), it can only be maintained by crossing it to heterozygous (Msms) plants. The progeny of this cross (msms × Msms) will segregate in to 50% male-fertile (Msms) and 50% male-sterile (msms) plants. Therefore, identification of the fertile segregants within female population was the primary requirement of large-scale seed production, and it was not found commercially viable.

21.13.2 CMS-Based Pigeonpea Hybrids

CMS results from an inherent disharmony between cytoplasmic (mitochondrial) and nuclear genomes. A range of mitochondrial genes have been reported in different

Table 21.2 Pigeonpea hybrids released in India

Name of the hybrid	Zone	Released	Days to maturity	Superiority over check (%)
GMS-based hybrids				
1 ICPH 8	Central	1991	125	30–40
2 PPH 4	Punjab	1994	137	14
3 CoH 1	Tamil Nadu	1994	117	19–22
4 CoH 2	Tamil Nadu	1997	125	35
5 AKPH 4104	Central	1997	135	64
6 AKPH 2022	Maharashtra	1998	190	25–35
CMS-based hybrids				
1 GTH-1	Gujarat	2006	140	32
2 ICPH 2671	Madhya Pradesh	2014	175–180	31–34
3 ICPH 2740	Telangana	2015	180	34
4 ICPH 3762	Orissa	–	180–190	36
5 IPH 15–03	NWPZ	2019	150–155	28–55
6 IPH 09–5	NWPZ(reconfirm trial)	–	138–140	33

Source: Project Coordinators (Pigeonpea) Report, 2019; Saxena et al. (2010)

crops that confer CMS trait. The fertility in CMS system is rescued because of fertility-restoring elements located in the nucleus. The first CGMS line GT 288A and its maintainer B line were registered by Pulse Research Station, SDAU, GAU, SK Nagar, Gujarat, in 2000. Consequently in 2006, the first CMS hybrid GTH 1 was developed by SDAU, Gujarat, and released by CVRC for cultivation in the central zone. There are 39 CGMS lines that have been registered with ICAR-NBPGR and six CMS based hybrids that are released for cultivation as of 2019.

21.14 Coordinated System and Programs for Pigeonpea

In India, the emphasis on pulse research including pigeonpea has been increasing since 1967. Systematic research on pulses was started with the establishment of the All India Coordinated Pulses Improvement Project (AICPIP) in 1967 with its headquarter at Indian Agricultural Research Institute, New Delhi. In 1978, the project was elevated to the status of Project Directorate (Pulses), while its headquarter was shifted to the then IARI Regional Research Station, Kanpur, Uttar Pradesh. The Project Directorate (Pulses) was further strengthened with the inclusion of basic and strategic research in its mandate and elevated as Directorate of Pulses Research (DPR) in 1984 at Kanpur, Uttar Pradesh. During VIII five-year plan, the AICPIP was divided into three projects, viz., chickpea, pigeonpea, and MULLaRP (Mungbean Urdbean, Lentil, Lathyrus, Rajmash and Pea), and the post of project coordinator was created to provide independent leadership to respective AICRPs. Subsequently, in the IX five-year plan, the project proposal on pigeonpea was proposed to nine

main centers (Badnapur, Varanasi, Coimbatore, Dholi, Gulbarga, Khargone, Warangal, Bengaluru, and S.K. Nagar) and 11 subcenters. Consequently, during X and XI plan, the subcenters were increased to 17 (Akola, Rahuri, Faizabad, Pantnagar, CSAUAT Kanpur, Berhampur, Hisar, Vamban, Sehore, Junagarh, Lam, Raipur, Ludhiana, Ranchi, Kota, Nagaland, and Tripura).

The organizational restructuring was done with renewed emphasis to cater the research and development, in which pigeonpea production needs to be increased in the country to meet the ever-growing demand. At present, AICRP on pigeonpea has 26 centers spread over 17 states comprising of 22 universities and 1 agricultural college covering the entire pigeonpea growing region of the country. In recent years, greater emphasis has been laid on the development of high-yielding varieties and hybrids coupled with appropriate production and protection technologies to bridge the gap between the potential and realized yields of pigeonpea. In this regard, our prime concern is also on developing early-duration varieties/hybrids suitable for breaking the rice-wheat cropping system with resistance to major biotic stresses like phytophthora stem blight (PSB), fusarium wilt, and sterility mosaic disease (SMD). Strengthening seed delivery systems, greater seed replacement rate, and strengthening of community-based services including seed production, input supply, and marketing support are expected to promote enhanced cultivation of pigeonpea, which in turn supports the cause of millions of livelihoods of dryland farmers.

The consumption and demand of pigeonpea in the rural India is unstoppable. However, the present popularity of pigeonpea as a healthy vegetable source of protein among the urban household created huge market elasticity for pigeonpea dal. Pigeonpea is nutritionally rich and highly digestible with less flatulence compared to chickpea and peas. It has been a recommended food for all age groups as a source of protein.

Pigeonpea and other pulses will very soon find its appropriate place especially in the context of future challenges such as global warming, scarce water supply, needs for new raw materials, and increasing health awareness among urban and rural public. In this circumstance, we need to reinforce our efforts in pigeonpea research and development by innovative research strategies and continue to develop cultivars and technologies suitable for specific end uses.

21.15 Future Prospects

Breeders have focused to increase the yield in pigeonpea since all time, and yield being a quantitative trait results from the combination G × E interaction and minor epistatic gene; therefore, to improve with respect to each and every factor, it becomes a tedious task (Saxena et al. 2020). The emergence of the omics technologies has enhanced and opened up the opportunities for rapid crop improvement and overcome the adverse effect of climatic change (Varshney et al. 2021b). The technologies such as marker-assisted backcrossing (MABC), genomic selection, rapid generation turnover with speed breeding, and haplotype-based breeding accelerated the current research program in pigeonpea. The whole genome resequencing and GBS

approaches have led to the discovery of SNPs and genome-wide variations (Bohra et al. 2020a) which has made possible to exploit "tailor-made" crops by using superior haplotypes. By using pangenome studies, the wild accession and their beneficial traits in breeding programs have also been incorporated. Future research is focusing to increase the protein yield by QTL analysis (Obala et al. 2019), and hybrid breeding has the potential to exploit more protein as the gene is controlled by additive gene action. Natural out crossing which is considered as a boon for hybrid production becomes a major challenge in pure line breeding. Thus, for the varietal development, it requires lot of resources such as isolation distance, insect proof cages, and labor cost for proper cleaning and care. These factors have shifting researcher attention towards cleistogamous trait. Moreover, studies need to be done on the area of photosensitivity and earliness, as their inverse relation has possibly made speed breeding impossible in medium and late maturity groups (Fig. 21.1). Therefore, researchers need to explore the candidate genes and factors responsible for this relation, and after that GS-based speed breeding can make future in pigeonpea. In hybrid production, three-line system has given so many potential hybrids for different geographical regions, but two-line system (TGMS and PGMS) is not so much explored.

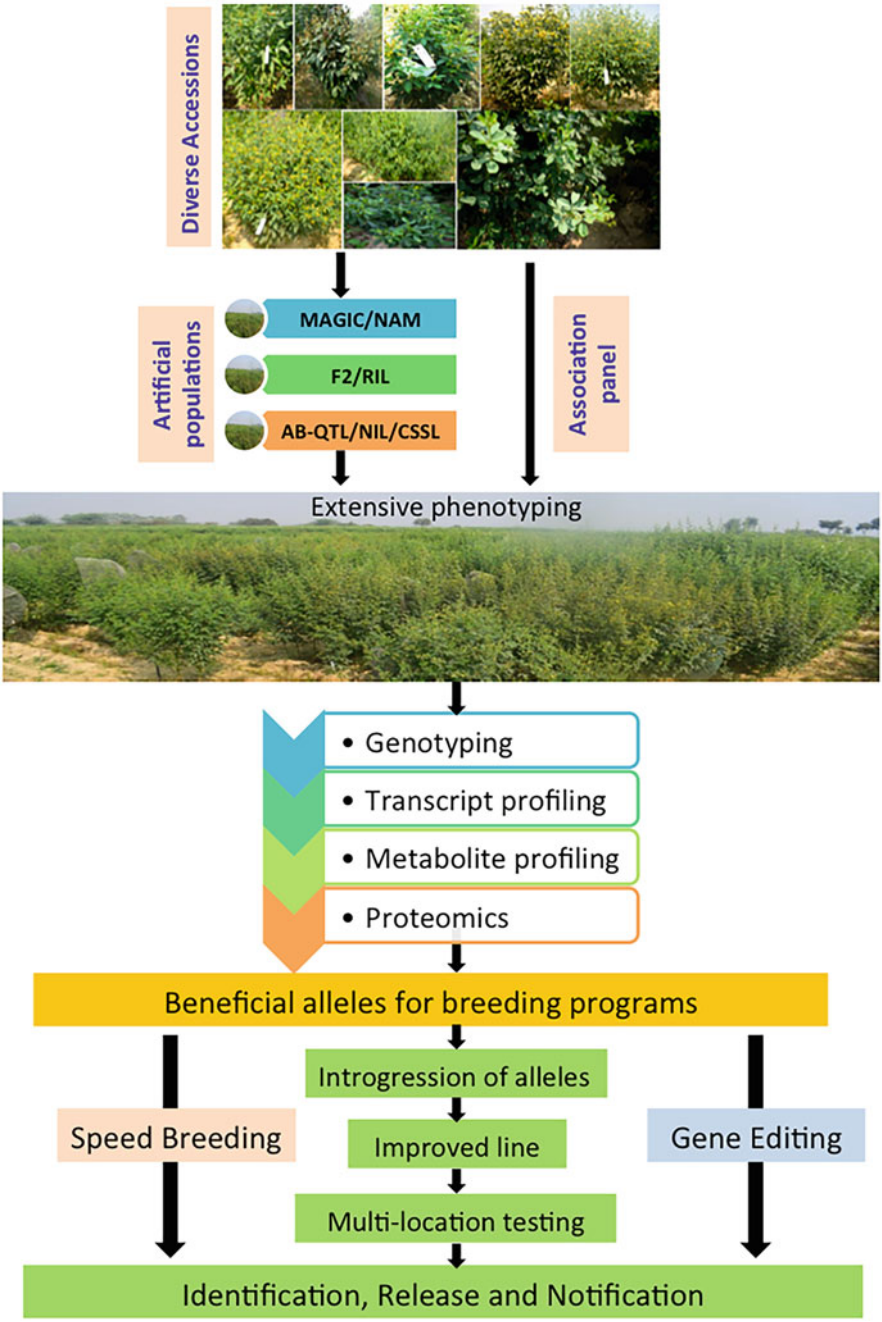

Fig. 21.1 Diagram highlighting the way ahead for pigeonpea breeding

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Mungbean Breeding

22

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Abstract

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*), which is also known as the green gram, or moong, is primarily grown in East Asia, Southeast Asia, and the Indian subcontinent. Mungbean has its own place due to some very unique features like short crop duration, low input requirement, wide adaptability, and tolerance to various abiotic stresses including heat and drought. Besides, mungbean is also very rich in overall nutrient content which makes it the most preferred crop for various culinary preparation including *dal*, soups, sprouts, etc. In addition to the biotic and abiotic stresses, breeding focus should be on other economic traits like extra-early maturity and photothermal insensitivity in the genotypes, which will help in suitable fitting of the developed varieties in various cropping systems and patterns. Due to its unique health and nutritional properties, along with soil and environment ameliorative ability, mungbean is one of the

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_22

preferred crops for food, nutrition, and environmental security and sustainability. In brief, this chapter deals with various aspects of the mungbean crop like breeding opportunities, varietal development, biotic and abiotic stress tolerance, nutritional and biofortification possibilities, along with emerging challenges and improvement opportunities at global level.

Keywords

Vigna radiata · Green gram · Breeding · Biofortification · Nutrition · Cultivation

22.1 Introduction

The mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) or green gram crop is the third most important grain legume after chickpea and pigeon pea. Mungbean is indigenous to India or Indo-Burma region and is known by various names like green gram, golden gram, green bean, green soy, etc. (Markam et al. 2018). Since various wild and cultivated mungbean species are extensively distributed in the central Asian region, it is considered as a primary center of genetic diversity. It is an important constituent of cereal-based farming systems of South and Southeast Asia and is also grown in several other parts of the world including east and central Asia, east Africa, and Australia. Globally this is being cultivated on nearly 7.2 million ha area with a productivity of nearly 750 kg per hectare (Nair and Schreinemachers 2020). Mungbean is being cultivated across a wide range of latitudes (40°N or S) covering tropical and subtropical regions of the world and is amicably adapted to a range of cropping systems (http://avrdc.org/intl-mungbean-network/). The mungbean grows on a wide range of soils but prefers well-drained loams or sandy loams, with a pH ranging from 5 to 8. Mungbean is reportedly growing up to an altitude of 1850 m and is known to be sensitive to both photoperiod and temperature (Lambrides and Godwin 2006).

Nearly 90% of world's production comes from Asian countries including India, Myanmar, China, Thailand, Indonesia, and Bangladesh (Schafleitner et al. 2015; Alam et al. 2014b). India is the largest producer, consumer, and importer of mungbean. In India, this is being grown on an area of nearly 4.32 million ha with a production of 2.17 million tonnes and an average productivity of 502 kg/ha (IIPR 2018). Mungbean is unique for various features including shorter crop duration, low input requirements, wider adaptability, tolerance to heat and drought, and high nutritional content which makes it an ideal crop for various cropping systems and patterns, especially for smallholder farmers (Pratap et al. 2020). The *Rhizobium* and *Bradyrhizobium* bacteria, which are present in the root nodules, fix the atmospheric nitrogen and thus improve the soil fertility and benefit the succeeding crops.

Mungbean belong to the genus *Vigna*, encompassing approximately 150 species, of which only 7 are cultivated species. The genus is subdivided into (1) Asiatic subgenus *Vigna* having *V. radiata* L. Wilczek, *V. mungo* L. Hepper, *V. aconitifolia* (Jacq.) Marechal, *V. angularis* (Willd.) Ohwi & Ohashi, and *V. umbellata* (Thunb.)

Ohwi & Ohashi and (2) African subgenus having *V. unguiculat*a (L.) Walp. and *V. subterranea* (L.) Verdc (Dikshit et al. 2012). The *Vigna* species is largely distributed in tropics and subtropics due to their wide adaptation range in different agroclimatic conditions. This chapter discusses on various aspects of mungbean including their cultivation, nutrition, genetic improvement, use of genomic tools, varieties, better crop management practices, and policy related issues.

22.2 Origin, Evolution and Distribution of *Vigna* Species

The center of origin of mungbean is believed to be the Indian subcontinent (de Candolle 1884; Vavilov 1926). Owing to the wide genetic diversity of cultivated and wild mungbean species found in India, it is also considered as the region of its first domestication (Baudoin and Marechal 1988). In cultivated and wasteland areas of India and also in the wetlands of subtropical, northern and eastern Australia (Lawn and Cottell 1988), *V. radiata* var. *sublobata*, the progenitor species of mungbean, is found growing naturally as a weed. In India, wild relatives were also documented from Western Ghats and Himachal Pradesh; *V. radiata* var. *sublobata* was from eastern and western peninsular tract, and carbonized grains of wild *Vigna* species from Daimabad, while the maximum diversity for *Vigna* species has been reported from Deccan Hills and upper Western Ghats (Chandel 1981; Kajale 1977).

Tomooka et al. (2002) reported West Asia as the region of diversity and proposed that from India mungbean spread to Southeast Asia, China, and Taiwan. *V. radiata* var. s*ublobata* is known to have moved from East to North Australia, Asia, New Guinea, Madagascar, and Central and East Africa (Tateishi and Maxted 2002). South and West Asian mungbean genotypes are small seeded with green, green mottled, black and brown seed color, whereas Southeast Asian germplasm possesses shiny green testa, tall plants, high branching habit, and late maturity. East Asian mungbean genotypes are of short stature and have early maturity having green to dull green testa.

22.2.1 Taxonomy and Gene Pool

- Family—*Leguminosae.*
- Subfamily—*Papilionoideae.*
- Tribe—*Phaseoleae.*
- Subtribe—*Phaseolinae.*
- Genus—*Vigna.*
- Subgenus—*Ceratotropis.*
- Section—*Ceratotropis.*
- Species—*Vigna radiata.*
- Variety—*Vigna radiata* var. *radiata.*

Table 22.1 The primary, secondary, and tertiary gene pools of *Vigna* species

Primary gene pool (GP-1)	Secondary gene pool (GP-2)	Tertiary gene pool (GP-3)	References
V. radiata var. *radiata;* *V. radiata* var. *sublobata;* *V. radiata* var. *setulose*	*V. mungo* var. *mungo;* *V. mungo* var. *silvestris; V. trilobata;* *V. subramaniana;* *V. grandiflora;* *V. stipulacea;* *V. tenuicaulis;* *V. trinervia;* *V. umbellata*	*V. angularis;* *V. dalzelliana;* *V. glabrescens;* *V. grandis;* *V. vexillate;* *V. aconitifolia;* *V. angularis*	Chandel et al. (1984), Smartt (1981), Smartt (1985), Kumar et al. (2004) and Tomooka et al. (2011)

Source: Kumar et al. (2011)

Harlan and de Wet (1971) postulated the gene pool concept which can be used to devise a pre-breeding program for directed crop improvement (Kumar et al. 2011). The details of primary, secondary, and tertiary gene pools of the genus *Vigna* is presented in Table 22.1. The distinct species have been grouped or pooled as per their cross compatibility along with their cytogenetic, phylogenetic, and molecular analyses. The existence of useful genes is persistent in the secondary and tertiary gene pools, but crossability barriers encountered during hybridization between various *Vigna* species of primary, secondary, and tertiary gene pools necessitates the use of novel techniques such as embryo rescue, polyploidization, reciprocal crossing, hormonal manipulations, use of bridge species, etc. for obtaining the viable progenies (Pratap et al. 2018; Nair et al. 2020).

However, some deviations have been reported like *V. aconitifolia* and *V. umbellata* in GP-2 and GP-3, respectively. Recent hybridization showed *V. glabrescens* forming fertile offspring upon crossing with *V. radiata* without any fertility/crossing barrier, though this is grouped in GP-3 (Pratap et al. 2014). Thus, there is a need to revisit the classification of *Vigna* gene pool using both molecular and conventional crossing-based tools for the more precise grouping of *Vigna* species.

22.3 Cytogenetics

The somatic chromosome number of mungbean is $2n = 22$ and the haploid genome size is approximately 579 mb (Lavania and Lavania 1983; Arumuganathan and Earle 1991). Due to its relatively small genome size, mungbean is considered as a model plant among *Vigna* species. Several studies have been conducted to measure the size and shape of mungbean chromosomes. Bhatnagar (1974) proposed $4L^{sm} + 4M^{sm} + 3M^{m}$ as karyotype formula for mungbean, where *L* is long (2.7–3.5 μm), *M* is medium (1.9–2.6 μm), sm is submedian centromere, and *m* is median centromere. The details of various chromosomes of mungbean are presented in Table 22.2.

Table 22.2 Details of mungbean chromosomes

Chromosome number	Chromosome length (μm)	Short arm length (μm)	Long arm length (μm)	Chromosome type
1	2.1	0.65	0.95	Metacentric
2	1.77	0.61	0.72	Submetacentric
3	1.62	0.62	1.00	Metacentric
4	1.53	0.69	0.84	Metacentric
5	1.40	0.63	0.77	Metacentric
6	1.35	0.60	0.75	Metacentric
7	1.27	0.57	0.70	Metacentric
8	1.20	0.44	0.76	Submetacentric
9	1.15	0.52	0.63	Metacentric
10	1.08	0.50	0.58	Metacentric
11	0.96	0.44	0.52	Metacentric

(Derived from Vinora 1974; Vinora et al. 1999)

22.4 Plant Genetic Resources

Overall, 6700 mungbean accessions are being maintained at the World Vegetable Center, Taiwan (erstwhile AVRDC), from which 1481 accessions are selected for the development of core collection through cluster analysis using eight descriptors. This core collection was then genotyped using 20 polymorphic simple sequence repeat (SSR) markers, and based on molecular diversity, 289 accessions were then selected as mini core set (Schafleitner et al. 2015). In India, mungbean germplasm collection was initiated by R. D. Bose during 1925, and he could identify 40 distinct genotypes based on seed and morphological traits.

In the year 1943, Coordinated Pulse Research Scheme was initiated by Indian Council of Agricultural Research in which promising genotypes were identified for large-scale cultivation. During the 1960s, efforts have been made at IARI, New Delhi, and a total of 1250 diverse mungbean accessions have been collected from various Indian states (Paroda and Thomas 1988). The National Genebank at ICAR-NBPGR has documented more than 11,000 accessions including 7453 indigenous and 3588 exotic accessions. Of these, nearly 3927 accessions, comprising of 3392 indigenous and 535 exotic collections which are from 12 countries, are stored in long-term storage (−20 °C). During the past four decades, NBPGR has introduced approximately 4000 diverse mungbean accessions from a number of countries.

22.5 Wide Hybridization in *Vigna* Species

Interspecific hybrids have been attempted in different *Vigna* species and F1s were found either sterile or partially fertile (Singh and Singh 2006). In some cases, pollen tubes were unable to pierce the stigma, whereas, in others, after fertilization embryo

Table 22.3 Crossability studies in *Vigna* species

Wide cross	References
V. radiata × *V. mungo*	Verma and Singh (1986a), Ravi and Minocha (1987) and Pande et al. (1990)
V. mungo × *V. radiate*	Gosal and Bajaj (1983a, b)
V. glabrescens × *V. Radiata*	Chen et al. (1990)
V. radiata × *V. trilobata*	Sharma and Satija (1996), Dana (1966) and Pandiyan et al. (2012)
V. radiata × *V. umbellate*	Pandiyan et al. (2008)
V. radiata × *V. radiata var. sublobata*	Sharma and Satija (1996)
V. radiata with *V. mungo, V. radiata var. sublobata, V. radiata* var. *setulosa, V. trilobata, V. trinervia, V. hainiana, V. dalzelliana* (incompatibility in chromosomal pairing)	Pandiyan et al. (2008)

(Derived from Dikshit et al. 2020; Gayacharan et al. 2020)

abortion was observed (Ahn and Hartman 1978a, b). In addition, very high flower drop under artificial pollination makes crossing in *Vigna* quite tedious. The anthesis timing (from 05:00 a.m. to 08:00 a.m.), dehiscence of anthers (10–14 h before anthesis), and stigma receptivity (from anthesis till 6 to 8 h after anthesis) are observed nearly identical in three crossable *Vigna* species, viz., *V. umbellata, V. mungo,* and *V. radiata* (Bhanu et al. 2018).

Mungbean produces successful hybrids when used as female and are crossed with *V. mungo* (urdbean), *V. umbellate* (rice bean), and *V. angularis* (adzuki bean) as male or pollen parent. Interspecific hybridizations between mungbean and urdbean have been attempted for the transfer of high methionine content from urdbean (Nair et al. 2013). Embryo rescue has been reported for the isolation of hybrids derived from such wide (mungbean × urdbean) crosses (Verma and Singh 1986a). Further, some reports mentioning successful mungbean × rice bean crosses have been reported especially for the transfer of mungbean yellow mosaic virus (MYMV) resistance in mungbean from rice bean (Verma and Singh 1986b). The details of possible wide hybridization are presented in Table 22.3. Further, the potential *Vigna* species and their useful traits for introgression are listed in Table 22.4. Till now, in India, there are three varieties which are reportedly developed through wide hybridization, viz., HUM 1, Pant mung 4, and IPM 99–125.

22.6 Genetic Studies for Breeding in Mungbean

Systematic crop improvement requires complete knowledge about the important traits, genetic resources available, and genetics underlying the target traits under consideration for improvement. Mungbean expresses wide variations for a number of morphological and biochemical traits. Genetics of any trait is very important as it helps in understanding the nature of gene action which in turn can be used to device the breeding strategy for the transfer of such economic traits in the elite genetic

Table 22.4 *Vigna* species and their useful traits for introgression

Vigna species	Useful trait(s)	Hybridization amenability
V. radiata var. *sublobata*	Salinity, alkalinity, and cold tolerance; MYMV and bruchid resistance	Crossable with mungbean
V. mungo	MYMIV and fungal disease resistance; shattering tolerance	Crossable with mungbean
V. umbellata	Bruchids and MYMV resistance; high productivity	Crossable with mungbean but F_{1s} are sterile
V. aconitifolia	High temperature and drought tolerance	No report
V. angularis	Determinate growth habit; early maturity; high productivity and high harvest index	Partially crossable
V. glabrescens	Bean fly resistance	F_{1s} can be obtained through embryo rescue

(Derived from Nair et al. 2020)

background. The genetics of various key traits in mungbean is presented in Table 22.5.

22.7 Floral Biology and Crossing in Mungbean

Flowers of mungbean are borne on an axillary or terminal raceme, and peduncles are up to 13 cm in length having clusters of 10–20 flowers. Corolla is yellow in color and papilionaceous, sometimes curved (5–10 cm). In general, small flowers are borne in capitate clusters on the end of long hairy peduncles. Petals are five in numbers, which are of three types, viz., 01 standard, 02 wings, and 02 keels. Androecium or the male reproductive part has 10 stamens in diadelphous condition (09 + 01). Gynoecium or the female reproductive part is made up of stigma, style, and ovary. Gynoecium is monocarpellary having superior unilocular ovary. The stigma is hairy and placentation is marginal in nature. Keel encloses the reproductive organs (10 stamens and one gynoecium).

The mungbean, urdbean, and rice bean plant flowers in phases with axillary or terminal racemes containing a cluster of 10–20 cleistogamous flowers. Flower shedding to the tune of 60% have been reported, while 2–5% outcrossing is found common in these *Vigna* species, which varies as per cultivar and season (Rheenen 1964; Bhadra and Shill 1986). The degree of outcrossing was found ranging between 0% and 8.26% in mungbean, and bold seeded varieties showed more outcrossing over small seeded ones while rainy season showed greater incidence of natural outcrossing (Dikshit et al. 2017). Boling et al. (1961) proposed the following hybridization procedure so as to achieve maximum success in both intra- and interspecific hybridization in *Vigna.* Mungbean must be taken as female or seed parent to make successful interspecific crosses with other *Vigna* species like urdbean and rice bean. In reciprocal crosses, either pod abscises in early stage or, if

Table 22.5 The genetics of various morphological traits in mungbean

S. no.	Trait	Genetics	References
1.	Twining habit	Single dominant gene	Sen and Ghosh (1959) and Khattak et al. (1999)
2.	Twining habit	Single recessive gene	Pathak and Singh (1963)
3.	Semi-spreading habit	Single dominant gene	Pathak and Singh (1963)
4.	Indeterminate growth habit	Single dominant gene	Talukdar and Talukdar (2003)
5.	Days to maturity	Epistatic gene action	Wilson et al. (1985)
6.	Purple hypocotyl color	Single dominant gene	Swindell and Poehlman (1978)
7.	Anthocyanin pigmentation (peduncle, petiole, stem, hypocotyl, and epicotyl)	Single dominant gene; dominant gene with pleiotropic effect	Pathak and Singh (1963), Virk and Verma (1977) and Dwivedi and Singh (1986)
8.	Anthocyanin pigmentation	Single recessive gene	Appa Rao and Jana (1973)
9.	Anthocyanin pigmentation	Recessive epistasis	Mukherjee and Pradhan (2002)
10.	Stem fasciation	Single recessive gene with pleiotropic effect on the number of floral organs	Dwivedi and Singh (1990)
11.	More branches per plant	Epistatic interaction	Malik and Singh (1983)
12.	Plant height	Additive and nonadditive effects	Yohe and Poehlman (1972), Malik and Singh (1983) and Wilson et al. (1985)
13.	Pentafoliate leaf shape	Single dominant gene	Chhabra (1990)
14.	Lobed trifoliate leaf	Dominant over entire leaf shape	Talukdar and Talukdar (2003) and Chhabra (1990)
15.	Trilobed leaves	Two dominant genes (*Tlb1 & Tlb2)*; Duplicate gene action	Sareen (1982)
16.	Narrow lanceolate leaf	Two recessive genes, *'nl1'* and *'nl2'*	Dwivedi and Singh (1986)
17.	Simple inflorescence	Two dominant genes (Í1′ and Í2′)	Sen and Ghosh (1959)
18.	Compound inflorescence	Double recessive homozygous lines	Sen and Ghosh (1959)
19.	Single cluster per node	Dominant gene "C"	Singh and Singh (1971)
20.	Three clusters per node	Recessive gene "c"	Singh and Singh (1971)
21.	Induced sterility	Monogenic recessive	Saini et al. (1974)
22.	Petal color (light-yellowish olive)	Singe dominant gene	Bose (1939)
23.	Days to 50% flowering	Additive gene action	Yohe and Poehlman (1972) and Wilson et al. (1985)
24.	Days to 50% flowering	Partial dominance	Luthra et al. (1979)

(continued)

Table 22.5 (continued)

S. no.	Trait	Genetics	References
25.	Pod shattering to non-shattering	Single dominant gene	Verma and Krishi (1969)
26.	Swollen pod tip over tapering pod tip	Single dominant gene	Sen and Ghosh (1959)
27.	Pod pubescence	Independent duplicate genes	Khadilkar (1963)
28.	Pod length	Additive gene effects	Luthra et al. (1979) and Wilson et al. (1985)
29.	Pod length	Partial dominance	Malhotra et al. (1980)
30.	Seed-coat color	Monogenic inheritance	Khattak et al. (1999) and Lambrides et al. (2004)
31.	Seed-coat color	Two dominant genes	Bose (1939)
32.	Seed-coat color	Dominant allele (*B*-) for black and (bb) for green	Chen and Liu (2001)
33.	Seed-coat color	Three gene pairs	Sen and Ghosh (1959)
34.	Seed-coat color	Dominant allele "A" (green) over "Sp" (spotted)	Rheenen (1965)
35.	Seed-coat color	Five major genes with non-allelic interactions	Chhabra (1990)
36.	Seed hardness	Four loci	Humphry et al. (2005)
37.	Seed weight	Additive gene effects	Yohe and Poehlman (1972) and Wilson et al. (1985)
38.	Seed weight	Dominant gene action	Singh and Jain (1971) and Rao et al. (1984)
39.	Photo-insensitiveness	Single dominant gen	Verma (1971)
40.	Earliness and photo insensitivity	Digeneic control	Tiwari and Ramanujam (1976)
41.	Sensitivity to photoperiod	Dominant or partially dominant gene	Swindell and Poehlman (1978)
42.	Photoperiod sensitivity	Two recessive genes	Islam et al. (1998)
43.	Seed yield	Additive gene action	Yohe and Poehlman (1972), Luthra et al. (1979) and Malik and Singh (1983)
44.	Seed yield	Nonadditive genes	Singh and Jain (1971)
45.	Seed yield	Partial dominance	Singh and Jain (1971)
46.	Seed yield	Overdominance	Malik and Singh (1983)
47.	Pods per plant	Additive gene action	Yohe and Poehlman (1972)
48.	Pods per plant	Partial dominance to overdominance	Luthra et al. (1979)
49.	Seeds per pod	Additive gene action	Luthra et al. (1979)
50.	Seeds per pod	Partial dominance to overdominance	Singh and Jain (1971) and Luthra et al. (1979)
51.	Seeds per pod	Epistasis	Malik and Singh (1983)
52.	Pods per cluster	Additive effects	Malik and Singh (1983) and Wilson et al. (1985)

(continued)

Table 22.5 (continued)

S. no.	Trait	Genetics	References
53.	Pods per cluster	Partial to overdominance	Singh and Singh (1971)
54.	Total phosphorus and phytate P	Dominant recessive epistasis	Sompong et al. (2010)

developed, it contains nonviable seeds. Flower buds having light-green color with optimum size should be selected and emasculated preferably in the evening around 16:00–18:00 h. During emasculation, it is advised to open only the upper half of the standard, wing, and keel petals with the help of dissecting needle or fine-tip forceps so as to expose the stigma and anthers. The anthers are then carefully removed with the help of forceps. This method increases the success rate of pollination/fertilization as there are fewer disturbances on style and ovary during emasculation and also pollination.

Pollination should be done in the next morning between 5:00 and 7:00 h. However, the time of pollination is directly associated with the prevailing weather conditions. For mungbean which is grown during summer season, the most optimum time for pollination is between 5:30 and 6:30 h, while during *kharif*, it should be performed between 6:00 and 7:00 h in the morning. It was observed that the pollination success rate decreases if it is performed after sun shines completely or if there are rains just after the pollination. For pollination, the feathery part of the stigma of the previous day emasculated bud is gently rubbed with dehisced anthers for getting maximum success rate (Fig. 22.1). To avoid any severe load, a total of 8–12 flowers per plant per day should be emasculated besides picking the self-pollinated flowers/pods (Bhanu et al. 2018).

22.8 Breeding in Mungbean

22.8.1 Breeding Objectives

Besides various biotic and abiotic stresses, there are a number of economic traits which seek immediate attention by the mungbean breeders. Development of extra-early and photothermal insensitive genotypes can suitably fit in various cropping systems. In addition, these can also avoid the terminal drought especially in rainfed areas. Traits like root architecture influences the nutrient and water uptake efficiency; dense leaf pubescence reduces leaf temperature and water loss by transpiration and enhances photosynthesis and vegetative vigor (Du et al. 2009); thus, work on these traits should also be initiated. The mungbean protein is deficient in sulfur-containing amino acids (methionine and cystine), and there is need to improve these by taking an important breeding target. Root extension and higher number of lateral roots can improve the possibilities of scavenging for P; thus, acquisition and mobilization of P within plants should also be considered as a key goal for mungbean breeder.

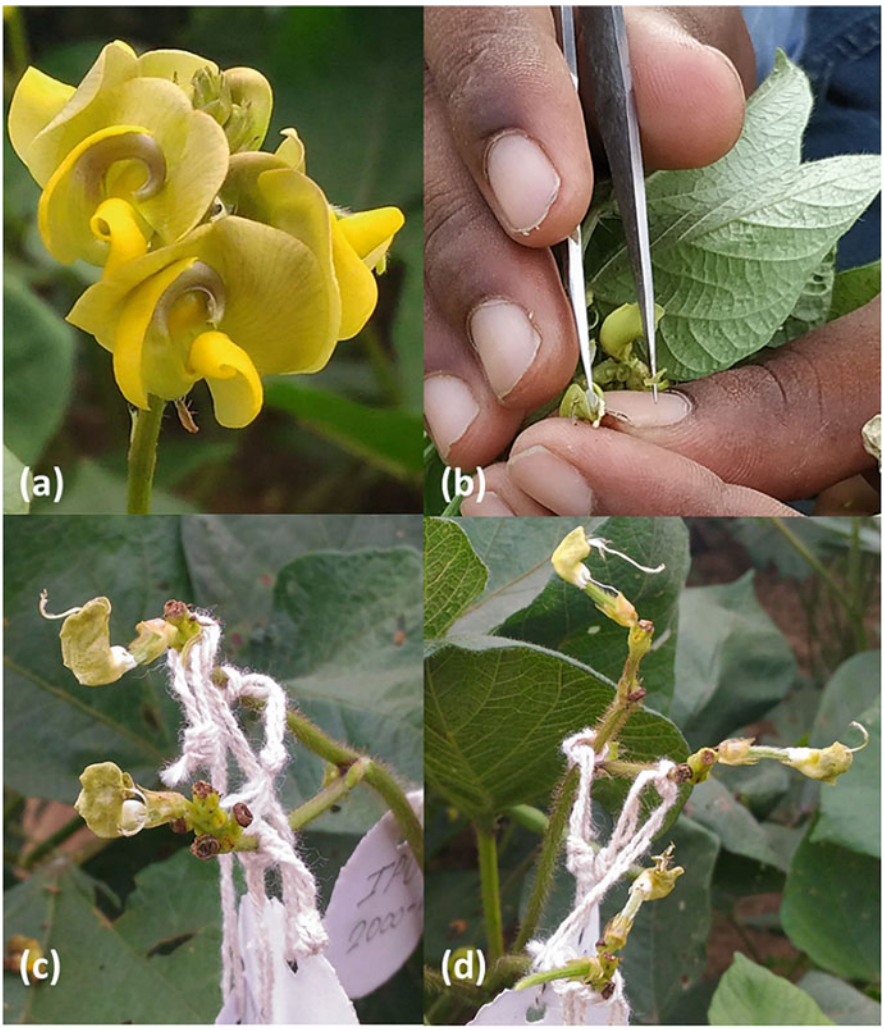

Fig. 22.1 Crossing in mungbean. (**a**) Flower; (**b**) emasculation of unopened flower bud (preferably in the evening); (**c**) flower buds with protruding stigma after pollination (next day morning); (**d**) pod setting in the pollinated flower buds (2 days after pollination)

Besides, there are certain traits which are season specific and require attention of the mungbean breeders: mungbean which are grown during kharif season should be of semi-determinate nature with 90–120 days maturity, medium plant height (70–75 cm), large inflorescence, more clusters/plant, 3–4 branches/plant, 8–10 seeds/pod, 100 seed weight of 3–4 g, tolerance to shattering, and moderate seed dormancy. However, the mungbean genotypes, targeted for spring or summer season, should be of short maturity duration (55–65 days), determinate growth habit and high harvest index, fast initial growth and reduced photoperiod sensitivity,

high pod set in first reproductive flush, long pod (>10 seed per pod), number of pods at top of plants that remain non-shattering, and vegetative growth should terminate with flowering.

22.8.2 Breeding Methods and Varieties Developed

There are a number of breeding methods (including introduction, hybridization, early generation selection, and mutation), which are being used for the development of high yielding varieties. In addition, a number of molecular markers are also used to enhance precision of trait selection during breeding for new variety (Ranali and Cubero 1997). The details of different breeding methods being used in the mungbean improvement and the varieties developed in India are discussed in this section.

22.8.2.1 Introduction

The introduction involves the direct release of introduced germplasm as a variety. This is considered as the primary approach in the crop improvement program. In India, during the last few decades, a number of mungbean germplasm and advanced breeding lines are being introduced from the World Vegetable Center (Erstwhile AVRDC), Taiwan. These introduced genotypes possessed a number of economic traits such as earliness, bold seed size, long pods having nearly 18 seeds per pod, etc. These unique germplasms were used in the Indian mungbean breeding program for broadening of the mungbean genetic base and also for their direct release as a variety (after intensive testing). The varieties like Pusa 105, Pusa 9531, Pant Moong 5, Pusa Vishal, and SML 668 are the examples of direct introduction. The details of the mungbean varieties developed directly from exotic germplasm sources are presented in Table 22.6.

22.8.2.2 Pureline breeding

Pureline breeding or pureline selection is a method commonly used for the self-pollinated crops like mungbean for the development of a variety. In this method, selection of desired plant types is made by the breeder from a genetically heterogeneous population including landrace (Gupta and Kumar 2006; Tickoo et al. 2006).

22.8.2.3 Recombination Breeding

Through hybridization (both intra- and interspecific), breeders aim to combine a number of traits of interest and desirable recombinants are selected in the segregating generations. Interspecific hybridization is mainly aimed to develop pre-breeding material for the transfer of some key traits which is otherwise not available in the cultivated genotypes. These pre-breeding materials are subsequently used for the development of improved varieties. Rare alleles can be identified from exotic lines, landraces, and primitive genotypes. After crossing, the segregating generations can be advanced following pedigree, bulk, recurrent, backcross, or single-seed methods of selection.

Table 22.6 Mungbean varieties developed using exotic germplasm in India

Germplasm	Origin/Country	Variety developed
V3484	AVRDC, Taiwan	Pusa101, WGG2
VC1137–213 (M 178)	AVRDC, Taiwan	Pusa105
VC 6368 (46–40-4)	AVRDC, Taiwan	UPM 98
VC 6370 (30–65)	AVRDC, Taiwan	UPM 98–1
V2164	AVRDC, Taiwan	SML134
VC 6368	AVRDC, Taiwan	Pant mung 5
VC6368 (ML 26)	AVRDC, Taiwan	Pant mung 2
NM94	Pakistan/AVRDC, Taiwan	SML688
NM9473	Pakistan/AVRDC, Taiwan	Pusa 9531
NM92	Pakistan/AVRDC, Taiwan	Pusa Vishal
China moong	China	Shining Moong 1, Sunaiana, RMG62, Jalgaon 781, DGGV-2
VC6367 (44–55-2)	Thailand	IPM 410–3 (Shikha)
Iranian germplasm	Iran	PS 16
CES44	Philippines	AAU34
MG50–10	Philippines	Co5, Co6

(Source: Gayacharan et al. 2020)

Recurrent selection is being used in case we need to break the undesirable linkage and accumulate the desirable linkage. Early generation testing was suggested by Burton (1997) for discarding the undesirable progenies. This involves early-generation selection in F_2, F_3, and even F_4 generations, and based on the expression of target traits, undesired families are rejected, which in turn reduces the overall population load. Interspecific hybridization involving *V. radiata* × *V. mungo* have led to the development of four mungbean varieties (Pant M4, HUM1, Meha, PM6) having better plant types. Some key traits like sympodial bearing, non-shattering, YMD resistance, etc. are targeted from urdbean to mungbean. The details of mungbean varieties developed before the inception of AICPIP program (Table 22.7), varieties identified/released by All India Coordinated Pulses Improvement Project (Table 22.8), and varieties released by the states of India (Table 22.9) are listed below. Two high yielding varieties are presented in Fig. 22.2.

22.8.2.4 Mutation Breeding

Mutations may occur naturally or these may be induced, especially for the traits which are lacking in the primary gene pool. Induced mutation effectiveness is dependent on the ability of the mutagen to cause the desired mutation(s) per unit dose of the mutagen, while efficiency of the mutagen is dependent on the undesirable

Table 22.7 State-wise list of varieties developed before the inception of All India Coordinated Pulses Improvement Project (AICPIP) in India

State	Varieties
Maharashtra	Sindhkheda 2–3, Chinamung 1/49, Jalgaon 17, Kopergaon, and Jalgaon 781
Madhya Pradesh	Krishna II, Gwalior 3, Ujjain 16, Kopergaon, Khargaon 1, Jawahar 45, Kachrod 5, and Bhilsa Green
Uttar Pradesh	T 1, T 2, T 44, and T 51
Tamil Nadu	Co 1, 367/2, 367/4, ADT 1, Co 2, and KM1
Rajasthan	R 288–8, D 66–26 ad RS 4
Bihar	Kanke, NP 23, BR 2, BR 1, BR 5, BR 6, BR 7
West Bengal	B1, B 105, T 10, Kharif Sona, Kulu type 1, No. 49
Punjab and Himachal Pradesh	Mung 54, Mung 305, Shining Mung 1
Gujarat	D 45–6, Gujarat 1 and Gujarat 2
Haryana	No. 305
Orissa	T 150, Utkal 2, Selections 196, 687, 855, 932, 946, T 1630, and T 2105

(Source: AICRP-MULLaRP)

changes it causes like sterility, injury, and lethality (Goud 1967). Among various chemical mutagens, ethyl methanesulfonate (EMS) showed higher mutagenic efficiency over others (Khan and Wani 2006). Gamma rays and ethidium bromide were used for the creation of variation for various agronomic traits (Gunasekaran et al. 1998).

Mutations for high protein and yield (Chakraborty et al. 1998), leaf and pod mutants and semi-dwarf plants (Srinives et al. 2000; Tah 2006), and resistance to powdery mildew, *Cercospora* leaf spot (CLS), and cowpea weevil (Wongpiyasatid et al. 1999) were reportedly induced in mungbean using gamma rays. In mungbean, a number of mutations were created for yield and other agronomic traits (Wani et al. 2017; Das and Baisakh 2018). This method of breeding has been used very effectively to develop new mungbean varieties (Ahloowalia et al. 2004; Gopalakrishna and Reddy 2009). The details of varieties developed using this approach is reported in Table 22.10. Most of the varieties developed have early maturing and high yielding and are tolerant/resistant to YMD (Ahloowalia et al. 2004). In addition, varieties like Pusa Vishal and SML 668 have been developed through selection in a mutant line NM 94. Mutant varieties NIAB Mung 92 and NIAB Mung 98 are very popular in Pakistan.

22.8.3 Breeding for Grain Quality

In the entire South Asian regions, mungbean is very frequently consumed as cooked/boiled dry grains with added spices known as *dal,* which is a kind of stew (Pratap et al. 2021). Till date, a few methodical efforts have been undertaken to improve the nutritional quality of mungbean. Mungbean grains contain very high protein

Table 22.8 List of mungbean varieties identified/released by All India Coordinated Pulses Improvement Project since 1985

S. no.	Name of variety	Developing center	Pedigree	Year of release	Average yield q/ha	Days to maturity	Area of adaptation
1.	ML 267	PAU, Ludhiana	ML1 × LM987	1987	10–11	75	NWPZ
2.	PDM 11	IIPR, Kanpur	Selection from LM595	1987	8.33	75	CZ
3.	PDM 54	IIPR, Kanpur	Selection from Kundawa Bahraich	1987	9.11	65	NEPZ
4.	Pusa 105	IARI, New Delhi	Selection from M178	1987	10.0	75	CZ
5.	Vamban 1	Vamban	S-8 × PIMS3	1989	8.0	65	Tamil Nadu
6	RMG 62	Durgapura	R 288–8 × China mung	1991	7.0	65–70	Rajasthan
7.	ADT 3	TNAU, Adutharai	(M70–16 × Rajendra) × G65	1991	10.7	65–70	Tamil Nadu
8.	Co 5	TNAU, Coimbatore	KM2 × MG50-10	1991	9.0	70–75	Tamil Nadu
9.	MUM 2	M.U., Meerut	Mutant of K851	1992	12.0	60–70	NWPZ
10.	BM 4	ARS, Badnapur	Mutant of T44	1992	10–12	65–70	CZ
11.	AKM 8803	PKV, Akola	PIM53 × MH1	1992	10.5	65–70	Maharashtra
12.	Narendra Mung 1	NDUA & T, Faizbad	G65 × UPM79-3-4	1992	10.0	60–70	Uttar Pradesh
13.	Phule M 2	MPKVV, Rahuri	J781 × ML	1992	6.9	65	Maharashtra
14.	AAU 34	AAU, Assam	CES 44/ML5	1992	10.0	60–65	Assam
15.	TARM 2	BARC/Akola	RUM5 × TPH1	1994	9.5	65	Maharashtra
16.	Pusa 9072	IARI	Pusa106 × 10–215	1995	9.0	65–75	SZ (Rabi)
17.	Warangal 2 (WGG 2)	APAU, Warangal	W 75–70 × Pusa101	1995	14.0	65–70	Andhra Pradesh

(continued)

Table 22.8 (continued)

S. no.	Name of variety	Developing center	Pedigree	Year of release	Average yield q/ha	Days to maturity	Area of adaptation
18.	LGG 407	APAU, Lam	Mutant of Pant M2	1995	14.0	70–75	Andhra Pradesh
19.	LGG 450	APAU, Lam	Mutant of Pant M2	1995	13.0	70–75	Andhra Pradesh
20.	Gujarat Mung 3	S.K. Nagar	–	1995	–	–	Gujarat
21.	Jawahar Mung 721	JNKVV, Indore	ML5 × PIMS3	1995	12.4	70–75	Madhya Pradesh
22.	ML 613	PAU, Ludhiana	ML2993 × ML229	1996	13.0	84	Punjab
23.	PDM 84–178	IIPR/ Kathalgeri	–	1996	8.1	65–70	Andhra Pradesh
24.	TARM 1	BARC/Akola	RUM5 × TPM1	1996	12.4	79	Maharashtra
25.	TARM 18	BARC/Akola	PDM54 × TARM2	1996	12.0	68	Maharashtra
26.	SML 134	PAU, Ludhiana	V2164 × ML258	1996	11.0	68	Punjab
27.	Pant Moong 4 (UPM 92–1)	Pantnagar	T44 × UPU2	1997	7.1	68	NEPZ
28	HUM1 (Malviya Jyoti)	BHU, Varanasi	BHUM1 × Pant U30	1999	9.4 8.1	65 60	CZ & SZ
29	RMG 268	RAU, Durgapura	R 288–8 × J 781	1997	8.9	60	Rajasthan
30.	CO6	TNAU, Coimbatore	WGG 37 × CO 5	1999	10.0	65	Tamil Nadu
31.	Pusa 9531	IARI, New Delhi	Selection from NM 9473	2000	9.0	60	CZ

32.	Pusa Vishal	IARI, New Delhi	Selection from NM 92	2000	11.0	62	NWPZ
33.	Ganga 8	ARS, Sriganganagar	K 851 × Pusa 105	2001	9.2	72	NWPZ
34.	OUM11–5	OUAT, Berhampur	Mutant of Dhauli	2002	7.3	62	SZ
35.	HUM2 (Malaviya Jagriti)	BHU, Varanasi	Selection from TVCM-3	2000	10.5	67	Uttar Pradesh & Uttaranchal
36.	HUM6 (Malaviya Janpriya)	BHU, Varanasi	Selection from BHUM 54	2001	10.0	68	Uttar Pradesh
37.	HUM12 (Malaviya Janchetna)	BHU, Varanasi	HUM 5 × DPM 90-1	2003	11.2	60–62	NEPZ
38.	Meha (IPM 99–125)	IIPR, Kanpur	PM3 × APM36	2004	9.8	66	NEPZ
39.	TM B 37	BARC, Mumbai	Kopergaon×TARM2	2005	11.0	65	NEPZ
40.	COGG912 (COGG 7)	TNAU, Coimbatore	MGG 336 × COGG 902	2005	8.0	62	SZ
41.	HUM16 (Malaviya Jankalyani	BHU, Varanasi	Pusa bold-1 × HUM8	2006	10.9	55–58	NEPZ
42.	MH 2–15 (Sattaya)	Hisar	PDM116 × Gujrat−1	2007	10.55	67	NWPZ
43.	Pant Mung-6	GBPUA & T, Pantnagar	Pant M2 × AMP36	2007	10.52	96	NHZ

(continued)

Table 22.8 (continued)

S. no.	Name of variety	Developing center	Pedigree	Year of release	Average yield q/ha	Days to maturity	Area of adaptation
44.	KM 2241	CSAU, Kanpur	Samrat × PDM 54	2008	10–11	65–70	NHZ
45.	IPM02–3	IIPR, Kanpur	IPM99–125 × Pusa bold2	2009	11.0	62–68	NWPZ
46.	PKVAKM 4	PKV, Akola	BM4 × PS16	2009	10.0	62–66	CZ & SZ
47.	Pusa0672	IARI, New Delhi	11/395 × ML 267	2009	10.0	64	NHZ
48.	IPM02–14	IIPR, Kanpur	IPM99–125 × Pusa bold2	2010	11.0	60–65	SZ
49.	DGGV-2	UAS Dharwad	Chinamung × TM-98-50	2014	11–14	70–75	Karnataka
50	MH421	CCSHAU, Hisar	Muskan × BDYR2	2014	10–12	60–61	NWPZ
51.	Pusa1371	IARI, New Delhi		2016	9–10	81–91	NHZ
52	IPM410–3 (Shikha)	IIPR, Kanpur	IPM03–1 × NM1	2016	11–12	65–70	NWPZ/CZ
53	IPM 205–7 (Virat)	IIPR, Kanpur		2016	10–11	52–56	Punjab, Haryana, Rajasthan, Uttar Pradesh, Bihar, Jharkhand, Madhya Pradesh, Gujarat, Tamil Nadu, Telangana, Andhra Pradesh and Karnataka
54	SML115	PAU, Ludhiana	SML134 × SML715	2016	11–12	65–70	NEHZ
55.	GM6	SDAU, S.K. Nagar	GM 9926 × Pusa Vishal	2018	11–12	70–75	NEHZ

56	IPM512–1 (Soorya)	IIPR, Kanpur	IPM 99–125 × Co 5	2020	12–13	60–65	NEPZ
57	MH1142	HAU, Hisar	MH 421 (Muskan × BDYR2)	2020	11–12	60–65	NEPZ & NWPZ

Source: Project Coordinators Report (2020) AICRP-MULLaRP. *CZ* central zone, *NEPZ* northeastern plain zone, *NHZ* northern hill zone, *NWPZ* northwestern plain zone, *SZ* southern zone, *AAU* Assam Agricultural University, *APAU* Andhra Pradesh Agricultural University, *ARS* Agriculture Research Station, *BARC* Bhabha Atomic Research Center, *BHU* Benaras Hindu University, *CCSHAU* Chaudhary Charan Singh Haryana Agricultural University, *GBPUA & T* G.B.Pant University of Agriculture and Technology, *HAU* Haryana Agricultural University, *IARI* Indian Agricultural Research Institute, *IIPR* Indian Institute of Pulses Research, *JNKVV* Jawaharlal Nehru Krishi Vishwavidyalaya, *MU* Meerut University, *MPKVV* MP Krishi Vishwa Vidyalaya, *NDUA & T* Acharya Narendra Deva University Of Agriculture And Technology, *OUAT* Odisha University of Agriculture and Technology, *PAU* Punjab Agricultural University, *PKV* Dr. Panjabrao Deshmukh Krishi Vidyapeeth, *RAU* Rajasthan Agricultural University, *SDAU* Sardarkrushinagar Dantiwada Agricultural University, *TNAU* Tamil Nadu Agricultural University, *UAS* University of Agricultural Sciences

Table 22.9 Details of mungbean varieties released by the Indian states

S. no.	Name of variety	Developing center	Pedigree	Year of release	Average yield q/ha	Days to maturity	Area of adaptation
1.	Asha	CCS HAU, Hisar	K851 × L24–2	1993	10–14	78	Haryana
2.	MGG 295	ARS, Madhira	Pims-4 × cO-3-5-2	1993	12–14	65–70	Andhra Pradesh
3.	LGG 410	RARS, Lam	Mutant from ML26–10-3	1994	14–16	65–70	Andhra Pradesh
4.	LGG 460 (Lam 460)	RARS, Lam	LamM2 × ML267	1997	15–16	65–70	Andhra Pradesh
5.	PBM 1	PAU	Induced mutation of ML131	1998	12.50	75	Punjab
6.	K1	ARS, Kovilpatti	Co4 × ML65	1998	–	70–75	Tamil Nadu
7.	Pratap	RARS, Shillongani/ AAU, Assam	ML56 × PIMS1	1999	12–14	60–70	Assam
8.	Pragya	IGKV, Raipur	Selection from germplasm	1999	9–10	90–100	Chhattisgarh
9.	Suketi	CSK HPKV, HAREC, Dhaulakuan	Selection from DPM8909	2000	10	85	Himachal Pradesh
10.	Samrat (PDM 139)	IIPR, Kanpur	ML20/19 × ML5	2001	10–11	60–65	Uttar Pradesh
11.	AKM - 8802	Dr. PDKV, Akola	MH-1 × PIMS-4	2000	10–11	61–63	Maharashtra
12.	RMG 344	RARI, Durgapura	Mung selection-1 × J-45	2001	8–9	62–74	Rajasthan
13.	G M- 4	S.K. Nagar, Gujarat	G M-3 × Pusa9333	2001	8.59	61 to 68	Gujarat
14.	VBN (Gg) 2	NPRC, Vamban	VGG4 × MH309	2001	9.00	65–70	Tamil Nadu
15.	VRM (Gg)1	ARS, Virinjipuram	Selection from K851	2001	–	60–70	Tamil Nadu
16.	PDM 139	IIPR, Kanpur	ML20–20/19 × ML5	2001	10.12	58–62	Uttar Pradesh and Uttrakhand
17.	Pant Mung 5	GBPUA & T, Pantnagar	Selection from VC6368	2002	12–15	60–65	Uttar Pradesh and Uttrakhand
18.	MGG 348	ARS, Madhira	MGG-329 × narp-1	2002	12–14	65–70	–
19.	RMG 492	RARI, Durgapura	Mutant of RMG 62	2003	9–10	65–70	Rajasthan

20.	Pusa Ratna (Pusa 9972)	IARI, New Delhi	VC6368 × ML267	2004	11–12	72	NWPZ, National Capital Region (NCR)
21.	Muskan	CCS HAU, Hisar	PDM116 × Gujarat–1	2004	12–15	75	Haryana
22.	Shalimar Moong-1	SKUAST-K	Selection from Jalgoan Mungbean	2005	8.0	105–115	Kashmir
23.	PKV Green gram (AKM-9911)	Dr. PDKV, Akola	BM-86 × MH-1	2007	10–12	64–72	Maharashtra
24.	TM-96-2	BARC & ANGRAU, LAM	Kopergaon×TARM-2	2007	9	65	Andhra Pradesh
25.	TJM-3	BARC & JNKVV, Jabalpur	Kopergaon×TARM-2	2007	9.5	65	Madhya Pradesh
26.	PAU 911	PAU	ML613 × K92–140	2007	12.25	75	Punjab
27.	MGG 347	ARS, Madhira	K-851 × PDM-54	2009	13–15	65–70	–
28.	MGG-207	ARS, Madhira	LBG-165 × LBG-637	2009	12–14	75–80	–
29.	VBN (Gg) 3	NPRC, Vamban	K1 × Vellore local	2009	9.75	65–70	Tamil Nadu
30.	Basanti	CCS HAU, Hisar	Asha × PDM90–1	2010	12–15	64	Haryana
31.	Pairymung	IGKV, Raipur	TARM1 × J781	2010	12–14	65–70	Chhattisgarh
32.	KM 2195	CSAUA & T, Kanpur	K92–140 × PDM54	2010	10–11	60–65	Uttar Pradesh
33.	TM-2000-2 Pairymung	BARC & IGKV Raipur	JL-781 × TARM-2	2010	10.9	88	Chhattisgarh
34.	SML 832	PAU, Ludhiana	SML302 × Pusa Bold-1	2010	11.60	61	Punjab
35.	DGGV-2	UAS Dharwad	Chinamung×TM-98-50	2012	11–14	70–75	Karnataka
36.	Shalimar Moong-2	Srinagar Centre, SKUAST-K	PS-7 × Larkipora Local	2013	10.0	99	Kashmir
37.	CO (Gg) 8	TNAU, Coimbatore	COGG923 × VC6040	2013	–	60	Tamil Nadu
38.	MH 318	CCS HAU, Hisar	Asha×BDYR1	2016	10–14	60–61	Haryana
39.	SGC 16	RARS, Shillongani/ AAU, Assam	PDM91–243 × WGG62	2014	13–14	60–65	Assam

(continued)

Table 22.9 (continued)

S. no.	Name of variety	Developing center	Pedigree	Year of release	Average yield q/ha	Days to maturity	Area of adaptation
40.	BGS 9 (Somnath)	UAS, Raichur	Selection from local land race	2014	12–13	65–68	Karnataka
41.	Utkarsh (KM 11–584)	Maharashtra State seed Corporation	Sel7-1-10 from Amainer	2016	12–13	60–65	Maharashtra
42.	Pant Mung 8 (PM 09–6)	GBPUA & T, Pantnagar	PM3 × NDM99–3	2016	10–11	78–83	Uttarakhand
43.	Yadadri (WGG 42)	PJTSAU, Hyderabad	–	2016	10–12	55–60	Telangana
44.	Sri Rama (MGG 351)	PJTSAU, Hyderabad	–	2016	12–14	60–65	Telangana
45.	MSJ 118 (Keshvanand mung 2)	RARI, Durgapura	Mutant of K851	2016	7–8	60–65	Rajasthan
46.	RMG 975 (Keshvanand mung 1)	RARI, Durgapura	ML613 × ML1189	2016	8–9	65–70	Rajasthan
47.	ML 2056	PAU, Ludhiana	ML1165 × ML1191	2016	11–12	70–75	Punjab
48.	GBM-1	NAU, Gujarat		2016	11–12	102–105	Gujarat
49.	KM2328	CSAUA & T, Kanpur	KM2241 × HUM16	2018	10–12	60–62	Uttar Pradesh
50.	Pusa1431	IARI, New Delhi	Pusa9531 × IPM02–19	2018	12–14	56–66	NCR Region, Delhi State
51.	SGC16 (Rupohi)	AAU, Jorhat, Assam	PDM91–243 × WGG62	2018	12–13	65–70	Assam
52.	GAM 5	AAU, Anand	Selection from VM6	2018	18–19	60–65	Gujarat
53.	Gujarat Mung-7	Navsari	Meha × GM4	2018	10–11	75–80	Gujarat

Source: AICRP-MULLaRP. *GAU* Gujarat Agricultural University, *ANGRAU* Acharya N. G. Ranga Agricultural University, *IGKV* Indira Gandhi Krishi Vishwavidyalaya, *CSK HPKV* Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, *HAREC* Hill Agricultural Research and Extension Centre, *NAU* Navsari Agricultural University, *NPRC* National Pulses Research Centre, *PJTSAU* Professor Jayashankar Telangana State Agricultural University, *RARI* Rajasthan Agricultural Research Institute, *RARS* Regional Agricultural Research Station, *SKUAST-K* Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu

Fig. 22.2 The seed and plants of high yielding variety Pusa 1371 and Pusa 1431.

(20.97–31.32%) (Anwar et al. 2007; Itoh et al. 2006), carbohydrates (62–67%), fat (1.4–1.85%), and fiber (3.5–6%) (Selvi et al. 2006). The details of nutrition present in the mungbean seed are presented in Table 22.11. Mungbean is also an excellent source of folate, potassium (K), and soluble fiber (Singh and Pratap 2016). Ebert et al. (2017) reported that the genetic enhancement of mungbean is possible in terms of protein quality, starch content and quality, mineral contents (iron and zinc), etc. Huge difference has been reported for the crude protein content in mungbean (Das et al. 2015). The seed storage proteins, viz., globulin (60%) and albumin (25%), are also present in good quantity. In addition, this also contains a good amount of essential amino acids like phenylalanine (1.443%), leucine (1.847%), isoleucine

Table 22.10 Mungbean varieties developed through mutation breeding

Country	Varieties
India	BM4, Co4, Dhauli (TT9E), LGG407, LGG450, MUM2, ML26–10-3, Pant mung2, TAP7, TARM1, TARM18, TARM2, TJM3, TM2000–2, TM96–2, TMB37
Bangladesh	Binamoog-1, Binamoog-2, Binamoog-3, Binamoog-4, Binamoog-5, Binamoog-6, Binamoog-7, Binamoog-8, Binamoog-9
Pakistan	AEM-96, NIAB Mung 121–25, NIAB Mung 13–1, NIAB Mung 19–19, NIAB Mung 2006, NIAB Mung 20–21, NIAB Mung 51, NIAB Mung 54, NIAB Mung 92, NIAB Mung 98, NIAB Mung 28
Indonesia	Camar
Thailand	Chai Nat 72, Chai Nut 84–1

(1.008%), valine (1.237%), tryptophan (0.26%), arginine (1.672%), methionine (0.286%), lysine (1.664%), threonine (0.782%), and histidine (0.695%) (Mubarak 2005). As mungbean is rich in lysine, thus it is an excellent complement to rice as balanced human nutrition (Vairam et al. 2016). A negative correlation has been noted between mungbean protein content and methionine content (Yi-Shen et al. 2018). The true digestibility of mungbean was reported to be 73% (Tsou and Hsu 1978; Mubarak 2005).

Mungbean carbohydrates are comprised of starch components (available, resistant), fibers (lignin, cellulose), monosaccharides (maltose, glucose, xylose), and oligosaccharides (raffinose, stachyose, verbascose). Total starch content ranges between 40.6% and 48.9% (Shi et al. 2016), and thus this is preferred for noodle preparation (Nair et al. 2020). Interestingly, mungbean starch has low glycemic index as this contains higher amylose content; thus these can be used in developing products to prevent the risk of diabetes (Hoover et al. 2010). Since this is also rich in iron (40–70 ppm), it is good for the lactating and pregnant women (Vairam et al. 2016). Seed size has been reported to have nonsignificant correlation with micronutrient content (Nair et al. 2015a, b). Varieties with better grain nutrient content would definitely have increased nutritive value as sprouts (Ebert et al. 2017). Nair et al. (2015a, b) have identified the genotypes having improved Fe uptake ability, and these were utilized in breeding for improving the iron content of commercial varieties.

Phytic acid (PA) has been reported to provide resistance to the grains against the bruchid beetle (Srinivasan et al. 2007), but it has negative impact on iron and zinc bioavailability. The low PA content (2.6–3.8 g/kg) and the presence of phenolic compounds such as ferulic acid (1540–3400 mg/g) in mungbean may lead to increased bioavailability of micronutrients (Nair et al. 2015b). Salunkhe et al. (1982) reported that polyphenols are present in higher amounts in colored and darker legume varieties than in the light-colored varieties. Muhammed et al. (2010) reported that the seed coat polyphenols can help the seed against pathogens and improve seed viability. Sprouting enhances the nutritional properties of mungbean significantly and makes sprouts a premium breakfast food across the world. The sprouts are quite high in vitamin C and folate (Nair et al. 2013), while its foliage is

Table 22.11 Nutritional composition of mungbean seed

Nutrition	Range	References
Protein	14.6–33.0 g/100 g	Harper et al. (1996), Mubarak (2005), Anwar et al. (2007), Dahiya et al. (2015) and Itoh et al. (2006)
Fat	1.45–1.85 g/100 g; 2.1–2.7%	Mubarak (2005) and Zia-Ul-Haq et al. (2008)
Fatty acids	Palmitic (2.8–4 g), stearic (1.4–1.7 g), oleic (2.1–2.9 g), linoleic (3.4–4.6 g), linolenic (1.9–2.4 g) and arachidic (0.23–0.25 g) per kg basis	Anwar et al. (2007)
Crude fiber	3.5–6.15 g/100 g	Mubarak (2005) and Dahiya et al. (2015)
Iron	1.15–12.63 mg/100 g	USDA 2010, Harper et al. (1996) and Dahiya et al. (2015)
Iron	3.5–8.7 mg/100 g (Indian lines/ varieties)	Nair et al. (2015a, b)
Zn	2.1–6.2 mg/100 g; 21–62 mg/kg DW	Taunk et al. (2011) and Nair et al. (2015a, b)
Carbohydrates	50–63.4%	USDA (2010), Mubarak (2005), Dahiya et al. (2015) and Kaur et al. (2020)
Starch	Total starch: 40.6–48.9% in seed; resistant starch: 16.1–22.3% (of total carbohydrates)	Shi et al. (2016)
Phosphorus	340–367 mg/100 g	Kaur et al. (2020) and USDA 2010
Phosphorus	2760–5170 mg/kg DW	Nair et al. (2015a, b)
Calcium	77.3 to 247.67 mg/100 g	Agugo and Onimawo (2009) and Kaur et al. (2020)
Calcium	1190–1580 mg/kg DW	Nair et al. (2015a, b)
Other minerals	Mg (970–1700 mg), Cu (7.5–11.9 mg), Mn (9.8–19.6 mg), Se (0.21–0.91 mg), K (8670–14,100 mg)/kg DW	Nair et al. (2015a, b)
Amylose	32%	Lang et al. (1999)
Ash	3.32–3.76 g/100 g	Mubarak (2005)
Energy	338–347 kcal/100 g	Dahiya et al. (2015)
Vitamins		
Carotenoid	0.5–0.8 mg/100 mg (cotyledons) and 0.07–0.44 mg/100 mg (seed coats)	Harina and Ramirez (1978)
Vitamin A	100 lg retinol activity equivalent (RAE) in sprouts; 70 lg RAE /kg grain	USDA (2010)
Vitamin C	0–10 mg/100 g; 1.38 g/kg (sprouts), 0.05 g/kg (grain) (DW basis)	Prabhavat (1990) and Nisha et al. (2005)
Riboflavin content	0.29 mg/100 g	Nisha et al. (2005)

(continued)

Table 22.11 (continued)

Nutrition	Range	References
Folate content	0.0069 g (grain) and 0.0064 g (sprouts)/kg DW basis; 0.0028 g/kg grain	USDA (2010) and Rychlik et al. (2007)
Tocopherol	12.5 mg/100 g	Zia-Ul-Haq et al. (2008)
Phytic acid	1.8–5.8 g/kg grain DW; 2.6–3.8 g/kg	Sompong et al. (2010) and Nair et al. (2015a, b)
Others		
Trypsin inhibitor activity	56–98 [trypsin inhibitor units (TIU)/ mg]	Philip and Prema (1998)
Tannin content	3.1–4 g/kg grain	Philip and Prema (1998)
Saponins	5.7 g/kg DW	Fenwick and Oakenfull (1983)
Total phenolic and flavonoid extracts	0.167–0.192 g ferulic acid equivalent (FAE)/kg DW (sprouts); 0.098–0.101 g FAE/kg DW (dry seed)	Kim et al. (2012)
2-Acetyl-1-pyrroline biosynthetic pathway	In aromatic mungbeans	Attar et al. (2017)
Phenolic components	Phenolic acids (1.81–5.97 mg rutin equivalent/g), flavonoids (1.49–1.78 mg catechin equivalent/g), tannins (1.00–5.75 mg/g)	Lee et al. (2011), Shi et al. (2016) and Singh et al. (2017a)

consumed as fodder and hay. Sprouting also reduces the indigestible oligosaccharides, tannins, and phytic acid, making it a preferred food (Savage and Deo 1989).

Due to the presence of easily digestible protein in the seeds, this is one of the highly recommended foods for the sick (Yi-Shen et al. 2018). Cereals when consumed in combination with mungbean are known to improve the protein quality and balances sulfur-containing amino acids (of cereals) with that of lysine in mungbean (Boye et al. 2010). A number of health-related properties known in the mungbean are presented in Table 22.12. Due to these properties, mungbean is also used as a component of infant's weaning food (Bazaz et al. 2016). Mungbean induces less flatulence and also has very less "anti-nutritive factors" which can be managed by various types of pre-consumption processing (Dahiya et al. 2014). Pressure cooking is known to degrade phytic acid, while dehusking and germination reduces the total tannin content (Wang et al. 2015). In addition, mungbean is being consumed across the world in different forms with some regional preferences (Table 22.13). Mungbean is also consumed as microgreens or nutrigreens in different parts of the world by the health-conscious people (Priti et al. 2021). Thus, while breeding for high nutrient, care should be taken to breed for the region-specific and preference-based traits for the wider acceptance of the developed variety.

Table 22.12 Health associated properties in mungbean

Compound	Health properties	References
Vitexin and isovitexin	Hypolipidemic (lowers inflammatory cytokines) and anti-melanogenic (tyrosinase inhibitor)	Inhae et al. (2015) and Yao et al. (2011)
Ligans and flavonoids	Hypoglycemic (Inhibits alpha-glucosidase and Alpha amylase activity; decreased fat accumulation)	Liyanage et al. (2018) and Jang et al. (2014)
Mungoin (protease inhibitor) and phenolics	Anticancerous	Ketha and Gudipati (2018), Yao et al. (2016) and Lee et al. (2013)
Vicilin protein hydrolysate	Antihypertensive (ACE-I inhibitory activity)	Xie et al. (2019), Gupta et al. (2018), Wang et al. (2006) and Xu and Chang (2012)
Arabinogalactan and saponins	Immunomodulation (induced release of NO, TNF-α, IL-6, and IL-1β)	Luo et al. (2016), Yao et al. (2011) and Ali et al. (2014)
Antityrosinase	Anti-melanogenesis (inhibited tyrosinase, monophenolase, and diphenolase activities)	Chai et al. (2018) and Kim et al. (2012)

Table 22.13 Preferred grain qualities by the consumers of different regions

Region	Preferred grain qualities by consumer
South Asian regions	Medium to bold grains for *dal;* dehusked and overnight soaked grains for making porridge, candies, etc.
Indian subcontinent	Shiny green and small to medium sized grains
Bangladesh, Sri Lanka, northeastern states of India	Shiny yellow and small grains (<3.0 g/100 seed)
Indonesia, Taiwan, Kenya, and Tanzania	Dull green seeds
India and parts of Southeast Asia	Medium-sized grains, thin seed coat, uniform texture for fried, salted and dehusked dry seeds
Kenya and several African countries	As thick bean stew
China	Cooked with rice and sugar to make a sweet desert soup
Throughout the world	Sprouts, soup mix, noodle garnish, etc.

(Derived from: Nair and Schreinemachers 2020)

22.8.3.1 Markers Associated with Mineral Biofortification

The process of enrichment in the content of some key micronutrients like Fe and Zn is called mineral biofortification. For developing a variety with high concentration of micronutrient like iron and zinc, it is important to identify the germplasm having high content of micronutrients (Singh et al. 2013a). Aneja et al. (2012) reported variation for Fe (29.95–00.97 mg/kg) and Zn (20.13–35.70 mg/kg) content in mungbean genotypes, while Taunk et al. (2012) reported iron and zinc content varying from 46.31 to 106.15 and 23.31 to 40.46 mg/kg, respectively. Singh et al. (2013a) reported wide variations for iron (1.6–9.3 mg/100 g) and zinc (1.5–3.9 mg/

Table 22.14 Molecular mapping for grain micronutrient concentration

QTLs/MTAs	LG/markers/population	References
17 QTLs (2 for Fe and 15 for Zn content)	LG4 (qZn-4-3 and qFe-4-1), LG6, LG7, and LG11 (qZn-11-2 and qFe-11-1)	Singh et al. (2017b)
43 marker-trait associations (MTAs) for Ca, Fe, K, Mn, P, S, Zn	6486 SNPs (explained 22% PVE) by GBS	Wu et al. (2020)
Vradi01g00820, *Vradi01g00830*, and *Vradi01g00840*	Chromosome 1 (K content)	Wu et al. (2020)
Vradi05g16350	Chromosome 5 (P content)	Wu et al. (2020)
Vradi07g26320 and *Vradi07g26340*	Chromosome 7 (P content)	Wu et al. (2020)
Vradi07g14180	Chromosome 7 (K content)	Wu et al. (2020)
Vradi08g22740 and *Vradi08g17100*	Chromosome 8 (K content)	Wu et al. (2020)
Vradi06g09900, Vradi06g10020, Vradi06g10060, Vradi06g10120, Vradi06g10210	Chromosome 6 (Fe content))	Wu et al. (2020)
QTLs for phytic acid P (PAP), total P (TP), and inorganic P (IP	–	Sompong et al. (2012)
QTLs for seed Fe and Zn content	RIL from ML776 × Sattya	Singh et al. (2017b)
Fe: 01 QTL (qFe-11-1) for Fe on LG11; Zn: 04 QTLs (qZn-11-4, qZn-11-5 on LG11 & qZn-4-1, qZn-4-2 on LG4)	RIL from ML446 (high iron content) × Sattya (low iron content)	Singh (2013)

100 g) content in RIL populations. Wu et al. (2020) conducted genome-wide association study (GWAS) for seven minerals which were analyzed using inductively coupled plasma (ICP) spectroscopy in 95 cultivated mungbean genotypes chosen from the United States Department of Agriculture (USDA) core collection representing accessions from 13 countries. The details of markers found linked with quantitative trait loci (QTLs) governing micronutrient content in mungbean is presented in Table 22.14.

22.8.3.2 Nutrient Bioavailability

Anti-nutritional factors present in the mungbean seeds like phytic acid can bind with iron, zinc, calcium, and magnesium and forms insoluble complexes, thereby limiting the mineral absorption in the small intestine (Weinberger et al. 2002). However, through various processing methods like fermentation, germination, dehulling, soaking, and cooking, these anti-nutritional factors can be reduced significantly (Hemalatha et al. 2007). In the sprouted mungbean, the phytic acid content has reportedly declined by 76%, which resulted in an increase in the bioavailability of zinc and iron by 3.0 and 2.4 times, respectively (Nair et al. 2012; El-Adawy 2002).

Similarly, trypsin inhibitors are low-molecular-weight proteins which adversely affect the protein digestion by inhibiting the proteolytic enzymes. Heat treatment, soaking, and sprouting of the seeds are known to lower the trypsin inhibitor activity in mungbean seeds (Chandrashekar et al. 1989).

22.9 Emerging Challenges

The productivity of different mungbean varieties depends upon their genetic constitution and environment in which they are cultivated. Favorable environment at different growth stages helps the mungbean plant in achieving maximum genetic potential. Favorable environment in terms of biotic and abiotic stresses is essentially required for achieving the full yield potential of mungbean crop. MYMV, powdery mildew, *Cercospora* leaf spot, bruchids, and sucking insect pests are all important biotic stresses. Drought, heat, and flooding are the main abiotic stresses affecting the crop.

Mungbean crop is being affected by a number of biotic stresses. Resistance to mungbean yellow mosaic virus (MYMV) was reported under the control of a number of genes including a single recessive gene, two recessive genes, trigenic, etc. (Mishra et al. 2020). Further, a number of weather parameters regulating vector activities are equally important factors for the whitefly-transmitted begomovirus disease management in mungbean. Powdery mildew is a foliar disease caused by *Erysiphe polygoni* which causes yield loss to the tune of 20–40%. For *Cercospora* leaf spot (CLS) resistance, a number of gene actions have been reported (AVRDC 1980; Lee 1980). Bruchid (*Callosobruchus chinensis* and *C. maculates*) may cause severe post-harvest losses, and they can cause complete destruction of stored seed in 2–3 months (Fernandez et al. 1988). Genetics of various biotic stresses in mungbean is presented in Table 22.15.

Mungbean is mostly grown as rainfed crop, and drought-tolerant varieties capable of withstanding soil moisture stress and produce better yield are required to be developed. Varieties that are found to germinate under reduced water potential do not usually fail to germinate and establish into seedlings (Kaur et al. 2017). Root length at seedling stage provides a fair estimate about the root growth in field (Ali et al. 2011; Vincent 2014). In general, deeper and more profuse root systems could be able to tap extra water from the soil profile and alleviate drought effects. Mungbean being rainfed crop sometimes also encounters excessive rains, which causes damage to the crop due to flooding (Miah et al. 1991). Stagnation of water impairs the root growth and this more frequently occurs in Eastern India and Southern Bangladesh. Variation for soil flooding tolerance among mungbean genotypes has been reported by various workers (Amin et al. 2016). The World Vegetable Center mungbean mini core collection consisting of 296 genotypes were screened for 14 root-related traits, and a number of genotypes displaying different root traits were identified as donors for breeding cultivars with enhanced adaptation to water-deficit stress and other stress conditions (Aski et al. 2021).

Table 22.15 Genetics of various key biotic and abiotic stresses in mungbean

Genetics of traits	References
YMD resistance	
Monogenic recessive	Malik et al. (1986), Reddy and Singh (1995), Saleem et al. (1998), Khattak et al. (1999), Khan et al. (2007), Reddy (2009a), Dhole and Reddy (2013) and Sudha et al. (2013)
Complementary recessive genes	Shukla and Pandya (1985) and Alam et al. (2014a)
Dominant and complementary recessive genes	Sandhu et al. (1985)
Trigenic recessive	Mishra and Asthana (1996)
Modifying genes	Khattak et al. (2000)
Digenic recessive	Dhole and Reddy (2012) and Singh et al. (2013b)
Trigenic (02 dominant +01 recessive)	Markam et al. (2018)
Digenic dominant	Mahalingam et al. (2018)
Powdery mildew resistance	
Monogenic dominant gene	Chaitieng et al. (2002)
Two dominant genes "Pm1" and "Pm2"	Reddy et al. (1994)
Single dominant gene	Khajudparn et al. (2007)
Single dominant gene (Pm3)	Reddy (2009b)
Quantitative and additive gene action	Kasettranan et al. (2009)
QTL (PMR1; PVE 65%)	Chaitieng et al. (2002)
QTL (PVE 86%)	Humphry et al. (2003)
Additive and dominance gene actions	Gawande and Patil (2003)
Cercospora leaf spot (CLS) resistance	
Single dominant gene	Chankaew et al. (2011) and Singh et al. (2017c)
Single recessive gene	Mishra et al. (1988)
01 major QTL on LG03	Chankaew et al. (2011)
Bruchid resistance	
Single gene	Miyagi et al. (2004) and Sun et al. (2009)
Br1 locus on LG11	Wang et al. (2016)
Seed hardness	
Single dominant gene 'Br' (*V. radiata* var. *sublobata* accession-TC1966) or few dominant genes	Kitamura et al. (1988), Young et al. (1992) and Humphry et al. (2005)
Mapped the gene on LG8	Kaga and Ishimoto (1998)
Calcareous soil (iron deficiency chlorosis)	
Two genes with inhibitory action	Nopparat et al. (1997)
Single dominant gene (IR) with a few modifying genes	Srinives et al. (2010)
Preharvest sprouting	
Additive and nonadditive gene action; high G × E interaction	Durga and Kumar (1997)

Rise in the temperature by 2050 by 3.2 °C in winter and 2.2 °C during summer as a result of climate change may cause flower drop and poor pod filling. Thus, to sustain the crop production we need to develop climate resilient varieties. Shift in area, especially total area reduction in northern India while an increase in area in Central and South India. In many areas, yields have started declining because of decline in organic matter content in soil and emergence of multi-nutrient deficiencies. Also, a widespread zinc and sulfur deficiency was reported, and nearly 50% pulse growing districts have Zn deficiency. Further, of 137 pulse-growing districts, 87 districts have reported 20–60% sulfur deficiency. Thus, a holistic approach is required for the realization of higher yield from this wonder crop.

22.10 Molecular Breeding and Genetic Engineering

In mungbean, a number of molecular markers have been developed and used for the mapping of various biotic and abiotic traits, with limited success. Various researchers have used SSR markers for deciphering genetic diversity in mungbean (Chen et al. 2015; Liu et al. 2017); however, low SSR polymorphism has been reported in mungbean (Tangphatsornruang et al. 2009). Transferable SSRs from cowpea and adzuki bean have also been used in mungbean for tagging of various QTLs (Kitsanachandee et al. 2013; Gupta et al. 2013). Van et al. (2013) have identified more than 300,000 SNPs in mungbean, and among them only 43 SNPs could be validated as competitive allele-specific polymorphism (KASP) markers (Van et al. 2013; Islam and Blair 2018). To date, a limited studies on GBS-based GWAS have been undertaken in mungbean for genetic mapping and diversity assessment (Noble et al. 2018; Reddy et al. 2020a, b, c). Previous genetic diversity studies of cultivated and wild mungbean germplasm, using both morphological and molecular markers, have highlighted low levels of genetic diversity in cultivated mungbean compared to wild *Vigna* (Pratap et al. 2015). Limited numbers of linkage maps have been developed in mungbean. A high-density map developed using whole genome sequences can enable further advancement in trait dissection (Noble et al. 2018; Sokolkova et al. 2020). The recent release of a reference genome for mungbean provides new opportunities for mungbean genomic research (Kim et al. 2015; Dasgupta et al. 2021). Therefore, quantitative trait locus (QTL) analyses through mapping populations or GWAS using molecular markers and high throughput sequencing techniques provide valuable ways of identifying the genes underlying various traits (Reddy et al. 2020a, 2021a; Aski et al. 2021; Mishra et al. 2020).

To decipher the genetic basis of PUE traits, 144 diverse mungbean genotypes were evaluated through GWAS using 55,634 SNPs. In total, 71 protein coding genes were identified, of which three potential candidate genes VRADI11G08340, VRADI01G05520, and VRADI04G10750 with missense SNPs in coding sequence region were identified (Reddy et al. 2020a, 2021b). RNA-seq analysis was conducted between a resistant and a susceptible mungbean genotype under infected and control conditions, and resistance to MYMIV showed a very complicated gene network, which begins with the production of general PAMPs (pathogen-associated

molecular patterns), then activation of various signaling cascades like kinases, jasmonic acid, and brassinosteroid, and finally the expression of specific genes such as PR-proteins, virus resistance, and R-gene proteins, leading to resistance response (Dasgupta et al. 2021).

Genetic transformation systems have been well developed in mungbean, and several transgenics targeting both biotic (Bhajan et al. 2019) and abiotic stresses (Kumar et al. 2017; Mekala et al. 2016) have been developed which are mostly at proof-of-concept level. Genome editing in mungbean is still in its initial phases, but researchers are aiming to use CRISPR/cas9 for developing virus-resistant transgene-free mungbean plants (Khatodia et al. 2018). With the availability of a deep sequenced reference genome of Asian mungbean, freely available SNPs data, tagged germplasm for specific traits, and easy regulatory policies, the SNP-trait associations will mark the future in mungbean breeding.

22.11 Mungbean Production Technology

The mungbean in India is being grown as kharif, spring, and summer crop. The details of mungbean production technology are described below:

22.11.1 Climate

Although mungbean can be grown in a wide range of climatic conditions, a warm humid climate with temperature ranging from 25°C to 35°C, with 400–550 mm rainfall, well distributed during the growing period of 60–90 days, is suitable for the cultivation.

22.11.2 Soil

Mungbean can be easily grown in a wide range of soils including red laterite soils, black cotton soils, and sandy soils. A well-drained loamy to sandy loam soil is best for its cultivation. The crop does not grow well on saline and alkaline soil or waterlogged soils.

22.11.3 Field Preparation

Mungbean requires proper drainage and ample aeration in the field so that the activities of nitrogen-fixing bacteria are not hampered at any stage of plant growth. In case of water-logging, ridges and furrows may be prepared and levelling and sowing can be performed on the ridges. Otherwise also, ridge sowing gives better yield over normal sowing.

22.11.4 Manures and Fertilizers

Well-decomposed farmyard manure (FYM) should be mixed at the rate of 10–-12 tonnes per hectare one month before sowing. This not only provides the nutrient but also the desired physical quality to the soil. In addition, 5 kg/ha of *Trichoderma viride* can be mixed with FYM, and the mixture is kept under partial shade for 4–5 days before its application to the soil. In general, 100 kg DAP per hectare is the recommended dose for mungbean. If soils deficient in Zn, then 25 kg $ZnSO_4$ per ha should be applied before sowing. Top dressing of N at 15 kg/ha should be done at flowering stage.

22.11.5 Seed Rate

For sowing, quality seeds which are healthy, undamaged, and free of insect pests should be selected. The seed rate depends on the seed size and sowing season. In case of bold-seeded varieties, seed rate of 20 kg/ha is considered optimum in spring and autumn, and 16 kg/ha during summer season. It is advised to have approximately 25 plants/m^2 of plant stand in the field for reaping the good yield.

22.11.6 Seed Treatment

Dried seed may be treated with captan or thiram at 3.0 g/kg of seeds against any seedborne fungal diseases. Seed inoculation with appropriate *Rhizobium* strain is highly recommended, especially in those fields where mungbean cultivation is taken up for the first time.

22.11.7 Isolation Distance

Although mungbean is a self-pollinated crop, some cross-pollination always happens under field conditions. Thus, an isolation distance of 3.0 m should be used so as to get the seeds of desired purity.

22.11.8 Method of Sowing

Line sowing is considered advantageous as it not only requires less seed but also produces more even crop, which is easier to manage and also gives more yield. The spacing between the ridges/rows should be kept as 25–30 cm, while plant-to-plant spacing is kept as 10.0 cm. Seed sowing through broadcasting makes various intercultural operations including weeding very difficult, making harvesting more labor intensive and thereby having significantly reduced crop productivity and poor economic return.

22.11.9 Irrigation

Frequency and number of irrigations required by mungbean crop depends upon season, weather, and soil and field conditions. Usually, first irrigation is required just after seedling emergence. Later, two to three irrigations may be applied at 10- to 15-day intervals depending on the dryness of the season. The last irrigation should be stopped at about 50 days after sowing. Generally, no irrigation is needed during rainy season except under drought-like situation.

22.11.10 Rouging

It is an important task especially in the seed crop to regularly inspect the seed crop and remove any off-type plants present in the field.

22.11.11 Weed Control

In mungbean, it is recommended to select the field with low weed pressure for sowing, as weed control options are very limited in this crop. Both manual weeding and use of herbicides are used for the weed control. The pre-sowing weedicides like Basalin45EC (fluchloralin) (5.0 mL/L) and Treflan48EC (trifluralin) (4.0 mL/L) or preemergence weedicide like Stomp 30EC (pendimethalin) (5.0 mL/L) or preemergence weedicide Stomp 30EC at 3.0 mL/L along with one hoeing, four weeks after sowing, can be used for the effective control of the weeds. Approximately 500 L of water is sufficient for one hectare, and herbicides should be sprayed immediately after sowing for preemergence application. In addition, it is recommended to perform inter tillage by hand or cultivator once or twice to promote healthy crop growth.

22.11.12 Diseases

The important diseases affecting mungbean crop and their control measures are briefly discussed below.

22.11.12.1 Seed and Seedling Rot

A number of fungi such as *Fusarium* sp., *Macrophomina phaseoli,* and *Rhizoctonia solani* cause seed and seedling rot which ultimately results in poor seed germination. These are serious disease and sometimes re-sowing of the crop has to be done if it is not controlled well on time. For the control of this disease, (1) treat the seeds with thiram or captan at 3.0 g/kg of seed, (2) sow fresh and clean seeds which are obtained from a healthy crop, and (3) adopt suitable crop rotation.

Fig. 22.3 Mungbean genotypes expressing resistant and susceptible reaction to yellow mosaic disease (YMD)

22.11.12.2 Mungbean Yellow Mosaic Virus

This virus causes yellow mosaic disease (YMD). The symptom starts as small yellow specks along the veinlets which then spreads over the lamina. In the case of severe infestation, the pods become thin and curl upward (Fig. 22.3). The disease is transmitted by an insect vector named whitefly (*Bemisia tabaci*). For the control of this disease, (1) spray the crop with neem oil at 20 mL/L or with Metasystox 25EC (oxydemeton-methyl) at 3.0 mL/L of water so as to control the whiteflies which are the actual vectors of this disease, (2) grow YMD-resistant varieties, and (3) use yellow sticky traps against whiteflies.

22.11.12.3 *Cercospora* Leaf Spot (*Cercospora canescens*)

This disease can be identified by the appearance of spots on the leaves which are circular to irregularly shaped, with grayish-white centers and reddish-brown to dark-brown margins. For the control of this disease, (1) spray the crop with Dithane Z-78 or Dithane M-45 at 3.2 g/L of water, (2) remove all the plant debris from the crop field, (3) remove all the infected plants and burn, and (4) avoid sowing of the seeds which are harvested last year from the infested field

22.11.12.4 Powdery Mildew (*Erysiphe* sp./*Podosphora* sp.)

This disease commonly occurs under cool weather conditions (20–26 °C) and is favored by cloudy weather. A white-gray powdery mass becomes visible first in circular patches on the dorsal leaf surface, but later this spreads to all over the leaves, stems, and pods (Fig. 22.4). For the control of this disease, (1) spray the crop with neem seed kernel extract (NSKE) at 50 g/L or neem oil at 20 mL/L twice at 10 days

Fig. 22.4 Powdery mildew on the mungbean leaf surface

interval from the initial disease appearance, (2) spray 10% eucalyptus leaf extract at the time of disease initiation and 10 days later, and (3) spray the crop with carbendazim at 1.0 g/L or wettable sulfur with 2.5 g/L of water.

22.11.13 Pests

The important pests infecting mungbean crop and their control measures are as follows:

22.11.13.1 Tobacco Caterpillar (*Spodoptera litura*)

The initial stage larvae are black in color, whereas grown-up larvae are dark green with black triangular spots on body. Its moth lays eggs in masses covered with brown hairs on the lower side of the leaves. After hatching, first and second instar larvae feed gregariously and skeletonize the foliage. Besides leaves, they also damage floral buds, flowers, and pods. For the control of this pest, (1) collect the egg masses and young larvae with leaves and destroy, and (2) spray the crop with commercial neem formulations (including neem oil or neem seed kernel extract), *Bacillus thuringiensis* formulations, *Spodoptera litura* nuclear polyhedrosis virus

(NPV), Novaluron 10EC at 1.5 mL/L or Acephate 75SP at 8.0 g/L or Chlorpyriphos 20EC at 15 mL/L of water.

22.11.13.2 White Fly (*Bemisia tabaci*)

The adults are tiny and very delicate and have white or smoke-colored wings with which they flitter away from plants on little disturbance (Fig. 22.5). Insects stick to the lower surface of leaves and leaves of infested plants show yellowish discoloration. For the control of this pest, (1) spray the crop with neem oil at 20 mL/L or with Metasystox (oxydemeton-methyl) 25EC at 3.0 mL/L of water, and (2) use yellow sticky traps in the field.

22.11.13.3 Bean Pod Borer (*Maruca testulalis*) and Asian Corn Borer (*Ostrinia furnacalis*)

In recent years, these have become very serious pest, causing substantial damage to the mungbean crop. They feed on buds, flowers, pods, and grains. The larvae may be pale green, yellow, brown, or black in color, 3–5 cm in length. Larva presence can be judged from dark-green feces under the plant canopy. For the control of this pest, consider the following: (1) Spray the crop with Acephate at 8.0 g/L or Spinosad at 0.6 mL/L or Indoxacarb at 2.0 mL/L of water. (2) Commercial neem-based formulations or neem oil or neem seed kernel extract or *B. thuringiensis* formulations can also be used as organic control.

22.11.13.4 Bean Fly (*Ophiomyia phaseoli, O. centrosematis, Melanagromyza sojae*)

This is the most important insect pest of mungbean which causes significant damage during the seedling stage. The adult flies are too tiny (2.0 mm) and cannot be recognized easily. The bean fly maggots feed inside the plant stem and their damage cannot be seen from the outside. For the control of this pest, consider the following: (1) Spray Metasystox (oxydemeton-methyl) 25EC at 3.0 mL/L of water. (2) Moth

Fig. 22.5 Whiteflies (in circle) on the dorsal surface of mungbean leaf

bean, chickpea, lentil, and cluster bean crop could be used as "dead-end trap crops"—the bean fly adults lay eggs on these crops, but the eggs fail to hatch.

22.11.13.5 Thrips (*Megalurothrips distalis* and *M. usitatus*)

Thrips are very small insects found in the flowers and causes flower drop, deformation of pods, and ultimately reduction in yield. For the control of this pest, (1) spray Spinosad at 0.6 mL/L of water at flower initiation stage, and (2) do not control the thrips infestation with broad-spectrum chemical pesticides, as this may cause the resurgence of thrips.

22.11.13.6 Cowpea Aphid (*Aphis craccivora*)

Sometimes this pest is also reported to attack mungbean crop. For the control of this pest, consider the following: (1) Spray Imidacloprid at 150–170 mL per 500 L of water per hectare. The spray must be done once we notice unusually high aphid populations (>20 insect per plant). (2) Neem oil may be used either alone or in combination with the entomopathogenic fungi biopesticides. The ladybird beetles and green lacewings are efficient predators of aphids. Protect the population of these predators by avoiding the use of broad-spectrum pesticides.

22.11.13.7 Bruchids (*Callosobruchus chinensis* and *C. maculatus*)

This is also known as pulse beetles or cowpea weevils, and they attack mungbean both in field and storage conditions, but the greater losses occur during storage. The nutritional quality of mungbean grains gets deteriorated rendering them unmarketable. For the control of this pest, consider the following: (1) Maintain proper cleaning of the storage area and proper drying of the seeds (9–10% moisture). For large-scale seed storage, fumigate with phosphine or other suitable fumigants. (2) Treat the grains with clays, sand, kaolin, and ash, as these were proven effective during storage. In addition, vegetable oils (e.g., olive oil or mustard oil at 15 mL/kg of seed) can also be used for the grain treatment. (3) Use traps such as pitfall trap or probe trap to monitor and mass-trap bruchids during storage. (4) Store the seeds in airtight containers and triple-bag the mungbean grains during storage.

22.11.14 Harvesting

Mungbean can be harvested when the pods get mature and dried, but before they start shattering. Manual harvesting is usually practiced, but mechanical harvesting can save labor cost and time. Desiccation of the plants is needed before mechanical harvesting, for which crop having mature pods and green leaves can be sprayed with Diquat (2–3 L/ha) or Glyphosate so as to desiccate the plants. In the case of manual harvesting, threshing must be done as soon as the pods get dried. Pods can be beaten with a stick until pods are opened by keeping them in a jute bag. All the foreign materials are removed by winnowing and are sun-dried for 3–5 days. Drying of seed to 9–10% moisture level is very important for good storage, which can be measured using seed moisture meter. Use of solar dryers is considered as a better option for

quick drying. It is advised to collect only good seeds which are free from diseases, seed coat cracking, split, or immature. If using a threshing machine, adjust the speed of the machine in order to avoid seed damage. Dried seeds can be safely stored for at least three years. Place the seeds in jars, manila envelopes, cloth or mesh bags, and plastic or foil envelopes. The best containers are airtight, such as a sealed glass jar, metal can, or foil envelope. Seeds should be protected from sunlight and stored in a cool (below 15 °C is ideal) and dried conditions. Seeds may be placed in a refrigerator for long-term storage. For short-term storage, seeds can be kept in a cool, shady and dry place. The recommended storage practices should be followed (as mentioned above in the pests section) for the control of storage pests especially bruchids (Tiwari et al. 2017).

22.12 Indian Minimum Seed Standards for Mungbean

The General Seed Certification Standards are basic and, together with the following specific standards, constitute the standards for certification of mungbean seeds.

22.12.1 Land Requirements

Land to be used for seed production of mungbean shall be free of volunteer plants.

22.12.2 Field Inspection

A minimum of two inspections shall be made, the first during flowering and the second after flowering and fruit setting stage.

22.12.3 Field Standards (General Requirements)

- *Isolation*: Mungbean seed fields shall be isolated from the contaminants shown in column 1 of Table 22.16 by the distances specified in column 2 and 3 for foundation and certified seed, respectively.

22.13 Social, Political, and Regulatory Issues

Legumes are the most important protein source for the vast majority of vegetarian population in Asia, which offers a great promise in achieving the nutritional security (Vision 2050). It has been envisioned to bring an additional area of three to four million ha under pulses in India including mungbean (nearly one million ha) by promoting mungbean in rice and wheat fallows, intercropping with sugarcane and vegetables and intensifying different cropping systems. Clearly, if a sustainable

Table 22.16 The details of contaminants, seed standards, and their respective isolation distances as required for the quality seed production in mungbean

Contaminants	Minimum distance (m)	
	Foundation	Certified
• Fields of other varieties	10	5
• Fields of the same variety not conforming to varietal purity requirements for certification	10	5
Seed standard factors	**Standards for each class**	
	Foundation	Certified
• Pure seed (minimum)	98.0%	98.0%
• Inert matter (maximum)	2.0%	2.0%
• Other crop seeds (maximum)	5/kg	10/kg
• Weed seeds (maximum)	5/kg	10/kg
• Other distinguishable varieties (maximum)	10/kg	20/kg
• Germination including hard seeds (minimum	75%	75%
• Moisture (maximum)	9.0%	9.0%

(Source: https://agricoop.nic.in/)

development of mungbean production has to be achieved, a three-pronged strategy needs to be adopted which mainly includes:

1. Vertical expansion of the crop by improving the yield potential of mungbean cultivars.
2. Horizontal expansion by extending its cultivation in new areas.
3. Intensifying a well-established cropping system with integration of shorter duration cultivars.

Timely availability of quality seeds of improved varieties is a serious issue in most of the mungbean-growing countries. Varietal mismatch and late arrival of quality seeds are the two most common problems in all mungbean growing areas which require an immediate attention. To address this issue, the Department of Agriculture and Cooperation (DAC), Government of India, has established 150 seed hubs in the country to ensure availability of 1000 quintals of quality seeds of pulses through each seed hub every year. Mungbean finds an important place in many seed hubs, and these have been producing seeds of mungbean varieties which have been developed in only last 10 years. Likewise, 12 "enhancing breeder seed production centers" have been established to ensure breeders seed production of mungbean and other pulses.

Fewer varieties for each pulse producing agro-climatic zones will ensure availability of pulses. Larger areas under single or fewer varieties will help in adopting suitable crop management and mechanization. Identification of varieties with uniform size and shape minimizes adjustments in machine parameters, thus minimizing the loss in form of breakage. Storage losses account to 15–30% loss in all stored grains and mungbean is no exception. The current storage protocols adopted for storage of mungbean are similar to those in major cereal. There is strong

need to develop specific storage protocol for mungbean. Jute bags prone to internal and external infestations are still being used for pulse storage, whereas for export and import PP woven bags are used. Adoption of polypropylene (PP) woven or high-density polyethylene (HDPE) bags at storage level will minimize chances of external infestation. Initial infestation can be curbed by fumigation of fresh arrival.

Buffer stock should be created for longer period, at least for 5 years and only 1/5 part needs to be replenished with fresh crop. This will minimize transportation cost and losses. Further the buffer stock should be converted into *dal* prior to release; otherwise millers will dictate the market. Nonetheless, for all of these targets, the cultivation of mungbean has to be made less cost intensive and profitable to farmers. Unfortunately, short duration crops like mungbean come associated with drudgery especially when most of the crop is still harvested by hand picking.

22.14 Future Thrust Area

Mungbean, as a crop, is so versatile that it can be grown under varying agro-ecological conditions and in different seasons (spring, summer, *kharif*, and winter). Extensive progress has been made for the development of high yielding short duration varieties which has resulted in significant increase in the production and productivity across the world. However, in the scenario of climate change, a constant pressure is there for the development of climate smart and resilient varieties. In addition, research is also required for the management of preharvest sprouting, bruchid resistance, and stacking of genes controlling resistance or tolerance to various biotic and abiotic stresses. Although much progress has been made for the development of short duration varieties in India, efforts are still needed to tap the huge potential which lies in summer mungbean cultivation especially in Indo-Gangetic plains and rice fallows. Out of 10.5 M ha rice fallows of eastern (Uttar Pradesh, Bihar, West Bengal, Assam), central (Chhattisgarh), and southern states (Andhra Pradesh, Karnataka, Tamil Nadu), 2.5 million ha can be utilized by expanding extra-short duration mungbean and urdbean cultivation. The summer mungbean is being grown as a bonus crop in a number of Indian states. Thus, very focused efforts are needed to develop suitable technology for rice fallow to expand the base of mungbean production in eastern states like Odisha, Chhattisgarh, Jharkhand, Bihar West Bengal, and Assam.

High yielding and input-responsive genes are yet to be identified and transgressed in common varieties. There is urgent need to broaden the genetic base by strengthening prebreeding and mapping of genes/QTLs and marker-assisted selection for resistance to insect pests and diseases, yield and grain quality, gene pyramiding for stable resistance, and development of transgenics in *Vigna* for problems hitherto unsolved through conventional means. Owing to its health and nutritional benefits as well as soil and environment ameliorative properties, mungbean promises to be a preferred candidate crop for food, nutrition, and environmental security and sustainability. Realizing its importance as a nutrition-rich crop, detail studies of the bioactive compounds were advocated. Postharvest

processing and value addition enhance its commercial value tremendously. Several in vitro and in vivo studies have indicated various health benefits of mungbean; nonetheless, the mechanisms involved in disease prevention and the metabolic processes leading them to become a functional food are essential to unravel.

Ample variability exists in different mungbean cultivars and germplasm accessions for almost all nutrition-related traits suggesting that improvement for most of these is possible through simple breeding methods. The sprouting segment represents the high-value segment of the market although the grains need to meet exacting quality attributes. Pesticide residue, nonuniform grain size, and hypocotyls pigmentation are some of the issues which need strict monitoring and quality standard compliance. For milling of pulses, such varieties must be identified which gives higher dal recovery in milling, indicating lower gum content in between husk and cotyledons. While efforts must be made to consume pulses as whole to prevent milling losses, pulses in form of *dal* are better protected from bruchid infestation as it does not provide hiding space to insect larvae and can be stored for longer duration. About 30% whole grain are lost in form of milling by-product which is rich in proteins and antioxidants. Presently it goes for low-value cattle feed. Powder component of the milling by-product can be separated and can be utilized as source for pulse proteins, whereas husk rich fraction can be used as nutraceuticals.

Postharvest storage has a great role to play in maintaining the nutritional and physical qualities of all pulses. Bruchids (*Callosobruchus* species) cause huge losses to the stored grains in terms of both physical and nutritional quality. These are reported to enhance trypsin inhibitor activity, saponins, and phytic acid in the stored grains (Modgil and Mehta 1994). Therefore, there is an urgent need to identify bruchid-resistant donors and molecular markers associated with resistance and which initiate host-plant resistance breeding immediately. There is a need of international collaborative efforts towards exploitation of biological variation for various nutritional parameters and deploying strong and reliable analytical methods to determine nutritional compounds in mungbean.

Acknowledgments This research was funded by Indian Council of Agricultural Research (ICAR), Indian Agricultural Research Institute (IARI), New Delhi, and SERB, New Delhi (CRG/2019/002024). The technical support received from Mr. Dilip Kumar is duly acknowledged.

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Urdbean Breeding

23

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Abstract

Urdbean (*Vigna mungo* L. Hepper) is a warm season food legume grown on a wide scale in South Asia. India is the largest producer and consumer for this pulse crop. Besides India, urdbean is grown profusely in ASEAN countries like Myanmar and Thailand as well as in Nepal, Pakistan, Bangladesh, and Afghanistan. It is consumed in the form of whole grain or splitted form or flour is used in different food preparation. Urdbean breeding was initiated formally since the inception of All India Coordinated Research Project on Pulses in India. Since then, a number of varieties were released for general cultivation which are high yielding and resistant to yellow mosaic, powdery mildew diseases, and crinkle virus. Seed production and distribution of recent varieties in traditional and newer niches resulted in the enormous production increase in this pulse crop. Urdbean has contributed to the ongoing pulse production boom in India. This chapter discusses different methods associated with urdbean breeding and their

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_23

potential impact in terms of increased production and productivity in this high protein crop. Starting from botanical descriptors, breeding objectives, hybridization techniques, selection methods to genetic resources, and use of molecular markers were discussed in detail.

Keywords

Urdbean · *Vigna mungo* · Phenotyping · Grain legumes · Genetic mapping · Genome

23.1 Introduction

Urdbean or uradbean or urid or urd or blackgram or kalai (*Vigna mungo* var. *mungo* L. Hepper) is a highly popular pulse crop in South and East Asia., particularly in India. It is a crop of tropical or subtropical growing environment. Indian subcontinent is considered as the centre of origin of this crop species based on the rich genetic diversity of this crop species (Arora 1985). The closest progenitor species of urdbean is *V. mungo* var. *silvestris*, which is still found in natural habitats (Singh and Ahuja 1977; Chandel 1984). This wild species is reported to be domesticated in one of the biodiversity hotspots, i.e. Western Ghats and northern hilly tracts of Maharashtra (Chandel 1984; Arora 1985). The wild type of urdbean was having smaller seed size and was having trailing kind of growth habit (Gupta et al. 2020a, b, c). The present-day cultivated urdbean varieties were developed through accumulation of recessive mutant genes, resulting in different plant characters which were not dominant in natural populations (Sen and Murty 1960; Smartt 1985). During this long man-made and nature-privileged selection process, many adaptive traits like the dehiscent pods and seed hardiness were negatively selected. Modern urdbean varieties are high yielding, resistant to multiple disease and insect pests, and shorter maturity duration compared to what was grown 50 years back (Rao and Jana 1974).

India is the largest producer and consumer as well as importer of urdbean. Myanmar contributes to 80% of total urdbean import in India. India produces about 2.45 million tonnes of urdbean annually from about 4.11 million hectares of area with an average productivity of 696 kg per hectare (second advance estimates, DES, DAC & FW, GOI 2020–21; Project Coordinator's Report 2020–2021). Urdbean production contributes to 14% of India's total pulses production (24.42 million tonnes in 2020–21). In Uttar Pradesh, it is also grown during Spring season and sown during the last part of February or the first part of March. Urdbean is grown as kharif or rainy season crop in Southern and Northern India. In Southern India, it is also grown as Pre-Rabi or Rabi crop. In Eastern and Southern growing regions of India, urdbean is predominantly grown as a rice fallow crop. Date of sowing of urdbean varies based on the local meteorological conditions, for example, in Uttar Pradesh and Madhya Pradesh state, it is sown in the month of early July to the first week of August. In many states in Southern India, it is sown in the last part of October or the first part of November, whereas, in many Southern states like Orissa,

it is sown in October or February. It requires moderate temperature (25–35 °C) with moderate to high humidity (70–90%) during vegetative growth. Bright sunshine hours during reproductive stage and well-distributed rainfall are key for the successful cultivation of urdbean. Weeklong continuous rainfall during pod filling or maturity phase damages the crop yield and quality of the seeds drastically (Gupta et al. 2020a, b, c). Urdbean has the better ability to withstand waterlogging condition. The hardy nature of this crop species towards draught or waterlogging condition makes it a unique crop for physiological studies. Limited irrigation during spring/summer season while cultivating urdbean in heavy black or brown soil gives satisfactory yield. Although the national average productivity is lower for this crop species compared to cool season pulses in terms of per day productivity and diverse growth ecologies, this makes it a popular pulse crop to compete with others.

23.2 Origin, Evolution, and Distribution of Species and Forms: Wild Relatives

Cultivated urdbean or urdbean (*Vigna mungo* var. *mungo* (L.) Hepper) is originated from wild progenitor, *Vigna mungo* var. *silvestris* Lukoki, Maréchal, and Otoul (Chandel et al. 1984). It was reported further based on excavations that urdbean originated in India (Zukovskij 1962), and, however, domestication of urdbean occurred about few thousand years ago (Fuller and Harvey 2006). Urdbean has a secondary centre of diversity in southeast Asia. It is cultivated throughout the southeast Asia including Northern Malaysia, Philippines, Thailand, and Myanmar (Gupta et al. 2020a, b, c). It is also grown in the neighbouring countries of India, i.e. Afghanistan, Bangladesh, Bhutan, Pakistan, and Nepal. DNA-based analysis revealed that cultivated urdbean was more closely related to wild urdbean from South Asia than that from Southeast Asia (Kaewwongwal et al. 2015).

The genus *Vigna* is a taxon in *Fabaceae* family with 104 species found in tropical and subtropical regions of Africa, Asia, America, and Australia (Schrire et al. 2005). Among these species, three are mostly popular as pulses; *V. radiata* (urdbean), *Vigna mungo* (mungbean), *V. unguiculata* (cowpea), and urdbean (*Vigna mungo* L. Hepper) varieties can be categorized into three groups, i.e. var. mungo, early maturing, and large seeded; var. *viridis* Bose, late maturing and greenish dull or shinning type seed; and var. *silvestris* Lukoki, Marechal, and Otoul, wild type. However, all possible types of recombinants are present today in cultivated types due to extensive breeding efforts done.

Botanical classification of urdbean:
Kingdom—Plantae.
Division—*Angiosperms.*
Subdivision—*Eudicots.*
Class—*Rosids.*
Order—*Fabales.*
Family—*Fabaceae.*

Mainly yellow-flowered 21 species of *Vigna*, with a diverse range throughout Asia, are known as Asiatic *Vigna* species. Among these, seven Asian *Vigna* species, i.e. mungbean (*V. radiata*), urdbean (*V. mungo*), adzuki bean (*V. angularis*), mothbean (*V. aconitifolia*), jungli bean (*V. trilobata*), rice bean (*V. umbellata*), and creole bean (*V. reflexo-pilosa*), are used as food crops. Taxonomically, cultigens and conspecific wild forms are recognized in all species except *V. aconitifolia* (Bisht et al. 2005). Based on morphological characterization and biochemical evidences, Asiatic *Vigna* species were grouped in separate subgenus *Ceratotropis* of the genus *Vigna savi* which were formerly under the genus *Phaseolus*. Present grouping of *Vigna* species is based on morphological, biochemical, and molecular characterization. These groupings of crop species are many a times modified based on the emergence of any new evidence or theories. However, these changes are not common and vary from species to species.

23.3 Plant Genetic Resources

National gene bank (long-term storage facility) (NBPGR, New Delhi) has 2246 accessions of urdbean. These were mostly germplasm collections made through explorations throughout India, breeding lines, primitive cultivars, and landraces over the years. Considerable morphological variability exists for different agronomic traits like flowering time, plant height, seed size, seed colour, disease resistance, and maturity duration. The wild species which are crossable to urdbean are also maintained in this gene bank. The primary crossing species are *Vigna sylvestris*, *Vigna umbellata*, *Vigna sublobata*, and *Vigna radiata* or mungbean. A medium-term gene bank facility is also present in ICAR-Indian Institute of Pulses Research in Kanpur City in India where different wild Vigna accessions are maintained for conservation and sharing with research institutions. Currently, more 200 wild *Vigna* accessions are maintained in that facility. Besides this, different state agricultural universities, which are plant genetic resource storage and maintenance facilities, like GBPUAT, Pantnagar, TNAU, Coimbatore, PAU, and Ludhiana have their own gene bank holding hundreds of accessions of urdbean.

23.4 Floral Biology: Emasculation and Pollination Technique

Urdbean has a typical cleistogamous flower which is bright yellow in colour. The cleistogamous flower denotes that here anthesis occurs before opening of the flower. Thereby, urdbean is a self-pollinated pulse crop. Urdbean flowers are emasculated, and 10 anthers (9 + 1) are removed and pollinated by the pollen dust from other desirable plants by handheld devices.

23.5 Breeding Objectives

23.5.1 High Yield

Systematic urdbean breeding effort was initiated with the inception All India Coordinated Pulse Improvement project in 1967. The main objective was development of high-yielding varieties in urdbean. The national urdbean productivity has improved from 300 kg/ha in 1960s to 842 kg/ha in 2020–2021. The yield potential of recent urdbean varieties is between 10 and 15 quintals per hectare. However, most of these high-yielding varieties are longer in duration (80–95 days).

23.5.2 High Yield Combined with Earliness

Many urdbean breeding programmes now targeting reduction in maturity duration by 10–15 days without much loss in productivity. Recently, Gupta et al. (2020a, b, c) evaluated 21 urdbean genotypes (14 advanced breeding lines, 7 released varieties) (IPU19–27, IPU19–11, IPU19–5, IPU19–6, IPU19–7, IPU19–8, IPU19–20, IPU19–24, IPU19–31, IPU19–44, IPU19–46, IPU19–51, IPU19–53, IPU19–55, IPU02–43, IPU11–02, IPU94–1, KUG479, WBU 108, Shekhar 3, WBU 109) for earliness and yield along with many other qualitative and qualitative traits including days to first flower, days to 50% flowering, days to first branch, days to first pod, plant height 30 days after sowing, plant height 45 days after sowing, number of pods per plant, number of seeds per pod, number of clusters per plant, and days to physiological maturity. Significant variation was observed for test entries as compared to released checks for yield and maturity duration. One advanced breeding line IPU19–27 matured early (60–65 days) and yield was at par with high-yielding checks. This line is the product of the cross "SPS 5 x IPU02-33". This kind of breeding lines of urdbean has a long way to go for varietal replacement in urdbean seed chain to provide growers with more time management options, thereby increasing the cropping intensity. In our personal experience, 60 days maturity urdbean with high yield potential will become popular among urdbean growers.

23.5.3 Quality Characters Including Biofortification

Food legumes are rich in protein, dietary fibres, folates, micronutrients, and many other nutrients or phytochemicals (Thavarajah et al. 2014; Sen Gupta et al. 2016; Maphosa and Victoria 2017; Siva et al. 2019; Jha and Warkentin 2020; Gupta et al. 2020a, b, c). Micronutrient or vitamin deficiency which is also known as hidden hunger is a global problem against achieving millennium developmental goals set by the United Nations worldwide, and it is estimated that, globally, more than two billion people are under malnutrition (FAO 2012; FAO, IFAD, UNICEF, WFP and WHO 2021). To ameliorate micronutrient deficiency, three basic procedures are operative: (1) direct micronutrient(s) supplementation in the form of tablet or capsule, (2) food fortification during processing, and (3) biofortification of crops

in which nutritionally rich crop varieties are developed (Singh et al. 2016; Gupta et al. 2015, 2020a, b, c). However, breeding for biofortified crop cultivars is a low-cost method for managing malnutrition or hidden hunger (Bouis and Saltzman 2017). After the release, biofortified crop cultivars can be grown with full potential in different regions with similar agroclimatic conditions. Further, recurrent cost of developing biofortified varieties are minimized worldwide as once the micronutrient trait becomes integral part of plant breeding programs (Bouis and Saltzman 2017).

The breeding objective of biofortified crop cultivars has already been set in few crops like rice, wheat, maize, pearl millet, and cassava; however many are still pending. Urdbean is a popular candidate for biofortification of crop varieties as it is consumed by millions throughout the country. In order to find out high sources for iron and zinc concentration, larger accessions should be tested under precise growing conditions. The status of released cultivars also needs to be known. In recent years, only one study has been conducted to know iron and zinc concentration among 26 urdbean genotypes that showed iron concentration ranging from 71 to 100 mg/kg and zinc concentrations ranging from 19 to 61 mg/kg (Singh et al. 2017). Gupta et al. (2020a, b, c) evaluated a larger set of urdbean genotypes which were grown over multilocations or environments to identify stable sources of high iron and zinc concentration in this crop species. Here, 83 urdbean genotypes of diverse origin were tested for iron and zinc concentrations over two locations. Analysis of variance showed that genotype effects were significant for both traits over both locations. Iron concentration ranged from 19 to 235 mg/kg (mean 117 mg/kg) and 16 to 255 mg/kg (mean 91 mg/kg) among tested genotypes at the first and second locations, respectively. For zinc concentration, it ranged from 5 to 134 mg/kg (mean 44 mg/kg) at the first location, while at the second location, it was between 12 and 59 mg/kg (mean 29 mg/kg). "Genotype (G)", "Location (L)", and "Genotype" (G) × "Location" (L) interaction effects were also significant for both micronutrient concentrations (Gupta et al. 2020a, b, c). This study has identified useful donors with high and stable sources of iron and zinc concentration.

23.6 Biotic Stress Resistance

23.6.1 Disease Resistance

23.6.1.1 Yellow Mosaic Disease Resistance

The begomovirus (family *Geminiviridae*) causing yellow mosaic disease (YMD) is the most important limiting factor in the production of urdbean in India (Anjum et al. 2010). YMD of black gram and in other grain legumes, mungbean, cowpea, and soybean causes yield losses of 100% under epidemic conditions (Nene 1973). The annual yield loss due to YMD was estimated to be $ 300 million in three leguminous crops, viz. urdbean, mungbean, and soybean (Varma and Malathi 2003). In the Indian subcontinent, YMD is caused by at least four different begomovirus species, mungbean yellow mosaic virus (MYMV), mungbean yellow mosaic India virus (MYMIV), horsegram yellow mosaic virus, and Dolichos yellow mosaic virus (Ilyas

et al. 2009). MYMIV and MYMV are the two most important begomoviruses in grain legumes in India. MYMV is the most prevalent in southern and western regions (Karthikeyan et al. 1996), whereas MYMIV is predominant in northern, central, and eastern regions of India (Usharani et al. 2004). The management of YMD is generally addressed through the control of whitefly (*Bemisia tabaci*) and use of resistant cultivar.

Though many resistant varieties of black gram and mungbean have been developed through conventional breeding (Gupta et al. 2005), the resistance does not hold for a long time due to the narrow genetic base of the cultivars as well as rapid emergence of new begomovirus species or strains (Varma et al. 2011). Most of the resistance screening/breeding and molecular work was conducted in mungbean in India (Mishra et al. 2020). However, with reference to urdbean, a limited study was conducted in India (Nene and Kolte 1972; Basandrai 1999; Bag et al. 2014). Presently, the genome sequence of urdbean has been generated in India. However, the sequence-based identification of resistant gene and validation of its function are yet to be demonstrated. The comparison of complete nucleotide sequence of DNA A component of yellow mosaic viruses with other begomoviruses led to clear differentiation of four species, MYMV, MYMIV, DoYMV, and HgYMV, on the basis of 91% species demarcation. The identity between the two species MYMV and MYMIV is 81%. The relationship is more or less similar with respective isolates of MYMV and MYMIV from Thailand, Pakistan, Bangladesh, Nepal, and Indonesia. In the DNA B component, the cognate DNA B of Thailand isolate MYMV-[TH-Mg1] and one urdbean isolate of Vamban MYMV-[KA 27] identity with DNA B of MYMIV-Bg3, MYMIV-Cp, MYMIV-Mg, and MYMI-Sb is only 67%.

The most unusual feature of MYMV is association of one DNA A with multiple DNA B. One urdbean isolate of MYMV from south India, MYMV-[IN:Vig] is associated with two distinct types of DNA B component. One type of DNA B MYMV-[KA 27] which shows 97% sequence identity with DNA B of Thailand isolate, and other set of DNA Bs, KA22, KA28, and KA34, which show only 71–72% identity with Thai isolate, but exhibited nearly 90–92% identity with DNA B of MYMIV (Karthikeyan et al. 1996; Balaji et al. 2004). Two types of DNA B components are also associated with a soybean isolate of MYMV [IN: Mad: Sb], one being closely related (96%) to DNA B of HgYMV.

Critical comparison of nucleotide sequence of the DNA B components of MYMV along with one DNA B variant cloned from Gujarat MYMIV-IN Anand 25 (John et al. 2008) revealed how the components have evolved. The four DNA B components, three associated with MYMV, one with MYMIV between themselves, share 96% identity in the coding region ORF BVI and ORF BCI. However, they differ in the non-coding region. While DNA B of MYMV- KA22, KA28, and KA34 exhibited similarity with CR of MYMV-[IN Vig], Gujarat isolate showed maximum identity with CR of MYMIV (John et al. 2008). These DNA B molecules referred to as DNA B variants may represent molecules generated by exchange of components between MYMV and MYMIV. Swapping of CR could have occurred from MYMV to MYMIV ("origin donation or regulon grafting") when both the viruses were

present together in mixed infection. This is well borne out by the divergence observed between A and B components in the CR region in MYMV and MYMIV.

Analyses of LYMV infected samples by RCA let to unexpected identification of both beta- and alphasatellites. Over the past few years, betasatellite has been found associated with MYMIV- (Rouhibakhsh and Malathi 2005) and MYMV-infected plants (Sathya et al. 2013). The symptoms in the presence of betasatellites are severe like crumpling and severe leaf curl. In all these cases, the betasatellite was identified as papaya leaf curl betasatellite. Sathya et al. (2013) found that samples of MYMV-infected urdbean revealed the presence of alphasatellites, which were identified to belong to the *Vernonia* yellow vein alphasatellite species. The importance of association of these satellites in LYMV pathogenicity is not understood, whether such tri/tetrapartite is stable and contributes to viral population evolution and diversity needs to be looked into. Several studies have been conducted to understand the genetics of resistance to YMV in urdbean. It has been reported to be inherited as monogenic dominant (Kaushal and Singh 1989a, b; Gupta et al. 2005, 2013a), monogenic recessive (Pal et al. 1991; Khattak 2000), oligogenic (Singh 1981), and complimentary recessive (Shukla and Pandya 1985) traits in different studies. These studies clearly indicate that resistance to this important disease depends upon combination of genotypes and the virus strains. It can be concluded that in most of the cases the host resistance is regulated by single gene in urdbean to yellow mosaic disease.

23.6.1.2 Powdery Mildew Disease Resistance

Powdery mildew is a major problem in coastal humid regions. In the case of mungbean, quantitative inheritance of resistance loci was reported (Chatieng et al. 2006). The inheritance of resistance is reported to be controlled by a single recessive gene in urdbean (Kaushal and Singh 1989a, b). Many workers reported resistance sources in urdbean like Pant U 30 (Jain and Yadava 1994), P 115, Line 6203, and LBG 642 (Parmeshwara and Setty 1993). Popular varieties or breeding lines such as LBG 17, LBG 402, Co 5, WBU 108, and WBU 26 combining resistance with high yield have also been developed. Among them, LBG 17 derived from two susceptible parents (Krishnaiah et al. 1978) has revolutionized urdbean cultivation in rice fallows of coastal Andhra Pradesh. Most of the recent varieties of urdbean grown in coastal regions are carrying this PMD resistance locus.

23.6.1.3 *Cercospora* Leaf Spot Resistance

Cercospora leaf spot is the most prevalent disease in kharif season causing leaf spotting and defoliation. Yield reduction from this disease was reported to be 25% when leaf defoliation reached 75%. The principal pathogen is *Cercospora canescens*, although *C. cruenza* was also identified to cause this disease. Resistant sources such as IC 11008, HPBU 51, HPBU 98, UPU 95–1, Pant U 26, and UG 407 have been identified, and prominent cultivars such as Jawahar Urd 2, Jawahar

urd 3, Pant U 19, Mash 48, Mash 21, RBU 38, and KB 512 combining resistance with high yield have also been developed.

23.6.1.4 Leaf Crinkle Virus Resistance

Leaf crinkle virus causes crinkling and rugosity of leaves and malformation of floral organs. Pollen fertility and pod formations are severely reduced on infected plants. Nene (1972) reported 62–100% yield reductions depending upon the stage of growth at which the plant becomes infected. Prasad et al. (1998) reported NDU 94–6 as a resistant source in India while Iqbal et al. (1991) found S 210, MM 5–60, S 250, and Mash Sialkot as resistant sources in Pakistan. Among the released cultivars, Pragati (US 131) and ADT 3 have shown field resistance.

23.6.2 Insect Resistance

The urdbean crop is infested by a range of insect pests. Twelve species of insect pests were observed to attack urdbean variety Pant Urd −31 at different stages of crop growth in an overlapping manner (Yadav et al. 2020). The spotted pod borer, *Maruca vitrata* (Geyer); gram pod borer, *Helicoverpa armigera* (Hubner); bihar hairy caterpillar, *Spilosoma obliqua* (Walker); and leafhopper *Empoasca kerri* (Pruthi) were recorded as major pests. Yadav et al. (2021) screened 15 urdbean genotypes against pod borers, i.e. *M. vitrata* and *H. armigera*. Two genotypes, viz. KU-99-05 and Azad Urd-1, were found with minimum pod infestation of 7.67% and 9.67%, respectively, and categorized as resistant against *M. vitrata*. The four genotypes KU-99-05, Azad Urd-1, Shekhar-2, and PU-6 were classified as resistant against *H. armigera* with minimum pod infestation of 5.83%, 6.17%, 8.50%, and 9.83%, respectively. Saleesha et al. (2019) observed that highly susceptible variety Co 5 had less number of trichomes (2.13/mm^2) and trichome length (3.95 mm), while the resistant variety VBN 6 had dense trichomes (4.13/mm^2) and trichome length (5.01 mm) which is considered to be the main factor to confer resistance in plants. A total of 17 blackgram genotypes were screened for resistance to major insect pests, including aphid (*Aphis craccivora* Koch.), whitefly (*Bemisia tabaci* Genn.), hairy caterpillar (*Spilosoma obliqua* Walker), and pod borer (*H. armigera* Hubner) during summer season of 2018 and 2019 (Neupane et al. 2021). It was reported that three genotypes BLG0069–1, BLG0036–1, and BLG0079–1 were having lower number of above-mentioned insect populations, exhibited more resistance in both years, and produced higher grain yield (~1.5 t/ha) than other genotypes. Naik and Mallapur (2019) screened 15 urdbean genotypes for their resistance against spotted pod borer, *M. vitrata*. Five genotypes with percent pod damage, LBG-685 (8.25%), WBU-108 (9.25%), COBG-653 (9.35%), VBN-05 (9.30%), and PU-31 (10.10%) were found as tolerant, and LBG-631 (32.35%), VBG10–024 (31.60%), RUG-10 (32.85%), KUG-586 (32.80%), and PUSA-9531(31.25%) genotypes showed susceptibility. The maximum pod damage was found in RUG-10 (32.85%), and significantly least pod damage was noticed in LBG-685 (8.25%). In urdbean, highly resistant lines such as UG 218, PDU 1, PDU 5, AKU 7, Co 305, UP

95–1, and LBG 707 have been identified against stem flies, *Ophiomyia* spp. (Gupta and Kumar 2006). At PAU, about 1400 urdbean genotypes have been screened against whitefly, *B. tabaci*, jassids, and MYMV and the genotypes LUs 15, 178, 190, 194, 196, 330, 397, 426, 434, UGs 119, 187, 218, 254, 302, 407, UL 29, UL 257, UL 310, UL 338, UL 557, UL 389, UL 407, UL 538, UL 597, UG 600, UG 633, UG 402, and UG 636 were identified as resistant (Chhabra and Kooner 1981, 1993, 1994, 1995a, b; Chhabra et al. 1984; Kooner et al. 1994). Taggar et al. (2013) categorized urdbean genotypes KU 99–20 and NDU 5–7 as moderately resistant to whitefly, whereas Chhabra et al. (1993) reported that urdbean entry LU 15, LU 178, LU 190, and LU 194 had resistance against black aphid, *A. craccivora* Koch. Chhabra et al. (1986) tested 30 urdbean genotypes and identified LU 335, LU 274, LU 332, and LU 470 as moderately resistant to *A. craccivora* and M 1–1 as highly resistant to the aphid. Highly resistant lines such as PDU 5, KB 63, UG 567, and UH 804 have been identified against thrips (Gupta and Kumar 2006). Lines such as UG 737, PLU 557, and TAU 1 have been identified highly resistant against pod borers (Gupta and Kumar 2006). VM 2011 and VM 2164 showed high resistance against bruchids (Nair et al. 2013). List of insect pests of urdbean and their tolerant genotypes are given in detail in Tables 23.1 and 23.2, respectively.

23.7 Abiotic Stress Tolerance Under Climate Change Scenario

23.7.1 Heat Stress

Urdbean is sensitive to abrupt climate changes like any other crop species. Although it is adapted to diverse agro-ecologies or growing conditions, the climatic fluctuations particularly during flowering or pod filing duration impacts the most. Discussing about the urdbean-growing ecologies, this crop species is grown mainly in rainy season (July–October), and in southern part, it is grown as winter season crop (November to February). However, its cultivation is limited during summer season cultivation. The scarcity of atmospheric humidity is also another reason for limited cultivation during summer season. Thus, development of heat-tolerant urdbean varieties can expand urdbean cultivation area in the country. Genetic variability for heat tolerance has been reported in many food legumes (Sita et al. 2017). However, there is no report of genetic variability study for heat tolerance in urdbean except done by Gupta et al. (2021) where they evaluated a panel of urdbean genotypes under field as well as laboratory testing for heat tolerance. Urdbean growth phenology includes warmer season (25–35 °C) along with high humidity for its normal growth and development. High temperature (>40 °C) during flowering results in deformation/abortion of flower parts or flower drop leading to negative impact on yield (Gupta et al. 2021). Similarly, in mungbean, higher temperature of >38/25 °C (day and night, respectively) markedly affected the yield under summer-season cultivation (Nayyar et al. 2017).

Current climate change scenario leads to abrupt changes in mean temperature during crop growth duration. The physiological processes are highly impacted by

Table 23.1 List of major insect pest of urdbean

S. No.	Common name	Scientific name	Family	Economic threshold level (ETL)
1	Gram pod borer	*Helicoverpa armigera* Hubner	*Noctuidae*	10% affected plants
2	Spotted pod borer	*Maruca vitrata* Fabricius	*Crambidae*	3/plant
3	Tobacco caterpillar	*Spodoptera litura* Fabricius	*Noctuidae*	8 egg masses/100 m^2
4	Red hairy caterpillar	*Amsacta moorei* Butler	*Noctuidae*	–
5	Bihar hairy caterpillar	*Spilosoma obliqua* Walker	*Erebidae*	–
6	Blue butterfly	*Lampides boeticus* L.	*Lycaenidae*	–
7	Stem fly	*Ophiomyia phaseoli*	*Agromyzidae*	5–10% incidence
8	Galerucid beetle	*Madurasia obscurella*	*Galerucidae*	–
9	Cowpea aphid	*Aphis craccivora* koch	*Aphididae*	20/2.5 cm length
10	Thrips	*Megalurothrips distalis*	*Thripidae*	–
11	Pod bug	*Clavigralla gibbosa*	*Coreidae*	–
12	Whitefly	*Bemisia tabaci*	*Aleurodidae*	–
13	Leaf hopper	*Empoasca kerri*	*Jassidae*	–
14	Blister beetle	*Mylabris pustulata*	*Meloidae*	–
15	Pulse beetle	*Callosobruchus* spp.	*Bruchidae*	–
16	Green stink bug	*Nezara viridula*	*Pentatomidae*	–
17	Red-banded stink bug	*Piezodorus hybneri*	*Pentatomidae*	–
18	Hawk moth	*Herse convolvuli*	*Sphingidae*	–

Table 23.2 List of insect-tolerant urdbean genotypes

Pest	Tolerant genotypes
Pod borer	Kalai, 338-3, Krishna, and Co 3, 4, and 5
	CBG 08-011 and PLU 54; UH 82–5, IC 8219 and SPS143
Stem fly	Killikullam, 338/3, P 58, Co 4 and Co 5
Jassid	Sinkheda 1, Krishna, H 70-3 and UPB 1
Thrips	PDU 5, KB 63, PDU 88-23, PDU 2, 5, UG 567, DU4, T9, UH80-4,UH90-9, and UH80-7

heat stress resulting in dramatic yield losses. The processes impacted are pollen or ovule activity, flower growth, and even growth and development of embryo or seed in many pulses (Sita et al. 2017). Taxonomically, urdbean is a close relative of mungbean and cowpea which are extensively cultivated under identical environments (Ehlers and Hall 1998; Basu et al. 2019). This further indicates the

possibility of identification of heat-tolerant urdbean genotype in germplasm collections. Trait discovery is an important tool for urdbean improvement, particularly newer morpho-physiological traits (Scafaro et al. 2010). Therefore, characterization of large number of germplasm under critical field and controlled condition is required (Gaur et al. 2019). In many field crops, various physiological and biochemical traits such as photosynthetic activity, membrane stability, pollen viability, and phenolic compounds have been used to identify heat-tolerant genotypes (Asseng et al. 2015; Murata et al. 2012; Allakhverdiev and Murata 2004; Sita et al. 2017; Challinor et al. 2007). Trait genetics underlying key morpho-physiological traits imparting heat tolerance helps researchers to make genetic improvement of field crops more precisely. In the recent years, molecular markers were used to find out genetics of morpho-physiological traits imparting heat tolerance in several crops (Argyris et al. 2008; Roy et al. 2011; Paliwal et al. 2012). However, in urdbean, use of molecular markers for genetic mapping and gene expression studies for heat tolerance are not yet in public domain.

In a separate study, a panel of 97 urdbean diverse genotypes was characterized for yield potential under stress and non-stress conditions to identify heat-tolerant urdbean genotypes (Gupta et al. 2021). Eight heat-tolerant and 35 highly heat-sensitive genotypes were identified based on heat susceptibility index (HSI). Heat susceptibility index was calculated for individual genotype based on the yield differences under heat-stressed and normal field conditions. Heat-stressed condition was created by adjusting the sowing date in a way that flowering or pod-filling stage coincides with critical maximum day temperature. Further, characterization of a group of heat-tolerant and sensitive urdbean genotypes based on physiological and biochemical traits showed genotypic variability for leaf nitrogen balance index (NBI), chlorophyll (SPAD), epidermal flavanols, and anthocyanin contents. It was found that heat-tolerant genotypes were having higher membrane stability index compared to sensitive urdbean genotypes. Significant differences among genotypes for ETR at varying levels of PAR irradiances were observed. PAR $\times$ genotypes interactions were also significant among tested urdbean genotypes. It indicated high photosynthetic ability of heat-tolerant genotypes under heat-stressed condition. Further, in the case of highly heat sensitive genotype, PKGU-1 showed distortion of photosystem II (PS II) as observed from the decrease in different fluorescence parameters. Fluorescence kinetics showed the delayed and fast quenching of Fm in highly heat-sensitive and heat-tolerant genotypes, respectively. Biochemically, heat-tolerant genotype (UPU 85–86) had higher antioxidant activities than sensitive one (PKGU 1). Molecular characterization placed heat-tolerant (UPU 85–86) and heat-sensitive genotype (PKGU 1) distantly from each other. These heat-tolerant genotypes can be used in breeding programmes.

23.7.2 Photosensitivity

Urdbean is a highly photothermosensitive crop. Therefore, its yield potential varies across locations due to variable day length in addition to varying thermal regimes.

Thus, minimizing the genotype × environment interactions can help to achieve stable yield of urdbean. The high temperature stress above the threshold across the locations during the summer season could be the compounding effects of both heat and photosensitivity. One of the strategies for selecting photothermo-insensitive lines is to evaluate different genotypes at multilocations having varying day length and thermal regimes. As a result, genotypes having stable yield across the locations could be identified as putative photothermo-insensitive lines. This strategy should be made to screen thermotolerant lines from the panel of photothermo-insensitive lines so that widely adapted stable heat-tolerant lines could be identified having less influence of photo-thermoperiods. In the present investigation, this approach has been followed to identify contrasting genotypes having high level of tolerance or sensitivity to high temperature.

23.8 Exploitation of Heterosis and Hybrid Development

Nonavailability of male sterile lines and cleistogamous nature of the urdbean plants impairs hybrid development or utilization of heterosis in this crop species. While hybridizing distant parents, many a times male sterile (MS) plants are recovered (personal communication, not published) (Fig. 23.1.). However, these MS genotypes are not stable and difficult to be maintained in the absence of any identified restorer. More research is required in this area for utilization of heterosis in urdbean.

23.9 Breeding Approaches: Conventional and Nonconventional Including Use of Genomic Tools

23.9.1 Precise and High-Throughput Phenotyping Protocols for Key Traits

Key traits in urdbean breeding are MYMIV/MYMV resistance, powdery mildew resistance, tolerance to different abiotic stresses including heat stress and salinity and tolerance to specific niche water logging. Precise phenotyping facilities are required for disease screening or evaluation of different key morpho-physiological traits which are key to imparting tolerance towards few abiotic stresses. From breeders' point of view, these phenotyping facilities are required to be high throughput in nature allowing hundreds of samples to be tested in a single run. In the following section, we will be discussing about various methods or phenotyping platforms available for increasing the selection efficiency in urdbean breeding:

23.9.1.1 Agroinoculation Method for MYMV/MYMIV Screening

The begomovirus (family *Geminiviridae*) causing yellow mosaic disease (YMD) is the most important limiting factor in the production of urdbean in India (Anjum et al. 2010). YMD of urdbean and in other grain legumes, mungbean, cowpea, and soybean cause yield losses of 100% under epidemic conditions (Nene 1973). The

Fig. 23.1 Male sterile urdbean plant with full blooming but without pod formation

annual yield loss due to YMD was estimated to be $ 300 million in three leguminous crops, viz. urdbean, mungbean, and soybean (Varma and Malathi 2003). In the Indian subcontinent, YMD is caused by at least four different begomovirus species, mungbean yellow mosaic virus (MYMV), mungbean yellow mosaic India virus (MYMIV), horsegram yellow mosaic virus, and Dolichos yellow mosaic virus (Ilyas

et al. 2009). MYMIV and MYMV are the two most important begomovirus in grain legumes in India. MYMV is most prevalent in southern and western regions (Karthikeyan et al. 1996), whereas MYMIV is predominant in northern, central, and eastern regions of India (Usharani et al. 2004).

The management of YMD is generally addressed through the control of whitefly (*Bemisia tabaci*) and use of resistant cultivar. Though many resistant varieties of urdbean and mungbean have been developed through conventional breeding (Gupta et al. 2005), the resistance does not hold for a long time due to the narrow genetic base of the cultivars as well as rapid emergence of new begomovirus species or strains (Varma et al. 2011). Most of the resistance screening/breeding and molecular work was conducted in mungbean in India. However, with reference to urdbean, limited studies were conducted in India (Nene and Kolte 1972; Basandrai 1999; Bag et al. 2014). YMV isolates from mungbean, moth bean, pigeonpea, cowpea, soybean, dolichos, and horse gram have been cloned and sequenced (Malathi 2007). Agroinfectious clones were developed against different isolates of MYMIV to prove Koch's postulates in urdbean (Mandal et al. 1997), mungbean (Sivalingam et al. 2022), cowpea (Malathi et al. 2005), pigeonpea (Chakraborty 1996), and soybean (UshaRani et al. 2005). The use of agroinfectious clones on a routine basis in breeding programmes needs to be adopted for MYMIV-/MYMV-resistant urdbean cultivar development with a durable resistance.

23.9.1.2 Screening for Heat Tolerance

Recently, Gupta et al. (2021) elaborated the high-throughput and precise phenotyping procedure of a large set of urdbean genotypes and about their biochemical as well as molecular validation to identify heat-tolerant urdbean genotypes. Heat susceptibility index (HSI) for each individual urdbean genotype was calculated using the equation by Fischer and Maurer (1978): HSI = (1−Yh/Y)/(1−Xh/X), where Yh and Y are the phenotypic means (Yield) for each genotype under heat-stressed and non-heat-stressed conditions, respectively, and Xh and X are the phenotypic means (Yield) for all lines under heat-stressed and non-heat-stressed conditions, respectively. This study identified 8 highly heat-tolerant and 35 highly heat-sensitive genotypes based on heat susceptibility index. Further, characterization of a group of six highly heat-sensitive and seven highly heat-tolerant urdbean genotypes based on physiological and biochemical traits showed genotypic variability for leaf nitrogen balance index (NBI), chlorophyll (SPAD), epidermal flavanols, and anthocyanin contents under 42/25 °C max/min temperature. Our results showed higher membrane stability index among heat-tolerant genotypes compared to sensitive genotypes. Significant differences among genotypes for ETR at different levels of PAR irradiances and PAR × genotypes interactions indicated high photosynthetic ability of a few genotypes under heat stress.

Further, the most highly sensitive genotype PKGU-1 showed a decrease in different fluorescence parameters indicating distortion of PS II. Consequently, reduction in the quantum yield of PS II was observed in a sensitive one as compared to a tolerant genotype. Fluorescence kinetics showed the delayed and fast quenching of Fm in highly heat-sensitive (PKGU 1) and heat-tolerant (UPU 85–86) genotypes,

respectively. Moreover, tolerant genotype (UPU 85–86) had high antioxidant activities explaining their role for scavenging superoxide radicals (ROS) protecting delicate membranes from oxidative damage. Molecular characterization further pinpointed genetic differences between heat-tolerant (UPU 85–86) and heat-sensitive genotypes (PKGU 1). These findings will contribute to the breeding towards the development of heat-tolerant cultivars in urdbean.

23.9.1.3 Screening for Salinity Tolerance

Recently, Shanthi et al. (2021) evaluated a set of urdbean genotypes under salinity stress condition to identify genotypes which are tolerant to salinity stress. Salinity is one of the most important abiotic stresses that affects the yield in most of the crops which are grown under degraded soil condition. The area under coastal cultivation of this crop is shortened due to increasing problem of soil salinity. In this report, fourteen urdbean genotypes, viz. VBN1, VBN2, VBN3, VBN(Bg) 4, VBN(Bg) 5, VBN(Bg) 6, VBN 7, and VBN 8 and VBG 12–034, VBG 12–062, VBG 12–110, VBG 12–111, VBG 13–003, and VBG 14–016, were screened under three EC level (4.0 EC, 11.0 EC, and 16.0 EC) and compared with 0.0 EC (control). The mean germination percentage of all the 13 genotypes studied illustrated reduced level of germination percentage with increasing salinity level.

At the highest salinity level (16.0 EC), the germination percentage was significantly affected compared to 4.0 EC and 11.0 EC. The grand mean of plumule length was more at 4.0 EC and was reduced to half (16.0 EC) as compared to control. The root grew longer at 11.0 EC (4.91 cm) as compared to 4.0 EC (4.83 cm) and 0.0 EC (3.02 cm), although it showed drastic reduction at 16.0 EC (1.92 cm). The grand mean value of dry matter weight increased concomitantly with salinity. The radical length had positive and significant correlation with dry matter weight at 11.0 EC (0.657), whereas positive and non-significant correlation with 4.0 and 16.0 EC suggested that radicle length is the most useful parameter to select salinity-tolerant urdbean genotypes. Genotypes VBG-14-016, Vamban 4, Vamban 8, and VBG-12-062 were found promising under salinity stress condition. It is pertinent to mention that a large set of urdbean accessions of diverse is needed to be phenotyped under soil salinity condition, so that a potential donor can be identified for this trait. The soil salinity condition should be monitored on a regular interval during the entire crop growth period.

23.10 Breeding Progress

23.10.1 Conventional Breeding

Predominant procedures in urdbean varietal development are mass selection, pedigree selection, back-crossing, and mutation breeding. Varieties released during 1940s were direct selections from local landraces. For example, Type 9 variety was first released by selection from local landrace. Urdbean varieties released in India from 1949 to 2000 more than 50% were developed from selections (Gupta

Fig. 23.2 (**a**) Main stem bearing urdbean. (**b**) Normal bearing urdbean

et al. 2020a, b, c). Later, after 2000, most of the urdbean varieties released were hybridization based followed by pedigree selection. Development of multiparent or MAGIC population is part of modern urdbean breeding in leading institutes. In India, mutation breeding is coordinated by Bhaba Atomic Research Center (BARC), Mumbai. Intra- as well as interspecific hybridization was initiated to combine traits in a single variety. Interspecific hybridization has become regular in most urdbean breeding programmes with the use of improved hybridization techniques using hormones or other chemicals during pollination which have increased the number of successful crosses. However, most of these interspecific crosses are with *V. radiata* or *V. mungo* var. *silvestris* (Gupta et al. 2020a, b, c). Other species are yet to be fully explored in distant hybridization of urdbean. During the last 10 years, more than nine urdbean varieties have been developed by interspecific hybridization in India. Presently, development of main stem bearing or sympodial (soybean type) type of urdbean is now a priority in urdbean breeding programmes. Recently, in ICAR-IIPR, we developed a main stem bearing urdbean variety, IPU 13–1 (Fig. 23.2.). The main purpose of manipulating plant types is to accommodate more number of plants in a same area, thereby increasing yield potential of a variety. Manipulation of plant type also facilitates mechanical harvesting. In crops like urdbean, mechanical harvesting is still an unexplored area and a greater number of varieties are required which will be amenable to mechanical harvesting.

23.10.2 Genomics-Assisted Breeding

23.10.2.1 Mapping or Tagging of Important Traits

Focus of use of molecular markers in urdbean breeding has been on MYMV or MYMIV resistance breeding. Limited research efforts in relation to the breeding for YMV disease resistance are in public domain. International efforts are mostly concentrated on another important food legume of South Asia, Mungbean (*Vigna radiata*). So far quantitative trait loci (QTLs) linked to MYMIV resistance loci were reported by few workers in mungbean (Kitsanachandee et al. 2013; Alam et al. 2014). Kitsanachandee et al. (2013) employed a F8 recombinant inbred line (RIL) mapping population generated in Thailand from a cross between NM10–12-1 (MYMIV resistance) and KPS2 (MYMIV susceptible). One hundred and twenty-two RILs and their parents were evaluated for MYMIV resistance in infested fields in India and Pakistan. Composite interval mapping identified five QTLs for MYMIV resistance: three QTLs for India (qYMIV1, qYMIV2, and qYMIV3) and two QTLs for Pakistan (qYMIV4 and qYMIV5). qYMIV1, qYMIV2, qYMIV3, qYMIV4, and qYMIV5 explained 9.33%, 10.61%, 12.55%, 21.93%, and 6.24% of variation in disease responses, respectively. Alam et al. (2014) used a F2 and BC1F1 population derived from a cross between susceptible (BARImung 1; BMl) and resistant (BARImung 6; BM6) mungbeans to identify quantitative trait loci (QTLs) associated with resistance to MYMIV.

Composite interval mapping consistently identified two major QTLs, qMYMM on linkage group 2 and qMYMVT on linkage group 7, conferring the resistance in both F2 and BC1F1 populations. qMYMIV2 and qMYMIV7 accounted for 31.42–37.60 and 29.07–47.36, respectively, of the disease score variation, depending on populations and locations. At both loci, the resistant alleles were contributed by the parent BM6. qMYMIV2 appeared to be common to a major QTL for MYMIV resistance in mungbean reported previously (Kitsanachandee et al. 2013), while qMYMIVT is a new QTL for the resistance. So, perusal of literature clearly indicates that no effort at the international level was made so far on mapping and tagging QTLs against MYMIV in urdbean. However, a field experiment was conducted at the Indian Institute of Pulses Research in Kanpur, Uttar Pradesh, India, during the rainy seasons of 2000 and 2001 to evaluate the inheritance of resistance to mungbean yellow mosaic virus (MYMV) in the F1, F2, and F3 populations of inter-varietal crosses of black gram involving eight highly resistant cultivars (DPU 88–31, NP 21, PLU 710, PDU 6, IPU 98–8, UPU 85–86, UG 27, and DUS 19) and six susceptible cultivars (PDU 1, IPU 99–182, IPU 99–168, PGRU 95013, UH 80–38, and UH 82–2) (Gupta et al. 2005). The highly susceptible cultivar for MYMV, PDU 1, was used as the indicator-infector and was also sown all around in the field to increase the MYMV incidence. The plants were classified as symptomless (R), and susceptible with typical symptoms of mosaic (S) and necrosis (N). Plants showing necrosis or no symptoms were classified as resistant. Each F3 family was classified as resistant (homozygous), susceptible (homozygous), or segregating (heterozygous). Disease severity on F2 plants segregated 3:1 (resistant:susceptible; R:S) as

expected for a single dominant resistant gene in all R/S crosses. The results of F3 analysis confirmed the presence of a dominant gene for resistance to MYMV.

ICAR-Indian Institute of Pulses Research (IIPR) has developed recombinant inbred line (RIL) populations by crossing resistant parent, DPU88–31, with the susceptible parent AKU9904 to identify and validate markers related to yellow mosaic disease resistance loci (Gupta et al. 2013b). Transferability of simple sequence repeat (SSR) markers was studied to increase the availability of molecular markers for germplasm evaluation, genetic analysis, and new cultivar development in urdbean. Three hundred sixty-one (361) simple sequence repeat markers developed for other food legumes were used to amplify genomic DNAs extracted from 24 diverse genotypes of urdbean. Out of these, 245 SSR markers (68%) amplified though only 39 (16%) were highly polymorphic among 24 diverse genotypes of urdbean (Gupta et al. 2013b). These identified SSR markers were used for genetic analysis of the RIL population. MYMIV resistance gene has been further mapped at a distance of 12.9 cM in LG10 of urdbean using SSR markers (Gupta et al. 2013b). This genomic region may be enriched with more closely linked molecular markers to make marker-assisted breeding for MYMIV in urdbean possible. Though various genomic resources have been developed for mungbean, urdbean, adzuki bean, and cowpea for enhancing the breeding efficiency, the limited marker polymorphism within the species makes it difficult to utilize marker-assisted selections in improvement of *Vigna* species. Comparative genome mapping holds a great opportunity as mungbean, adzuki bean, common bean, cowpea, and lablab bean exhibited high level of genomic collinearity (Han et al. 2005; Somta and Srinives 2007). Recently we have developed a set of polymorphic SSR markers in urdbean mapping population (DPU88–31 × LBG685) (personal communication from D. Sen Gupta, IIPR, Kanpur, not yet published) which showed high polymorphism percentage between the parental lines of the RIL population. This RIL population has 350 lines which are either resistant or susceptible to MYMIV infection. Polymorphic markers identified may be used to fine map the region of interest in urdbean.

23.10.2.2 Genome Sequencing

Recently, Jegadeesan et al. (2021) developed the genome assembly and draft genome sequence of urdbean variety, Pant Urd 31. They used Illumina and Oxford Nanopore sequencing technology to develop this draft sequence. The assembled de novo whole genome of urdbean is ~475 Mb (82% of the genome) and has maximum scaffold length of 6.3 Mb with scaffold N50 of 1.42 Mb. Bioinformatic analysis identified 42,115 genes with mean coding sequence length of 1131 bp. Nearly half of the assembled sequence was repeat elements. From this data, primer pairs for 34,816 SSRs were designed. Out of the 33,959 proteins, 1659 proteins showed the presence of R-gene-related domains (Fig. 23.3). KIN class was found in majority of the proteins (905) followed by RLK (239) and RLP (188). Previous to this work, Pootakham et al. (2021) employed the 10X Genomics linked-read technology to obtain a de novo whole genome assembly of urdbean cultivated variety Chai Nat 80 (CN80). The draft assembly contained 12,228 contigs and had an N50 length of

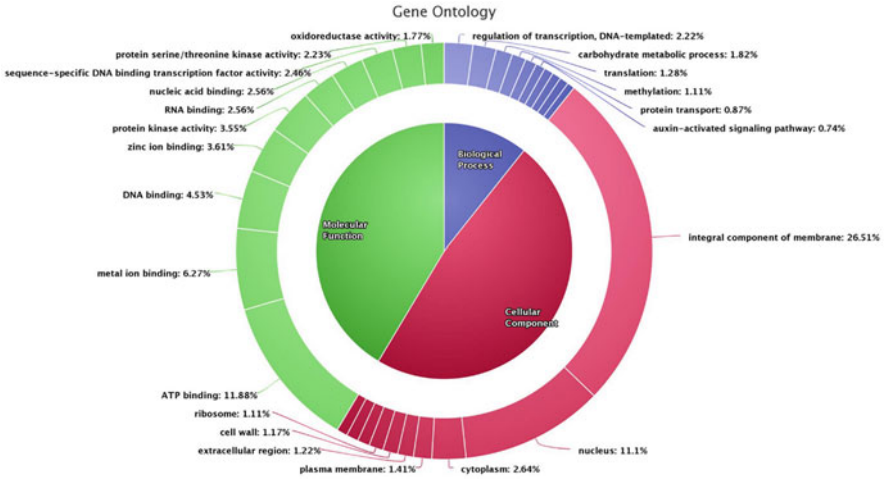

Fig. 23.3 Gene ontology chart of *Vigna mungo* depicting R-gene-related domains (adopted from Jegadeesan et al. 2021, Draft genome sequence of the pulse crop urdbean [*Vigna mungo* (L.) Hepper] reveals potential R-genes. Scientific reports, 11, 11,247)

5.2 Mb. Subsequent scaffolding using the long-range Chicago and HiC techniques produced chromosome-level assembly of 499 Mb comprising 11 pseudomolecules.

23.11 Status of Varietal Development and Maintenance Breeding

Sincere efforts of varietal development in urdbean had been started in the first quarter of twentieth century at Pusa, Bihar, with the endeavour of improving locally adapted but genetically variable populations mainly through the pure line and mass selection method for yield contributing traits. Numbers of genotypes/cultivars were collected from all important urdbean-growing areas of India and Burma (Myanmar) in 1925 (Bose 1932). Subsequently, large number of cultivars (15 small, green seeded, and 10 large black seeded) comes out, some of which are still popular in certain areas of the country (Table 23.3). Only two cultivars had a semi-erect plant type and the rest were spreading type. The breeding work on urdbean accelerated with the inception of AICRP on pulses in 1967. But before that during 1943–1950, a large number of urdbean varieties such as Mash 48, Pusa 1, T-122, T 27, T 77, HPU 6, and Amoo 46–5 were also developed in different states. Later on, during 1950–1970, limited numbers of varieties were developed like Gwalior-2, T-65, CO-1, ADT 1, T-9, and D 6–7. Before 1970, the varieties were developed by research scientists working in the state department of agriculture and/or in agriculture colleges in different parts of India, for example, popular variety T 9 was developed at Kanpur. It was an early maturing (80–90 days) and higher yielding variety. This variety was ruling variety for several years in areas and season where yellow mosaic disease was not a big

Table 23.3 Decade-wise list of urdbean varieties developed in India after 1920

S. no.	Years	Names of varieties
1	Before 1950	Amoo 46-5, HPU 6, Mash 48, Pusa-1, T-122, T-27, T-77
2	1950–1970	Gwalior-2, D 6-7, T-65, Co-1, T-9,ADT-1
3	1970–1990	Mash 2, Mash 1-1, Keishna, Kulu Mash No-4, , Khargone-3, KM-1, Co-2, C0-3, G-75, Naveen, Co-4, ADT 2, Kalindi, KM-2, N0.55, Pant U-19, Pant U-30, Sindhkeda 1-1, TMV, 1, ADT 3, Co-5, Zandewa, Pragati, Sarla, LBG 402, Mash 218, TAU-1, Jawahar Urd-2, Jawahar Urd-3, Pant U-35, LBG 17
4	1990–2010	PDU-1, LBG 402, LBG 20, Vamban 1, ADT 4, ADT-5, TPU 4, TAU 2, APK 1, LBG 22, K-1, Narendra Urd 1, WBU 108, PDU 1, UG 218, LBG 648, Pant U 19, LBG 623, LBG 685, VBN 3, AKU-15, LBG 645, PBG 1, PBG 107, KU 309, VBN(Bg)4, GU-1, Mash 1008, P-93, LBG 709, VBN(Bg) 5, HM-1, DU-1, Mash 114, LBG 752, CO 6, LBG 611, Mash 338, Birsa Urd 1, Mash 414, AKU 4, KBG 512, Vamban 2, WBG 26, TU 94-2, KU 301, KU 92-1, IPU 94-1, RBU 38, KU 300, NDU 99-2, KU 96-3, Pant U-31, Pant U 40, WBU 109, IPU02-43, NUL 7
5	2010–2020	LU 391, KUG 479, IPU 07-3, VBG 04-008, TU 40, VBN-8, VBN-9, VBN-10, LBG 787, KPU 405, PU-10, Kota Urd 4, VBN 6, UH-1, DBGV 5, Pratap Urd-1, SBC 40,MDU-1, Vallabh Urd 1, Indira Urd Pratham, TBG 104, AKU 10–1, ADT 6, KKM-1, TRC Urd 99-2, Pant U-7, Pant U-8, Pant U-9, IPU 13-1, IPU 10-26, IPU 11-02, GBG 1, VBN-11, OBG 33, Kota Urd-3, C0-7

issue. Consequently, it became very popular and used as national check in AICRP trials for several years.

Out of aforementioned cultivars, T 9, ADT 1, and Co 1 were most important lines as they were not only preferred by farmers' community but also used extensively in breeding programme to develop many varieties of urdbean. With the inception of AICRP on pulses during 1970–1990, many varieties developed were KM-1, khargone, ADT-2, Pant U-19, Pant U-30, Mash 218, Sarala, and LBG 17. Of which, Pant U-19, Pant U 35, and TAU 1 are still popular in certain areas and existed in seed chain. Amid 1970–1980 in collaboration with AICRP, hybridization work was taken up by different research stations to evolve varieties having characteristics such as high yield potential, short duration, non-shattering, resistance to pest and diseases, wider adaptability, and high protein content (Kumar and Singh 2009). It was necessary to diversify the agronomic bases with incorporating YMV resistance. KM 1 was the first variety developed through hybridization in the year 1977. Other varieties, Pant U19 and Pant U 30, were developed from a cross of UPU 1, a selection from T 9 and UPU2. These varieties are short duration and YMV resistance and released in 1981. The hybridization followed by selection has played an important role in the development of resistant varieties in 1980s. This had led to the development and release of several MYMV-resistant/MYMV-tolerant varieties like Pant U 19, Pant U 30, Pant U 35, PDU 1, etc. in urdbean. Type 9 in combination

with L 64, Sel. 1, Line 400, NP 19, and 7378/2 leads to development of urdbean varieties KM 2, Narendra Urd 1, WBG 26, IPU 94–1, and KU 300, respectively. Simultaneously, it was observed that the productivity of urdbean under rice fallow is very poor, and a need was felt to enhance the productivity through incorporation of powdery mildew resistance in the varieties. The first powdery mildew resistance urdbean variety LBG 17 was developed in 1983 for rice fallow situation. This has revolutionized the urdbean cultivation in the coastal regions of A.P. Later, few more urdbean varieties like LBG 20, LBG 625, LBG 685, LBG 402, and LBG 611 led to its area expansion in rice fallows of coastal peninsular.

Similarly, LBG 17, Pant U 35, and KU 301 are also derived from cross Netinminumum × Chikkuduminumu, UPU 3 × Pant U 19, and 7570/7 × Sel.1. Varieties KM 1 and ADT 4 are the product of three-way crosses (G 31 × Khargone 3) × G 31 and (T 9 × ADT 2) × Pant U 19.

Furthermore, national crossing programme was initiated in 1980 in order to create new variability (Singh and Satyanarayana). The number of crosses has been assigned to different centres. The impact of this programme could be judged from the fact that about 67% varieties were developed by hybridization during the 90s. By the mid of the 1990s, substantial reduction in crop duration coupled with photothermo-insensitivity and synchronous maturity, resistance to major disease, and large seed size were achieved. During the first decade of twenty-first century, many varieties have been developed with high yielding potential and biotic stress resistance (YMV, CLS, and PM). AKU-15 is the product of hybridization for Vidarbha region of Maharashtra, and it has tolerance to powdery mildew and dry root rot. Systematic breeding efforts for incorporating MYMV resistance resulted in development of two resistant cultivars, Pant U 31 and IPU 02–43, which were bred in northern Indian conditions but had shown resistance against MYMV across the locations and found promising in southern peninsula. Pant U 31 was released in 2005 and became popular among farmers in 2009. Other varieties like KU 300, NDU 99–2, Pant U-40, and WBU 108 developed through hybridization for different agro-climatic conditions with MYMV resistance. After hybridization and selection, mutation with gamma rays was also most frequently used in urdbean for varietal development. Many varieties like Co 4, Manikya, TAU 1, TPU 4, TAU 2, and TU 94–2 have been developed through mutation breeding approach. The mutant variety TAU 1 was the most popular variety particularly in Maharashtra.

However, pedigree analysis of urdbean revealed that a small number of parents with high degree of relatedness were repeatedly used in crossing programme which narrows down the genetic base. Sixty seven percent of varieties in urdbean have Type 9 as one of the ancestors in their pedigree. Therefore, keeping in mind this issue, wide hybridization has been started in urdbean to develop varieties with wider adoptability and improved plant types. Initially the progress in urdbean has been very slow, but recently some urdbean lines isolated from mungbean x urdbean and urdbean × *Vigna mungo* var. *silvestris* crosses have been found very promising with respect to wider adoptability and degree of sensitivity to abiotic stresses.

Till date, a total of nine urdbean cultivars have been developed using interspecific approach (Table 23.4), and several promising lines are being evaluated in

Table 23.4 Varieties developed through interspecific hybridization

Crop	Species used	Cultivar developed
Urdbean	*Vigna silvestris* *Vigna radiata* *Vigna umbellata*	VBN 7, Vamban 5, Vamban 6, Mash 1008 Mash 114, VBG04-008, TU 40, VBN 8, Mash 1137 (09)

multilocation trial under AICRP. Recently the incidence of MYMV has become serious in rice fallows of south India, and efforts are being diverted towards incorporation of MYMV resistance genes along with powdery mildew. During the last two decades, major emphasis has been to breed short-duration, photo- and thermo-insensitive varieties of urdbean coupled with resistance to biotic stresses, viz. yellow mosaic virus and powdery mildew, which contributed significantly to the national production. Consequently, more than 100 varieties of urdbean have been developed till date in India.

23.12 Coordinated System of Testing

The coordinated research programme on urdbean along with other pulses started with the establishment of All India Coordinated Pulse Improvement Project (AICPIP) in the year 1966 at Indian Agricultural Research Institute, New Delhi. Later in 1978, the headquarter of AICPIP was shifted to the then regional station of IARI at Kanpur. The Project Directorate (Pulses) was further strengthened with the inclusion of basic and strategic research in its mandate and elevated as Directorate of Pulses Research in 1984. It was also delinked from IARI and started functioning independently under ICAR. To provide focused attention on each crop, MULLaRP crops MULLaRP (Mungbean Urdbean, Lentil, Lathyrus, Rajmash and Pea) were separated from the rest of the pulses under independent coordinated project AICRP on MULLaRP during VII Plan in 1993 with 6 mandatory and 8 verifying centres. The post of Project Coordinator (MULLaRP) was created to provide independent leadership to AICRP on MULLaRP. These centres were also allowed for varietal testing of one or two other pulse crops besides carrying out the intensive research on mandated crops of MULLaRP which includes generation of breeding material, development of location specific production, and protection technologies. During IX Plan, all 8 verification centres were converted to sub-centres through redeployment of manpower from other projects and ten new sub-centres, i.e. Kanpur and Varanasi (Uttar Pradesh), Dholi (Bihar), Sehore (Madhya Pradesh), Dharwad (Karnataka), Imphal (Manipur), Agartala (Tripura), Ludhiana (Punjab), Hisar (Haryana), and Coimbatore (Tamil Nadu), were added. Additionally, 10–15 voluntary centres were also included in the network to test the location-specific genotypes and production and protection technologies by providing the additional contingency grant. This was the great expansion of this AICRP. During X Plan, a new sub-centre at Kota (Rajasthan) was added to cater the need of southern Rajasthan. During XI Plan, more emphasis was laid on location-specific focused research activities. The

two sub-centres, one each in Sagar (Madhya Pradesh) and Keonjhar (Odisha), were created. To give impetus on rice fallow pulses, two sub-centres, one each in Ghantasala (Andhra Pradesh) and Aduthurai (Tamil Nadu) in southern peninsula, were added to the project. At present, the project operated through 6 main centres and 22 sub-centres.

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Lentil Breeding

24

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Abstract

Lentil (*Lens culinaris* Medikus) is important rainfed winter season grain legume for diversification of cereal-based cropping system worldwide. The crop originated in Near East and spread to different region establishing in wide range of agro-ecology. Lentil is cultivated in more than 50 countries. Lentil grains are rich sources of protein, prebiotic carbohydrates, micronutrients, and vitamins. Lentil is important staple food in regions with low income. The productivity of lentil is low due to poor seedling vigour, high flower drop, low pod set, poor dry matter accumulation, and susceptibility to biotic and abiotic stresses. Biotic and abiotic stresses induced by climate change pose challenge to lentil cultivation. Discovery of new genes and quantitative trait loci offer opportunity to breeders for improving lentil varieties for higher grain yield, nutritive value, and tolerance to biotic and abiotic stresses. In this chapter, we discuss the present challenges and opportunities for lentil improvement.

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_24

Keywords

Lens culinaris · Origin · Hybridization · Breeding · Biotic and abiotic stresses · Nutritional quality

24.1 Importance of Crop

Lentil (*Lens culinaris* subsp. *culinaris*) is self-pollinated diploid crop grown in cool season. Lentil is Old World legume domesticated with wheat and barley. From Fertile Crescent, lentil cultivation proliferated westwards to the Mediterranean region, the Nile Area, and Central Europe and in east towards Asia. Later the lentil was introduced to North America and Australia (Cubero 1984; Matny 2015). Cultivated lentils are known as masur (Hindi), adas (Arabic), heramane (Japanese), and mercimek (Turkish). Cultivated lentils are classified in two groups: macrosperma types and microsperma types (Cubero 1981). Macrosperma types have large and flat seeds and are predominantly grown in North Africa, Southern Europe, and North and Latin America. Microsperma types are grown in Afghanistan, Egypt, Ethiopia, and South Asia. Lentil is grown in 52 countries with global area of 6.1 Mha and production of 6.33 Mton (FAO 2018). Canada, India, Australia, Turkey, and Nepal were the leading producers of lentil. Canada and Australia produce lentil for export to South Asia. Nutritive lentil grains are grown for human consumption and lentil straw is valuable animal feed. Lentil is rich in protein, carbohydrate, fibre, vitamins, and micronutrient and provides nutritional security (Kissinger 2016). Lentil straw is nutrient rich with higher ruminal degradation in comparison to cereal straw and has been successfully utilized for ration of large ruminants and sheep (Mudgal et al. 2018). In South Asia, lentil is grown in different cropping systems (rice/lentil, fallow/lentil, and pearl millet/maize/cotton/lentil).

Major lentil-producing regions in South Asia are Northwestern Plains, Northeastern Plains, Central Highlands, and *Terai* region. Northwestern plains include Western Uttar Pradesh, Haryana, Punjab, and Punjab province of Pakistan. Small-seeded lentil varieties with bushy growth habit, profuse branching, and maturity duration of 130–140 days are grown. This region occupies 10% of total area under lentil. Vascular wilt (*Fusarium oxysporum* f.sp. *ciceri*), ascochyta blight (*Ascochyta lentis*), and rust (*Uromyces fabae*) are the major diseases (Mishra et al. 2007). Northeastern Plains include Bangladesh, Myanmar, Eastern Uttar Pradesh, West Bengal, and Bihar. This region accounts for 35–40% of area. Small-seeded lentils are grown on rice fields under rainfed conditions, on low-lying areas (Tal area), or on uplands after jute. Wilt, stemphylium blight, and rust are the major diseases. Central highlands occupy 35–40% area of South Asia including Madhya Pradesh, Uttar Pradesh, and Maharashtra. Here lentil is monocropped in kharif rice fallows. Bold seeded, early maturing varieties with long tap roots, and upright growth habit are preferred. Wilt and rust are the major diseases. *Terai* region includes Himalayan foot hills of Nepal, Pakistan, and India occupying about 15% of total area in South Asia. This region has high humidity, rainfall, and severe winter. Frost, rust, and wilt are abiotic and biotic constraints.

24.2 Origin and Distribution

The genus *Lens* Miller is part of the family *Fabaceae* (*Leguminosae*), subfamily *Faboideae*, tribe *Fabeae*, or alternatively in subfamily *Papilionaceae*, tribe *Vicieae*. *Lens culinaris subsp. culinaris (Bioss.) Ponert* is divided into two groups: the small-seeded, red cotyledon var. *microsperma* and the large-seeded, red, yellow, green var. *macrosperma*. *Lens culinaris* ssp. *culinaris* is native to near East and Central Asia. *Lens orientalis* is the probable progenitor (Ladizinsky 1979) of lentil. It was found in the field of farmers cultivating lentil in the Middle East, and plant characteristic and pollen morphology resembles lentil. Initially Cubero (1981) classified genus *Lens* in five species *Lens culinaris*, *Lens orientalis*, *Lens ervoidis*, *Lens nigricans*, and *Lens montbretti*. Later this genus was divided in seven species/subspecies (Ferguson 2000):

(a) *L. culinaris*.
ssp. *culinaris,*
ssp. *orientalis* (Boiss) Ponert,
ssp. *tomentosus* (Ladizinsky) Fergusan, Maxted, van Slageren and Robertson,
ssp. *odemensis* (Ladizinsky) Fergusan, Maxted, van Slageren and Robertson.
(b) *L. ervoides* (Brign.) Grande.
(c) *L. nigricans* (M.Bieb.) Godron.
(d) L. *lamottei* Czefr.

The *Lens* wild relatives originated from areas with variable climatic conditions and soils. The distribution of CWR in genus *Lens* is reported by Singh et al. (2014a). *Lens culinaris* ssp. *orientalis* exists in the entire Fertile Crescent including Jordan, Syria, Israel, Lebanon, and Cyprus and in Armenia, Azerbaijan, Czech Republic, Iran, Russia, Turkmenistan, Tajikistan, and Uzbekistan in open or shaded habitats on stony calcareous to basalt soils at 500–1700 m. *L. culinaris* ssp. *tomentosus* is reported from Syria and Turkey. *L. culinaris* spp. *odemensis* is distributed in Israel, Syria, and Palestine in grassy habitat and in calcareous soils of pine groves and on basaltic gravel, at 700–1400 m. in Turkey. *L. ervoides* is found in Jordan, Syria,and Palestine and westwards to Montenegro, Italy, and Croatia and northwards to Russia, Ukraine Armenia, and Azerbaijan under trees and shrubs in shady and partially shady habitat. *L. lamottei* is reported from Spain and France. *L. nigricans* extends in open or shady stony habitats in granitic or limestone soil or in terraces, settlements, and plantations up to 1200 m in Bahrain, Crimea, Croatia, France, Italy Montenegro, Spain, and Ukraine.

Lentil was domesticated by selection of plants from their wild species. The initial selection was made primarily for seeds in pod and seed size. Archaeological evidence indicates the presence of lentil in Syria-Turkey-Iraq region around 8500–600 BC. The crop was probably domesticated in this region. From here the crop spread to Greece, Nile, Central Europe, and South Asia (Nene 2006). Later lentil spread to Afghanistan, China, Ethiopia, India, and Pakistan. Invaders of the Hamites introduced lentil as part of Near Eastern crop assemblage to Ethiopia. From

Bronze Age, lentil was grown with wheat and barley throughout the Mediterranean region (Erskine et al. 1989). Lentil cultivation as part of farming system began in 5th–4th millennium BC.

24.3 Plant Genetic Resources

24.3.1 *Lens* Gene Pool

Cubero et al. (2009) noticed hybridization barriers in the genus *Lens*. They classified *L. culinaris* and *L. orientalis* in primary gene pool and *L. odemensis* in secondary gene pool due to some restrictions in hybridization. *L. nigricans* and *L. ervoides* were grouped in tertiary gene pool. Later, Tullu et al. (2013) reported that *L. nigricans* and *L. ervoides* can be part of secondary gene pool using embryo rescue. Wong et al. (2015) classified seven taxa in four gene pool (primary, *L. culinaris*; secondary, *L .orientalis*; tertiary, *L. tomentosus* and *L. lamottei*; and quaternary, *L. ervoides and L. nigricans*).

Several reports are published (Ladizinsky et al. 1984; Abbo and Ladizinsky 1991, 1994; Fratini et al. 2004; Fratini and Ruiz 2006; Muehlbauer et al. 2006) on crossability of *L. orientalis* and *L. odemensis* with *L. culinaris*. Hybrid embryo abortion, hybrid sterility, and albino seedling are major constraints in the interspecific hybridization of *Lens* species. Abbo and Ladizinsky (1991, 1994) and Gupta and Sharma (2005) reported hybrid embryo abortion in crosses involving *L. culinaris* and *L. nigricans* and *L. ervoides*. Application of gibberellic acid (GA3) has led to success of *L. culinaris* crosses with *L ervoides, L. odemensis,* and *L. nigricans.* Protocol to recover embryo of crosses between *L. culinaris* and *L. odemensis, L ervoides*, and *L. nigricans* has been reported by Fratini and Ruiz (2006). The details on leading gene banks and PGR conserved are mentioned in Table 24.1.

Taxonomic classification of the genus *Lens* Miller has gone through several modifications, initially with five species, *Lens culinaris, Lens orientalis, Lens ervoides, Lens nigricans*, and *Lens montbretii* (Cubero 1981), to the present seven species/subspecies, *L. culinaris* ssp. *culinaris*, *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *tomentosus*, *L. culinaris* ssp. *odemensis*, *L. ervoides*, *Lens lamottei*, and *L. nigricans* (Ferguson 2000). Despite the taxonomic reclassifications, all studies indicated *L. culinaris* ssp. *orientalis* as the most closely related wild progenitor of *L. culinaris* ssp. *culinaris* (Cubero et al. 2009).

During domestication, successive selection rounds have reduced genetic variation and the allelic diversity of crops in comparison to their wild progenitor. The reduced diversity during domestication is the major bottleneck in improving crop productivity. Effective adaptation strategies are required to meet biotic and abiotic stresses due to climate change and variability of weather within growing season. Crop wild relatives (CWRs) have tremendous potential to enhance and sustain optimum productivity. Search, characterization, and conservation of CWR is important in lentil

Table 24.1 Leading gene banks and PGR conserved

Gene bank	Country	Wild relatives	Land races	Total accessions	Reference
ICARDA	Global	4%	80%	13958	Coyne et al. (2020)
Australian Temperate Field Crops Collection	Australia	4%	54%	5254	Singh et al. (2017a, b)
Seed and Plant Improvement Institute	Iran			3000	
USDA	USA	1%		2875	
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry	Russian Federation		80%	2556	
National Bureau of Plant Genetic Resources	India	1%	42%	2285	
Inst de Inv. Agropecuarias, Centro Regional de Investigación Carillanca	Chile			1345	
PGRC	Canada		56%	1139	
Plant Genetic Resources Dept. Aegean Agricultural Research Inst.	Turkey	1%	99%	1095	
General Commission for Scientific Agricultural Research General Commission for Scientific Agricultural Research	Syria			1072	
Research Centre for Agrobotany	Hungary		3%	1061	
NGB	Egypt		5%	875	
Institute of Crop Germplasm Resources	China		60%	855	
Plant Genetic Resources Institute, National Agricultural Research Center	Pakistan	8%	91%	805	
Bangladesh Agricultural Research Institute	Bangladesh			798	
Centro de Recursos Fitogeneticos, INIA	Spain	10%	87%	703	
Biodiversity Conservation and Research Institute	Ethiopia	70%		678	

breeding programme. CWRs are the most potential sources for additional genetic variation in crop plants.

24.3.1.1 Wild Relatives as Source of Novel Variation

Variation of yield traits (secondary branches, crop duration, biological yield, number of pods, seed size, and grain yield) in different *Lens* species have been reported

(Kumar et al. 2011; Singh et al. 2013a). *L. culinaris* ssp. *orientalis* accession ILWL 118 is very valuable source for earliness. Earliness is important for rainfed lentil in Central India and rice fallows in Eastern India. The utility of *L. ervoidis* has been reported as source of seed traits, growth habit, and biomass production (Tullu et al. 2011, 2013). Gorim and Vandenberg (2017a) investigated root trait variation for improving biomass and grain yield of *L. orientalis, L. tomentosus, L. odemensis, L. lamottei*, and *L. ervoides*. Ferguson et al. (1998) and Gupta and Sharma (2006) reported that *L. lamottei* and *L. culinaris* ssp. *orientalis* are the potential sources of genes for seed size and number of seeds and pods. Singh et al. (2014a) evaluated a large number of *Lens* species accessions over the years and reported variation for yield contributing traits and multiple disease resistance in *L. ervoidis* and *L. nigricans*. The study of Singh et al. (2020) exhibited significant variation for agromorphological traits in *Lens* wild species.

24.3.1.2 Wild Relatives as Source of Tolerance to Abiotic Stresses

Lentil and its wild relatives evolved in low rainfall areas on marginal soil. The most important abiotic stress is drought. Accessions in primary gene pool exhibit poor water extraction and lower growth rate than accessions in secondary and tertiary gene pool. The potential of *L. nigricans, L. odemensis,* and *L ervoides* for drought tolerance has been reported (Hamdi and Erskine 1996; Gupta and Sharma 2006). The *L. culinaris*. spp. *orientalis* from low rainfall environments in Syria, Azerbaijan, Jordan, Tajikistan, and Turkmenistan provides sources of drought tolerance. Gorim and Vandenberg (2017a) studied lentil CWRs for root traits under water stress and controlled environment. Omar et al. (2019) examined interspecific derivatives and reported that drought tolerance was associated with pubescent leaves, relative leaf water content, increased root-shoot ratio, cell membrane stability, and transpiration, reduced wilting, and canopy temperature. Sanderson et al. (2019) evaluated RILs of crosses of *L. culinaris* with *L. odemensis, L. orientalis*, and *L ervoides*. Stress tolerance was associated with reduced transpiration, delayed flowering, deep roots, and reduced plant height.

Genotypes of *L. orientalis* and *L. odemensis* with deep roots exhibited drought tolerance. Delayed flowering permitted root exploration in deeper soil; however pod number and seed yield were reduced. *L. tomentosus* exhibited reduced transpiration rate. Hamdi and Erskine (1996) reported cold tolerance in *L. culinaris* ssp. *orientalis*. *L. lamottei* accession, evolved from frost-prone area, exhibited trichomes on leaves, stems, and pods (Gorim and Vandenberg 2017a). Baute et al. (2015) reported that the reproductive period in May–June of *Lens orientalis* genotypes from Tajikistan, Turkmenistan, and northern Syria makes it potential source for heat tolerance.

24.3.1.3 Wild Relatives as Source of Tolerance to Biotic Stresses

Donors for important biotic stresses (fusarium wilt, ascochyta blight, stemphylium blight, rust, anthracnose, powdery mildew, bruchids, *Sitona* weevil, and *Orobanche*) have been reported from different *Lens* ssp. (Meena et al. 2017). Bayaa et al. (1994) screened 248 accessions of wild germplasm from ICARDA for ascochyta blight resistance. Resistance was reported in 12 accessions of *L. culinaris* ssp. *orientalis*, *3*

accessions of L. culinaris ssp. *odemensis,* 36 accessions of *L. nigricans*, and 03 accessions of *Vicia montbretti.* Gupta and Sharma (2006) characterized 70 accessions of wild species and reported resistance for both biotic and abiotic stresses in *L. nigricans*. Tullu et al. (2006) evaluated 564 accessions of six *Lens* species for resistance to anthracnose (*Colletotrichum truncatum*). Among the studied species, *L. ervoides* revealed high resistance to race Cti and Cto followed by *L. lamottei*. It was reported that the *L. ervoides* accessions exhibiting resistance originated at Turkey and Syria. Cultivated lentil exhibits susceptibility to *Ascochyta lentis.*

Fernández-Aparicio et al. (2009) studied screened wild *Lens* accessions and reported high level of resistance to broom rape in accessions of *Lens odemensis*, *Lens ervoides*, and *Lens orientalis.* Tullu et al. (2010) studied 375 accessions of six wild *Lens* ssp. for resistance against *A. lentis*. Several accessions of *L. ervoides* and *L. nigricans* belonging to secondary gene pool exhibited resistance. Laserna-Ruiz et al. (2012) evaluated 571 accessions of wild *Lens* against seed bruchids. Relatively less infestation was recorded in 32 accessions of *L. culinaris* ssp. *culinaris*, *L. culinaris* ssp. *orientalis*, *L. nigricans*, and *L. lamottei*. Podder et al. (2013) characterized 70 accessions of six *Lens* species for resistance to *Stemphylium botryosum*. High frequency of resistance was reported in *L. lamottei* followed by *L. ervoides*. Resistance to multiple diseases in *L. nigricans* and *L. ervoides* accessions had been reported by Singh et al. (2014a). Dadu et al. (2017) screened 30 genotypes of five species (*L. orientalis, L. odemensis, L. ervoides, L. nigricans,* and *L. lamottei*) against *Ascochyta lentils*. About one-third of the studied genotypes exhibited resistance to ascochyta and *L. orientalis* accession ILWL 180 exhibited stable resistance against highly aggressive isolates. Barilli et al. (2020) reported high level of resistance in *L. ervoides* accession PI 572330, PI 572334, and PI 572338 against *Colletotrichum lentis.*

24.3.2 Germplasm Evaluation for Agromorphological Traits

Several reports are available on characterization of germplasm. Few recent reports on evaluation of germplasm indicating the range of variability in studied germplasm are presented in Table 24.2.

24.4 Floral Biology: Emasculation (Pollination Techniques)

24.4.1 Flower Structure

The inflorescence is an axillary raceme with 1–4 flowers borne on slender peduncle (Fig. 24.1a, b). However, 5–7 flowers per peduncle are also reported (Mishra et al. 2020). A genotype bearing up to 15 flowers per peduncle (fasciated stem mutant line) was also reported recently (Tripathi et al. 2021). The flower consists of five unequal sepals, five petals (standard petal broadly obviate, two wing petals oblong to obovate, and keel formed by fusion of two petals), androecium, and gynoecium.

Table 24.2 The range of variation for agromorphological traits in lentil

Traits	Anjam et al. (2005)	Kumar and Solanki (2014)	Mekonnen and Hoekstra et al. (2014)	Pouresmael et al. (2018)
Plant height (cm)	28–45	14.60–45.20	20.6–44.7	16.67–33
Days to 50% flowering	–	59.00–107	42–78	62–77
Days to pod initiation	–	71.00–115	–	61–73
Days to maturity	–	101.00–145	77–138	81–103
Secondary branches	–	5.80–49.00	–	–
Pods/plant	23–160	7.20–302	1.6–73.3	1.42–14.78
Seeds/pod	–	1.00–2.00	0.5–2.0	1–4.33
100 seed weight	–	0.49–5.17	1.38–4.9	0.21–6.59
Seed yield/plant	–	0.19–3.84	–	0–6.33
Pod dehiscence (1–9 scale)	2–9	–	–	–
Seed yield (kg/ha)	333–1544	–	0–347.2	–
Biomass (kg/ha)	1667–7292	–	–	–
Straw yield (kg/ha)	1333–6133	–	–	–
Viral disease (1–9 scale)	1–9	–	–	–
Pods/peduncle	2–4	–	–	–

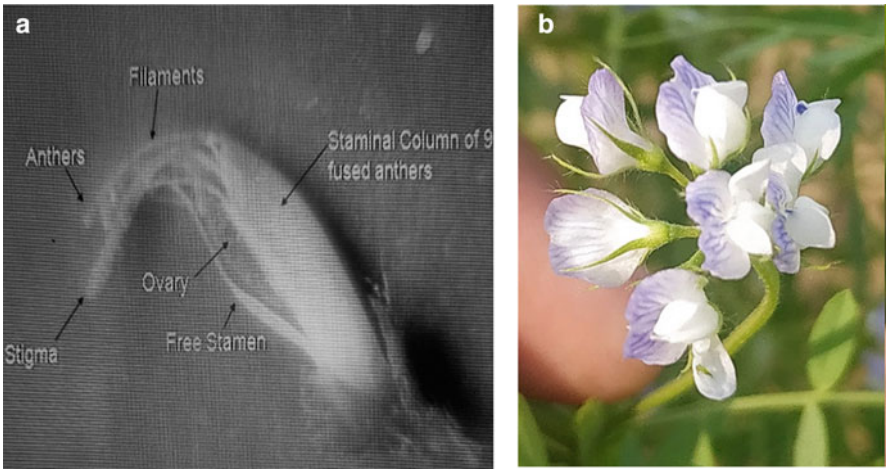

Fig. 24.1 (**a**) Details of a lentil flower; (**b**) a peduncle bearing seven flowers in cultivated lentil

Gynoecium includes laterally compressed sub-sessile ovary, glabrous style (1–5 mm long, flat, and abruptly unturned), and stigma (glandular papillate and spatulate). The stamen is diadelphous (9 + 1), axillary stamen is free, and stamens are winged

towards base. Spherical anthers are basifixed with 0.2 mm diameter and light yellow in colour.

24.4.2 Hybridization Technique

Lentil is a self-pollinated crop with less than 0.8% natural outcrossing. Emasculation of lentil flower prevents self-pollination. The highest percentage of crossed seed is produced when pollination is done immediately after emasculation. The average temperature range of 15–25 degree centigrade is suitable for hybridization. Relative humidity of 50% and 12–15 h of light ensure good seed set. Hybridization is carried out in forenoon. The buds with three-fourth length of calyx lobes are selected and are held between the thumb and the forefinger with the suture of the keel. Pointed forceps are used to remove one or two calyx lobes adjacent to the suture side of the keel. The forceps is inserted in keel to split it without damaging it. The stamens are carefully removed with help of forceps and pollination is done manually immediately after emasculation. Pollen is collected from young flowers immediately after anther dehiscence. Interspecific hybridization is vital for broadening the genetic base of cultivated lentil. Hybridization of lentil with species of secondary gene pool results in degraded and shrivelled embryos and non-viable seeds. To overcome the problem, embryo rescue technique was employed but the success was low. Embryo rescue is time consuming and requires controlled growing environment and highly skilled technical personal. The initial work on interspecific hybridization in lentil has been reported by Cohen et al. (1984) and Fratini and Ruiz (2006).

24.5 Cytogenetics

Lentil is a self-pollinated crop with $2n = 2x = 14$. Sindhu et al. (1983) reported one metacentric, three acrocentric, and three sub-metacentric chromosome pairs in karyotype of lentil from somatic tissues of *L. nigricans*, *L. orientalis,* and *L. culinaris*. Ladizinsky et al. (1984) reported a similarity in karyotype of *L. culinaris* and *L. orientalis.* Three interchange differences were recorded by Gupta and Bahl (1983) between *L. culinaris* and *L. nigricans*. Different workers have reported different total chromosome length varying with species and seed size. Gupta and Singh (1981) reported total chromosome length of 39.31 μm for PL 639, (Dixit and Dubey 1986), 31.77 μm for Type 36, and 30.4 μm for *L. orientalis* and 21.47 μm for *L. ervoides* (Sindhu et al. 1983). Dixit and Dubey (1986) reported a range of 28.2–72.3 μm in different small-seeded lentil cultivars. Mishra et al. (2007) reported a range of 3.0–9.2 μm.

24.6 Genetic Studies for Morphological Traits

24.6.1 Growth Habit

Ladizinsky (1979) reported the incomplete dominance of erect growth habit and proposed gene symbol *Gh*. Emami and Sharma (1999) reported recessive gene *ert* for erect growth habit. Later Kumar (2002) and Mishra (2004) reported that erect growth habit was completely dominant over the prostrate growth habit and proposed gene symbol *Ert*.

24.6.2 Foliage Colour

Kumar (2002) and Mishra (2004) reported that dark-green foliage was dominant over the light-green foliage, and the gene symbol *Dgl* was proposed. Red pigmentation on plant parts has been studied (Ladizinsky 1979, epicotyl (gene Gs for green stem); Emami and Sharma (1996a), brown leaf (gene Bl); Vandenberg and Slinkard (1989), pods (gene Grp green pod); and Emami (1996), pods (anthocyanin developments gene Rdp)).

24.6.3 Leaflet Size, Shape, and Number

Mishra (2004) reported that the higher number of leaflets is dominant trait and proposed gene symbol *Hl*. They also reported incomplete dominance of broad leaflets (gene symbol *Blf*) over narrow leaflets. Leaflet size varies between microsperma and macrosperma genotypes.

24.6.4 Plant Pubescence

Pubescent pod (gene symbol *Glp*) is dominant over the glabrous pod (Vandenberg and Slinkard 1989). Kumar (2002) reported that plant pubescence is controlled by single dominant gene (*Pub*).

24.6.5 Tendril

The genetics of inheritance of tendril in lentil was studied by Vandenberg and Slinkard (1989). They reported that this trait is governed by single dominant gene (*Tnl*).

24.6.6 Plant Height

Hadded et al. (2004) reported quantitative inheritance for plant height. Tahir and Muehlbauer (1994) reported that tallness in lentil is dominant (*Ph*) over dwarfness.

24.6.7 Stem Fasciation

Stem fasciation is a recessive trait (Sharma and Sharma 1978).

24.6.8 Flowering Time

Sarker et al. (1999) reported that earliness is a recessive trait and proposed gene symbol *sn*. Transgressive segregation in F_2 is due to interaction of *sn* with minor genes for earliness.

24.6.9 Flower Colour

The flower colour depends on colour of standard, wing, and keel. Lal and Srivastava (1975) reported two genes for flower colour in lentil and suggested gene symbols VVPP, VVpp, and vvPP for violet white and pink flower. Bluish flower colour in lentil is governed by single dominant gene (Ladizinsky 1979). Red flower with gene symbol P was dominant over the white flower (Kumar 2002; Mishra 2004).

24.6.10 Number of Flowers/Peduncle

The number of flowers/peduncle is an important trait influencing grain yield in lentil. The trait is influenced by environment and declines with plant age. Gill and Malhotra (1980) reported that flower number is monogenic trait and two flowered phenotypes is dominant over three-flowered phenotype. However, the studies by Emami (1996) and Kumar (2002) revealed that higher number of flowers/peduncles was dominant.

24.6.11 Pod Size

Pod size is related to seed size and is a variable trait in lentil. Kumar et al. (2005) reported the monogenic inheritance of pod size with incomplete dominance.

24.6.12 Pod Dehiscence

Ladizinsky (1985) reported that pod dehiscence in *L. culinaris* ssp. *orientalis* is governed by two complimentary genes.

24.6.13 Seed Size

Cultivated lentils based on seed size/weight are divided in *microsperma* and *macrosperma*. Indian lentils are characterized as microsperma types with 100 seed weight between 12 and 35 g. Ladizinsky (1979) studied microsperma × macrosperma cross (*L. orientalis* × *L. culinaris*) and reported intermediate seed size in F_1 and continuous distribution in F_2. Abbo and Ladizinsky (1991) reported quantitative inheritance for this trait.

24.6.14 Seed Coat Colour

Different seed coat colours (black, grey, green, and brown) have been reported in lentil. The inheritance of black seed coat colour in lentil was studied by Vandenberg and Slinkard (1987). He reported one dominant gene with codominant expression. However, Vandenberg and Slinkard (1990) reported two recessive genes for black seed coat. Studies by Vaillancourt and Slinkard (1992), Emami (1996), Emami and Sharma (2000), and Sharma et al. revealed monogenic inheritance. Emami and Sharma (2000) proposed gene symbol Blt for this trait. The dominance of brown testa over yellowish-grey testa was reported by Ladizinsky (1979). Slinkard et al. (1990) reported digenic dominance of brown testa over green. Both grey and tan testa were reported as dominant over green.

24.6.15 Tannins in Seed Coat

The inheritance of tannins in seed coat was studied by Vaillancourt et al. (1986). They reported that the presence of tannins in seed coat was dominant trait. Bezeda (1980) reported that the presence of tannins (proanthocyanidins) in seed coat causes the discolouration of seed coat.

24.6.16 Seed Coat Spotting

The presence of spotting on seed coat is dominant over spotless seed coat (Ladizinsky 1979; Vandenberg and Slinkard 1990; Emami 1996). Vandenberg and Slinkard (1990) identified multiple alleles for spotting (*Scp* locus). They identified two types of spotting: spotting (*Scps*) and dotted (*Scpd*). Later Emami (1996)

confirmed two types of seed coat (mottling-*Mot* and speckling-*Spt*) in lentil both dominant over spotless.

24.6.17 Seed Hardness

Seed hardness in lentil is associated with poor water absorption. Ladizinsky (1985) reported that hard seed coat is monogenically controlled in cross between *L. ervoidis* and *L. culinaris* and proposed gene symbol *Hsc*. Vaillancourt and Slinkard (1992) reported that seed hardness is a recessive trait in cross involving *L. culinaris orientalis* and *L. culinaris.*

24.6.18 Cotyledon Colour

Microsperma types are called red lentils due to orange cotyledons, and macrosperma types have yellow and green cotyledon. Cotyledon is sporophytic tissue and colour can be recorded in freshly harvested seed. Hybridization of red and yellow cotyledons (Tschermak-Seysenegg 1928; Wilson et al. 1970; Singh 1978; Slinkard 1978; Sinha et al. 1987; Emami 1996; Kumar 2002) revealed that orange is dominant over yellow (Slinkard 1978 designated the gene as *Yc*). In addition to yellow cotyledon colour, there is phenotype with brown or pink tinge called as brown. Emami and Sharma (1996b) reported independent nature of Y and B. Double dominant YB results in orange cotyledon and yybb results in greenish cotyledon due to the absence of pigment. Emami (1996), Emami and Sharma (1999), and Sharma and Emami (2002), identified another gene Dg controlling the cotyledon colour.

24.6.19 Protein Content

Lentil is a rich source of dietary protein. The quantitative inheritance of protein content was reported by Hamdi et al. (1991), Chauhan and Singh (1995), and Tyagi and Sharma (1995). Seed protein content is negatively correlated with seed size.

24.7 Breeding Objectives

24.7.1 Breeding for Yield-Related Traits

24.7.1.1 Prebreeding for Broadening the Genetic Base

The genetic base of released cultivars in the Indian subcontinents is narrow. Sustained use of few genotypes in hybridization programme is the main reason for yield stagnation. Diverse Mediterranean germplasm and wild species is valuable repository of useful genes not available in cultivated lentil. Under DAC-funded

ICARDA-ICAR project "Broadening the genetic base: Pre-breeding and genetic enhancement in breaking yield barriers in kabuli chickpea and lentil", Mediterranean germplasm lines were evaluated and important donors were identified for different traits (Table 24.3).

24.7.1.2 Breeding for Mechanised Harvesting

Manual harvesting of lentil is expensive and often adequate number of labourers is not available for harvesting at crop maturity. Delay in harvesting results in shattering of pods. The use of combined harvestor is hampered due to loss of pods because of low plant height. New plant types with erect and non-lodging growth habit will suit mechanical harvesting. ICARDA can facilitate the national research programmes by providing non-lodging plant types with erect growth habit. For mechanical harvesting, ground clearance of about 15 cm is required. ICARDA-developed lentil variety Idlib is suited for machine harvesting (El-Ashkar et al. 2003).

24.7.1.3 Breeding for Herbicide Tolerance

Lentil production suffers parasitic and nonparasitic weeds. The non-parasitic weeds cause yield loss up to 40% due to competition with crop for nutrient and light (Tepe et al. 2005). The weeds are controlled by manual hand weeding. Manual weeding is not cost effective and sometimes adequate labourers are not available for manual weeding. Herbicide resistance is an alternative strategy to reduce the cost of production. Herbicide-tolerant lentil varieties have earlier been developed in Canada and Australia (Slinkard et al. 2007). In addition, these parasitic weeds like *Orobanche* are major threat to lentil cultivation under conservation agriculture in the Mediterranean region (Rubiales et al. 2006). Fernández-Aparicio et al. (2008, 2009) have screened *Lens* germplasm for orobanche resistance.

24.7.1.4 Breeding for Nutrient Use Efficiency

Lentil is grown in marginal soils deficient in micronutrients. From India, Syria, and Ethiopia, iron and boron deficiencies are reported. Fe deficiency in high pH soil causes yield. Yield loss in susceptible genotypes of 25% and 47% has been reported from India and Syria (Ali et al. 2000; Erskine et al. 1993). B deficiency is reported from northern Bangladesh, eastern Terai plains of Nepal, and Eastern India.

24.7.1.5 Breeding for Early Maturity and Bold Seed Size

Several studies have been conducted to decipher the genetics of flowering time in lentil (Tullu et al. 2008). A number of QTLs controlling flowering duration have been reported in lentil by Tullu et al. (2008). Several short-duration lentil varieties escaping terminal heat have been developed in Bangladesh (BARI M4, BARI M5, BARI M6 and BARI M7, BARI M8 and BARI M9) and India (L 4717). L 4717 is the first extra-early variety of lentil maturing in less than 100 days. Early maturing lentil varieties with stemphylium blight resistance are required for rice fallow cultivation in Eastern India.

Conventionally small-seeded types (100 seed weight 2.5 g/100 seed) are grown in India. Small-seeded types have shown wider adaptability to different agroclimatic

Table 24.3 Donors for important traits identified from Mediterranean germplasm from ICARDA

Trait	Donors identified	Trait	Donors identified
Drought tolerance	ILL 10040, ILL 10044	Pods/cluster	ILWL 15
Heat tolerance	ILL 10174, FLIP 2009-55 L, IG 2507, IG 4258, IG 2503, IG 3101, IG 3563, IG 4079, IG 4098, IG 4312, IG 4380, IG 1539, LC 279–1296, LC 270–804, LC 284–1206	Short internode	ILWL 17
Salinity resistance	ILWL 297, ILWL 368, ILWL 371, ILWL 417, IG 136670	Higher primary branches	ILWL 117, ILWL 398
Fusarium wilt resistance	ILWL 76, ILWL 79, ILWL 37, ILWL 1, ILWL 138, ILL 3492, ILL 4385, ILL 10956, ILL 6449, ILL 4649, ILL 3796, ILL 2502, ILL 2505, ILL 5714	High biomass	IL 10732, ILL 10733, ILL 10734, ILWL 30
Rust resistance	ILWL 15, ILWL 17, ILWL 19, ILWL 30, ILWL 62, ILWL 81, ILWL 97	Large seed size	ILL 1005, ILL 4559, ILL 4637, ILL 4649, ILL 5488, ILL 5628, ILL 8072, ILL 8595, ILL 7946, ILL 7678, ILL 7943, ILL 9890, ILL 10734, ILL 10736, ILL 10741, IPLS -09-17, IPSL-09-23, IPLS-09-33, IPLS-09-35, ILL 45113, ILL 45101
Powdery mildew resistance	ILWL 55, ILWL 59, ILWL 334, ILWL 359, ILWL 450	High grain Fe and Zn concentration	ILWL 74, ILL 8095, ILL 7981, ILL 10854, ILL 10072, ILL 88527, IG 135395, IG 135403
Root knot nematode resistance	ILWL 18, ILWL 72	Early maturity	ILL 6002, ILL 7663, ILWL 118, ILL 2585, ILL 4605, ILL 10848, LIRL-21-50-1-1-1-0, LIRL-22-46-1-1-10, LIRL-22-46-1-1-1-0, LIRL-22-46, ILL 45101, ILL 45107, IPLS-09-17, IPLS-09-35

Unpublished data from DAC-funded ICARDA-ICAR project "Broadening the genetic base: Pre-breeding and genetic enhancement in breaking yield barriers in kabuli chickpea and lentil"

conditions. In India, large-seeded types are confined to Central India, and small-seeded types are preferred in eastern, northern, and northern hills. Seed size in lentil is a quantitative trait controlled by several genes (Abbo and Ladizinsky 1991). Fedoruk et al. (2013) and Verma et al. (2015) have identified QTLs controlling seed size in lentil.

24.7.2 Diseases

24.7.2.1 Wilt

Fusarium wilt is most important disease of lentil caused by *Fusarium oxysporum* Schlecht.:Fr. f. sp. *lentis* Vasudeva and Srinivasan. The disease is reported from most of the lentil-producing regions. The symptoms of this disease are produced at pre- and post-emergence stage. Browning of micropylar end occurs in infected seeds. After seed germination, the radicle browning results in death of seedling. Plant growth is reduced, leaves become yellow and curled, crown droops, and plant dies. Roots become yellowish/dark brown. The wall of xylem vessel is discoloured/turns brown due to the presence of fungal hyphae. Macroconidia, microconidia, and chlamydospores are the asexual spores produced by the pathogen (Meena et al. 2017). Pathogen survives as dormant mycelium and chlamydospores on seed surface or as systemic infection besides infected plant debris. Soil water holding capacity of 25% and soil temperature of 17–31 °C favour the disease development (Khare 1981). Fusarium wilt occurs both at seedling and reproductive stage.

Screening for fusarium wilt in sick plot using infector row technique was proposed by Bayaa et al. (1995) following a rating scale of 1–9. Limited wilt sick plots are available for screening against wilt in lentil. Efforts have been made to screen lentil varieties and germplasm for identification of donors. Kannaiyan and Nene (1975, 1976) reported that LWS 14 and LWS 21 were immune to all 5 strains of pathogen, and Pant 209, Pant 220, Pant 234, Pant 538, Pusa 3, Pusa 4, JL 80, and UPL 175 also exhibited substantial resistance. Screening of 2000 lentil lines in field and pots at Jabalpur led to identification of 11 resistant lines (LWS numbers 4, 6, 10, 14, 15, 20, 23, 24, 25, 26, and 50) exhibiting less than 5% wilt incidence (Khare et al. 1979). Saxena and Khare 1988 reported that lentil varieties with shorter roots or few secondary roots show low disease incidence. Kamboj et al. (1990) and Abbas (1995) reported that inheritance of the resistance to vascular wilt is controlled by the monogenic dominant gene, Fw. Eujayl et al. (1998) and Hamwieh et al. (2005) identified molecular marker linked to wilt resistance gene in lentil.

24.7.2.2 Collar Rot

Collar rot in lentil is caused by *Sclerotium rolfsii*. Infection caused by pathogen at collar region of plant results in yellowish-brown colouration and rotting of tissue. Damping off symptoms are seen in younger seedlings. Fungus survives in soil in the presence of organic matter. High soil moisture, high temperature, and sunshine after rain favour the disease development. Seedlings are more susceptible as compared to adult plants. Khare et al. (1979) screened lentil germplasm for resistance against *S. rolfsii* in the field and under inoculated condition and reported LWS numbers 2, 4, 7, 9, 11, 12, 22, 35, 37, 40, 47, 51, 52, 53, 55, 59, 60, 61, 63, 66, and 74 to be resistant. Resistance in Pusa 3, Pant 234, and JL80 was recorded by Kannainyan and Nene (1976) and by Mohammad and Kumar (1986) in Pusa 1, LP 18, and Pant 638.

24.7.2.3 Root Rot

Root rot in lentil is caused by *Rhizoctonia solani* Kuhn (Shukla et al. 1972) and *Rhizoctonia bataticola* (Taub) Butler (Khare et al. 1979). The typical symptoms include change of foliage colour from dull green to reddish brown and then to yellow. Roots show infection in all tissues except xylem. Roots are ashy brown with minute sclerotia in the case of *R. bataticola*. *R. solani* isolated from field exhibited yellowish brown to hyaline mycelia and sclerotia of about 2 mm, and *R. bataticola* exhibited minute black sclerotia. This disease development is favoured by high temperature.

24.7.2.4 Stem Rot

Stem rot in lentil is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. The disease is favoured by high humidity during plant growth. The affected plant exhibits white cottony fungal growth, and sometimes light-brown to dark-brown sclerotia are seen.

24.7.2.5 Rust

Rust is an important disease in lentil caused by autoecious fungus *Uromyces fabae* (Pers) de Bary. Singh and Solanki (1980) reported six pathotypes of this fungus. Initially the development of pycnidia and aecia is seen on the lower surface of leaflets and pods. Later uredial pustules emerge on leaflets, stem, and pod. Pustules are nearly 1 mm in size with oval to circular shape. Telia are produced on stem and branches. Lentil rust occurs in the form of aecia and pycnidia. At a low temperature of 17–22 °C, aeciospores germinate forming secondary aecia or uredia. A Temperature range of 20–22 °C, high humidity, and cloudy weather favour disease development. From urediniospores, telia develop. Teliospores survive in soil in the form of broken leaves and stem and are the source of basic inoculum. Teliospores germinate at 17–22 °C. Nene et al. (1975) reported rust resistance in improved varieties (L 9–12, T 36, and Bombay 18), germplasm lines (LP 846, UPL 172, UPL 175, and BC 10) and both wilt and rust resistance in JL 599, JL 632, JL 674, and JL 1005. Agrawal et al. (1976) identified JL 599, 632, 674, and 1005 as resistant sources. Pandey (1981) identified Pant L 236 as resistant and Pant L 406 as susceptible. Khare and Agrawal (1978) reported T 36, BR 25, Pusa 10, and UPL 175 and 22 other germplasm lines free from rust. Sinha and Yadav (1989) and Singh and Singh (1990) reported single dominant gene for rust resistance in lentil.

24.7.2.6 Powdery Mildew

Powdery mildew caused by *Erysiphe polygoni* DC was first reported from Rajasthan by Sankla et al. (1967). Plants can be attacked by powdery mildew at any stage but symptoms are often seen at flowering stage. Initially small white powdery patches appear on lower leaves and later they become amphigenous. Mishra (1973) reported resistance to powdery mildew in five lentil lines 10,511, 10,526, 10,528, 10,536, and 10,537.

24.7.2.7 Ascochyta Blight

Ascochyta blight in lentil is caused by *Ascochyta lentis* (Kulshrestha and Vallabhacharyulu 1985). *Ascochyta* sp. has been detected in lentil seeds imported from the USA and Syria (Lambet et al. 1985).

24.7.2.8 Stemphylium Blight

Stemphylium blight in lentil is caused by *Stemphylium botryosum* Waltr. Initially this disease emerged in West Bengal, and now it is spreading in Indo-Gangetic plains and even up to Himachal Pradesh. In South Asia, a temperature of 18–22 °C and relative humidity of over 85% favour disease development (Erskine and Sarker 1997). Das et al. (2017) screened lentil germplasm at two locations and reported immunity in P 3235, LL 1122, and ILL 10832.

24.7.2.9 Nematodes

Root knot (*Meloidogyne* sp.) nematode is an economically important pest of lentil. Symptoms include yellowish plant with stunted growth and irregular swelling of roots. The root knot nematodes of lentil were classified as *Meloidogyne incognita* (Mishra and Gaur 1980) and *Meloidogyne javanica (*Prakash 1981).

24.7.3 Insects Pests

24.7.3.1 Black Aphids

Aphis craccivora Koch is a threatening pest worldwide. Aphids deplete assimilates and increases respiration rate of plant (van Emden 1973). Lentils are prone to aphid damage at seedling, flowering, and podding stage. Severe insect infestation causes stunting and deformation of leaves and shoot and black mould grows on plant on honey dew deposits of insect. Dry weather favours multiplication and spread of aphids.

24.7.3.2 Pea Pod Borer

Etiella zinckenella (Treit.) is an important pest damaging 5–15% of pods (DPR 1988). Young larvae feed on flowers and young pods. Older pods with brown spot can be identified as infested pod. Seeds are consumed by borer and fross and silk can be seen in damaged pods.

24.7.3.3 Bruchids

Three bruchid species – *Callosobruchus chinensis, C. analis, and Bruchidius minutus F.* Lentil seed inpods are attacked by the insects in the field. Eggs are glued to seeds/pods or lie loose. The preharvest oviposition on pod is an important source of infestation in storage. Bruchid-infested lentil seeds are not suitable for consumption and lose germinability.

24.7.4 Abiotic Stress

24.7.4.1 Heat Tolerance

Lentil is a sensitive crop to the rising temperature (Choudhury et al. 2012; Bhandari et al. 2016; Sehgal et al. 2017), and its growth and development is hindered due to high temperature. At various stages, temperature requirement varies, and it ranges from 18 to 30 °C, and cooler temperature is required at vegetative stage, while warmer temperatures are needed at the time of maturity (Choudhury et al. 2012). Under the changing climatic scenario, the length of heat period has been increased in comparison to chilling period. Some of the responses of plants exposed to heat stress are sterility of pollen grain and flower drop crops, which are exposed to increasing temperature stress specifically at reproductive stage (Hasanuzzaman et al. 2013).

The maximum temperature and above 32/20 °C (the ratio represents max/min temperature) at flowering and pod filling stage in lentil can sharply deteriorate the quality of the grain and also reduce the grain yield (Delahunty et al. 2015). According to the reports, heat waves (35 °C) consecutively for 6 days have a repercussion on lentil yield, which results in 70% reduction across southeastern Australia (Delahunty et al. 2015). Heat stress during germination decreases the germination rate in lentil (Covell et al. 1986). The consequence of heat stress is reduction in percentage of germination, abnormal growth of seedlings, degeneration of nodules, loss in cell membrane stability, reduction in plant biomass, early flowering, increase in lipid peroxidation, and reduction in photosynthetic efficiency (Ellis and Barrett 1994; Muehlbauer et al. 2006; Chakraborty and Pradhan 2010; Sehgal et al. 2017).

Studies in lentil report that genotypes tolerant to heat stress exhibit superiority in functionality of pollen and enhanced level of antioxidants (Sita et al. 2017a, b) and reduced reproductive period, reduced seed set, pod abortion, and forced maturity (Gaur et al. 2014; Bhandari et al. 2016). The genetic variability for heat tolerance has been reported in few studies; however limited studies have been performed for screening of lentil genotypes for heat tolerance (Gupta et al. 2019).

Screening System for Heat Stress

Screening of lentil genotypes for heat stress can be done by growing them in late sown conditions so that the temperature at reproductive stage is above 35 °C (Delahunty et al. 2015; Kumar et al. 2016a, b; Sita et al. 2017a, b; Singh et al. 2019b). However screening under field condition is extremely difficult, and hence precise phenotyping techniques (phenomics) can be employed for evaluating heat-tolerant traits in the field (Basu et al. 2015). Lentil genotypes have been evaluated for traits such as rate of photosynthesis, pollen viability, and membrane stability with high precision laboratory techniques (Kumar et al. 2018a, b). Heat-tolerant genotype has been also identified by evaluating the genotypes in laboratory and hydroponics conditions under high-temperature conditions (Roy et al. 2011).

However, the best practice for identifying heat-tolerant genotypes is to combine the screening under field and controlled conditions as merely screening under controlled condition would not reproduce similar results in field condition. Some

other researchers followed different method wherein plants were grown in pots in field conditions and later on at flower initiation stage transferred into controlled conditions to expose it to suitable temperature during anthesis (Chen et al. 2019; Sita et al. 2017a, b).

Donors

The donors for the heat tolerance have been discovered in a wide range of studies IG 3263, IG 3745, IG 2507, IG4258, and FLIP 2009 (Kumar et al. 2016a, b; Sita et al. 2017a, b). Rajendran et al. (2020) screened the FIGS set of lentil germplasm for tolerance to terminal heat and combined heat stress tolerance at two locations, namely, Marchouch and Tessaout, in Morocco. Accession ILL 7835 was identified as a good source of stable tolerance to heat stress and combined heat-drought stress at both locations (Rajendran et al. 2020). However, there is a need to use these donors in a breeding program, which is otherwise still limited so far. Simple monogenic inheritance has been reported for pod setting and seedling survival in heat stress environment in lentil. However more research is required to confirm the inheritance of heat tolerance in lentil.

24.7.4.2 Cold Tolerance

Lentils exhibit sensitivity to frost as all the legumes (Murray et al. 1988). Exposure to frost stress in the early stages of plant growth leads to instant recovery from underground axillary bud, but if plant gets exposed to frost at maturity stage, then it results in ceasing of initiation of axillary buds and further restricts the plant to go further to reproductive stage and consequently to the death of the plant. In lentil, frost stress hinders the flower initiation and pod development, injures the vegetative tissue, damages seed coat and leaf (Gupta et al. 2019), and hence impedes the overall development of seed. Maximum injury due to frost occurs at flowering stage due to exposure of flowers to frost. Plant exposed to frost continuously becomes susceptible to disease such as anthracnose botrytis grey mould and pest infestation (Gupta et al. 2019). Frost tolerance in lentil has been characterized in lentil (Erskine et al. 1981; Summerfield et al. 1985; Murray et al. 1988; Spaeth and Muehlbauer 1991; Kusmenoglu and Aydin 1995; Ali and Johnson 1999). In some of the recent studies, frost injury and winter hardiness have been studied in lentil (Kahraman et al. 2004; Barrios et al. 2007, 2010, 2016).

Donor

The characterization of lentil genotypes for cold tolerance identified genotypes WA8649041 (Barrios et al. 2017) and WA8649090, ILL-1878, and ILL-669 (Kahraman et al. 2004). Hamdi and Erskine (1996) evaluated lentil accessions for winter hardiness and reported that *L. culinaris* ssp. *orientalis* LC9978057, LC9977006, Kafcas, Cifei, ILL1878, ILL4400, ILL7155, LC9977116, LC9978013, ILL759, ILL8146, ILL8611, ILL9832, and Ubek as frost/cold tolerant. Some of the tolerant genotypes LL1878, ILL662, ILL857, ILL975, ILL1878 (Sarker et al. 2002), and ILL5865, Balochistan local (Ali and Johnson 1999), have been reported by the researchers.

Genetics

The inheritance of radiation frost tolerance has been suggested to be monogenic in lentil (Eujayl et al. 1999). In another study, inheritance for winter hardiness has been reported to be controlled by quantitative traits (Kahraman et al. 2004).

24.7.4.3 Drought Tolerance

Lentil can be grown in dry regions and among all the legumes it exhibits moderate tolerance (Abraham 2015). It requires minimal water for its growth and development, but the productivity gets impeded by variable annual rainfall which magnifies the incidence of prolonged drought periods (Dai 2011). Drought stress also affects the metabolism, osmoregulation, and concentration of photosynthetic pigments, in lentil (Gökçay 2012; Muscolo et al. 2014; Mishra et al. 2016; Biju et al. 2017). The impact of drought stress varies at different stages of crop development (Shrestha et al. 2006; Allahmoradi et al. 2013). Drought stress occurring at flowering or podding stage affects vegetative and reproductive growth leading to reduced leaf area (48–55%), flower production (22–55%), number of pods and seeds (27–66%), and total dry matter (32–50%) with significantly higher abortion of pods and flower drop and aborted pods (Shrestha et al. 2006).

Drought avoidance and tolerance are the two mechanisms involved in lentil for combating drought stress. Traits related to root are the important components of drought avoidance mechanism (Idrissi et al. 2016; Biju et al. 2017), and selection for root-related traits provides drought tolerance to the crop and further improves the production and productivity of the crop (Gahoonia et al. 2005; Chen et al. 2015). Drought tolerance in wild lentil species has been evaluated by analysing root-related traits to understand the underlying mechanism of drought (Gorim and Vandenberg 2017a). In lentil, early-maturing varieties (Precoz, Bakaria, BARI M4, BARIM5, BARI M6, and Idlib 3) show adaptation to drought stress and reflect drought avoidance (Erskine et al. 1994; Shrestha et al. 2005).

Above ground level, some of the traits such as early or delayed flowering and pubescence play a key role in shielding plants exposed to water-deficient conditions. Singh et al. (2014b) exhibited the association of agro-morphological traits with drought tolerance. To breed for traits for drought tolerance, two strategies can be applied: short-term strategy for assessing the genetic variability for drought tolerance in lentil germplasm and long-term strategy to introgress desirable traits from wild species to the cultivated one.

Screening System for Drought Stress

Osmotic adjustment (increase in solute concentration in cell) for maintaining turgor at low water potential (Kumar and Elston 1992) has been reported as an adaptation to drought. Osmotic adjustment has been reported to enhance in yield of field crop by maintaining the turgor pressure (Munns 2002; Morgan and Condon 1986; Passioura 1986). Estimation of drought tolerance can be also carried out by evaluating traits like relative water content (Sinclair and Ludlow 1985; Schonfeld et al. 1988), stomatal conductance (Terzi et al. 2010), seedling vigour, and water use efficiency (WUE) (Nagarajan and Rane 2000; Dhanda et al. 2004). Screening methods based

on biochemical, morphological, and physiological traits have been reported by Hura et al. (2009) under soil condition. In lentil, rapid screening method was reported by Singh et al. (2013b). Screening for dehydration avoidance can be done by characterization of root and shoot attributes at seedling development and plant growth stages (Kumar et al. 2012).

Morphological and physiological parameters related to root (depth, length, and density), shoot (rapid ground cover, early growth vigour), and leaf characteristics are related to drought tolerance and assist in maintaining the transpiration demand of plant (Passioura 1981). Root attribute traits, for instance, highly branched roots, strengthen the capacity of absorption of nutrient and water from the soil (Gahoonia et al. 2005, 2006). However, limited studies were performed to characterize legume root system, and in lentil, few researchers reported their work on root and shoot attributes (Sarker et al. 2005; Gahoonia et al. 2005).

Donors

Lentil genotypes IPL 98/193, DPL 53, and JL 1 have been identified with excellent root parameters which give these genotypes the ability to sustain in drought environment (Kumar et al. 2012). Some genotypes with greater drought escape mechanism (ILL7618, ILL9921, ILL7981, ILL9830, ILL9922, ILL9844, ILL9850, ILL9920, ILL6024, ILL7504, ILL8095, ILL8138, ILL8621, and ILL9923) have been reported at ICARDA (Malhotra et al. 2004). Wild species have adapted to a broad range of environments and developed rich genetic diversities for drought tolerances. Hamdi and Erskine (1996) exhibited that wild *Lens* species have greater tolerance to drought. The primary strategies identified for drought stress are escape, avoidance, and tolerance, and screening of cultivated (*Lens culinaris* Eston) and wild lentils (*Lens odemensis* IG 72623, both L. erv. Genotypes (IG 72815 and L-01-827A), *Lens lamottei* IG 110813, *L. ori.* PI 572376, *L. ori.* PI 572376, *L. ori.* IG 72643, and *L. erv*. L-01-827A) identified accessions tolerant to drought stress (Gorim and Vandenberg 2017b; Fang and Xiong 2015). In a recent study, Rajendran et al. (2020) identified accession ILL 7835 as a good source of stable tolerance to combined heat-drought stress at different environments in Morocco.

Genetics

Simple inheritance of drought tolerance (Morgan 1991; Monneveux and Belhassen 1996; Tomar and Kumar 2004) has been reported by several researchers, and few studies also demonstrated complex inheritance (Ekanayake et al. 1985) for the trait.

24.7.4.4 Flooding and Submergence Tolerance

Submergence and flooding drastically affect the yield of legume crops (Solaiman et al. 2007; Kang et al. 2017). Lentil cannot withstand flooding or waterlogging; the production is hindered specifically in poor drainage and fine-textured soil or in conditions of persistent extreme rainfall (Wiraguna et al. 2017). The amount of injury occurred is dependent on its severity and growth stages and duration of the stress, and it further causes absolute loss of crop in the severely affected conditions (Toker et al. 2011). Waterlogging will affect all the development stages in lentil and

subsequently reduce the yield (Materne and Siddique 2009). At germination stage, it delays the seed germination, and hence leads to suppression in the root growth and development (Jayasundara et al. 1997), and transient waterlogging is a major impediment for production of lentil, specifically in early vegetative growth stages (Materne and Siddique 2009). At flowering stage, waterlogging causes abortion of pods and flowers and restricts the pod filling, which is referred to as the most sensitive stage in waterlogging. The plant exhibits variable symptoms such as leaf senescence and stunted growth, and the plant withers, ultimately causing death of the plant.

Screening for Flooding and Submergence Tolerance

Waterlogging-tolerant genotypes were identified by its high root porosity, low biomass, higher stomatal conductivity, early flowering, and maturity (Ashraf and Chishti 1993; Malik et al. 2015; Erskine et al. 2016). The adverse effect of waterlogging in lentil can be managed by applying few management practices such as drainage, paddock selection, seeding rate, and time of sowing (Toker and Mutlu 2011). Selections for adventitious root growth and more aerenchyma have been suggested as possible solutions for increased tolerance to flooding (Materne and Siddique 2009; Jayasundara et al. 1997). Screening of lentil germplasm against waterlogging tolerance has been studied by Wiraguna et al. (2017), and results exhibited that genotypes from Bangladesh are more adapted to waterlogged soil at germination. In rice and *Arabidopsis*, flooding stress and its subsequent derivatives like waterlogging, hypoxia, submergence, and anoxia were studied exhaustively, but in lentil, it is still required to do more research in order to understand the underlying molecular mechanism related to flooding or submergence tolerance.

Donor

Genotypes identified for the waterlogging tolerance in several studies are ILL6439, ILL6778, and ILL6793 (Ashraf and Chishti 1993), Nugget (Malik et al. 2015), and BINAmasur1 (Islam et al. 2009) and can be utilized in future breeding program for development of tolerant genotypes.

24.7.4.5 Salinity Tolerance

Lentil roots are extremely sensitive to the salinity stress, which results in arresting the root growth and restricting the rhizobium infection (Rai and Singh 1999; Van Hoorn et al. 2001). The effect of salinity on different stages of plant growth and development varies according to the growth stages (Munns and Tester 2008). Germination stage is less sensitive than the early stages of vegetative growth, and the most critical stage sensitive to salinity is reproductive stage (Vadez et al. 2007; Sakina et al. 2016). The response towards stress also varies with the level of salinity, available nutrients, relative humidity, soil-water status, and temperature (Lachaâl et al. 2002). Salinity stress involves changes in plant's metabolic, biochemical, and the physiological mechanisms such as photosynthesis (AL-Quraan et al. 2014), γ-Aminobutyric acid (GABA) accumulation (Al-Quraan and Al-Omari 2017), oxidative and membrane damage, ion homeostasis, osmolyte accumulation, and proline

metabolism (Hossain et al. 2017) depend upon the intensity of the stress and eventually inhibit crop growth. In lentil, salinity stress results in reduction in flowering and pod setting and anthocyanin pigmentation in stems and leaves (Van Hoorn et al. 2001); reduces total biomass, plant height, and biochemical and enzymatic activity; and further reduces grain yield by 90 to 100% (3 dS/m) and 20% (2 dS/m) at variable electrical conductivity (Tewari and Singh 1991; Van Hoorn et al. 2001; Ghassemi-Golezani and Mahmoodi-Yengabad 2012). Strategies need to be evolved to overcome the yield loss due to salinity with the emphasis on management of soil and water in the salinity-affected regions, but amelioration processes are very expensive, and more cost-effective methods need to be identified. Therefore, developing an effective breeding program for the development of salinity stress varieties is the most effective and sustainable strategy, for stabilizing and improving yield in salinity-affected areas.

Screening System for Salinity Tolerance

In general, field and hydroponic screening methods are in practice for salinity stress. But due to lack of homogeneity in the environment and soil conditions, it is difficult to perform screening in field conditions, and it can be resolved by screening in hydroponic system. Traits such as reduction in seedling growth; germination; visual salt injury; biomass accumulation; seedling survival; proline and antioxidant activities; Na^+, Cl^-, and K^+ contents, and hydrogen peroxide (H_2O_2) production have been studied by various researchers for the evaluation of salinity tolerance in lentil and other crops (Singh et al. 2019b). Recently, an image-based screening method has been developed by Dissanayake et al. (2020) to screen lentil genotypes for salinity tolerance.

Donor

The genotypes reported for salinity tolerance are PDL-1 and PSL9 (Singh et al. 2019b) SAPNA, RLG-258 and RLG-234 (Kumawat et al. 2017), PDL1, PSL9, ILWL95, Jordan 1 (Al-Quraan and Al-Omari (2017), Flash (CIPAL0411), Bounty (CIPAL0415), Nipper (CIPAL0203) (GRDC, 2013), Masoor2002, NL20–3-3, LN0188, M93, NL9775 (Aslam et al. 2017), Siliana, Local oueslatia, Nefza (Ouji et al. 2015), Nipper, PBA Flash, ILL2024 (Siddique et al. 2013), Çagıl, AltınToprak (Kokten et al. 2010), Ustica, Pantelleria (Sidari et al. 2007), DL443, Pant L406, ILL3534 LG 128, ILL6796 (Materne and Reddy 2007). Crop wild relatives ILWL 368, ILWL 297, ILWL 371, IG 136670, and ILWL 417 of *L. culinaris* ssp. *orientalis* (DAC-ICAR-ICARDA, Annual Progress Report, 2014) were identified as the salt-tolerant genotypes in various studies for introducing the novel allelic diversity in breeding programmes.

Genetics

In lentil also, monogenic inheritance was reported by Singh et al. (2019b). Inheritance of traits associated with salinity tolerance has been studied in chickpea, soybean, and pigeonpea. These traits showed monogenic inheritance pattern and mapped major QTLs associated with the salt tolerance (Abel 1969; Shao 1994; Lee

et al. 2007; Hamwieh and Xu 2008; Hamwieh et al. 2011; Liu et al. 2016; Guan et al. 2014).

24.8 Nutritional Quality of Lentil

24.8.1 Protein and Amino Acid Content in Lentil

Lentil grains are excellent sources of high-quality protein. The average protein concentration in lentil grain is around 26% crude protein. Lentil protein is comprised of 70% globulins, 16% albumins, 11% glutelins, and 3% prolamins (Boye et al. 2010). There is extensive variation in the protein content in lentil seeds as suggested by many studies. One of the primary studies for the protein content in lentil varieties was performed by Barulina (1930) which exhibited a variation from 27.5% to 31.7% in lentil varieties (Barulina 1930). The lentil global germplasm was evaluated for protein content by Hawtin et al. (1978), and it showed broader variation in the protein content which varies from 23.4% to 36.4% among the 1688 accessions of lentil.

The variation is also reported by Hamdi et al. (1991) and Kumar et al. (2016a, b). Cultivated lentils are rich in amino acids like arginine, aspartic and glutamic acids, and leucine, and they are low in some of the essential amino acids such as tryptophan, threonine, phenylalanine, histidine, methionine, isoleucine, valine, and leucine except lysine, while they have inadequate level of sulphur-containing amino acids like methionine and cysteine (WHO 2007). As compared to the animal-based protein, lentil proteins are low in methionine (0.9%) (Van Vliet et al. 2015). Anabolic properties of diet based on plants can be balanced by combining cereals and legumes, as cereals are higher in methionine and lower in lysine and legumes are lower in methionine and higher in lysine (Van Vliet et al. 2015). Some of the important non–amino acid proteins in lentil seeds are trigonelline (Rozan et al. 2001), erythro-γ-hydroxyarginine 2(S), 4(R)-4-hydroxyarginine γ-hydroxyarginine, γ-hydroxyornithine, and homoarginine (Sulser and Sager 1976). Grusak (2009) reviewed the nutritional quality of lentil and reported the protein content range of 15.9 to 31.4%.

24.8.2 Folates/Vitamin B9

The insufficient folate intake affects millions of people globally (De Benoist 2008). Inadequate intake of folic acid drastically affects pregnancy and raises the risks of preterm delivery, foetal growth retardation, low birth weight of newborn, and developmental neural tube defects (NTDs). Low intake of folate along with the increase in the level of homocysteine levels is closely linked with the occurrence of cardiovascular diseases, neurodegenerative disorders, and a range of cancers, while proper intake of folates and folic acid in diets reduces total homocysteine levels in plasma (Blancquaert et al. 2010). Staple crops such as rice, potato, maize, and

plantain are low in folate (USDA-ARS, 2012), while legume crops such as lentil (*Lens culinaris L.*) and common beans (*Phaseolus vulgaris L.*) are rich in folates (Singh 2018). Lentil is a rich source of folate, and according to a study, lentil has a folate concentration of 255 μg/100 g (on average) which makes it a whole food source of folates (Sen Gupta et al. 2013).

Moreover, it has been also revealed that lentil has higher folate concentration in comparison to green field pea, yellow field pea, and chickpea. Rychlik et al. (2007) also reported variability in the folate concentration of green lentils (110–154 μg/100 g). The recommended dose of folate for adult is 400 mg, while for pregnant women, it is much higher around 600 mg (Institute of Medicine, Food and Nutrition Board, 1998). Serving of 100 g lentil provides 54–73% of folate RDA (Thavarajah et al. 2008). Characterization of wild species and cultivated lentil quantifies eight folate monoglutamates, and higher folate concentration in wild lentil species (195–497 μg/100 g) has been reported than cultivated genotypes (174–361 μg/100 g) (Zhang et al. 2019). Several studies on lentil and other pulses reported 5-formyltetrahydrofolate (5-FTHF), 5-methyltetrahydrofolate (5-MTHF), and tetrahydrofolate (THF) as the most prominent folate (Jha et al. 2015; 2020; Fernandez-Orozco et al. 2013). Along with this, several studies have also revealed the predominance of 5-MTHF in lentils (Hefni et al. 2010; Rychlik et al. 2007).

24.8.3 Prebiotic Carbohydrates

Prebiotic carbohydrates are the form of complex carbohydrates with poor digestibility in upper portion of the gastrointestinal tract. They play a major role in stimulation of health-promoting bacteria. Lentil is rich in prebiotic carbohydrates (Bhatty 1988; Johnson et al. 2013) and has showed significant variation for prebiotic carbohydrates (Chung et al. 2009; Tahir et al. 2011; Johnson et al. 2013). Lentils are low in mono- and disaccharides and oligosaccharides, and starch is the main polysaccharide in lentil. Resistant starch value of 3.7 g/100 g dry matter of lentil was reported by De Almeida Costa et al. (2006).

Lentil starch is digested slowly releasing glucose. Lentil has low glycemic index among food crops (Jenkins et al. 2012). The average concentration of prebiotic carbohydrates in lentil has been reported around 7,500 mg of resistance starch (RS), 4071 mg of raffinose family oligosaccharides (RFOs), 62 mg of fructooligosaccharides (FOSs), and 1423 mg of sugar alcohols (SAs) per 100 g (Johnson et al. 2013). Siva et al. (2019) analysed the simple sugar content in lentil and revealed that the maximum concentration is of sucrose, followed by glucose, fructose, mannose, and rhamnose. The concentration of stachyose was the maximum followed by verbascose and raffinose in RFOs, while in SAs the highest concentration is of sorbitol followed by mannitol and xylitol. Considering lentil FOSs, kestose levels were higher than nystose levels. Other prebiotic carbohydrates present were arabinose, xylose, and cellulose. The concentration of lentil prebiotic carbohydrate also varies by the growth environment (Johnson et al. 2015).

24.8.4 Phenols

Dietary phenolics include polyphenols, phenolic acids, and flavonoids. Of the total dietary intake, phenolic acids account for about one-third, and the remaining two-thirds is accounted by flavonoids (Scalbert and Williamson 2000). The antioxidant activities of phenolic compounds and their potential use in processed food as natural antioxidants have attracted the attention of researchers (Gupta et al. 2021b). Twenty lentil cultivars have been evaluated and revealed that the major phenolic compounds in lentil are flavonoids, which includes catechin/epicatechin glucosides, kaempferol glycosides, and procyanidins (Zhang et al. 2015). The phenolic composition varies with differentiation in seed coat colours of lentils (Mirali et al. 2017; Gupta et al. 2018). The total polyphenol content in Canadian lentils ranges from 1.22 to 7.45 mg/g and has exhibited positive correlation with antioxidant activity (Padhi et al. 2017). The total phenolic content of lentil hulls was reported to be 3 to 8 times higher in comparison to the whole lentil seeds (Oomah et al. 2011), and hulls have more diverse phenolic composition than the cotyledon. In lentil seed coat, 43 phenolic compounds have been reported including three anthocyanidins, four proanthocyanidins, six flavones, one stilbene, one phenolic acid, two flavanones, seven flavanols, and 15 flavonols (Mirali et al. 2014, 2017). Lentil microgreens are also found as the rich sources of antioxidants including minerals (Priti et al. 2021).

24.8.5 Micronutrients

A number of studies have been conducted for the estimation of micronutrients in a wide set of cultivated lentil gemplasms (Gupta et al. 2021a, b; Kumar et al. 2019). However, significantly low efforts have been done to discover the wild relatives with high concentration of micronutrients and vitamins. The study conducted by Sen Gupta et al. (2016) reported wide variability of micronutrient concentrations in *L. lamottei* (Fe = 64–80, Zn = 26–40, Ca = 311–434, Cu = 2–6, Mg = 754–839-mg kg^{-1}), *L. nigricans* (60–70, 33–39, 508–590, 3–4, 445–738 mg kg^{-1}), and *L. ervoides* (65, 37, 339, 6, 638 mg kg^{-1}). One of the recent studies reported 150 ppm Fe in a wild accession of *L. orientalis*, ILWL 118, and this accession is being broadly employed in pre-breeding programs (Sarker et al. 2016). Ninety-six wild accessions were analysed to determine its biofortification potential (Kumar et al. 2018a, b). Significant variation was observed in species for various mineral concentrations. *L. culinaris* ssp. *odemensis*, *L. culinaris* ssp. *orientalis,* and *L. ervoides* showed extensive variability for Fe, Zn, Mo, Cu, and Mn, *L. ervoides* and *L. culinaris* ssp. *orientalis* for Mg content, *L. nigricans* and *L. culinaris* ssp. *orientalis* for Ca content. This may be occurred due to diverse genetic makeup of these species or may be due to the sound expression of respective genes of particular species under given environmental conditions.

However, *L. culinaris* ssp. *culinaris* had the minimum variability for some of the minerals as it is a cultivated species. Significant variability for Zn concentration was reported in lentils (36.7–50.6 mg kg^{-1}) (Ray et al. 2014), and the effect of genotype

Fig. 24.2 L 4717: An extra-early and biofortified variety of lentil from India

and environment interaction has been also observed (Khazaei et al. 2017; Vandemark et al. 2018). Selenium concentration in lentil has been also evaluated in lentil grown in dark-brown and brown soil of Western Canada, and high concentration of Se (425–672 μg kg^{-1}) has been observed (Thavarajah et al. 2008). In another study, lentils grown in six major lentil-producing countries, Syria (22 μg kg^{-1}), Northwestern USA (26 μg kg^{-1}), Morocco (28 μg kg^{-1}), Turkey (47 μg kg^{-1}), Southern Australia (148 μg kg^{-1}), and Nepal (180 μg kg^{-1}) and had a significantly less concentration of selenium (Thavarajah et al. 2011). Recent study by Bansal et al. (2021) indicates that biofortified variety tolerates heavy metal (Cadmium) stress in soil. Recently two biofortified lentil varieties L 4717 and IPL 220 were released from Indian programme. L 4717 is extra-early lentil variety maturing in 100 days (Fig. 24.2).

24.9 Emerging Challenges at National and International Level

24.9.1 Climate Change

The average global temperature rose by 0.74 °C during the last century but is expected to increase by 2.6–4.8 °C by the end of the twenty-first century (Leisner 2020). Climate changes may affect the global food and nutritional security. The impact of climate change on productivity, biotic and abiotic stresses, and nutritional quality requires the attention of researchers.

24.9.2 Nutritional Quality Improvement

Lentils are low in saturated fat and sodium and are rich in folate, fibre, potassium, and polyphenols. Lentil is low in glycemic index due to slow-digesting resistant starch. Lentils are valuable sources of prebiotics. There is increasing interest in

legume protein for environmental sustainability and nutritional security. Lentil lowers BP, cholesterol, and blood sugar (Ganesan and Xu 2017; Hanson et al. 2014).

24.9.3 Mechanical Harvesting

Mechanical harvesting is important for reducing the cost of production of lentil. Scarcity of labourers at maturity sometimes causes shattering losses. For mechanical harvesting, tall, erect, non-lodging plants with pod set of 10 cm above ground are required. Such plant types ensure both good straw and seed yield. Erskine et al. (1991) compared hand harvesting with cutting by mower and cutting with angled blades. Both mechanical harvest methods resulted in harvest loss as compared to hand harvesting. Grain yield loss was about 8.6% and straw loss was 16.6%. Labour harvest cost compensated loss due to mechanical harvest. Sidahmed and Jaber (2004) developed a experimental harvester for harvesting lentil. Mechanical harvestor harvests lentil 5.9 cm above ground at 22% moisture with 20% straw loss and 2% seed loss as reported by Sidahmed and Jaber (2004). Jawad et al. (2019) evaluated 36 genotypes for mechanical harvesting. Two genotypes from ICARDA nurseries 09S 83,184–10 and 2009S 96,518–1 were found superior. These can be used to improve the existing cultivars. In the Mediterranean region and Canada, lentil varieties are harvested using combined methods. Efforts are being made to develop suitable varieties for South Asia. In the recent year, ICARDA has developed a breeding material suitable for mechanical harvest and is providing this material to national programmes.

24.9.4 Herbicide Tolerance

Lentil was recently introduced in Australia and Canada. Lack of registered and safe post-emergence weedicide is major bottleneck in controlling the weeds. Attempts were made to utilize metribuzin (aminotriazinone) as option for weed control. Metribuzin is a broad-spectrum weedicide used for grasses and broad leafed weeds. Mutant lines exhibiting herbicide tolerance to metribuzin were reported in Australia and Canada. Very low mutation rate of 9.4×10^{-8} was recorded. Development of lentil cultivars with metribuzin tolerance would ensure crop safety and reduce crop phytotoxicity providing option of controlling broad leafed weeds.

24.10 Breeding Approaches (Conventional and Non-conventional)

24.10.1 Conventional Breeding

The first lentil collection was made by N.I. Vavilov and his colleagues working at the All-Union Institute of Agricultural Sciences in Leningrad, USSR, in 1920–1930. Vavilov's wife, Elena Barulina, led the programme on collection and characterized the diversity of cultivated and *Lens* species. The collection made in Europe saved the genetic variability. N.I. Vavilov Research Institute of Plant Industry, the first gene bank, retains large *Lens* collections. At global level, lentil breeding received attention with the establishment of ICARDA at Aleppo, Syria, in 1977. Collection of cultivated and wild species and their ex situ conservation was the top priority. The Crop Trust supports this international collection in the form of in-perpetuity grant. ICARDA has established gene bank, above the Arctic Circle in the Svalbard Global Seed Vault. As the lentil demand increased, this crop entered new areas like Australia and Canada. Breeder in these countries used both wild and cultivated lentils to meet the challenges faced in new environment. In Canada, anthracnose resistance from *Lens ervoides* was introduced in cultivated varieties.

The lentil collections of ICARDA were supplied to different National Research Partners. These genetic resources are valuable sources of tolerance to drought, cold, and diseases. Large numbers of lentil varieties were released for North Africa, Middle East, and Central Asia. Bangladesh Agricultural Research Institute developed around ten short duration and high yielding varieties from ICARDA material. The varietal improvement in lentil was initiated in 1924. Single plant selections were made from local mixed lots. NP1, NP47 (IARI), BR25 (Bihar), L12 (Punjab), T8, T38 (Uttar Pradesh), and B77 (West Bengal) are the earliest developed lentil pure lines. With initiation of All India Coordinated Pulses Improvement Project in the 1960s, research work on lentil was intensified involving multidisciplinary team of researchers. The initial emphasis was on population improvement through pure line selection. The lentil varieties released in India till 1985 are presented in Table 24.4.

Later the focus shifted to pedigree, bulk, and single seed descent method. The lentil varieties developed from 1985 to 2020 are presented in Table 24.5. The lentil varieties released have poor seedling vigour and low pod set. High flower drop and lodging besides biotic and abiotic stresses are the major bottlenecks in enhancing productivity. Indian programme has released two biofortified varieties L 4717 and IPL 220. L 4717 matures in less than 100 days and is the first extra-early lentil variety of the country.

Table 24.4 Lentil varieties released in India up to 1985

S. no.	Variety	Pedigree/year of release	Area of adaptation
1	Type 36	Selection from Badaun local 1956	UP, MP
2	No. 26	Selection from local germplasm 1956	Bihar
3	No. 27	Selection from local germplasm 1956	Bihar
4	C 31	Selection from Sonadanger local 1956	West Bengal
5	BR 25	Selection from local germplasm 1956	Bihar, Northeastern Hill Region
6	L 9–12	Selection from local germplasm 1962	Punjab, UP, Jammu, Kashmir, Assam
7	Type 8	Selection from local germplasm 1967	UP
8	Pusa 4	Selection from indigenous germplasm 1973	Northwestern Plains, Teria Central zone
9	Pant L 406	Selection from P 495	Assam, UP, Bihar, Punjab, NEH region
10	Asha (B77)	Local selection from Jorhat	West Bengal, assam
11	Pant L 639	L 9–12 × T 8	UP, Bihar, MP, Punjab, Haryana, Delhi, Gujarat, Maharashtra
12	LL 56	L 9–12 × L 32–1	Punjab
13	Ranjan	Selection from local germplasm/1983	Bengal, Punjab
14	VL Masoor 1	Selection from Hill landrace/ 1983	UP
15	PL 77–2	Mutant of BR 25/1984	Eastern UP, Bihar, Bengal
16	LL 147	PL 284–67/1987	Punjab
Bold seeded varieties			
1	L 4076	PL 639 × PL 234	North West Plain Zone Central Zone
2	K 75	Selection from Bundelkhand local	North East Plain Zone Central Zone
3	Sehore 74–3	Selection from Sehore local	Central Zone
4	Vipasha	Unknown	Himachal Pradesh
5	JL 1	Pure line selection from MP local	Madhya Pradesh
6	Type 8	Pure line selection from Bihar local	UP

Table 24.5 List of varieties of recent lentil released by All India Coordinated Pulses Improvement Project (1985 to 2020)

S. no.	Name of variety	Centre responsible for developing	Pedigree	Year of release	Average yield (q/ha)	Days to maturity (days)	Reaction to major diseases	Area of adaptation	Any other relevant information
1.	K 75 (Malika)	CSAU, Kanpur	Local sel. From Bundelkhand region	1986	13–14	130–135	–	NEPZ, CZ	Foliage dark green; semi-spreading seeds grey mottled large (2.7 g/100 seed wt)
2.	LH 84–8 (Sapna)	CCS HAU, Hissar	L 9–12 × JLS 2	1991	15–16	130–135	Resistant to rust	NWPZ	Plant semi-spreading; seeds grey mottled bold (2.7 g/100 seed)
3.	Pant lentil 4	GBPUAandT, Pantnagar	VPL 175 × (PL 184 × P 288)	1993	16–17	135–140	Resistant to rust and wilt	NWPZ	Plant semi-spreading, dark-green foliage, small seed
4.	Lens 4076	IARI, New Delhi	PL 234 × PL 639	1993	14–15	135–140	Resistant to rust	NWPZ and CZ	Dark-green foliage, semi-spreading, large seed
5.	DPL 15 (Priya)	IIPR, Kanpur	PL 406 × L 4076	1995	15–16	135–140	Resistant to rust and tolerance to wilt	NWPZ	Large seeded
6.	PusaVaibhav (L 4147)	IARI, New Delhi	(L 3875 × P 4) × PKVL	1996	17–18	130–135	Resistant to rust	NWPZ	Small seed
7.	DPL 62 (Sheri)	IIPR, Kanpur	JLS 1 × LG 171	1997	17–18	130–135	Resistant to rust and tolerance to wilt	NWPZ	Large seed
8.	JL 3	JNKVV, Sehore	Land race Sel. From Sagar	1999	14–15	110–115	Resistant to wilt	CZ	Large seed

9.	IPL 81 (Noori)	IIPR, Kanpur	K 75 × PL 369	2000	12–13	110–115	Tolerant to rust and wilt	CZ	Large seed
10.	KLS 218	CSAU, Kanpur	KLS 133 × L 9362	2005	13–14	120–125	Resistant to rust	NEPZ	Small seed
11.	HUL 57	BHU, Varanasi	Mutant of HUL −11	2005	14–15	120–125	Resistant to rust	NEPZ	Small seed
12.	VL 507	VPKAS, Almora	Sel. From ILL-7978	2006	12–13	160–170	Resistant to wilt	NHZ	Large seed
13.	VL 126	Almora	LL 498 × LH 84–8	2006	13–14	160–170	Resistant to rust	NHZ	Small seed
14.	IPL 406	IIPR, Kanpur	DPL 35 × EC 157634/382	2007	17–18	125–130	Resistant to rust	NWPZ	Large seed
15.	WBL 77	Berhampore (W.B)	ILL 7723 × BLX 88176	2008	14–15	115–120	Resistant to rust	NEPZ	Small seed
16.	Pant L 6	GBPUA & T, Pantnagar	Pant L 4 × DPL 55	2009	16–18	125–145	Resistant to rust	Uttarakhand	Small seed
17.	Pant L 7	GBPUAandT, Pantnagar	L 4076 × DPL 15	2009	16–18	125–145	Resistant to rust	Uttarakhand	Large seed
18.	Pant L 8 (Pant L 063)	GBPUA & T, Pantnagar	DPL 59 × IPL 105	2010	15–16	130–135	Moderately resistant to rust and wilt	NWPZ	Small seed
19.	IPL 316	IIPR, Kanpur	Sehore 74–3 × DPL −58	2013	14–15	110–115	Tolerance to wilt and rust	Central Zone	Large seed
20.	RVL 11–6	RAK College, Sehore	JL 3 × DPL 62	2017	11–12	107–113	Tolerant to wilt.	Central Zone	Large seed
20.	L 4717 (Pusa Ageti Masoor)	IARI, New Delhi	ILL 7617 × 91,516	2016	12–13	96–106	Resistant to wilt and Ascochyta blight	Central Zone	Extra-early type
21.	RKL 14–20 (Kota Masoor 2)	AU, Kota	LL 1049 × RKL 11	2018	12–15	97–104	Tolerant to drought, high temperature	Central Zone	Large seed

(continued)

Table 24.5 (continued)

S. no.	Name of variety	Centre responsible for developing	Pedigree	Year of release	Average yield (q/ha)	Days to maturity (days)	Reaction to major diseases	Area of adaptation	Any other relevant information
22	L 4727	IARI, New Delhi	Sehore 74–3 × Precoz	2018	11–15	92–128	Moderately resistant to wilt	Central Zone	Suitable for timely planting under rainfed conditions, large seeded
23	IPL 220	IIPR, Kanpur	(DPL 44 × DPL 62) × DPL 58	2018	14–18	119–122	Resistant to rust and Fusarium wilt	NEPZ	Suitable for normal sown conditions, small seeded
24	Kota Masoor 1 (RKL 607–1)	AU, Kota	KLB 339 × SL 94–09	2018	10–14	98–107	Tolerant to drought and high temperature	Central Zone	Suitable for normal sown conditions
25	L- 4729	IARI, New Delhi	SKL 259 × L 4147	2020	17–18	96–110	Moderately resistant to wilt	Central Zone	Suitable for timely planting under rainfed conditions. Large seeded
26	Kota Masoor 3 (RKL 605–03)	AU, Kota	L 4682 × SL 73–3	2020	18–19	105–110	Moderately resistant to wilt and tolerant to drought and high temperature	Central Zone	Suitable for normal sown conditions. Large seeded
27	LL 1373	PAU, Ludhina	IPL406 × FLIP 2004-7L	2020	15–16	125–130	Moderately resistant to wilt and rust	NWPZ	Suitable for rabi season under irrigated conditions. Large seeded

28	VL Masoor-148	VPKAS, Almora	DPL-15 × L-4076	2020	11–12	145–160	Moderately resistant to wilt and rust	NHZ	Suitable under rainfed conditions. Small seeded
29	RKL 58 F 3715 (Kota Masoor 4)	AU, Kota	Mutant of DPL 62	2020	18–19	110–115	Resistant to rust and stemphylium blight, moderately resistant to wilt and less incidence of pod borer and aphids	CZ	Suitable for normal sowing conditions (tenth November)

24.11 Genomics-Assisted Breeding

Significant achievements have been made in lentil using conventional means. For further gains, the strengthening of conventional breeding programmes is required with new tools and techniques. Genomics has emerged as potential tool for enhancing genetic gains in lentil.

24.11.1 Molecular Marker Development

The earliest linkage maps in lentil (Eujayl et al. 1998; Havey and Muehlbauer 1989) were developed using RFLP/AFLP markers. RFLP markers require sound technical skill for their development. Later PCR-based markers like random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR), and simple sequence repeats (SSR) were developed. PCR-based markers were used extensively in lentil breeding programmes (Gupta et al. 2016; Singh et al. 2016a, b, 2019a; Ates et al. 2016, 2018; Tsanakas et al. 2018; Mbasani-Mansi et al. 2019; Polanco et al. 2019). Verma et al. (2014) developed genomic SSR markers.

Following the advent of next-generation sequencing technologies, single-nucleotide polymorphism (SNP) markers have been used in lentil to describe genetic variation of germplasm collections and link-specific markers to phenotypic traits, including seed quality, disease resistance, and micronutrient concentration (Lombardi et al. 2014; Sudheesh et al. 2016; Khazaei et al. 2016, 2017, 2018). The NGS technologies have licensed quick and cost-effective SNP mining for mapping genes and QTLs. Now the SNP markers are preferred by breeder's over the PCR-based markers. Different strategies are available for identifying and substantiating SNP markers.

Sanger sequencing technology is being utilized to resequence unigene-derived amplicons, and the gene-based SNPs were discovered expressed sequence tags (EST) which were converted to PCR-based markers. Sharpe et al. (2013) reported generation of 44,879 SNPs using Illumina Genome Analyzer, and Tamel et al. (2014) reported discovery of 50,960 SNPs for construction of linkage map in lentil. The use of NGS platforms for transcriptome analysis (Sharpe et al. 2013; Kaur et al. 2014; Singh et al. 2019a) has generated large number of SNPs from the coding region of lentil. These SNPs have been utilized to characterize the genetic diversity and for development of linkage maps.

24.11.2 Molecular Markers in Lentil Improvement

Molecular markers decipher the gene networks controlling the quantitative traits, and the molecular markers have been linked to several traits of economic importance in different crops. The enrichment of genomic resources, development of PCR molecular markers, and next-generation sequencing have increased the utilization of

molecular markers for crop improvement in lentil. Limited numbers of molecular markers linked to trait of economic importance have been identified in lentil (Table 24.6). The utilization of bi-parental populations with poor marker density has not identified very tight association of molecular markers with QTLs/genes in these studies.

24.12 Modernization of Crop Improvement Programme

24.12.1 Speed Breeding

Plant breeders improve productivity of crop by fast-tracking research. Conventional breeding techniques require long time for development of improved varieties. The reduction of generation time can accelerate the development of improved varieties. Speed breeding is an excellent tool for rapid generation advancement utilizing extended photoperiod (22 h). Sodium-vapour lamps (SVL) are used in glass houses and light-emitting diodes (LED) and metal halide lighting in growth chambers (Ghosh et al. 2018). Controlled temperature and 22-hr photoperiod reduced generation time in barley, chickpea, canola, pea, and wheat. Up to 6 generations/year have been obtained using speed breeding. Limited reports on the use of extended photoperiod are available in lentil (Idrissi and van Damme 2018; Idrissi et al. 2019; Mobini et al. 2016). Recently Idrissi et al. (2019) reported that efficient protocol of extended light period to speed breeding in lentil can reduce crop duration and also shorten the flowering time of wild types (for synchronization of flowering in cultivated and wild for hybridization). However, there is need to optimize light intensity, spectral composition (light-emitting diodes [LED], red, blue light) and red/far-red light ratio for this crop.

24.13 Future Thrust Area

Lentil is grown as rainfed crop on marginal soils. The yield potential realization depends on residual soil moisture of rainy season and rainfall during the crop season. Climate-resilient varieties are required to stabilize the production. Thrust is required for a number of parameters listed below:

24.13.1 Prebeeding

Lentil is grown in different agroclimatic conditions. Hybridization with Mediterranean lines will introduce earliness, seedling vigour, bold seed size, and larger secondary and tertiary branches. Using ICARDA germplasm, bold small and large-seeded varieties have been developed. Systematic prebreeding efforts being made at IARI, New Delhi; NBPGR, New Delhi; and IIPR, Kanpur, have led to identification of new sources of resistance for biotic and abiotic stresses. Efforts are

Table 24.6 Molecular markers linked to desirable genes/QTLs in lentil

Trait	Molecular markers	Mapping population	Phenotypic variance	Reference
Wilt resistance	SSR 59-2B P17m30710	ILL 5588(R) × L 692–16-1(S) RIL	8 3.5	Hamwieh et al. (2005)
	RAPD marker OPK-15_{900} OP-BH_{800} and OP-$DI5_{500}$ found to be associated in the coupling phase with the resistance trait	ILL 5588(R) × L 692–16-1(S) $F_{2:4}$ progenies and $F_{6:8}$, $F_{6:9}$ recombinant inbred line	– -	Eujayl et al. (2006)
Stemphylium blight resistance	QTL QLH4 $_{80–81}$ SSRs ME5XR10 and ME4XR16C RAPD marker UBC 34	ILL 6002(R) × ILL 5888(S) F7 RIL	46	Saha et al. (2010)
Rust resistance	SSR Gllc 527	PL 8 (S) × L 4149 (R) $F_{2:3}$	–	Dikshit et al. (2016)
Ascochyta blight resistance	Seven RAPD markers linked to the AbR1 gene	ILL 5588 (R) × ILL 6002(S) $F_{2:3}$	89	Ford et al. (1999)
	Three QTLs (using RAPD, ISSR, and AFLP analysis)	ILL7537 (S) / ILL6002(R) F2	50	Rubeena et al. (2006)
	QTL on LG 6 AFLP and random amplified polymorphic DNA (RAPD) markers localized around the resistance region	Eston(S) × PI 320937(R) RILs	41	Tullu et al. (2006)
	Three QTLs	Northfield (ILL5588) × Digger (ILL5722) F5 RILs	34–61	Gupta et al. (2012)
	Two QTLS Three QTLs	Indianhead × Digger [IH × DIG] Indianhead × Northfield [IH × NF]	52 69	Sudheesh et al. (2016)
Anthracnose resistance	Five QTLs with a significant association with resistance to C. lentis race 0	L01-827A and IG 72815 (*L. ervoidis* accessions) RIL	8.9–24.8	Bhadauria et al. (2017)
	A major-effect QTL (qAnt1.Lc3) conferring resistance to race 1 was mapped to lentil chromosome 3	200 genotypes AM Panel	66.6–69.8%	Tadesse et al. (2021)

(continued)

Table 24.6 (continued)

Trait	Molecular markers	Mapping population	Phenotypic variance	Reference
Seed size	One QTL	Precoz × L 830 F_8 RILs	27.5	Verma et al. (2015)
Seed weight	One QTL	Precoz × L 830 F_8 RILs	48.4	Verma et al. (2015)
	Three QTLs for seed weight in linkage groups 1, 4, and 5	*Lens culinaris* × *Lens orientalis* F_6 RILs	--	Tahir and Muehlbauer (1995)
	Three QTLs located in linkage groups I, III, and VI	*Lens culinaris* ssp. *culinaris* Medik. cv. "Lupa" × *L. c.* ssp. *orientalis* Boiss (BG 16880) F_2	18.2	Fratini et al. (2007)
Seed diameter	Three QTLs located in linkage groups I, III, and V		37	
Seed thickness	Seed thickness QTL were detected on all LGs except LG 3. The QTL that was most stable throughout the different site-years was located on LG 7, explaining an average of 8.4% of the variation in three site-years	CDC Robin × 964a-46 RILs	8.4	Fedoruk et al. (2013)
Seed plumpness	Seed plumpness QTLs were present on LG 1, LG 2, and LG 4		50	
Days to flowering	Three QTL for days to flowering in linkage groups 1, 4, and 10	*Lens culinaris* ssp. *culinaris* Medik. cv. "Lupa" × *L. c.* ssp. *orientalis* Boiss (BG 16880) F_2	90.4	Fratini et al. (2007)
	Three significant QTLs were detected with two on linkage group 4 and one on linkage group 13.	ILL 5888 × ILL 6002	15.6–24.2	Saha et al. (2013)
	Single QTL was identified for the character flowering time located on LG6	*Lens culinaris* cultivar Alpo × *L. odemensis* accession ILWL235 RILs	–	Polanco et al. (2019)
Seed cotyledon colour (Yc)	QTL for cotyledon colour locus Yc. LcC13114p356, located 1 cM away	CDC Robin × 964a-46 RILs	23	Fedoruk et al. (2013)

(continued)

Table 24.6 (continued)

Trait	Molecular markers	Mapping population	Phenotypic variance	Reference
Seed coat spotting pattern(Scp)	*Scp* locus was mapped onto LG6	CDC Robin × 964a-46 RILs	–	Fedoruk et al. (2013)
Plant height	One QTL	ILL 5888 × ILL 6002 RILs	15.3	Saha et al. (2013)
100 seed weight	Five QTLs		17.5	Saha et al. (2013)
Early maturity	Quantitative trait loci affecting earliness and plant height were identified on LG1, LG2, LG4, LG5, LG9, and LG12	Eston × PI320937 RILS	37–46	Tullu et al. (2008)
Plant height			31–40	
	Three QTLs for plant height in linkage groups 1, 3, and 5	*Lens culinaris* ssp. *culinaris* Medik. cv. "Lupa" × *L. c.* ssp. *orientalis* Boiss (BG 16880) F_2	38.2	Fratini et al. (2007)
Branches at first node	Four QTLs for branches at first node in linkage groups 3, 5, 5, and 8		91.7	
Pod dehiscence	Three QTLs for plant height in linkage groups 2, 3, and 5		81.3	
Height at first node	Two QTLs for height at first node in linkage group 1 and 5		33.3	
Total no. of branches	Two QTLs for total number of branches in linkage group 3 and 10		54	
Boron tolerance	Two SNP loci were found to be associated with B tolerance through marker regression, and a single genomic region was detected in the interval between these SNP loci on LG4.2	Cassab × ILL2024 RILs	–	Kaur et al. (2014)
Fe concentration	21 QTL regions explaining 5.9%–14.0% of the phenotypic variation were identified on six linkage groups (LG1, 2, 4, 5, 6, and 7)	ILL 8006 × CDC Milestone RILs	5.9–14.0	Aldemir et al. (2017)
	Three SSRs (PBALC 13, PBALC 206, and GLLC 563) associated with grain Fe concentration	Association mapping panel	9–11	Singh et al. (2017a, b)

(continued)

Table 24.6 (continued)

Trait	Molecular markers	Mapping population	Phenotypic variance	Reference
	Two SSR markers, GLLC 106 and GLLC 108	Association mapping panel	17 and 6	Kumar et al. (2019)
Zn concentration	Four SSRs (PBALC 353, SSR 317–1, PLC 62, and PBALC 217)	Association mapping panel	14–21	Singh et al. (2017a, b)
Winter hardiness	One major QTL on linkage group 6	WA8649090 × Precoz RIL	20.45	Kahraman et al. (2010)
Mn concentration	6 QTLs for Mn concentration identified using composite interval mapping (CIM).	CDC Redberry × ILL7502	15.3–24.1%	Ates et al. (2018)
Milling quality	Multiple QTLs for milling traits were detected in six of seven linkage groups (LGs). The most stable QTLs governing dehulling efficiency and milling recovery were clustered on LGs 1, 2, 3, and 7, whereas football recovery QTLs were clustered on LGs 4, 5, 6, and 7	CDC Robin × 946a-46 RILs	–	Subedi et al. (2018)
Seed coat spotting Seed size Ascochyta blight	The *Scp* locus was mapped to a 3.3 Mb region of LcChr6 comprising 46 annotated genes including a candidate gene (Lc25388) Three QTLs were detected for the seed size trait in LG1, LG5a, and LG5b, corresponding to one genomic location in LcChr1 and two in LcChr5 SNP flanking markers of AB_NF1 in the genes Lc28181 and Lc25002 located in the LcChr6	*Lens culinaris* cultivar Alpo × *L. odemensis* accession ILWL235 RILs	–	Polanco et al. (2019)

being made to map the genes utilizing the available genomic resources. Marker-assisted selection is required to develop climate-resilient varieties.

24.13.2 Early Maturity

Earliness is required to escape terminal moisture stress in Central India and in rice fallows of Eastern India. Among biotic stresses, wilt is important for Central India, whereas stemphylium blight and rust for Eastern India. Tolerance to high temperature at reproductive stage and terminal soil moisture stress are of extreme importance. Herbicide tolerance will reduce the cost of manual weeding. Efforts should be made to develop new lentil varieties for rice fallows.

24.13.3 Nutritional Dense Varieties

To address the micronutrient deficiency, micronutrient dense varieties are required. Fe-rich lentil varieties L 4717 and IPL 220 have been released for cultivation in Central and Eastern India, respectively. Efforts are required for reducing the phytic acid. Enhancement of protein concentration is desired.

24.13.4 Restructuring Plant Type

Plant types ensuring higher solar light interception are required to have partitioning of photosynthates. Mechanized harvesting is required to reduce the harvesting losses caused by shattering and cutting the cost of harvesting and threshing. Tall, erect, and non-lodging plants are required for mechanized harvesting.

24.13.5 Climate-Resilient Varieties

IARI, New Delhi, has developed protocols for screening for abiotic stresses. Donors for drought, heat, salinity, and salinity have been identified. QTLs governing tolerance to abiotic stress have been identified, and transcriptome analysis has deciphered the mechanism involved for tolerance. Utilizing the available information, varieties tolerant to abiotic stress can be developed utilizing genomic resources. Phosphorus usage efficiency in lentils is being studied to produce phosphorus absorption and utilization efficient varieties. The virulence of pathogens under climate change requires close monitoring.

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Field Pea Breeding

25

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Abstract

Field pea (*Pisum sativus* L.) is a nutritionally dense winter season pulse crop, consumed worldwide as food, feed and fodder and offers nutritional security to low-income folks of various developing countries. It is an excellent source of protein and carbohydrate in juxtaposition with vitamins, essential amino acids, and macro- and micronutrients. In addition, it plays an important role in management of Type 2 diabetes and body weight, blood cholesterol reduction, improves cardiovascular health and gastrointestinal function. It is susceptible to many biotic and abiotic stresses that seriously hinder its sustainable production. Over the years, sincere efforts have been made toward the genetic improvement of field pea to subsidize antinutritional components and elevate production potential. In this book chapter, the importance of the crop, its common uses, origin, evolution, gene pool, botanical description, floral biology, cytogenetics and molecular cytogenetics, genetic variability for important agronomic traits, inheritance of qualitative and quantitative traits, and brief account of genetic resources have been illustrated. The achievement made in field pea through conventional and nonconventional breeding approaches, that is, hybridization, distant hybridization, and mutation breeding, have been reviewed. The current scenario of genomics resources and marker-assisted breeding has also been deliberated.

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_25

Moreover, the breeding objectives, major constraints, and future perspectives in order to explore cutting-edge tools and technique for enriching field pea genomic resources have been outlined. Furthermore, the currently existing coordinated testing system for new entries and quality seed production has also been described in short. Overall, to accelerate genetic gain in field pea along with nutritional enrichment, there is urgent need of exploitation of recent advanced tools and techniques such as transcriptomics, proteomics, metabolomics, small RNAomics, epigenomics, interactomics, bioinformatics, genomic selection, genome editing, and speed breeding to bolster the field pea breeding program.

Keywords

Pisum sativum · Cytogenetics · Germplasm · Breeding strategies · Breeding objectives · Coordinated system of testing

25.1 Introduction

Field pea (*Pisum sativum* L.) is an imperative cool season grain legume cultivated around the globe as food, feed, and fodder (Rubiales et al. 2019; Parihar et al. 2020a, b). Field pea with different cotyledon color, that is, yellow, green, and orange, is an important ingredient of various culinary and confectionary stuffs such as *soup, chat, chhola, dal*, *stew, snacks,* vegetables, and flour, while the integral seeds are usually accepted as animal feed (Dahl et al. 2012; Singh et al. 2018; Mahajan et al. 2018; Parihar et al. 2021a, b). Pea hay is used as fodder for animals (Bastida Garcia et al. 2011). In terms of nutritional composition, it is an excellent source of protein (21.2–32.9%) and carbohydrate (56–74%) in juxtaposition with vitamins, essential amino acids, macro- and micronutrients and plays a decisive role in nutritional security of resource poor folks (Harmankaya et al. 2010, Shivay et al. 2016, Parihar et al. 2016). Furthermore, it is a good source of arginine, valine, and methionine (Tömösközi et al. 2001). Carbohydrates are the main constituent of pea dry matter, which exists in variable amounts. The high amylase content in seed results in the slow digestion of starch (Chung et al. 2009). In addition, the seed coat and cotyledon are excellent source of dietary fiber, that is, water-insoluble and water-soluble fiber, respectively (Guillon and Champ 2002; Tosh and Yada 2010).

In case of mineral content, field pea seed predominantly contains potassium and phosphorus, whereas it is comparatively low in the calcium. The microelements, especially iron, manganese, copper, cobalt, selenium, molybdenum, and zinc, are present in substantial amount in pea seeds (Reichert and MacKenzie 1982; Savage and Deo 1989; Sommer et al. 1994; Amarakoon et al. 2012). Additionally, field pea has considerable amount of folate, vitamins (C, B1, B2, B3 and B6) (Dang et al. 2000; Hedges and Lister 2006). Corresponding to other pulses, it has an array of phytochemicals: carotenoids, β-carotene, chlorophyll, and phenolic compounds (Campos-vega et al. 2010). The carotenoids cannot be synthesized in human and animal body; therefore, it is accessible solely from an external source (plant or an animal) an animal (Fraser and Bramley 2004). The prevalent carotenoids in peas are

xanthophylls, lutein, and zeaxanthin in addition to α and β-carotene (Hedges and Lister 2006). A green cotyledon field pea cultivar contains 10 times higher b-carotene concentration than yellow or orange cotyledons (Holasová et al. 2009). Likewise, the lutein and zeaxanthin concentration in peas is many folds higher as compared to other legumes (Hedges and Lister, 2006). The phenolic acids and flavonoids are available in good quantity in seed coat and cotyledon, especially pea seeds pigmented seed coat (Duenas et al. 2004; Campos-vega et al. 2010; Xu et al. 2007). Since, the pigmentation of a seed coat is imparted by the phenolic compounds and tannins, seeds with dark seed coat are reported to have higher antioxidant activities (Hagerman et al. 1998; Devi et al. 2019). In addition, a subgroup of the flavonoid category compounds, well-known as Isoflavones, also exists in sizeable amount in peas (Hedges and Lister 2006). Saponin is a diverse group of biologically active glycosides, which are distributed comprehensively in the plant kingdom (Curl et al. 1985). Several saponins have been isolated in peas of which Soyasoponin-I is predominant (Murakami et al. 2001). Being a cheaper and rich source of protein, vitamin, minerals, and prebiotic carbohydrate particularly for poorer consumers, it is often acknowledged as "poor man's meat" (Amarakoon et al. 2012). The nutritionally dense field pea is a valuable international food commodity, well equipped to cater to the daily nutritional requirements of malnourished resource poor folks worldwide (Food and Agriculture Organization 2011). Most interestingly, its consumption is reported to minimize the risk of occurrence of Type 2 diabetes (Marinangeli et al. 2009; Marinangeli and Jones 2011), reduces blood cholesterol (Ekvall et al. 2006), improves cardiovascular health (Singh et al. 2013), cancer-combating and antioxidant properties (Kalt 2001; Kleijn et al. 2001; Boker et al. 2002; Steer 2006), body weight management, and improves gastrointestinal function (Fernando et al. 2010; Tosh and Yada 2010; Lunde et al. 2011).

25.2 Common Uses

Field pea is used in varied ways, the tender green pods and seeds are used as vegetables (Duke 1981), while the mature dry seed in different forms (whole, split, flour) is used for human consumption or as animal feed (Elzebroek and Wind 2008; Bastida Garcia et al. 2011). It is an important ingredient of various culinary and confectionaries such as *soup, chat, chhola, dal*, *stew, snacks,* vegetables, and flour (Dahl et al. 2012; Singh et al. 2018; Mahajan et al. 2018; Parihar et al. 2020a, b). It is an ideal candidate crop to be used in crop rotation, as green manures, and cover crops, since it grows fast and contributes to soil nutrient enrichment owing to its inherent ability fixing atmospheric nitrogen (Ingels et al. 1994; Biederbeck et al. 2005; Chen et al. 2006; Clark 2007).

25.3 Area, Production, and Productivity: World Scenario

Field pea or dry pea is widely cultivated across the continents, and is cultivated in nearly 94 countries (Parihar et al. 2021a, b). The dry pea area has been oscillated between 6.58 to 8.09 mha and production between 10.44 and 16.21 mt during 2010 to 2019 (Fig. 25.1). The leading countries in terms of production are Canada, Russian Federation, China, India, and United States of America (Parihar et al. 2020a, b). The region-wise production scenario at global level demonstrated that the Americas with 39.33% share hold highest contribution in total production followed by Europe (36.98%) and Asia (18.09%). The global dry pea productivity is currently around 2.0 tonnes per hectare. The countries having highest productivity are Netherland (5005 kg/ha), Denmark (4463 kg/ha), Belgium (3824 kg/ha), Germany (3486 kg/ha), and Finland (3450 kg/ha). Contrarily, in India and other dry pea-growing countries like China, Australia, and Myanmar, productivity is quite low than aforesaid countries, which fluctuate between 1000 and 2000 kg/ha (FAOSTAT 2021).

25.4 Origin, Evolution, Distribution, and Gene Pools of Field Pea

Field pea is an important member of third largest flowering plant family (*Leguminosae*) and largest subfamily (*papilionoideae*), and tribe Fabeae. The *Leguminosae* family and *papilionoideae* subfamily comprised of 800 and 476 genera, respectively (Doyle et al. 1997; Lavin et al. 2005; Nemecek et al. 2008; Smýkal et al. 2013; Mahajan et al. 2018). The tribe *fabeae* comprises of five genera (*Lathyrus*, *Lens*, *Vicia*, *Pisum,* and *Vavilovia formosa*) (Smýkal et al. 2011;

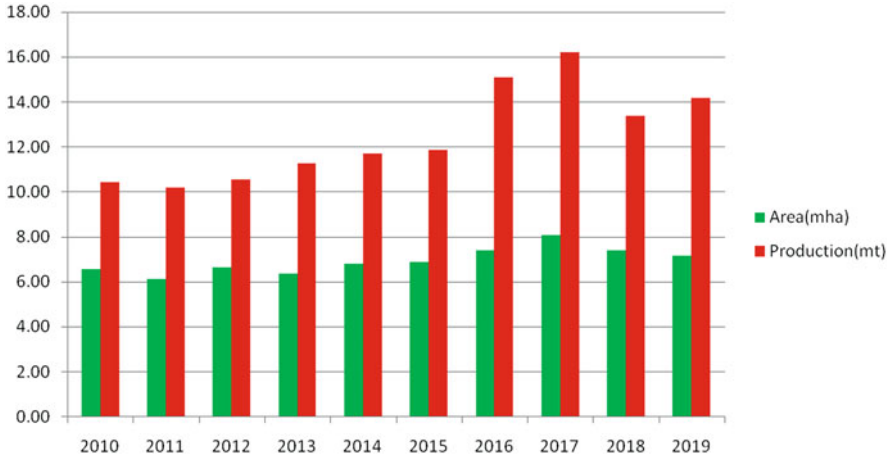

Fig. 25.1 Worldwide area and production scenario of dry pea during 2010–2019

Schaefer et al. 2012; Mikić et al. 2013; Warkentin et al. 2015). The genus *Pisum* L., is comprised of three species that is cultivated pea (*P. Sativum* subsp. *sativum*), Ethiopian pea (*P. abyssinicum*), and *P. fulvum.* The cultivated pea is further classified into five subspecies (*elatius*, *sativum, humile, arvense, and hortense*) (Maxted and Ambrose 2001; Warkentin et al. 2015; Trněný et al. 2018), of which, *arvense* and *hortense* subspecies are considered as varieties under species *sativum* (Govorov 1928; Nasiri et al. 2009). All the given species could successfully generate hybrids, though the fertility status may be constricted due to karyological and nuclear–cytoplasmic incongruity (Ben Ze'ev and Zohary 1973; Bogdanova et al. 2015). Based on morphological variation and wide geographical range, *P. sativum/elatius* complex and the more distant *P. fulvum*are are considered in secondary genepool, whereas the phylogenetically related *V. formosa,* a perennial mountain species, harbored in tertiary genepool (Ben Ze'ev and Zohary 1973; Warkentin et al. 2015). Taking into consideration the crossing potential, the genus *Pisum sativum* is further grouped into following subspecies, which are recognized as varieties, that is, *P. sativum* L. var. *hortense* (garden pea), *Pisum sativum* L.var. *arvense* (field pea), *Pisum sativum* L.var.*macrocarpon* (whole pod edible pea), *Pisum sativum* L.var. *syriacum* (wild form) (Gaskell 1997; Nasiri et al. 2009; Mohan et al. 2013).

The name *Pisum* is originated from the Greek word "*pisos*," which in Rome become *Pisum* and in English as *peason*, then *pease* or *peasse*, which later became the universal name (Peas) (Mikicˇ 2012). Pea had penetrated in China through India by the first century BC (Makasheva 1973). In the first century BC, pea was mentioned by the Romans Collumela, Pliny, and Virgil (Smýkal et al. 2013). The primary center of origin for pea is Near East and Mediterranean region where two wild species, that is, *P. fulvum* and *P. sativum* subsp. *Elatius*, are still being grown. However, the distribution of *P. fulvum* is constrained to the Middle East region (Ladizinsky and Abbo 2015; Smýkal et al. 2015), whereas the wild pea (*P. sativum* subsp. *elatius*) is distributed across the Mediterranean region with greatest diversity in the Near East region, which is considered as the center of diversity (Smýkal et al. 2017). The upland Asiatic region of the Hindu Kush, Ethiopia, and Yemen are considered as secondary center of diversity (Rubiales et al. 2019). Vavilov (1949) has considered Ethiopia together with the Mediterranean countries and Central Asia as primary centers, with Near East secondary. Moreover, pea has been cultivated since the Fertile Crescent to today's Russia, north and West Europe, Greece, Rome, Persia, India, and China (Makasheva 1979; Chimwamurombe and Khulbe 2011). The archeological evidence supported pea as one of the world oldest domesticated grain legumes (Smýkal et al. 2018). The *P. humile*, which has been recently incorporated as additional taxa, currently subsist only at secondary habitation (Abbo et al. 2013). The literature witnessed that ssp. *elatius* and ssp. *humile* are the progenitors of pea ssp. *Sativum* (Zohary and Hopf 1973; McPhee 2003). Other species, for instance, *P. jomardi, P. transcaucasicum,* and *P. arvense*, have also been incorporated within *P. sativum* (Jing et al. 2007; Zaytseva et al. 2012).

25.5 Botanical Description

Pea is an annual herbaceous plant with hollow, succulent, slender, trailing, or climbing stems ranging from 0.3 m to 2.0 m in length with a bluish-green waxy layer (Elzebroek and Wind 2008; Laber 2014; Parihar et al. 2014a). The plant type is either indeterminate (often referred as tall type) or determinate (bushy or dwarf type) (McKay et al. 1994, 2013). The leaves are alternate, pinnately compound with the rachis terminating in a single or branched tendril and consist of large stipules at the base, one to several pairs of oval leaflets (Yaxley et al. 2001; Pavek 2012). Several modern cultivars have a semi-leafless or "*afila*" type leaf, wherein the leaflets are transformed into additional tendrils (Parihar and Dixit 2017; Parihar et al. 2019a). In semi-leafless types, the leaflets are replaced by tendrils with intact stipules, whereas, in leafless types, the stipules become rudimentary and are replaced by tendrils (*afila* type). The "*afila*" type leaves have good standing ability and are comparatively tolerant to lodging to some extent than the leafy types owing to interlocking of tendrils among plants (Banniza et al. 2005; Dixit and Parihar 2014; Singh and Srivastava 2018; Parihar et al. 2019a; Dixit et al. 2014). The plants have tap root system with high root surface area and nodules on the surface (Gupta and Parihar 2015; Rao et al. 2021). The flowers are typical papilionaceous type and inflorescences developed in the leaf axils and consist of racemes with one to five flowers and are highly self-pollinated (Cooper 1938).

A flower contains five green compound sepals and five white, purple or pink petals of different sizes (Gritton 1980; Duke 1981; Majumdar 2011). The top petal is called the "standard," the two small petals in the middle are amalgamated together and called the "keel" (due to their boat-like appearance), and the two shrinked bottom petals are called the "wings" (Elzebroek and Wind, 2008). Flower color ranges from reddish purple, white, pink, and blue (Sattell et al. 1998; MacKay 2013). The stamens are diadelphous (9 + 1), with nine of them fused to form a staminal tube, while the tenth is free throughout its length (Ferrandiz et al. 1999). Gynoecium is monocarpellary and contains up to 15 ovules that is alternately attached to placenta. Style bends at right angle to ovary with sticky stigma (Gupta and Parihar 2015). The first flower-bearing node is characteristic of a given variety; in temperate regions, the first flower-bearing node is reported to vary from 4 to 25 under field conditions (Gritton 1980). The pod or fruit encapsulates about 5–10 seeds, which remains attached to the fruit by funiculus. Pods are straight or curved, and its length may reach up to 8–9 cm. The seed consists of an embryo, two cotyledons, and the seed coat. The mature seeds are round, smooth or wrinkled, and can be creamy, green, white, light brown, brown, red-orange, blue-red, dark violet to almost black, or dotted in color (Duke 1981; Kalloo 1993; Peter and Kumar 2008). The cotyledon colors of the mature round seed may vary from yellow, green, and red (Warkentin et al. 2015; Rubiales et al. 2019). Its cotyledon remains buried inside the soil during germination, characteristic of a typical hypogeal germination (Torrey and Zobel 1977).

25.6 Floral Biology, Emasculation: Pollination Techniques

It is a sexually propagating crop and flowers are cleistogamous and self-pollinated, that allows breeder to breed true breeding lines (Gill and Vear 1980; Suso et al. 2016; Smýkal et al. 2018). However, occurrence of crosspollination (0–60%) has been reported in pea (Kosterin and Bogdanova 2014; White 1917; Harland 1948; Myers and Gritton 1988), which may be due to competent pistil even after anthesis (Bogdanova and Berdnikov 2000; Kosterin and Bogdanova 2014). However, the proportion of out-crossing reported in the commercial cultivars is less than 1.0% (Gritton 1980; Davis 1993). Pollen grains of pea has been observed to disperse over several hundred meters (Dostálová et al. 2005), which could be by the pollinators as several insects belonging to Diptera, Hymenoptera, Lepidoptera, and Coleoptera are reported to be a pollinator in pea (Saboor et al. 2016).

Anthesis and anther dehiscence in field pea occurs during morning hours between 5 and 8 AM. The stigma becomes receptive to pollen few days prior to anthesis and a day after flower opening (Warnock and Hagedorn 1954). Pollen remains viable from the time of anther dehisce to several days thereafter. Pollination happens 24 h before flower opening and subsequently pollen germinates on the stigma in about 8–12 h following fertilization (Cooper 1938; Gritton and Wierzbicka 1975; Gritton 1980; Peter and Kumar 2008; Mohan et al. 2013). The selection of flower bud for emasculation should be done at the stage just before anther dehiscence, which is indicated by expansion of petals beyond sepals. In the process of emasculation, first the sepals and standards are removed from the selected bud. Then the keel petals are removed from the base of the bud, or slit is made in the keel to expose the stigma and anthers using a forcep, followed by removal of ten male stamens and anthers without touching or damaging the pistil. The following precautions must be taken during emasculation

- The flower buds to be chosen for emasculation should be of appropriate stage: unopened standard petal.
- To avoid damaging the pistil, the flower bud must be handled gently and disturb the flower as little as possible.
- Confirm that no anthers are left on the pistil.

The stigma of the emasculated female flower is highly receptive during morning hours. For pollination, male flowers about to open should be collected; the standard and wings and keel removed to expose the pollen-rich anthers. Gently brush the pollen onto the stigmatic surface of the emasculated flower bud. To increase the pod setting, the old flowers and other nonpollinated flower buds should be removed after crossing. Normally emasculation is executed in afternoon followed by pollination in next morning. Proper cleaning of the forceps to be used for pollination should be done by dipping them into 95% ethyl alcohol before changing pollen donor. After pollination, proper labelling of the crossed bud should be done. Proper care must be taken to put the pollen on the stigmatic surface, which is situated at the extreme tip of the style, not on the hairs on the hollow side of the style, which might appear to be

the suitable spot (Warnock and Hagedorn 1954). Pollen must be applied carefully to avoid injury to the style by forcing it too far backward. Fix a tag on the pollinated flower with date of emasculation and pollination, name of the parents (female and male) written on or tie different color thread of about 10 cm on the peduncle of each pollinated flower to discriminate the crossed flower.

The ease with which all forms of peas except *P. fulvum* can be intercrossed provides plant breeders access to the array of variation that exists in the wild, primitive, and cultivated forms (Davis, 1993). Some of the wild forms and cultivars demonstrate chromosomal changes, which may cause partial sterility of hybrids (Casey and Davies 1993). Initially, through physical mutagenesis (x-ray irradiation), a total 13 genetic male sterile lines were developed from "Dippes gelbe Viktoria" genotype of*Pisum sativum* and subsequently several studies were carried out by many researchers (Gottschalk 1968, 1971; Gottschalk and Baquar 1971; Gottschalk and Kaul 1974; Klein 1969). The tests of allelism of these lines revealed that nine unique *ms* genes responsible for male sterility and all *ms* genes segregated like single recessive genes. Interestingly, out of nine, two ms genes *(ms-3* and *ms-10)* exhibited reduced female fertility in addition to male sterility (Myers and Gritton 1988). However, a spontaneous mutant plant with male sterility was noticed in "*Longittee*" cultivar and, inheritance study explained that the sterile character is genetically governed and controlled by a recessive gene (Singh and Singh 1995). However, natural hybridization has not so far been used in pea breeding programs due to lack of pollination control and the pollen transfer would be dependent upon insects.

In pea, nuclear–cytoplasmic incompatibility has been observed in the crosses of wild species *P. sativum* subsp. *elatius* accession VIR 320 with cultivated forms (Bogdanova and Berdnikov 2001; Bogdanova and Kosterin 2006, 2007; Yadrikhinskiy and Bogdanova 2011). Further, inheritance analysis revealed that it is governed by two complementary unlinked nuclear genes, *Scs1* and *Scs2*. In crosses of cultivated peas as male and wild accession (VIR320) as female the F_1 was highly sterile and displays chlorophyll deficiency, reduction of leaflets, and stipules, while the reciprocal cross produces normal hybrids. The gene *Scs1*is closely linked to the *PhlC* gene on linkage group III, and other gene*Scs2 is* linked to *gp* gene on linkage group V (Bogdanova et al. 2009). Heterozygotes for either of the genes Scs1 or Scs2 decrease pollen fertility about 50% and 30%, respectively, whereas in homozygote, *Scs2* reduced 20%pollen fertility. In contrast to Scs2, Scs1 allele from the cultivated parent was shown to be both sporophyte and male gametophyte lethal in the background of the alien cytoplasm (Bogdanova et al. 2012). Recently, the plastid accD gene coding for the acetyl-CoA carboxylase beta subunit and the nuclear gene bccp coding for the biotin carboxyl carrier protein of acetyl-CoA carboxylase have been designated as candidate genes underlying nuclear–cytoplasmic incompatibility in peas (Bogdanova et al. 2015).

25.7 Cytogenetics and Molecular Cytogenetics

Pea is a valuable model plant in genetics since the days of Gregor Johann Mendel. Peas are diploid with a chromosome number of $2n = 2x = 14$ (Yarnell 1962). The standard karyotype of pea was described by Levitskii (1934) and Blixt (1959). Contrary to the other major legumes crops such as *M. truncatula* and *Lotus japonicus*, pea has a large genome of 4300 Mb organized in seven chromosome pairs ($2n = 2x = 14$) (Smýkal et al. 2013; Praca-Fontes et al. 2014). Out of seven, five are acrocentric and two with a secondary constriction corresponding to the 45S rRNA. The chromosomes in pea are distinguishable on the basis of morphology and can be identified with linkage groups. The chromosome and linkage group numbers have been depicted utilizing Arabic and Roman numerals, separately such as 1 = VI, 2 = I, 3 = V, 4 = IV, 5 = III, 6 = II, and 7 = VII (Fuchs et al. 1998; Ellis and Poyser 2002). The pea genome consists of about 37.7% GC and 30% of cytosine are 5methyl-cytosine (5meC), and 50% of 5meC contain the sequence of C(A/T)G (Murray et al. 1978; Thompson and Murray 1980; Salinas et al. 1988).

The DNA-related studies witnessed about 75–97% of genome to be of repetitive sequences (Flavell et al. 1974), which was further confirmed by next-generation sequencing (NGS), which reports presence of 50–60% highly to moderately repeated sequences (Novák et al. 2010). The grouping of sequence reads depicted that *Ty3/gypsy* LTR-retrotransposons are the most important components of the pea repeats. Ogre elements accounted 20–33% of the pea genome, while *Ty1/copia* and other sorts of repeats were noticed at low proportion (Macas et al. 2007). The pea repeats have been embraced as the important part of many studies targeting mainly on LTR-retrotransposons (Cyclops), MITE elements (Zaba), PIGY, Angela, PDR, Stowaway and centromeric retrotransposons (Chavanne et al. 1998; Neumann et al. 2011). These repeats could be used as valuable markers for cytogenetic chromosomal discrimination within its karyotype, for instance, the satellite repeat PisTR-B the most convenient cytogenetic marker of all pea chromosomes (Smýkal et al. 2012).

25.8 Genetic Resources Panorama at National and International Level

The success of any crop breeding program in terms of development of high-yielding varieties with targeted traits primarily hinged on the availability of genetic resources and their proper exploitation. In India currently, approximately 4484 accessions of pea are conserved in national genebank housed at ICAR-NBPGR, New Delhi, of which about 1118 accessions are exotic, while 3366 are indigenous to India. The Geo-referenced map illustrating the coverage of pea germplasm collections executed across the states in India is depicted in Fig. 25.2. Around 98,000 accessions consist of released varieties, advanced breeding lines, land races, mutants and wild species: these are being maintained in various gene banks worldwide. Of the total collections, wild pea accessions contribute only 2%, highest by land races (Smýkal et al. 2013;

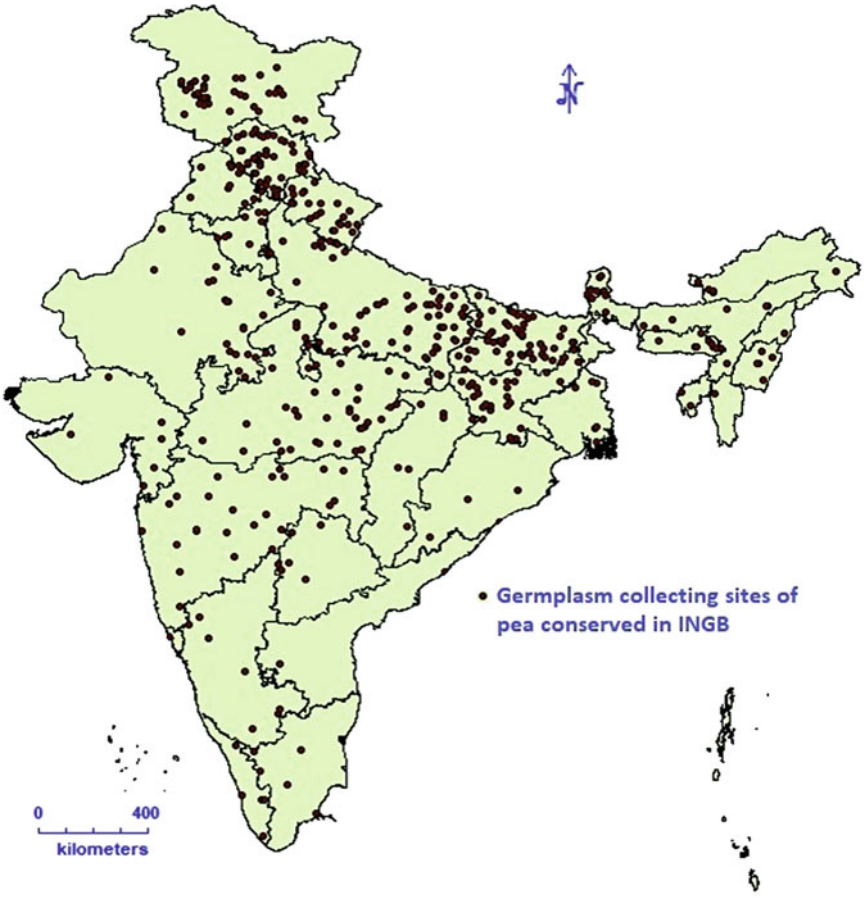

Fig. 25.2 Georeferenced map showing the distribution of pea germplasm collections in India

Warkentin et al. 2015; Rubiales et al. 2019; Parihar et al. 2020a, b). Among wild collection, 706 accessions belong to *P. fulvum,* 624 to *P. s*. subsp. *elatius,* 1562 to *P. s*. subsp. *sativum* (syn. *P. humile/syriacum*) and 540 to *P. abyssinicum* (Smýkal et al. 2013).

National Institute for Agricultural Research, Dijon, France (8839 accessions); Australian Temperate Field Crop Collection, Horsham (7432 accessions); N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia (6790 accessions); The United State Department of Agriculture (USDA) (6827 accessions); Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (5343 accessions) and International Center for Agricultural Research in the Dry Areas (ICARDA) (4596 accessions) are the six leading institutions, which holds around 40% of total pea germplasm collections. However, collections of pea germplasm are also available in Italy (4558 accessions), China (3837 accessions), UK

Table 25.1 Major dry pea germplasm holding organizations worldwide (Source: Warkentin et al. (2015); https://www.genesys-pgr.org/)

S. no.	Name of institutions/organization	Number of accessions
1	INRA CRG Légumineuse à grosses graines, Dijon, France	8839
2	Australian Temperate Field Crop Collection, Horsham, Australia	7432
3	Plant Germplasm Introduction and Testing Research Station, Pullman, USA	6827
4	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russia	6790
5	Leibniz Institute of Plant Genetics and Crop Plant Research, Gaterleben, Germany	5343
6	International Center for Agricultural Research in the Dry Areas, Lebanon	4596
7	Istituto del Germoplasma, Bari, Italy	4558
8	ICAR-National Bureau of Plant Genetic Resources, New Delhi, India	4484
9	Institute of Crop Sciences, CAAS, China	3837
10	John Innes Centre, Norwich, UK	3562
11	Science and Advice for Scottish Agriculture, Scottish Government, UK	3298
12	Plant Breeding and Acclimatization Institute Blonie, Radziko, Polland	2896
13	NordGen, Nordic Genetic Resource Centre, Alnarp, Sweden	2849
14	Yurjev Institute of Plant Breeding, Kharkov, Ukraine	2311
15	Genetic Resources Unit, Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK	2110
16	National Center for Vegetable Crops Research (CNPH)/EMBRAP, Brasil	1958
17	Junta de Castilla y León. Instituto Tecnológico Agrario de Castilla y León, Spain	1772
18	Ethiopian Biodiversity Institute, Ethiopia	1768
19	Institutot Nacional de Investigacion y Tecnologia Agraria y Alimantaria, Spain	1648
20	Institute of Plant Introduction and Genetic Resources, Sadovo, Bulgaria	1570
21	Centre for Applied Research of Vegetables and Special Crops, Olomouc, Czech Republic	1414
22	AGRITEC, Research, Breeding and Services Ltd., Sumperk	1326

(3562 accessions), Poland (2896 accessions), and Bulgaria (2100). Also, countries such as Brasil, Spain, Ethiopia, Ukraine, Czech Republic, Hungry, and the Netherlands also conserve substantial germplasm accessions of *Pisum* in their national gene banks (Smýkal et al. 2012; Warkentin et al. 2015). Detail list of major dry pea germplasm holding at global level is given in Table 25.1. Among the above-mentioned organizations, the Vavilov Research Institute is the oldest and the John Innes (JI), Norwich, is having very representative and perhaps the best studied *Pisum* collection with 1200 *P. sativum* cultivars, 600 landraces, 750 genetic stocks, and reference lines. An estimated 20% of the world's ex situ pea germplasm is duplicative, and a total 59,000 of accessions are designated to be unique (Warkentin et al. 2015; Smýkal et al. 2013).

During recent times, several online portals have been developed by different national and international organization to store and share the important information of pea. The web portal of John Innes Centre, Norwich, UK (JIC; http://www.jic.ac.uk/germplasm/) and the USDA (http://www.ars-grin.gov/npgs/) are the most advanced websites involved in supply of germplasm accessions as per requisitions. In case of wild species, Israel gene bank possesses a collection of wild relatives of *P. fulvum* and *P. sativum* subsp. *elatius* var. *pumilio* collected from the Middle East. To share important information of pea related to phenotypic and genotypic data sets, there are a number of international collection web databases: for instance, European Cooperative Program on Plant Genetic Resources (ECPGR), International Legume Database and Information Service (ILDIS), Legume Information System (LIS), Legume phylo-informatics database, INRA Dijon Legume genetic and genomic resources, INRA LegumeBase, LegumeIP, Genetic Resources Information Network (GRIN), System-wide Information Network for Genetic Resources (SINGER), GRIN-Global, Cool Season Food Legume Database (https://coolseasonfoodlegume.org; Washington State University), and KnowPulse (https://knowpulse.usask.ca; University of Saskatchewan). Genesys (https://www.genesys-pgr.org/) is an online platform where information about 21,693 pea accessions are available, which provides information about plant genetic resources conserved in gene banks worldwide.

So far, eight core collections have been developed in Australia, China, Czech Republic, France, Poland, Spain, the United Kingdom, and the United States to accelerate germplasm evaluation and their judicious utilization (Warkentin et al. 2015; Rubiales et al. 2019). The first pea core set was constructed at JIC following Brown (1989) recommendations and consists of representatives from all the *Pisum* taxon phenotyped for nine seed quality characteristics (Matthews and Ambrose 1994). Simon and Hannan (1995) developed in the USDA core collection of *P. sativum* with a few *P. sativum* ssp. *elatius* based on geography and flower colour. The USDA core was further refined to 310 accessions using 26 quantitative traits (Coyne et al. 2005). The Spanish core is a landrace collection based on passport and quantitative data (Martin-Sanz et al. 2011). The Polish core was developed considering diversity of type, taxon, and described genes studied using isozymes (Swiecicki et al. 2000). Baranger et al. (2004) reported the INRA core collection of 148 accessions wherein 43 *Pisum* accessions were selected based on protein and DNA marker diversity. With further utilization of molecular data, Zong et al. (2009) developed a core of Chinese landraces considering molecular diversity data. Smýkal et al. (2011) proposed the use of a model-based method for the creation of a molecular/ecogeographically diverse international pea core collection for a realistic initiation toward phenotyping of the nonduplicative pea germplasm.

25.9 Brief of Existing Genetic Variability for Agronomic and Nutritional Parameters

Genetic variability is quintessential to increase yields and sustainable production in the face of anticipated climatic changes. In addition, knowledge of genetic diversity plays an important role in gene bank management and in planning experiments, as it enables efficient sampling and utilization of germplasm through identification and elimination of duplicates (Ghafoor et al. 2005). In order to maintain, evaluate, and utilize germplasm efficiently and effectively, the proper investigation of the magnitude of genetic diversity is indispensable (Smith and Smith 1989).

Morphological characterization is the first step toward the categorization of crop germplasm (Smith and Smith 1989; Ghafoor et al. 2003). Considerable morphological variation is reported in pea by several research groups (Blixt 1974; Hoey et al. 1996; Kumar and Jain 2003; Sharma et al. 2003; Singh et al. 2003a, b; Parihar et al. 2014b). The significant and positive association of traits like percent emergence, plant height, pod length, number of pods/plant, number of seeds/pod, number of locules per pod, primary branches, number of nodes, seed set, 100-seed weight, flowering time, days to maturity, biological yield, and harvest index with seed yield has been reported (Singh and Singh 2006; Sardana et al. 2007; Nisar et al. 2008, 2011; Gatti et al. 2011; Basaiwala et al. 2013; Ofga and Petros 2017; Pratap et al. 2021). Also, significant morphological variation in terms of flower color, seed coat color, cotyledon color, leaflet shape, pod shape, pod length, plant height, numbers of pods per plant, number of seeds per pod, seed size, hilum color and seed shape, stipule length, stipule width, intermodal length, number of nodes, seed diameter (Ghafoor et al. 2005; Smýkal et al. 2008; Umar et al. 2014; Handerson et al. 2014). A large number of field pea genotypes have been evaluated for major agronomic traits, and nutritional parameters and plenty of variability was observed (Table 25.2).

Similarly, in case of nutritional value abundant amounts of variability exist in field pea for protein, starch, minerals, and antinutritional factors (Table 25.2), that usually is influenced by both environments and genetic factors (Ray et al. 2014; Hood-Niefer et al. 2012; Bourgeois et al. 2011; Harmankaya, et al. 2010; Wang et al. 2008a, b). Carbohydrates are the main constituent of pea, which is available in substantial amounts (Dahl et al. 2012). Starch content fluctuates from 27.6% to 57.23% (Tzitzikas et al. 2006; Holasová et al. 2009). Likewise, the amylose content is reported to vary widely among varieties and mutant lines (Guillon and Champ 2002), with a mean of 27.8% of total starch (Holasová et al. 2009). However, no effect of genotype or environment was demonstrated on the amylose content (Hood-Niefer et al. 2012). The seed coat, hull, and cotyledon are the source of dietary fiber in peas (Martens et al. 2017), with water-insoluble polysaccharides (mainly cellulose) predominant in seed coat, while the cotyledon consists of hemicellulose, pectin, and cellulose (Martens et al. 2017; Dahl et al. 2012; Tosh and Yada 2010; Guillon and Champ 2002; Reichert and MacKenzie 1982).

Like other legumes, pea contains considerable amount of raffinose-family and other galactose-containing oligosaccharides (Tosh and Yada 2010) of which stachyose is reported to vary from 0.7% to 4.1%. (Jones et al. 1999), total

Table 25.2 Genetic variation on important agronomic and seed quality traits in pea

Traits	Range	References
Days to 50% flower	41.00–103.00	Parihar et al. (2014b)
	45–100	Handerson et al. (2014)
	60–120	Rana et al. (2013)
	62.08–86.08	Singh et al. (2018)
	56–168	Rana et al. (2017)
	55.00–73.67	Basaiwala et al. (2013)
Days to maturity	73.00–135.00	Parihar et al. (2014b)
	128–212	Rana et al. (2017)
	96.33–132.33	Singh et al. (2018)
	81.0–121	Jha et al. (2012)
	94.33–128.67	Basaiwala et al. (2013)
Plant height (cm)	13.33–209.33	Parihar et al. (2014b)
	33.4–197.2	Ali et al. (2007)
	47.6–175	Yadav et al. (2010)
	43.20–180.40	Basaiwala et al. (2013)
	14.39–245.53	Umar et al. (2014)
	51.7–85.3	Annicchiarico et al. (2017)
	37.9–75.3	Gatti et al. (2011)
	19–117	Handerson et al. (2014)
	38.66–279.8	Rana et al. (2017)
	30—132	Jha et al. (2012)
	30.0–132	Nisar et al. (2011)
100 seeds weight	3.03–31.6	Nisar et al. (2008)
	3.2–23.27	Ali et al. (2007)
	3.03–26.32	Ghafoor et al. (2005)
	13.40–28.00	Handerson et al. (2014)
	13.3–24.0	Gatti et al. (2011)
	15.88–22.22	Jeberson et al. (2017)
	15.68–23.14	Basaiwala et al. (2013)
	4.26–25.65	Singh et al. (2010)
	6.12–20.27	Ouafi et al. (2016)
	6.2–32.3	Azmat et al. (2011)
	5.6–28.9	Rana et al. (2013)
	11.18–31.43	Singh et al. (2018)
	2.99–47.25	Burstin et al. (2015)
Pod length (cm)	2.94–8.02	Nisar et al. (2008)
	3.5–7.98	Ali et al. (2007)
	2.90–12.10	Rana et al. (2017)
	1.73–8.55	Umar et al. (2014)
	4.66–9.99	Parihar et al. (2014b)
	4.9–12.8	Azmat et al. (2011)
Pods/plant	7.67–89.00	Parihar et al. (2014b)
	12.00–29.67	Basaiwala et al. (2013)

(continued)

Table 25.2 (continued)

Traits	Range	References
	8.33–53.50	Rana et al. (2017)
	3.17–10.87	Jeberson et al. (2017)
	3.5–195	Ali et al. (2007)
	2–210.	Umar et al. (2014)
	8.5–18.3	Yadav et al. (2010)
	11–59	Rana et al. (2013)
	4.25–179.33	Ghafoor et al. (2005)
Seeds/pod	2.2–7.8	Nisar et al. (2008)
	2.2–8.0	Ali et al. (2007)
	2.0–8.0	Rana et al. (2017)
	3.40–6.53	Parihar et al. (2014b)
	3.7–5.4	Yadav et al. (2010)
	4.60–10.33	Ouafi et al. (2016)
	2.1–5.6	Gatti et al. (2011)
	3.0–7.5	Rana et al. (2013)
	3.0–9.1	Azmat et al. (2011)
	3.01–6.47	Singh et al. (2018)
	2.2–6.8	Ghafoor et al. (2005)
Seed yield (g/plant)	0.24–213.4	Nisar et al. (2008)
	14.27–112.13	Parihar et al. (2014b)
	2.28–34.42	Rana et al. (2017)
	2.0–49.0	Ali et al. (2007)
	8.7–17.7	Yadav et al. (2010)
	7.10–19.17	Basaiwala et al. (2013)
	4.15–12.82	Handerson et al. (2014)
	10.1–45.6	Rana et al. (2013)
Biological yield (g/plant)	9–452.66	Nisar et al. (2008)
	9–170	Ali et al. (2007)
	7.20–28.00	Parihar et al. (2014b)
	9.13–103.25	Ghafoor et al. (2005)
Harvest index (%)	24.75–63.87	Parihar et al. (2014b)
	9.74–47.23	Ghafoor et al. (2005)
	0.16–50.60	Nisar et al. (2008)
	23.23–54.10	Basaiwala et al. (2013)
	23.45–60.00	Singh et al. (2018)
	1.81–55.71	Nisar et al. (2011)
Protein	17.5–27.8	Annicchiarico et al. (2017)
	18.1–29.4	Jha et al. (2012)
	21.13–27.05	Harmankaya et al. (2010)
	15.8–32.1	Burstin et al. (2007)
Iron (ppm)	*21.90–58.40*	Harmankaya et al. (2010)
	23.16–105.2	Kwon et al. (2012)
	23–105	Grusak and Cakmak (2005)

(continued)

Table 25.2 (continued)

Traits	Range	References
	45–53	Amarakoon et al. (2014)
Zinc(ppm)	*21.0–57.10*	Harmankaya et al. (2010)
	16.10–106.63	Kwon et al. (2012)
	11.3–82.9	Demirbas (2018)
	39.0–63.0	Amarakoon et al. (2012)

a-D-galactosides from 22.6 to 63.4 g/kg, verbascose from 0.0 to 26.7 g/kg; raffinose from 4.1 to 10.3 g/kg, and sucrose from 11.6 to 25.4 g/kg (Vidal-Valverde et al. 2003). Most recently, Gawłowska et al. (2017) reported highest content of total soluble carbohydrates and total RFOs in wrinkled seeds and lowest in *P. fulvum*. It has also been observed that the total RFOs content was positively associated with stachyose and verbascose. Noteworthy, all oligosaccharides contents were low in lines having dominant alleles of pea seed genes (*R*, *A*, and *I*). The recessive mutations for these genes resulted in an increased content of RFOs. Consequently, detection of mutant lines having very low concentrations of oligosaccharides is prerequisite toward the development of field pea cultivars with low RFOs that would prevent flatulence and related issues.

The protein content varied from 13.7% to 30.7% of seed dry matter (Tzitzikas et al. 2006). Similarly, Harmankaya et al. (2010) reported noteworthy variations for protein (21.13 to 27.05%), potassium (562.8 to 937.8 mg/100 g), phosphorus (163.4 to 374.2 mg/100 g), calcium (45.91 to 157.40 mg/100 g), magnesium (47.31 to 102.81 mg/100 g), sulfur (75.69 to 194.4 mg/100 g), iron (2.19 to 5.84 mg/100 g), and zinc (2.10 to 5.71 mg/100 g) content. The pea proteins are predominantly storage proteins, or globulins, and their amino acid matrix plays pivotal role in nutritional significance (Boye et al. 2011; Bourgeois et al. 2011). Recently, number of pea germplasm lines with more than 30% protein in the seeds have been identified and used in breeding program (Bing 2015; Shen et al. 2016; Demirbas 2018). Pea seed is considered as good source of several macro- and micronutrients and significant amount of its variability has been noticed (Table 25.1). The yellow peas are reported to have higher levels of Fe, Mg, and Mn, but lower levels of K, as compared to green peas (Gawalko et al. 2009). The abundant variability for Fe (46–-54 mg kg^{-1}), Zn (39–63 mg kg^{-1}), and Mg (1350–1427 mg kg^{-1}) was reported in a multilocation evaluation (Amarakoon et al. 2012). Similarly, Kwon et al. (2012) noticed plenty of variation for most of the micronutrients especially Fe (23.16–105.2 ppm) and Zn (16.1–106.63 ppm) in USDA core collection. While the Fe content of dry pea seeds is reported to vary from 45 to 58 mg/kg in commercial cultivars (Ray et al. 2014).

Recently, Amarakoon et al. (2015) observed that the Fe concentration varied from 45 to 53 mg/kg, and reported existence of substantial concentrations of Fe promoters like xanthophyll (17 mg/100 g), canthaxanthin (68 mg/100 g), beta-carotene (680 mg/100 g), kestose (1433 mg/100 g), quercetin (51.7 mg/100 g), and ferulic acid (56.1 mg/100 g). The phytic acid concentration was low (2.7–3.2 mg/g) and the

phytic acid:Fe molar ratio ranged from 5.0 to 5.6. Ma et al. (2017) have registered enormous genotypic variability for several minerals (Fe, Zn, Ca, K, and S) in a RIL population, the iron and zinc content ranged from 37.3 to 71.2 and 30.7 to 64.9 μg/g DW), respectively. Demirbas (2018) reported tremendous diversity for nitrogen (22.3–66.7 g kg^{-1}), phosphorus (1.48–8.47 g kg^{-1}), potassium (6.7–18.7 g kg^{-1}), iron (38.6–320.9 mg kg^{-1}), zinc (11.3–82.9 mg kg^{-1}), copper (10.5–50.8 mg kg^{-1}), and manganese (10.2–37.9 mg kg^{-1}) in Turkish pea germplasm. In addition to other micronutrients, field pea is an important dietary source of selenium and is reported to range from 373 to 519 mg/kg (Thavarajah et al. 2010).

Pea is also rich in vitamins, particularly thiamin (B1) and folate (B9), although limited research efforts are made so far to explore the diversity in vitamin B concentrations in field pea (Sierra et al. 1998; Jha et al. 2015a, b). The folate content in yellow and green seeded genotype is reported to range from 23·7 to 55·6 and 24·9 to 64·8 mg/100 g (Han and Tyler 2003), 41 − 55 μg/100 g, and 50 − 202 μg/100 g (Gupta et al. 2013), respectively. Recently, Jha et al. (2015a, b) reported total folate concentration to vary from 23 to 30 mg/100 g in pea, of which 5-methyltetrahydrofolate (5-MTHF) and tetrahydrofolate (THF) were the predominant forms. Significant effects of environment and cultivar were also observed for the majority of the folates. The lutein concentration in green cotyledons ranged from 0.768 to 1.394 mg/100 g, whereas yellow cotyledon has lower amount of lutein. The highest variation in lutein content was recorded in orange cotyledon lines. Besides, β-carotene in green cotyledon genotypes fluctuates between 0.1 and 0.2 mg/100 g, whereas yellow and orange cotyledon contains 10 times lower concentration of β-carotene.

A strong positive association among lutein and chlorophyll content was obtained (Holasová et al. 2009). Most recently, Bangar et al. (2017) reported that cotyledon pigmentation (green and yellow) had no association with total carotenoid concentration, but β-carotene concentration was greater in green cotyledon genotypes. However, Ashokkumar et al. (2014, 2015) have reported total carotenoid to vary with cotyledon pigmentation; the genotypes with green cotyledon had approximately twice the amount of total carotenoids (16–21 μg g^{-1}) as compared to yellow cotyledon (7–12 μg g^{-1}). Peas also contain various phytochemicals including phenolic compounds, phytates, saponins, and oxalates. The prevalent phenolic compounds in pulses are tannins, phenolic acids, and flavonoids (Campos-Vega et al. 2010). The highest concentrations of most phenolics exist in the seed coat, especially in dark-seeded genotypes (Campos-Vega et al. 2010; Troszynska and Ciska 2002; Duenas et al. 2004). Likewise, Xu et al. (2007) also noticed that the antioxidant activity is associated with seed coat color.

The results pertaining to crop wild relatives revealed that wild *Pisum species* and *subspecies* are a source of many desirable traits, like resistance to pea weevil in *P. Fulvum* (Teshome et al. 2015a, b; Clement et al. 2009; Byrne et al. 2008a, b). Sincere efforts have been made for the transmission of powdery mildew (Fondevilla et al. 2007b; Mishra et al. 2007) and bruchid (Byrne et al. 2008a, b; Clement et al. 2009) resistance from *Pisum fulvum* into cultivated pea along with the incorporation of PSbMV and *Fusarium* resistances from primitive landraces (Smýkal et al. 2013;

McPhee et al. 1999). The value of CWR has been demonstrated by novel dominant gene (*Er3)*, which was identified in *Pisum fulvum* conferring resistance to *E. Pisi* and has been introduced successfully in adapted Pea (Sharma and Yadav 2003; Fondevilla et al. 2008). Moreover, some *P. fulvum* accessions were found to show resistance to *Mycosphaerella pinodes* and *Orobanche crenata* and subsequently involved in crossing with cultivated pea (Fondevilla et al. 2005; Pérez-de-Luque et al. 2005). On similar note, high level of resistance was reported in *P. fulvum* for rust (Barilli et al. 2010, 2018) and ascochyta blight (Fondevilla et al. 2005; Jha et al. 2012).

Interestingly, the commercially least accepted pigmented flower and seed coat are an outstanding sources of *Aphanomyces* root rot resistance (Hamon et al. 2011) and *Fusarium* root rots (Weeden and Porter 2007; Grunwald et al. 2003). Most importantly, the valuable resistance for biotic and abiotic stresses can also be embraced from *Lathyrus species*, which is harbored in tertiary pea gene pool (Patto et al. 2007), preferably through the use of modern biotechnological techniques. Based on germplasm characterization and evaluation for various yield attributing traits, nutritional parameters and biotic and abiotic stress resistance potential donors have been identified by different researchers. The details of trait specific potential donors are given in Table 25.3.

The morphological traits are limited and most of them are multigenic, quantitative or continuous characters, and their appearance is influenced by environmental conditions. As a complementary approach, biochemical analysis of isozyme markers proves its diagnostic potential in pea (Swiecicki and Wolko 1987; Swiecicki et al. 2000; Pošvec and Griga 2000; Ali et al. 2007; Smykal et al. 2008), but a limited degree of polymorphism and potential sensitivity to environmental and developmental variation prevented its broad application. In addition to morphological and biochemical marker, various DNA-based markers have been successfully used to compute genetic variations among closely related pea germplasm, such as STMS (Baranger et al. 2004; Haghnazari et al. 2005); ISSR (Lázaro and Aguinagalde 2006), SNP (Duarte et al. 2014a, b), SRAP (Esposito et al. 2007), IRAP (Smýkal et al. 2008), RBIP (Smýkal et al. 2008), EST-SSR (Teshome et al. 2015a, b), and SSR (Handerson et al. 2014; Negisho et al. 2017; Mohamed et al. 2019). Among them, SSRs have gained popularity because of cost effectiveness, speed, reproducibility, and polymorphism (Snowdon and Friedt 2004). More recently, next -generation sequencing has allowed rapid SNP discovery and genotyping array development in pea (Deulvot et al. 2010; Duarte et al. 2014a, b; Leonforte et al. 2013a; Sindhu et al. 2014).

25.10 Inheritance of Important Qualitative and Quantitative Traits

To accelerate the success of any breeding program, the information about nature and magnitude of gene action is mandatory (Shashikumar et al. 2010), as the selection of suitable parent for hybridization and breeding procedure for the improvement of trait

Table 25.3 Potential source for field pea improvement

Trait	Germplasm/variety/wild relatives	Country	Reference
Earliness	DDR-30	India	Bhuvaneswari et al. (2017) and Handerson et al. (2014)
	DDR 23	India	Handerson et al. (2014)
	IPFD 18–14, IPFD 18–16, IPFD 18–17, IPFD 18–11, IPFD 18–13, IPFD 18–18, IPFD 18–19, IPFD 18–12, IPFD 18–20, IPFD 18–22	India	Parihar et al. (2021a, b
	P 1613, P 73–1, P 118–1, P 1343	India	Dixit and Gautam (2015)
	Spring pea	Argentina	Gatti et al. (2011)
Pods /plant	10,622, 11,116, 10,476, 10,479	Pakistan	Ghafoor et al. (2005)
	P 1541–33, P 1548–2, P 108, P 996	India	Dixit and Gautam (2015)
100-seed weight	10,603, 10,609, 10,623, 10,625, 11,083, 11,089	Pakistan	Ghafoor et al. (2005)
	KPMR-747	India	Handerson et al. (2014)
Root length and volume	PI 261631	USA	McPhee (2005)
Harvest index	10,603, 10,607, 10,609, 11,052, 11,094, 11,100, 11,114, 10,479	Pakistan	Ghafoor et al. (2005)
Protein	PS3045 (27%)	Turkey	Harmankaya et al. (2010)
	MI3391 (32%), CDC647–1(26%)	Canada	Bing (2015)
Iron content	Tekirdağ2, Tokat1, Konya3, İzmir4, Giresun, Elazığ, Adıyaman2	Turkey	Demirbas (2018)
Zinc content	Agassiz	USA	Amarakoon et al. (2012)
	PS3029–2	Turkey	Harmankaya et al. (2010)
	Tekirdağ2, Konya3, Elazığ, Adıyaman2	Turkey	Demirbas (2018)
Insect and pest resistance germplasm/wild relatives			
Powdery mildew	9057, 9370, 9375, 10,609, 10,612, 18,293, 18,412, 19,598, 19,611, 19,616, 19,727, 19,750, 19,782, 20,126, 20,152, 20,171, It-96, no. 267, and no. 380	Pakistan	Azmat et al. (2012)
Powdery mildew	*P. fulvum* (P660–4)	Spain	Fondevilla et al. (2007a, b, c)
Powdery mildew	HFP4, EC598878, EC598538, EC598757, EC598704, EC598729, EC598535, EC598655, EC598816, EC381866, IC278261, IC267142, IC218988, IC208378, IC208366	India	Rana et al. (2013)

(continued)

Table 25.3 (continued)

Trait	Germplasm/variety/wild relatives	Country	Reference
	HFP 9907 B, Pant Pea −42, VL Matar 42, IPFD 99–13, IPFD 1–10, IPF 99–25, Pusa prabhat, Ambika	India	Dixit and Gautam (2015)
	GPHA-9 and GPHA-19	Ethiopia	Assen (2020)
Rust	IPF-2014-16, KPMR-936 and IPF-2014-13	India	Das et al. (2019)
	PJ 207508, C 12, Wisconsin, DMR 3, Pant P 5, Pant P 8, Pant 9, HFP 8711 and HUDP 15, IPFD 1–10	India	Chaudhary and Naimuddin (2000) and Dixit and Gautam (2015)
Downey mildew	Mukta, Snowpeak	Australia	Davidson et al. (2004)
Pea seed-borne mosaic virus (PSbMV)	PI 193586, PI 193835	Ethiopia	Hagedorn and Gritton (1973)
Salinity tolerance	ATC1836	Australia	Leonforte et al. (2013a)
Pseudomonas syringae pv. *Pisi* (race 6, 8)	JI0130	Spain	Martín-Sanz et al. (2012)
Pseudomonas syringae pv. *Pisi* (race 8)	Forrimax, JI2546, PI-277852, ZP1328, Cherokee, Corallo, Lincoln, JI2385, PM29, PM232, PM33, JI1829, ZP1282, ZP0104, ZP1301, ZP0123, ZP0168	Spain	Martín-Sanz et al. (2012)
Mycosphaerella blight (*Mycosphaerella pinodes)*	CN 112432, CN 112441, CN 112513	Canada	Jha et al. (2012)
	P. Fulvum (P651), Radley	Spain	Fondevilla et al. (2005)
Stem fly (*Melanagromyza phaseoli*)	P-4039, P-4107	India	Vishal and Ram (2005)
Leaf miner (*Chromatomyia horticola*)	P-4107	India	Vishal and Ram (2005)
Pea weevil (*Bruchus pisorum*)	*P. fulvum* (ATC113)	Australia	Hardie et al. (1995) and Byrne et al. (2008a, b)

of interest mainly hinged on the understanding of gene action/effects operating in a particular breeding population (Sharma et al. 2013). The study of trait inheritance pattern has been the major aim of myriad studies since early days (Knight 1799; Mendel 1866). Notably, pea has been acknowledged as the original model organism and used in the discovery of the Mendel's laws of inheritance, which makes it the founder of modern plant genetics (Smýkal et al. 2012). The inheritance of numerous morphological, physiological, quality, and resistance attributes has been elaborated

by several workers (Lamprecht 1948; Yarnell 1962; Blixt 1974; Kalloo and Bergh 1993; Gritton 1980; Kumar et al. 2006a, b; Amin et al. 2010). Mendel (1866) was the first to study the inheritance of seed forms, that is round versus wrinkled and observed it to be governed by a single gene *R*. Round form was dominant over wrinkled. Due to spontaneous mutation in the wild-type, round (*RR*) seeded at the beginning of seventeenth century, the wrinkled (*rr*) seed developed (Lamprecht 1956; Bhattacharyya et al. 1993).

Wrinkling of the seed was one of the characters used by Mendel in experiments, which led him to formulate the laws of inheritance. This character or locus was later named as rugosus (*r*) from the Latin for wrinkled or shriveled (White 1917). The wrinkled-seed mutant *(rr)* arose through mutation of the gene encoding starch-branching enzyme isoform I (SBE1) by insertion of a transposon-like element into the coding sequence (Bhattacharyya et al. 1993). Much later, a second locus *rb* causing wrinkled seeds was identified (Kooistra 1962). The *rb* mutation of peas causes structural and regulatory changes in ADP glucose pyrophosphorylase from developing embryos, but this mutation has only become available in Europe since the 1930s (Hylton and Smith 1992; Reid and Ross 2011, Rayner et al. 2017). These two loci are known to affect the development of embryo, and mutants at both loci behave as single gene recessives. Despite the large number of genes that affect the seed (Blixt 1972), very few have been identified that influence the development of the embryo (Hedley and Wang 1987).

Recessive alleles at the *r* locus not only have a profound effect on the shape of the seed but also have numerous effects at all levels of seed development. Later the role of interaction of gene pair *Aa*, *Rr*, and *Didi* in determination of seed surface was examined (Wellensiek 1943) and noticed that the gene *A* and *a* controlled indented and smooth testa, respectively. The gene *R* and *r* was responsible for smooth and wrinkled surface, respectively, and *di* gave rise to dimpling in presence of *r* gene. The *a* and *di* were episatic to the smooth phenotype of *R*. Single recessive genes determine flattened seed shape (*com)*, gritty seed surface (*gty*), and green cotyledons (*i*), while dominant gene *pi* along with *ar* and *b* determined black hium. Gene pair *Rr*, which is responsible for seed shape, also governed the starch and amylase content (Amin et al. 2010; Mohan et al. 2013). Round seed shape was monogenic and dominant over wrinkled seed shape (Rastogi and Saini 1984). Recently, Rayner et al. (2017) reported that the wrinkled-seeded phenotype is maternally determined in JI2110 genotype, which is controlled by two genetic loci, and the extent to which it is manifested is very sensitive to the environment.

As flowering time is associated to maturity, the investigation of inheritance pattern of flowering is quintessential to develop the required cultivars. Mendel (1865) reported the flowering time of hybrids to stand almost exactly between the times of the two parents. During the early days of modern genetics, the flowering time evoked the interest of investigators and Hansel (1954) reported flowering time to be governed by two major genes with unspecified number of modifiers. The polygenic system of inheritance of flowering was reported wherein lateness was dominant to earliness and gene effects were additive (Rowlands 1964; Watts et al. 1970). Floral initiation and development in pea have been studied for many decades

(Murfet and Reid 1993) and based on physiological and mutational analyses, a model for flowering that involves both a floral inhibitor and a stimulus has been developed (Reid et al. 1996; Weller et al. 1997b). The stimulus is specific to flowering and is under the control of GIGAS (Beveridge and Murfet 1996). The synthesis of the floral inhibitor is controlled by different genes (STERILE NODE, HIGH RESPONSE, PHOTOPERIOD, DAY NEUTRAL, and EARLY) and is strongly regulated by photoperiod (Foucher et al. 2003). Earlier reports have identified about ten genes, that is, *Lf*, *E*, *Hr*, *Sn, Ppd*, *Dne, FUN1, LV, GI*, and *VEG1*, involved in controlling flowering time (Murfet 1971a, 1990a, b; Murfet and Reid 1993, Weller et al. 1997b). The *Lf* (late flowering) was the first major gene identified in pea (Hoshino 1915; White 1917).

The ability to respond to photoperiod in pea requires the presence of the dominant alleles for three complementary genes: *Sn* (sterile node), *Dne* (day neutral), and *Ppd* (photoperiod response) (Murfet 1971b; King and Murfet 1985; Arumingtyas and Murfet 1994). The genes *E* (early) and *Hr* (high response) influence expression of the *Sn Dne Ppd* system during different stages of ontogeny (Murfet and Reid 1993). So far, twenty loci pertaining to flowering time and inflorescence development have been identified in pea. Initial work on genetic control of flowering recognized several loci in existing variation among various cultivars of pea, whereas other loci have been subsequently identified through characterization of induced mutants and specific mutant screens (Murfet, 1985; Weller et al. 1997a, 2009; Weller and Ortega 2015). Flowers are borne on axillary racemes in the pea and the wild type pea cultivars usually produce two flowers per raceme; however, multiflowered types have also been identified (Lamprecht 1947; Hole and Hardwick 1976; Gritton 1980; Murfet and Reid 1993; Devi et al. 2018, 2021). Some reports have proposed a polygenic control (Ibarbia and Bienz 1970; Snoad and Arthur 1973), whereas others proposed control by two genes, *Fn* and *Fna.* The plant with *FnFna* produces one flower per raceme, while *Fnfna* and *fnFna* produces two flowers, and *fnfna* produces three or more flowers per raceme (Lamprecht 1947; Murfet and Reid 1993). This trait is certainly influenced by some major flowering genes: *Sn*, *Hr*, *Veg*-2^{inc}, and *pim* (Maki et al. 1993; Singer and Maki 1993; Alcalde et al. 2000).

The genetic basis of the tall (*Le*)/dwarf (*le*) difference was first identified by Mendel (1866). The *Le* locus is probably the best known of the internode length loci (White 1917). The principal effect of *le* is to reduce the length of the upper internodes by 40–60% by making the stem to appear in zig-zag (Blixt, 1972). However, the *Le* locus is also reported to have a minor pleiotropic effect on flowering behavior (Rasmusson 1935; Barber 1959; Marx 1975). Overall, five major gene loci, *Le/le*, *La/la*, *Cry/*cry^{c}*/*cry^{1}, *Na/na*, and *Lm/lm* are known to administrate internode length in peas. Combinations of the different alleles at these loci decide the phenotype of a plant (tall, dwarf, cryptodwarf, slender, nana, and micro). A new phenotype cryptotall (*Le la* cry^{c} *Na Lm*) was described in which the *na* mutant was completely epistatic to the *Le/le* gene pair (Reid et al. 1983).

The branching is controlled by two single recessive genes, *Fr* and *Fru (fructicosa)* in presence of each other, which are located in chromosome 3 and 4, respectively (Blixt 1968; Lamprecht 1950). The *ramosus* mutant *rms* was

obtained after X-irradiation treatment (Blixt 1976). Six *ramosus* loci have been identified among 16 induced, single-gene recessive, branching mutants: *ram*, *rms-1, rms-2, rms-3, rms-4*, *and rms-5*. The *ram* mutant is characterized by profuse branching and a large number of poorly fertile flowers. *Rms1* is one of the series of six *ramosus* loci in pea in which recessive mutant alleles confer increased branching at basal and aerial vegetative nodes. The *rms2*, *rms3*, and *rms4* mutants differ from wild-type plants mainly in regard to increased lateral bud release and growth. All mutants are responsible for the increase in number of branches (Blixt 1976; Arumingtyas et al. 1992; Murfet and Reid 1993; Beveridge et al. 1994, 1996, 1997). In addition, most flowering and internode length genes have significant effect on branching habit. Further, three loci have been identified that influence the angle of growth of stem braches. The dominant alleles *ASc* (*Ascendens*) cause braches to grow semiprostrate, the recessive *ho* (*horizontalis*) causes lateral branches to grow horizontally, and the *pro* (*procumbens*) is said to cause stem branches to grow at first horizontal but subsequently at approximately 45° angle (Lamprecht 1963; Murfet and Reid 1993). In the beginning of twenty-first century a further *Ramosus* locus, *Rms6*, with two recessive or partially recessive mutant alleles, *rms6*–1 and *rms6–2* have been identified, which is characterized by increased branching from the basal node (Rameau et al. 2002) and is reported to be derived from dwarf and tall cultivars, respectively.

Generally, the stem of peas is either round or angular and hollow (Mohan et al. 2013), and rarely fascinated. Stem fasciation in peas is reported for the first time in 1597 (Święcicki 2001), and is reported to change the stem architecture along with the physiology of flowering and maturity (Gawłowska and Święcicki 2016). It has been shown that this character is controlled by one to four independent genes or multiple alleles of a single locus (Scheibe, 1954; Marx and Hagedorn 1962; Blixt 1972; Lamprecht 1974; Gottschalk 1977; Święcicki 2001). The most popular was the acceptance of two independent genes—*fa* in LG IV and *fas* in LG III responsible for fasciated character (Lamprecht 1974; Blixt 1977). White (1917) was the first to ascribe a gene symbol, in this case *Fa* for the wild-type form. Since then, another gene for fasciation (*FAS*) has also been documented (Sinjushin and Gostimskii 2008). Additionally, a similar mutation type, *dichotomous branching*, was selected and reported as a character governed by two polymeric genes *bif1* and *bif2* (Gottschalk and Wolf 1983); this alteration was associated with a fasciation of only a few upper nodes that results in a forked stem (Gawłowska and Święcicki 2016).

The wild-type pea leaf is pinnately compound and consists of basal, foliaceous stipules, proximal leaflets, and distal tendrils (Yaxley et al. 2001). The character of leaves, leaflets, stipules, and tendrils are governed by single recessive gene (Amin et al. 2010). The replacement of the tendrils in the leaf of wild type by leaflets is controlled by a single recessive gene tendril (*tl)* and creates "*acacia"* phenotype (de Vilmorin and Bateson 1911). On the contrary, the single recessive gene *afila* (*af*) converted leaflets into tendrils (Kujala 1953; Goldenberg 1965). The two recessive genes *af tl* altered the identity of leaf pinnae, afila (*af*), and acacia (*tl*) (Villani and DeMason 1997, 2000). The gene *stipuleless* (*st*) reduces stipule size dramatically

resulting in a narrow strap-like organ (Pellew and Sverdrup 1923; Yaxley et al. 2001). Another allele *st*^{bs} (butterfly stipules) causes intermediate size of stipules between WT and *st* (Apisitwanich and Swiecicki 1992). Several other genes have been identified such as *uni*, *uni*^{tac} (*uni-tacluni*), and *apu*, which modify the form of the leaflets or tendrils.

The gene *unifoliata* (uni) replaces multiple leaflets and tendrils by single leaflet. In *uni*^{tac} plants, the terminal tendril is replaced by a laminate leaflet (Marx 1986, Sharma 1972, Sharma 1981, DeMason and Schmidt 2001; Yaxley et al. 2001). In *apu* (*apulvinic*) plants, the leaflets are produced on stalk or petiolules (Harvey 1979; Marx 1987; Naidenova 2000). In the *cochleata* (coch) phenotype stipules is often replaced by stalked leaflet, while in *coch*^{het} (*heterophyllus*), the stipules differ in size and are often reduced (Rozov et al. 1992; Wellensiek 1959). There are other genes like *sil*, *cri*, *Arg*, and *Td* that influence both the stipules and leaflets, like the *sil* causes undulation in the margins of leaflet and stipule (Marx 1977); the *cri* mutation causes crinkle in leaflet and stipules (Lamm 1949); the *Arg* causes the leaves to appear silvery grey (Hoch et al. 1980), while the incompletely dominant gene *Td* causes dent on the stipule and leaflet margins (Wellensiek 1925).

The seed coat pigmentation in peas is due to photosynthetic pigments (Chlorophyll, carotenoids, and xanthophylls) as well as phenolic pigments, notably flavonoids (Marx 1977; McCallum et al. 1997). The gene responsible for cotyledon color was designated as *I* by White (1917), which is reported to retain chlorophyll in the seed. Therefore, the mature wild-type (*II*) seeds are yellow because of degradation of the chlorophyll, whereas the seed remains green in genotype with *ii allele*. In dried field pea, the seed color is contributed by the seed coat and cotyledons. Several other loci have been reported to affect seed senescence and color retention in pea, notably *pa*, *gla*, and *vim* (Blixt 1962; Weeden and Wolko 1990). Lamprecht (1959) suggested that seed coat and cotyledon color in dry seed of *aa* (white flowered) pea genotypes are determined by the action of the genes *I*, *o*, and *gla*. Dribnenki (1979) concluded that at least three genes were involved in imbibition rate, green seed coat color, and cotyledon bleaching resistance (Blixt 1962; Lamprecht 1959; Marx 1977; McCallum et al. 1997).

The phenotype is somewhat variable: wild-type seeds that dry out early sometimes retain green color, whereas green *ii* seeds can sometimes bleach (Ellis et al. 2011). It was also observed that not only cotyledon in dry seed exhibit a green color but also senescing leaves remain green, as do detached leaves placed in the dark (Armstead et al. 2007; Sato et al. 2007; Aubry et al. 2008). This was the result of reduced chlorophyll breakdown during dark incubation (Sato et al. 2007). The corresponding gene, homolog of *Stay-Green* (SGR), has been identified based on candidate gene approach using knowledge from rice and *Arabidopsis* SGR appears to direct chlorophyll to the degradation pathway (Armstead et al. 2007; Sato et al. 2007). Mendel noted that colored seed coats were always associated with colored (purple) flowers. White flower in cultivated forms of pea is common but wild type had purple flowers. On the other hand, a clear or colorless testa was always associated with white flowers and the absence of pigmentation in the leaf axils, suggesting that these were pleiotropic effects of a single gene. The symbol for gene

that determines the accumulation of anthocyanin pigmentation throughout the plant, most notably in flowers, is *A* (von Tschermak 1912; White 1917; Hellens et al. 2010).

The purple color that accumulates in the flower of wild type is due to the anthocyanin (compounds derived from phenylalanine). The mutation in (*a*) gene abolishes anthocyanin pigmentation throughout the plant (Symkal 2012). A second locus conferring white flowers, *a2*, has been identified from a mutagenesis study (Marx et al. 1989). Previous investigations in pea have suggested that the white flower is determined by the recessive allele *a* (Harker et al. 1990), while the pink and rose color flower trait is governed by gene *b* and *ce,* which is dependent on "*a*" for manifestation of color (Amin et al. 2010; Mohan et al. 2013).

The color of the immature pods is controlled by gene *GP* (White 1917), which imparts green (*GpGp*) or yellow (*gpgp*) color to the pods (Ellis et al. 2011). The structural analyses revealed the role of plastids in differences such as the plastids of yellow pod (*gp*) had single and paired membranes, while the plastids of green pods (*GP*) were lacking grana and contained only 5% of the chlorophyll of the wild-type green pods. However, the *gp* mutation did not change the chloroplasts in the endocarp of the pods (Price et al. 1988; Reid and Ross 2011; Smýkal 2014). In contrast to *I* locus where the wild-type dominant form is yellow and the recessive mutant form is green, for the *Gp* locus, the wild-type dominant form is green and the recessive mutant form is yellow. This suggests that the mutant form *i* represents a failure of chlorophyll degradation, whereas the mutant form *gp* fails to develop a normal chlorophyll complex in the pods (Price and Hedley 1988; Ellis et al. 2011). The purple pod color is governed by two dominant genes *pu*and *pur* (Amin et al. 2010).

The pods of wild type dehisce at maturity, scattering the seeds, whereas the pods of cultivated types are usually nondehiscent, which is determined by *Dpo* (dehiscent) and *dpo* (non-dehiscent) alleles (Marx 1971). Mendel (1866) referred to the form of the ripe pod as either inflated or deeply constricted (with the pod being quite wrinkled in appearance). Wild-type pods are inflated, with a complete layer of sclerenchyma on the inside of the pod wall. The complementary genes *P* and *V* interact to control formation of a tough, sclerenchymatous layer inside of the pod wall: *P V* has a complete membrane, *P v* has patches of sclerenchyma, *p V* has a band of sclerenchyma along the region near the ovule-bearing suture and recessive mutants of both *p v* lack a complete layer of sclerenchyma in the endocarp of the mature pod, and their pods are deeply constricted, because they are inflated only in those areas where the seeds have filled (Murfet and Reid 1993).

The inflated versus constricted pod phenotype refers to the presence or absence of a layer of lignified cells (sclerenchyma) near to the epidermis of the pod wall and is referred to as parchment (Ellis et al. 2011). The *n* mutation results in a thick, fleshy pod wall (Amin et al. 2010). Pea pods have a cord of lignified sclerenchymatous fibers along both sutures. Two recessive mutations, that is, *sin* and *sin-2*, results in stringless pods (Wellensiek 1971; McGee and Baggett 1992). The expression of *sin-2* is dependent on high temperature and the combination of *p v n sin-2* results in a snap pea with a pod that can be eaten even when fully inflated (Wellensiek 1971;

Murfet and Reid 1993). The shape of the pod apex is controlled by the genes *Bt* (blunt) and *bt* (pointed). The recessive gene *te* reduces pod breadth by 25% and allele *Te* is incompletely dominant. The genes *con* and *cp* control curvature of pods in convex and concave pattern, respectively (Amin et al. 2010; Mohan et al. 2013; Lamprecht 1936, 1953). The *twp* causes twisting of the immature pod due to arrested growth in patches of tissue (Marx 1973). The single dominant gene *Np* causes a pustule-like growth on the external surface of the pod (Nuttall and Lyall 1964). The waxiness in pod is operated by single recessive gene, like *wa* causes lack of wax on pods or upper and lower surfaces of stipule and underside of leaflets, *wb* for pods devoid of wax or little wax on rest of plants, and *wel* for no wax on any aerial parts of the plant.

The color of different plant parts, such as foliage, flower, and seed, is also governed by single recessive genes, like for absence of anthocyanin in plants, flower and seed, *ch-l* for light yellow green plant, *d* for green leaf axil, *pa* and *vm* for dark green immature seed and foliage (Amin et al. 2010; Mohan et al. 2013). The two single recessive gene *ar* and *def* influences development of funiculus. The *ar* causes reduced diameter of funiculi and *def* developed a funiculus that remains attached to the seeds even after harvesting (Khangildin and Khangildin 1969; Lamichaney et al. 2021c).

The most appropriate approach to combine various desirable quantitative traits is recombination breeding, which is completely hinged on the genetic architecture of the traits (Cockerham 1961; Sood and Kalia 2006). Therefore, intensive efforts have been made to understand the inheritance pattern of different traits and consequently noticed that additive and nonadditive gene effects are instrumental in the inheritance of various yield components (Singh and Sharma 2004; Avcı and Ceyhan 2006; Burstin et al. 2007; Beeck et al. 2008; Ceyhan et al. 2008). In pea, grain yield is predominantly controlled by additive gene action but nonadditive factors also play significant role (Singh et al. 2006; Kumar et al. 2006a, b). The heritability of yield varies widely depending on the choice of parents, the environmental conditions, and the efficacy of field plot techniques. Various studies suggested positive association of plant height, pods per plant, seeds per pod, seed weight with grain yield. Therefore, selection of these traits may be more effective for breeding point of view (Dixit and Gautam 2015; Singh et al. 2004, 2007; Sharma and Khan 1996; Pandey and Gritton 1975; Krarup and Davis 1970). Likewise, various research groups have accorded high heritability and genetic advance for yield-attributing traits like pod yield/plant, plant height, number of primary branches/plant, indicating suitability of its improvement through hybridization (Sharma et al. 1997; Singh et al. 2007). Sharma et al. (1999) demonstrated the predominant role of nonadditive gene action for pod yield, pods/plant, grain weight, whereas additive gene action for plant height.

High broad sense as well as narrow sense heritability was observed by different research groups for plant height, biological yield, number of pods per plant, and 100-seed weight (Lal et al. 2011; Punia et al. 2013; Kumar et al. 2013). Similarly, Lal et al. (2011) considering association reiterated that pod per plant and harvest index were the most important yield components that could be used as selection

indices for further improvement in field pea. Seed per pod and seed weight are the key yield components after the pod number (Krarup and Davis 1970; Sancha and Singh 1973; Pandey and Gritton 1975). The additive gene effects play predominant role in the inheritance of both seed number and seed weight (Kumar 1973; Snoad and Arthur 1974; Venkateswarlu and Singh 1982). In many reports, ovule number is controlled by a simple, additive genetic system (Marx and Mishanec 1967; Krarup and Davis 1970), while the seed number per pod is under control of a polygenic system of an additive nature (Snoad and Arthur 1973). Thus, such traits can be effectively improved by adopting standard selection procedures like pedigree and pure line breeding methods. Singh and Singh (1990) reiterated the importance of dominance (h) gene effect for yield/plant, pods/plant, and plant height. However, additive, dominance, and epistatic interactions were significantly evident for this attribute. Sharma and Rastogi (2001) recorded significant additive and dominance gene effects for all the traits. However, duplicate type of epistasis was more prominent for plant height and leaf area, whereas complementary type of epistais was also recorded. The preponderance of nonadditive gene effects for plant height and leaf area indicates that a poor gain under selection may be expected for these traits. Dixit et al. (2006) reported that additive, dominance, and epistatic gene effects play important role in the inheritance of these traits.

Punia et al. (2011) indicated dominance and epistatic gene interactions to play major role in the inheritance of yield and yield-attributing traits. The additive × additive (i) and dominance × dominance (l) digenic interactions are important as compared to additive × dominance (j) for seed yield and its component traits. Duplicate-type epistasis played a bigger role than complementary epistasis. Overall, the nonadditive types of gene action are important for most of the traits, thereby suggesting that selection at later segregating generations could provide better results. In another study Punia et al. (2013) reported that days to flowering, days to maturity, pods per plant, seeds per pod, seed yield per plant, and harvest index are controlled by more of dominant gene. Similarly, Rebica et al. (2013) noticed, nonadditive gene effects for pods per plant, pod length, seeds per pod and seed yield per plant, but additive gene effects for days to 50% flowering, plant height and 100-seed weight.

Kosev (2013) reported that the additive gene effects play important role in the inheritance of seeds per pod, seed weight per plant, and seed weight whereas plant height, first pod-bearing node, pods per plant, seeds per plant, nodes per plant, fertile nodes per plant have an influence of nonadditive genetic interactions. Kosev (2015) reported that epistatic gene effects controlled all traits except plant biomass and number of fertile nodes per plant, suggesting selection in later generations for plant biomass. The characters, such as seeds per pod, seed yield per plant, pod length, and harvest index, showed high GCV, heritability, and genetic advance would be more helpful in formulation of selection strategy for prediction of the gain under selection (Lal et al. 2019). Overall to exploit all three types of gene effects (additive, dominance, and epistatic) reciprocal recurrent selection may be adopted for developing elite population for selection of high-yielding lines in advanced generations. It will also lead toward an increased variability in later generations for effective

selection by maintaining considerable heterozygosity through mating of selected plants in early segregating generations.

In case of biotic stresses, host plant resistance is the most appropriate, efficient, and economic strategies. Therefore, extensive efforts have been made to understand the inheritance of biotic stresses. Among biotic stresses, powdery mildew (Smith et al. 1996; Kraft and Pfleger 2001), rust (Singh et al. 2015a, b; Rubiales et al. 2019), ascochyta blight (Liu et al. 2013; Tran et al. 2014), fusarium root rot (Hamid et al. 2013; Porter et al. 2015), fusarium wilt (Sharma et al. 2010; Rubiales et al. 2015), and common root rot (Pilet-Nayel et al. 2005; Desgroux et al. 2016) are the serious constraints affecting field pea across the countries of the resistance to powdery mildew and are reported to be governed by two recessive (*er1 and er2*) and one dominant (*Er3*) gene (Heringa et al. 1969; Fondevilla et al. 2007a). A recent study indicates that resistance provided by *er1* is due to a loss of function of PsMLO1, an MLO (Mildew Resistance Locus O) gene (Humphry et al. 2011). The gene *er2* (Heringa et al. 1969) confers complete resistance that was effective in found to be location specific (Tiwari et al. 1997; Fondevilla et al. 2006). Gene Er3 has been recently identified in *Pisum fulvum* and successfully introduced into adapted *Pisum sativum* material (Fondevilla et al. 2007a, 2010). Resistance toward rust is governed by single dominant gene (*Ruf*) (Katiyar and Ram 1987; Tyagi and Srivastava 1999; Vijayalakshmi et al. 2005). In addition to the reported oligogene *Ruf*, the polygenic nature of gene action has also been reported for rust resistance (Singh and Ram 2001). Recently, partial dominance of single gene along with minor and 2–3 additive genes has been reported (Singh et al. 2012).

The nature of inheritance for ascochyta blight (AB) and fusarium root rot resistance is reported to be polygenic (Fondevilla et al. 2007b; Carrillo et al. 2014; Jha et al. 2017; Kraft 1992). The resistance to pea enation mosaic virus and *Fusarium oxysporum* f. *pisi* (race 1 and 2), brown root of peas, *Fusarium solani* f. sp. *Pisi*, downy mildew, bacterial blight (race 1), and pea root rot is governed by single dominant gene. On the contrary, resistance to pea seed-borne mosaic virus (*sbm*), bean yellow mosaic virus (*mo*), pea mosaic virus (*pmv*), and bean virus is operated by recessive gene (Mohan et al. 2013, Amin et al. 2010; Kalloo 1993). The pod resistance for pea weevil is quantitatively controlled, whereas the seed resistance is operated by three major recessive alleles (*pwr1*, *pwr2*, and *pwr3*) (Byrne et al. 2008a, b). The inheritance pattern of different biotic and abiotic stresses is briefly given in Table 25.4. In case of abiotic stresses, heat, drought, and frost are the important stress, which substantially affects the yield potential of field pea (Parihar et al. 2020a, b), which are reported to be controlled quantitatively (Iglesias-Garcia et al. 2015; Huang et al. 2017; Klein et al. 2014).

Table 25.4 Inheritance pattern of biotic and abiotic stresses in filed pea

Traits	Inheritance pattern	References
Powdery mildew resistance *(Erysiphe pisi)*	Single recessive gene *er1*	Heringa et al. (1969), Harland (1948), Pierce (1948), Saxena et al. (1975), Tiwari et al. (1997), Fondevilla et al. (2006) and Humphry et al. (2011)
	Single recessive gene *er2*	Heringa et al. (1969), Ali et al. (1994a, b), Tiwari et al. (1997) and Fondevilla et al. (2006)
	Single dominant gene *Er3*	Fondevilla et al. (2007a, b, 2010), Fondevilla et al. (2008) and Fondevilla and Rubiales (2012)
Rust (*Uromyces viciae-fabae)*	Single dominant gene (*Ruf*)	Katiyar and Ram (1987), Tyagi and Srivastava (1999) and Vijayalakshmi et al. (2005)
	Polygenic	Singh and Ram (2001), Singh et al. (2012), Rai et al. (2011) and Barilli et al. (2018)
Ascochyta blight (*Mycosphaerella pinodes*)	Polygenic	Fondevilla et al. (2007b), Prioul et al. (2004, 2007), Xue and Warkentin (2001), Carrillo et al. (2014), Timmerman-Vaughan et al. (2016) and Jha et al. (2017)
Fusarium root rot (*fusarium solani* f. sp. *pisi)*	Polygenic	Lockwood (1962), Muehlbauer and Kraft (1973), Kraft (1992), Hance et al. (2004) and Feng et al. (2011)
Fusarium wilt (*Fusarium oxysporum*. f. Sp. *pisi)*	Races 1, 5, and 6 (single dominant genes) and race 2 (quantitative)	Mcphee (2003), Hagedorn (1989), McPhee et al. 1999, Bani et al. (2012, 2018), McPhee et al. (2012) and Rispail and Rubiales (2014)
Common root rot (*Aphanomyces euteiches)*	Polygenic	Marx et al. (1972), Pilet Nayel et al. (2002, 2005), Hamon et al. (2011, 2013) and Lavaud et al. (2015)
Heat tolerance	Polygenic	Huang et al. (2017)
Drought tolerance	Polygenic	Iglesias-Garcia et al. (2015)
Frost tolerance	Oligogenic and polygenic	Lejeune-Henaut et al. (2008), Dumont et al. (2009) and Klein et al. (2014)

25.11 Major Constraints of Field Pea Production at National and International Level

Like any other crop, field pea is susceptible to many biotic and abiotic stresses that seriously hinder its sustainable production (Parihar et al. 2020a, b). Field pea are prone to number of diseases of which fungal diseases, powdery mildew, rust, ascochyta blight, wilt, and root rots like are most widespread (Parihar et al. 2013; Mahajan et al. 2018). Powdery mildew, potential of reducing seed yield by 25–80% (Munjal et al. 1963; Singh et al. 1978; Warkentin et al. 1996; Ghafoor and McPhee

2012), is caused by *Erysiphe pisi, Erysiphe baeumleri*, and *Erysiphe trifolii* (Attanayake et al. 2010; Fondevilla and Rubiales 2012; Sun et al. 2016). Rust is incited either by *Uromyces viciae-fabae or* U. *pisi* and causes more than 30% yield loss (Barilli et al. 2010, 2018; Singh et al. 2015a, b). Ascochyta blight caused by a complex of fungal species (*Ascochytapisi*, *Peyronellaea pinodes*, *Phomamedicaginis* var. *pinodella*, *Ph. koolunga*and *Ph. Glomerata)* is the most severe disease of field peas distributed worldwide (Bretag et al. 2006; Liu et al. 2013; Tran et al. 2014) with a potential of reducing grain yield by 60 percent (Liu et al. 2016). Fusarium root rot, incited by *Fusarium solani* f. sp. *pisi*, may develop in both dry and wet field conditions and reduces yield significantly under suitable circumstances (Chang et al. 2004; Porter 2010).

Fusarium wilt is caused by *Fusarium oxysporum*. f. sp. *Pisi*, which has about 11 different races (Armstrong and Armstrong 1974; Gupta and Gupta 2019). Of them, races 1 and 2 are widely distributed, while races 5 and 6 are scattered only in some specific regions (Infantino et al. 2006; Bani et al. 2018). Another important soilborne disease of pea is common root rot caused by *Aphanomyces euteiches,* prevalent in USA, Europe, and Canada (Wicker et al. 2003; Pilet-Nayel et al. 2005; Chatterton et al. 2015; Desgroux et al. 2016; Wu et al. 2018), that causes wilting of the roots (Wu et al. 2018). Field pea crop is also damaged by a number of insect pests like pod borer complex, seed damaging pests, leaf feeders, leaf miners, stem fly, aphids, cut worms, etc., which appears in different stages of the crop growth period (Sharma 2000; Yadav and Patel 2015; Yadav et al. 2019). Pod borer, *Helicoverpa armigera*, a pest blessed with a diverse range of host plants, also infests field pea and can readily adapt to new environment which is one of the reasons for its pervasiveness (Djihinto et al. 2012). The pest is widely distributed over Asia, Africa, the Mediterranean region, and Oceania (EPPO 2006). The larvae of pulse pod borer (*Etiella zinckenella)* feeds on seed by boring the pods. Pod damage in field pea by pod borer complex has been reported to be 13.45–40.38% (Dahiya and Naresh 1993).

The pea weevil (*Bruchus pisorum* L.) is another important insect that lays their eggs on pods and larvae bore into the pods and damaged the seed (Brindley et al. 1956). The best way to manage this pest is to develop weevil-resistant cultivars (Clement et al. 2009). The resistant lines and non-host-like *Vicia faba*, *Lathyrus sativus*, *P. fulvum*, and *P. sativum* ssp. *syriacum* holds great promise toward development of pea weevil resistance varieties (Teshome et al. 2015a, b; Mendesil et al. 2016; Fernandez and Rubiales 2019). Bean α-amylase inhibitor has also shown promising results in developing transgenic lines against the pest (Schroeder et al. 1995; Morton et al. 2000). The pea leaf miner, *Phytomyza horticola* Goureau, taxonomically described under the family Agromyzidae of the order Diptera, is a pest of high economic importance (Spencer 1973). It is one among the major insect pests of pea crop (Singh et al. 1992) widely distributed over Africa, Asia, and Europe (Crop Protection Compendium 2007). The pea leaf miner is a serious and persistent pest of peas in northern India (Atwal et al. 1969; Bhalla and Pawar 1977; Prasad et al. 1984). Few species of aphids do infest the field pea among which *Acyrthosiphon pisum* (Bieri et al. 1983) is major one, which sucks the sap mainly

from growing tender shoots, lower side of the leaves, buds, and pods, which debilitate the plant. Ali et al. (2005) reported the field pea line "061 K-2P2/9/2" as the most resistant genotype against this aphid. Melesse and Singh (2012) recommended "Milky" and "Adi" cultivars and NSKE to manage pea aphid. In case of nematodes, *Heterodera species* (cyst nematode), *Meloidogyne* sp. (root knot nematode), *Rotylenchulus species* (reniform nematode), and *Ditylenchus species* (stem nematode) are the important limiting factor and cause severe loss (Vovlas et al. 2011; Lombardo et al. 2011; Leach et al. 2012; Ahmad and Prasad 2012).

In case of abiotic stresses, extremities of temperature (low and high), moisture (drought and flood), and salinity have becomes major concern in sustainable production of dry pea (Guilioni et al. 2003; Karatas et al. 2012; Sadras et al. 2012; Liu et al. 2019; Rubiales et al. 2019; Lamichaney et al. 2021a). This crop has relatively low heat tolerance compared to other cool-season legumes like chickpea and lentil (Siddique 1999), and so very often, the production starts to decline when the maximum day-time air temperature during flowering exceeds 25 °C (Guilioni et al. 2003; Sadras et al. 2012). Several studies have addressed the impact of high temperature on crop growth, physiology, and yields of field pea across the different agroregions (Sadras et al. 2013; Liu et al. 2019; Jiang et al. 2019; Mohapatra et al. 2020). Most recently, Lamichaney et al. (2021a) observed reduction in the seed yield (24–60%), seed germination (4–8%), seed set (7–14%), and 100-seed weight (6–16%) under elevated ambient temperature during vegetative and reproductive stages. Frost stress is another major abiotic stress causing significant problem at vegetative and reproductive stage (Shafiq et al. 2012; Liu et al. 2017). Drought or water stress is a critical environmental constraint that declines quality and quantity of the produce (Ali et al. 1994a, b). The reduction in grain yield by 25% due to moisture stress is reported under field conditions (Sánchez et al. 1998).

25.12 Breeding Progress/Varietal Development

Grain yield improvement is indispensable and continued objective for field pea breeding program along with improvement in plant type, earliness, and resistance/tolerance to multiple biotic and abiotic stresses, like diseases, insect pests, plant parasites ((broomrape), drought, heat, frost, and salinity. Breeding for resistance/tolerance against such stresses has been core objectives for field pea breeders in order to increase and stabilize grain yields. In recent years, efforts have been made toward development of biofortified genotypes especially for protein, iron, and zinc (Parihar et al. 2021a, b). To attain above targets, concentrated efforts have been made in field pea using different breeding approaches, which in detail are elaborated.

25.12.1 Accomplishment Through Conventional Breeding

The overall improvement in productivity of dry pea has been mainly achieved through conventional breeding for tailoring plant type (lodging resistance and

plant height), resistances to key biotic (powdery mildew, rust, ascochyta blight, etc.), and abiotic (heat, drought and cold) stresses (Rubiales et al. 2019). In field pea, plant stature has dramatically been changed from tall and high biomass to dwarf type. Earlier, maximum cultivars were of tall type with high biomass, which caused severe lodging problems that leads to disease induction (Donald and Hamblin 1983). Concentrated efforts were made to incorporate the dwarf gene (*le-1*), resulting in modern dwarf plant type varieties. The dwarf gene resulted due to developmental mutation that shortened the internode length by reducing 3β-hydoxylation of GA_{20} to GA_1 (Ingram et al. 1984; Ross et al. 1989; Martin et al. 1997). An analogous experience was earlier exploited in wheat and rice during *Green Revolution* period, which has association with gibberellin (GA) pathway (Martin et al. 1997). The short internode length also improved the standing ability significantly (Burstin et al. 2007). Inspite of the incorporation of dwarfing traits, the pea plant still lodged due to high biomass (Amelin et al. 1991). Therefore, to reduce lodging, an alternative tactic, that is, development of "semi-leafless" pea cultivars using "*afila*" leaf type, was used, which is considered to be a greatest accomplishment in pea breeding (Amelin et al. 1991; Duparque 1996).

The semi-leafless plant type considerably improved standing ability of pea genotypes, which ultimately condensed grain yield losses (Wang et al. 2002; Banniza et al. 2005, Singh and Srivastava 2015). The crop with better standing ability resulted in proper aeration and reduced humidity, which otherwise remained very high in lodged crop and was very favorable for the development of various diseases like *Ascochyta blight* (Banniza et al. 2005). The first commercial semi-leafless (*afila*) variety was "Solara" developed in 1970s in Europe. The "semi-leafless" cultivars are accountable for about 95%, 80%, and 30% of the total dry pea production in Canada, European Union, and Russia, respectively (Tayeh et al. 2015a, b, c, d). An interesting achievement has been made through trait pyramiding for lodging resistance and reduced pod shattering and consequently the first broadly adapted semi-dwarf cultivar "Kaspa" has been developed, which dominated production across southern Australia (Leonforte et al. 2006; Warkentin et al. 2015).

In last 20 years, a large number of varieties were developed with semi-leafless trait, which helped in enhancement of production of dry pea in India (Dixit and Parihar 2014; Dixit et al. 2014; Gupta and Parihar 2015; Parihar and Dixit 2017; Parihar et al. 2019a). In India, *dwarfing* and *afila* plant type has been extensively used in pea breeding programs and both the traits were successfully transferred in conventional cultivars through hybridization, which ultimately enhanced productivity. The dwarf varieties are more responsive to fertilizers, irrigation, and could be densely planted. In India, the first dwarf and semi-leafless variety HFP 4 (Aparna) was developed in 1988. Toward the end of the twentieth century, a dwarf and landmark variety Malviya matar-15 (HUDP-15) was developed, which is derived from the three way cross (PG 3/S 143)/FC 1, which also showed resistance against powdery mildew and rust (Dixit et al. 2014).

In central part of India and in rice fallows, a short cropping window is available, which however is prone to terminal drought and heat stresses. In such conditions, extra early varieties are the best alternative that matures before the onset of terminal

stresses, and also the field remains available for timely planting of subsequent crops (Dixit et al. 2014). Therefore, breeding efforts were made to reduce the duration of crop, resulting in the development of short duration (100–105 days) high-yielding dwarf varieties like DDR 23, DDR 27, IPFD 99–13, IPFD 11–5, and IPFD 2014–2 (Anonymous 2021). However, there lies scope for further reduction in the duration of field pea crop as Parihar et al. (2021a, b), have identified lines with extra earliness (<100 days), which could be utilized as donor in future breeding program to develop extra early and high-yielding varieties.

In addition to plant type, the productivity of dry pea is limited largely by different biotic and abiotic stresses. The powdery mildew remains a severe bottleneck in dry pea production in most of the pea-growing ecologies (Tayeh et al. 2015a, b, c, d; Sun et al. 2019; Parihar et al. 2020a, b). Considering these facts, with extensive breeding efforts, the PM resistance was first noticed in the landrace "Huancabamba," which is genetically operated by a single recessive gene*er1* (Harland 1948). Further, many resistant accessions were identified over the period and subsequently characterized their gene(s) for resistance to *E. pisi*. Hitherto, three genes *er1*, *er2*, and *Er3* were reported for PM resistance, of which *er1*, *er2* are recessive and *Er 3* is dominant *Er3* (Heringa et al. 1969; Fondevilla et al. 2007c; Parihar et al. 2013). Among these genes, *er1* provides resistant in maximum accessions followed by e*r2*, which operates only in few accessions, whereas *Er3* has been recently identified in *P. fulvum*, a wild relative (Tiwari et al. 1997, Fondevilla et al. 2007a, b, c; Fondevilla and Rubiales 2012). Ascochyta blight (AB) is another serious disease of field pea distributed worldwide (Li et al. 2011; Tran et al. 2014; Rubiales et al. 2019). Till date, none of the cultivated pea could exhibit complete resistance against AB. However, few genotypes were identified with low to moderate level of resistance in cultivated pea (Kraft et al. 1998; Zhang et al. 2006). Interestingly, accessions of wild relatives, that is, *P. fulvum*, *P. sativum ssp. Elatius,* and *P. sativum ssp. Syriacum*, have demonstrated high level of resistance and have been utilized for resistance breeding (Fondevilla et al. 2005; Jha et al. 2012, 2016; Sindhu et al. 2014).

Fusarium root rot is another major limiting factor in dry pea production (Grunwald et al. 2003; Hamid et al. 2013; Porter et al. 2015) and till date source for its complete resistance is not reported, while some sources for partial tolerance have been found (Gretenkort and Helsper 1993; Hwang et al. 1995; Grunwald et al. 2003; Porter et al. 2015). Interestingly, the accessions with pigmented flower are associated with enhanced resistance to root rot (Kraft 1975; Grunwald et al. 2003). Fusarium wilt is a serious production threat worldwide and causes huge loss in dry pea production (McClendon et al. 2002; Sharma et al. 2010; Rubiales et al. 2015; Aslam et al. 2019). The resistance accessions for race 1 and 2 have been reported by McPhee et al. (1999) in pea core collection. Common root rot (CRR) (caused by *Aphanomyces euteiches)* is yet another serious disease of pea. The accessions showing partial resistance to CRR have been reported (Kraft 2000; Kraft and Coffman 2000; Pilet Nayel et al. 2007; Conner et al. 2013), which have been used in breeding programs to develop breeding lines (Roux-Duparque et al. 2004; Moussart et al. 2007) and various experimental populations (Pilet Nayel et al.

2002, 2005; Hamon et al. 2011, 2013; McGee et al. 2012; Lavaud et al. 2015). However, breeding for tolerance to CRR always remains complicated owing to polygenic nature and other associated unwanted traits (Mark et al. 1972; Pilet Nayel et al. 2002). Thus, deployment of modern technologies in regular breeding program is essential to accelerate breeding for CRR-resistant varieties.

Rust, incited either by *Uromyces viciae-fabae* or *U. pisi*, is also an important disease scattered in all pea-growing countries (Barilli et al. 2010, 2018; Rubiales et al. 2011; Singh et al. 2015a, b). Dedicated efforts have been deployed toward screening of germplasm for rust, but unfortunately, so far, none of the genotypes has shown complete resistance (Gupta 1990; Anil-Kumar et al. 1994). However, numerous genotypes have been identified with partial resistance for rust (Vijayalakshmi et al. 2005; Chand et al. 2006; Kushwaha et al. 2006; Barilli et al. 2009). The reported partial resistance sources have been inculcated in pea breeding and developed some high-yielding and partial rust-resistant varieties such as HUDP 15, Prakash, Swati, Aman, Pant P 42, IPF 5–19, IPFD 11–5, IPFD 12–2, and IPFD 9–2.

In case of abiotic stresses, high ambient temperature of more than 25 °C during pea life cycle is reported to negatively affect its production owing to reduced plant growth, flowering nodes, pods per plant, seed set (%), vegetative and reproductive phase, seed weight, and pollen viability (Sadras et al. 2012; Bueckert et al. 2015; Jiang et al. 2015, 2018; Lamichaney et al. 2021a). Considering traits like membrane stability index, plant height, biomass, seed yield, and harvest index, few genotypes were identified showing resistance to high temperature stress (Vijaylaxmi 2013). Lamichaney et al. (2021a) reported that terminal heat stress not only reduced seed yield but also seed quality in terms of its planting value and also identified few promising genotypes considering mean performance and stability for yield and germination efficiency. Drought is an imperative environmental limitation that reduces quality and quantity of the produce (Boyer 1982; Ali et al. 1994a, b; Sánchez et al. 1998). In general, the ability of plants to combat with moisture stress is judged through its yield potential in a specific environment. There are primarily three ways, that is, escape, avoidance, and tolerance of crops to sustain in moisture-restricted conditions (Turner et al. 2001). The mentioned strategies can be used to develop genotypes that would perform well under limited water conditions. The avoidance via escape approach is primarily considering earliness in case of flowering and maturity and as a result, it has become the preferred approach for breeders. But early flowering–early maturing crops cannot respond well under normal moisture conditions and shows a significant reduction in the yield (Khan et al. 1996).

Therefore, early vigor and flowering and good pod setting are important criteria for development of genotypes with drought tolerance (Khan et al. 1996; Turner et al. 2001). Currently, selection pressure is targeted toward high yield potential with earliness and prolonged flowering duration for development of drought resistance genotypes. The drought avoidance is mainly hinged on delayed water loss by various means of, for example, stomatal conductance, leaf area and any nontranspirational water loss from leaves. Due to reduced leaf area, the semi-leafless type has many advantages in water-deficit situations (Rodríguez-Maribona et al. 1990; Alvino and

Leone 1993; Sánchez et al. 2001). The elevated ABA content was also used as a scale for selection of drought-tolerant genotypes in 1980s and successfully adopted in maize and wheat but only in restricted environments (Read et al. 1991). The correlation between growth and osmotic adjustment and turgor maintenance has been observed at seedlings stage in induced water stress condition. The turgor maintenance at the early stages of development could be used to identify drought-tolerant genotypes (Sanchez et al. 2004). So far, limited study has been conducted to address the inheritance pattern of adaptation to drought in pea; however, the drought adaptation in pea is reported to be quantitative and also identified the genomic regions controlling the trait (Iglesias-Garcia et al. 2015).

Frost stress is one of the major abiotic stresses causing significant problem at vegetative and reproductive stage in pea (Shafiq et al. 2012; Liu et al. 2017). Genotypic variation was noticed for frost tolerance in dry pea at seedling (Bourion et al. 2003), vegetative (Lejeune-Henaut et al. 2008), and reproductive stage (Shafiq et al. 2012). Interestingly, few varieties of pea with winter hardiness are found, which are capable of adapting in a wide range of temperature from −8 to −12 °C (Homer and Sahin 2016). The genotypes belonging to winter production regions recorded better cold tolerance as compared to genotypes from spring production regions (Zhang et al. 2016). Efforts were made to incorporate the delayed flowering locus *Hr*, resulting into initiation of flowering after passage of main winter freezing periods may improve the cold tolerance (Lejeune-Henaut et al. 2008, Avia et al. 2013; Dhillon et al. 2010). Liu et al. (2017) have identified a number of accessions tolerant to frost based on their ability to survive. These winter-hardy accessions will play a vital role in breeding of winter-hardy pea cultivar.

The root parasitic weed *Orobanche crenata* is widely distributed in the Mediterranean region and the Middle East and severely affects dry pea production (Rubiales and Fernández-Aparicio 2012). Some levels of resistance were recognized, which is operated by quantitative gene action (Rubiales et al. 2005) and has been successfully transferred to cultivated pea by crossing and selection, resulting in the release of the first resistant cultivars (Rubiales et al. 2009; Fondevilla et al. 2017; Rubiales 2018). Very restricted attempts have been employed toward resistance to insect pest in pea. However, some level of resistance for pea weevil infestation has been found in the cultivated pea and wild relatives (Clement et al. 2002; Teshome et al. 2015a, b; Aznar-Fernández et al. 2018) and successfully transferred into cultivated pea (Clement et al. 2009; Aryamanesh et al. 2012). Likewise, few sources of intermediate resistance against aphid have been reported (Aznar-Fernández and Rubiales 2018). Achievements are in premature stage of exploitation in case of salinity, boron toxicity, and iron deficiency. However, some landrace with improved stress tolerance has been identified in case of boron toxicity (Bagheri et al. 1994; Paull et al. 1992), salinity (Leonforte et al. 2013a) and iron deficiency (Kabir et al. 2012).

Quality attributes of peas cultivated for dry seed have been focused primarily on the visual appearance of the seed, that is, uniformity and intensity of seed color as well as shape. Mainly two types of field pea, that is, seed with yellow and green cotyledons are available in market, of which green seeded dominates the market especially in Canada (Ubayasena et al. 2010). To get highest market grade, green pea

seeds should be naturally green in color with less than 2% bleached seeds. Consequently, cotyledon bleaching during seed maturation or seed storage is a critical factor that determines the value of green pea (Holden 1965; McCallum et al. 1997; Cheng et al. 2004). Bleaching is reported to have negative effect on seed germination and early seedling vigor (Maguire et al. 1973; Loria 1979). Thus, the improvement in bleaching resistance has been an objective of pea breeding worldwide. Sincere efforts have been made to understand the genetics of bleaching and quantitative inheritance, transgressive segregation, and moderately high heritability were observed for seed color, shape, and surface dimpling (Ubayasena et al. 2010, 2011). However, due to dearth of information regarding inheritance of this trait, accurate phenotypic characterization and effects of the environment on the trait have slowed down efforts to deliver improved cultivars.

During recent years, greater attention is being given to improve the nutritional composition of the pea due to its importance in food and feed. In field pea, restricted attempts have been invested to screen the existing released varieties and germplasm for various nutritional parameters. However, ample amount of genetic variability was noticed for Fe, Zn, and Mg (Gawalko et al. 2009; Amarakoon et al. 2012). Notably, numerous promising genotypes with high iron and zinc content have been identified and being used in conventional breeding for development of high-yielding nutritionally rich genotype and development of mapping populations (Parihar et al. 2021a, b). The nutritional quality upscaling may be done by selection of locality specific genotypes and their judicious deployment in conventional breeding for developing location specific biofortified varieties. In addition to the enhancement of seed micronutrient status, the bioavailability of micronutrients could be improved by reducing antinutritional compounds, for instance, phytate, and escalating levels of absorption-promoting compounds, such as xanthophyll, ascorbate, and betacarotene (Hurrell and Egli 2010; Lockyer et al. 2018). The genotype, environment, and their interactions affect the concentration of phytate phosphorus, inorganic phosphorus, and concentration of iron (Delgerjav 2012; Warkentin et al. 2012; Shunmugam et al. 2015). The high carotenoid concentration is part of a biofortification strategy and substantial variability has been reported, higher in seeds with green cotyledon (Ashokkumar et al. 2014). The genotypic and environmental significantly influenced carotenoid content, the amount of which is higher in cotyledon followed by the embryo axis and seed coat (Liu et al. 2015a, b).

In India, rigorous attempts have been made and numbers of high-yielding varieties with dwarf/tall plant type, resistance to powdery mildew, and rust have been developed. For example, in tall category, IPF 99–25 (Adarsh), IPF 5–19 (Aman), IPF 4–9, IPF 16–13, TRCP-9, TRCP 8, Pant Pea 243, VL-42, Pant P-42, and Ambika have been developed during last 20 years. Similarly, in dwarf class varieties such as IPFD 99–13 (Vikas), IPFD 1–10 (Prakash), IPFD 10–12, HFP 9907B, Pant P-74, SKNP 04–09, HFP 529, HFP 715 and Pant P 250, IPFD 12–2, IPFD 11–5, IPFD 2014–2, IPFD 9–2, IPFD 6–3, IPF 16–13, IPFD 12–8, IPFD 13–2 have been developed (Parihar and Dixit 2017; Dixit et al. 2017, Parihar et al. 2019a, b, 2020a, b, 2021a, b; Anonymous 2021).

25.12.2 Distant Hybridization

Crop wild relatives (CWRs) are extensively recognized as a valuable resource for crop improvement, because they are reservoir of genetically important traits due to their wider adaptation to a diverse range of habitats (McCouch et al. 2013; Dempewolf et al. 2014; Smýkal et al. 2017). Currently, main focus of any breeding program is development of high-yielding and climate-resilient genotypes with resistance to prevailing disease and pest. As the genetic variability in domesticated pea has reported to be low, recently attention is being given to prebreeding to induce and increase the variation for further genetic enhancement (Sharma et al. 2013). In case of pea, approximately 98,000 accessions of pea germplasm are available worldwide, of which, only a small proportion (less than 1%) represent wild pea (Smýkal et al. 2013). The genetic diversity of cultivated pea germplasm has been extensively studied during recent past (Jing et al. 2010; Smýkal et al. 2011, 2013; Holdsworth et al. 2017); however, a limited number of wild peas have been evaluated (Kosterin and Bogdanova 2008; Polans and Moreno 2009; Jing et al. 2010; Holdsworth et al. 2017).

Wild pea forms have large potential to be used as a donor of several agronomically important traits such as *P. fulvum,* which has resistant toward the pea weevil (Clement et al. 2002, 2009; Byrne et al. 2008a, b; Aryamanesh et al. 2012, 2014), rust (Barilli et al. 2009, 2010), powdery mildew (Fondevilla et al. 2007b) and ascochyta blight (Fondevilla et al. 2005; Carrillo et al. 2013). Notably, *P. sativum* subsp. *elatius* also showed resistance for pea weevil (Berdnikov et al. 1992). In addition, some of the wild forms of the cultivated pea (*P. sativum* subsp. *elatius*) showed resistance against nematode *Heterodera goettigniana* (Vito and Perrino 1978), broomrape *Orobanche crenata* (Valderrama et al. 2004), powdery mildew (Tiwari et al. 1997; Fondevilla et al. 2007a, 2008, 2011a, b; Fondevilla and Rubiales 2012; Cobos et al. 2018), rust (Barilli et al. 2010, 2018), *Fusarium* wilt (McPhee et al. 1999; Hance et al. 2004), PSbMV virus (Konečná et al. 2014), and white mold (Porter et al. 2009). Likewise, accession AWP 442 (*P. elatius*) and AWP 600, AWP 601 ((*P. fulvum*) have been identified, which show complete resistance against pulse beetle (*Callosobruchus chinensis* L.), could be used in breeding programs for development of resistant cultivars (Esen et al. 2019).

To enhance seed protein quality, a double null mutant for the two closely linked genes encoding TI1 and TI2 seed protease inhibitors has been identified in *Pisum elatius*. This mutant has extremely low seed protease inhibitor activity and introgression of the mutation into cultivated germplasm has been achieved (Clemente et al. 2015). The CWR, for instance, *P. fulvum* and *P. sativum* subsp. *Elatius*, are recognized as promising genetic resources for abiotic stress tolerance including drought and temperature extremities (Ali et al. 1994a, b; Coyne et al. 2011; Naim-Feil et al. 2017).

The use of natural pea diversity from CWR in pea breeding is hampered by reproductive barriers existing not only between different species but even within *P. sativum*. However, interspecific crosses have been attempted between the cultivated pea (*Pisum sativum)* and wild relatives such as *P. elatius*, *P. humile* and

P. fulvum, and observed that *P. elatius* and *P. humile* are conspecific with *P. sativum*. Due to chromosomal incompatibility between *P. fulvum* and *P. sativum*, hybrid seed could not be produced (Ben Ze'ev and Zohary 1973; Smartt 1984; Errico et al. 1996), while fertile hybrids seed can be produced when *P. fulvum* serves as male parents (Ben Ze'ev and Zohary 1973; Muehlbauer and Kaiser 1994). Later, intergeneric and interspecific crosses of *P. sativum* × *L. sativus* and *P. sativum* × *P. fulvum,* respectively, have been attempted, which recorded strong cross-incompatibility. Conversely, the interspecific crosses successfully produced hybrid without bridging cross, which has also been confirmed through different in vitro techniques such as flow cytometry, isoenzymes, molecular approaches, and GISH (Campbell 1997; Ochatt et al. 2004). Advanced introgressed populations using *P. fulvum* as pollen donor with pea weevil resistance have also been developed (Fondevilla et al. 2007a, b, c; Byrne 2005). The *P. fulvum* accession ATC113 (PI 595933) has been successfully crossed with *P. sativum* and produced interspecific pea weevil-resistant lines (Byrne 2005; Byrne et al. 2008a, b). The introgression of pea weevil resistance into cultivated field pea was further demonstrated in advanced backcross lines of the original population (Aryamanesh et al. 2012). Therefore, an interspecific hybridization approach has potential for developing pea cultivars with resistance to pea weevil.

Unfortunately, the nuclear–cytoplasmic incompatibility has been displayed in crosses of *P sativum* subsp. *elatius* accession VIR320 when used as a cytoplasm donor with most of the cultivated *P. sativum* representatives. The produced hybrids were sterile with chlorophyll deficiency, chlorophyll variegation, and reduced leaflets and stipules (Bogdanova and Berdnikov 2001). Analysis of plastid DNA markers showed that the incompatibility is mainly owing to improper functioning of plastids rather than mitochondria (Bogdanova and Kosterin 2006, 2007). The nuclear–cytoplasmic incompatibility is operated by two unlinked nuclear genes *Scs1* and *Scs2* and located on LG III and LG V, respectively (Bogdanova et al. 2009). Some progress has been made in the introgression of genes for resistance from *P. fulvum* into the genome of the cultural species (Fondevilla et al. 2010). When wild pea VIR320 was used as cytoplasm donor, the *Scs1* allele from the cultivated pea is gametophyte lethal and sporophyte recessive lethal. The *Scs2* allele from the cultivated pea reduced male gametophyte viability. In homozygous situation, *Scs2* from cultivated parent bring nuclear–cytoplasmic inconsistency and reduced pollen fertility by 20%, while in heterozygous condition for either of the genes *Scs1* and *Scs2* had subsidized pollen fertility by 50 and 30%, respectively. Genetic mapping demonstrated that the gene *Scs1* has flanking marker located at 2.5 cM on LG III and *Scs2* gene has flanking markers positioned at varied distance from cross to cross in the range of 2.0–15.1 cM on LG V (Bogdanova et al. 2012). Interestingly, the different wild peas differ in hybrid sterility in reciprocal crosses with cultivated pea depending on alleles of a nuclear "speciation gene" involved in nuclear–cytoplasmic compatibility (Bogdanova et al. 2014). Bogdanova et al. (2015) reported that the nuclear–cytoplasmic conflict is associated with nuclear and plastid candidate genes acetyl-CoA carboxylase beta subunit.

In recent past, Bobkov and Selikhova (2017) had successfully made interspecific crosses between *P. sativum* × *P. fulvum*, while the crosses *P. fulvum* × *P. sativum* resulted in the formation of seeds with unfilled embryos. The hybrid nature of *P. fulvum* × *P. sativum* plants was confirmed using biochemical and morphological markers. Kosterin et al. (2019) shed light on reproductive compatibility of *P. fulvum* in both directions with *P. abyssinicum*, *P. sativum L. subsp. elatius* and *P. sativum L. subsp. sativum*. They noticed that the cross ability with *P. fulvum* as the female parent was very poor, but at least some viable seeds were obtained in all crosses. The sum of evidence available suggests that *P. fulvum* does not differ from *P. sativum* by reciprocal translocations. Most recently, the presence of identified cultivated isoforms of storage proteins in all studied lines of BC_2F_5 interspecific hybrids of *P. sativum* × *P. fulvum* indicated the possibility of using the *P. fulvum* in pea breeding program (Bobkov et al. 2020). The realistic exploitation of CWR of peas is hindered by inadequate knowledge of their diversity (Kosterin 2016). Therefore, there is urgent need of investment of more intensive efforts to study the useful traits of CWRs and their diversity in natural habitats, which is currently being vanished because of transformation and degradation of plant communities because of direct and indirect human influence. Most importantly, the interspecific hybridization leads to the transfer of both desirable and undesirable genes. Thus, during transfer of genes of interest, utmost care should be taken to stop the transfer of worthless genes. The backcross with cultivars and elite breeding lines encourage the selective introgression of valuable genes. These tactics for transferring desirable alleles are not exclusive, and there are good predictions for their joint use.

25.12.3 Mutation Breeding

Mutation breeding being cheaper, fast, and robust approach has great promise toward creation of quantitative and qualitative variability in crop plants, which along with hybridization leads to creation of new genetic variation that is crucial for the genetic improvement as well as evolution of crops (Sharma and Sharma 2004; Solanki et al. 2011). In plant breeding programs, physical and chemical mutagens have been successfully applied for the development of new varieties with enhanced traits. Till now, more than 3200 mutant varieties have officially been released for commercial use in more than 210 plant species from over 70 countries (FAO/IAEA Mutant Varieties Database). The mutation studies in pea were mainly concerned with spontaneous mutations initially, but subsequently shifted toward induced mutations. In field pea, limited efforts have been invested in mutation breeding as compared to other pulse crops. Nevertheless, several biologically interesting mutants in pea have been released as variety with improvements including increased yield, lodging resistance (*afila* leaf trait), larger seeds, increased protein content, modified maturity, disease, and insect pest resistance and less toxic compounds (Jaranowski and Micke 1985; Micke 1988, 1993; Micke et al. 1985, 1990; Gottschalk 1991; Naidenova and Vassilevska-Ivanova 2004a, b; Vassilevska-Ivanova and Naidenova 2006).

So far, more than 30 varieties of pea have been developed through exploitation of mutation breeding. The highest number of varieties through mutation breeding has been developed in Poland followed by Russian Federation. In addition, few mutants with super-nodulation and nonnodulation have also been reported. So far, natural variation and induced mutation have identified 50 symbiotic regulatory genes in pea (Tsyganov and Tsyganova 2020). Of which, many pea mutants have been characterized at the morphological level (Borisov et al. 1992; Markwei and LaRue 1992; Postma et al. 1990; Sagan et al. 1993, 1994; Tsyganov et al. 1999) and a block at a specific stage of nodule development has been delineated (Voroshilova et al. 2001). Two powdery mildew-resistant mutant, that is, S(*er1mut1*) and F(*er1mut2*), have been induced from Solara and Frilene cultivars, respectively, with ethylnitrosourea (Leitão et al. 1998; Pereira et al. 2001). The further genetic analysis of the novel PMR mutant lines showed that both resistances are inherited as monogenic recessive traits (Pereira and Leitão 2010). Vassilevska-Ivanova and Naidenova (2006) estimated stability and adaptability of waxbloom and waxless pea (*Pisum sativum* L.) mutant lines. The investigated mutants demonstrated diverse response to environments, thus suggesting development of targeted breeding program. Recently, Singh et al. (2015) irradiated two cultivars of field pea viz. HFP-4 and Rachna with different doses of gamma-rays, wherein a considerable amount of genetic variability and heritability recorded for all the traits in both cultivars, which can be exploited through selection. Mutagenesis is also a promising means to identify novel genes and their functional regulations.

The mutants are also playing crucial role in development of various genomic tools such as fast neutron and TILLING populations that have been developed for reverse genetics approaches (Dalmais et al. 2008; Ambrose et al. 2008; Wang et al. 2008a, b; Le Signor et al. 2009a, b). A fast neutron (FN)-mutagenized population was developed in pea using recombinant inbred line, JI 2822 as a parent, to facilitate the identification and isolation of genes underlying the trait (Hofer et al. 2009; Domoney et al. 2013). To develop a *Pisum sativum* TILLING platform, an EMS mutagenized library of 8000 M_2 families derived from the cultivar "Terese" was used (Rameau et al. 1997; Triques et al. 2007). A sufficiently large TILLING population has been developed from the genotype "Cameor" and subsequently the characterization data have been stored in an online database, that is, UTILLdb, which provides phenotypic as well as sequence information regarding mutant genes in public domain. At present, the population size is 4817 lines: 1840 lines have been characterized for phenotypes and 464 mutations have been identified (Ambrose et al. 2008; Dalmais et al. 2008).

Unlike traditional screening methods, TILLING focuses on identification of mutation within gene of interest and subsequent linking of those mutations to a specific phenotype. Though this technique is only feasible when a gene correlated to the trait of interest is well known and the gene sequence is available (Sikora et al. 2011). The major pea mutant collections institutions are as follows: John Innes Collection, Norwich, UK (575 accessions); IPGR collection, Plovdiv, Bulgaria (122 accessions); a targeted-induced local lesions in genomes (TILLING) population of 4817 lines (1840 described by phenotype), and 93 symbiotic mutants for

26 genes involved in nitrogen fixation (Duc et al. 1994; Duc and Sagan 1996; Ambrose et al. 2008; Le Signor et al. 2009a, b). In addition, fast neutron produced deletion mutant resources (around 3000 lines) are available for pea, which have been used in identification of several developmental genes (Ambrose et al. 2008; Le Signor et al. 2009a, b, Jacobs et al. 2010; Smýkal et al. 2012). In addition, mutagenesis approach needs to embrace PCR-based screening techniques and mapping techniques (NGS techniques) for the characterization of mutant lines as well as for the mapping of causal mutations. Furthermore, new approaches such as mapping by mutation (MutMap), mutant chromosome sequencing (MutChromSeq), exon capture, whole genome sequencing, MutRen-Seq, and different tilling approaches need to be used for the detection of induced mutation.

25.13 Breeding Objectives

The main objectives or goals of any crop breeding program are to increase the production of crop with sustainability. The major breeding objectives in field pea are elaborated below:

25.13.1 High Grain Yield

Improvement in grain yield and its stability are considered as key goals in most of the plant breeding programs. Yield is a cumulative effect of many yield attributes such as number of pods per plants, numbers of seeds per pods, pod length, productive nodes per plants, biomass, and seed weight. Therefore, in addition to high grain yield, the above-mentioned yield attributes need adequate attention.

25.13.2 Short Duration or Earliness

Development of extra early and high-yielding cultivars would allow expansion of field pea toward new niches with short season and prone to terminal stresses like rice fallows (Parihar et al. 2021a, b). In addition, such type of varieties will very well fit in different cropping systems, ultimately increasing per unit time and area productivity.

25.13.3 Resistance to Biotic Stresses

The productivity of dry pea is negatively influenced by a large number of biotic stresses including fungal, viral, bacterial pathogens causing diseases and various insect pests and nematodes. Of them, powdery mildew, rust, root rots, wilt, and ascochyta blight are the important fungal diseases affecting field pea across the globe (Mahajan et al. 2018, Parihar et al. 2020a, b) and need continuous attention for

further improvement. In case of insect pest pea weevil, aphids and leaf miner are the serious menace; therefore, resistance and tolerant varieties are the need of the hour.

25.13.4 Resistance to Abiotic Stresses

Temperature extremities (low and high), drought, and frost are the major limiting factors in pea production (Parihar et al. 2020a, b). Therefore, intensive efforts need to be made for identification of resistance and tolerant genotypes and their judicious utilization in regular breeding program.

25.13.5 Biofortified Genotypes

To combat the hidden hunger among the resource poor vegetarian populations of developing and underdeveloped nations, cultivar with high iron, zinc, and protein needs to be developed (Parihar et al. 2021a, b). The use of pea seeds in human and animal nutrition is limited by the presence of antinutritive substances: for example, trypsin inhibitors, raffinose family of oligosaccharides and phenolic compounds (Dvorak et al. 2005; Parihar et al. 2016; Gawłowska et al. 2017). Therefore, reduction of such antinutritional compounds would increase the acceptance of this crop among consumers.

25.13.6 Mechanical Harvesting Amenable Genotypes or Lodging Resistance

Owing to the succulent and hollow stem, field pea lodged at maturity. Therefore, breeding for erect type genotypes has remain a key objective in pea breeding programs (Zhang et al. 2006), which could also help in mechanized harvesting in addition to reduction in disease pressure (Smitchger et al. 2020).

25.13.7 Seed Quality Improvement

Visual appearance of seed is one of the most imperative determinants of market value of the harvested crop. Seed coat color, seed shape, and seed dimpling are the major components of visual seed quality and are considered as important breeding objectives (Ubayasena et al. 2011). Therefore, development of varieties having a potential of resisting the weather damage caused by unfavorable weather variables during maturity is the need of an hour to harvest a seed with good physical appearance.

25.14 Brief of Available Genetic and Genomic Resources

Pea genome is comprised of 7 pairs of chromosomes with haploid size (1C) of 4.45 GB and of which about 50–60% is of high to moderate repetitive sequences with preponderance of Ty3/gypsy family (Novák et al. 2010; Dolezel and Greilhuber 2010; Praca-Fontes et al. 2014; Gali et al. 2018). Unquestionably, the big genome size and high transposable elements have played decisive role in impediment of the development and availability of genomic tools in pea as opposed to other major food legumes (Kreplak et al. 2019). In recent past, considerable improvement has been made in molecular marker development, which to a great extent facilitated diversity analysis, genetic mapping, QTL analyses, and marker-assisted breeding. Several types of markers like morphological, isozymes, RFLP, AFLP, RAPD, SCAR, SSR, IRAP, RBIP, ESTs, and more recently, high-throughput parallel genotyping have been developed and deployed in genetic studies and improvement of pea (Blixt 1972; Hall et al. 1997; Aubert et al. 2006; Weeden and Marx 1987; Timmerman et al. 1994; Tiwari et al. 1993, 1998; Irzykowska et al. 2001; Laucou et al. 1998; Gilpin et al. 1997; Weeden and Boone 1999; Timmerman-Vaughan et al. 2000; Ford et al. 2002; Janila and Sharma 2004; Ek et al. 2005; Konovalov et al. 2005; Smýkal et al. 2008; Katoch et al. 2010; Deulvot et al. 2010; Bordat et al. 2011; Kaur et al. 2012; Mishra et al. 2012; Teshome et al. 2015a, b). Genomic and EST-based SSRs were extensively used for studying genetic diversity and building different genetic maps (Mishra et al. 2012; Sun et al. 2014; Tayeh et al. 2015b).

There is a long history of genetic mapping studies in pea. In 1912, the first genetic linkage map of pea was reported, and the first genetic map was constructed in 1925 with six-linkage group (Wellensiek 1925). Lamprecht (1948) developed a full map with 7 LGs using 37 morphological markers (Rozov et al. 1999). The plenty of genetic markers, such as AFLP (Vos et al. 1995), RAPD (Laucou et al. 1998), retrotransposon (Flavell et al. 1998; Pearce et al. 2000), or EST based (Gilpin et al. 1997), has paved the way toward the development of moderate density linkage maps. The availability of common markers has permitted the integration of the maps derived from different crosses and a consensus map was synthesized from an RIL population, by positioning classical mutants, isozymes, RFLP, RAPD, EST, SSR, and STSM markers on the *Pisum* genetic map (Gilpin et al. 1997; Weeden et al. 1998). On similar note, pea consensus linkage maps were generated using three RIL populations (Hall et al. 1997). Later, a composite genetic map of 1430 cM (Haldane) was built using 239 microsatellite markers in three RIL populations (Loridon et al. 2005), which has been deployed to localize numerous QTLs for disease resistance as well as quality and morphology traits.

Over the years, several linkage maps have been generated using F_2 populations (Dirlewanger et al. 1994; Timmerman-Vaughan et al. 1996, 2005; Hunter et al. 2001; Sun et al. 2014; Timmerman-Vaughan et al. 2004; Barilli et al. 2010) and RIL populations (Pilet Nayel et al. 2002; Hamon et al. 2013; Krajewski et al. 2012; Tayeh et al. 2015a; Bourgeois et al. 2011; Bourion et al. 2010). More recently, a number of functional maps composed of genes of known function have been developed by many researchers (Deulvot et al. 2010; Bordat et al. 2011; De Caire et al. 2012;

Hamon et al. 2013; Sindhu et al. 2014; Duarte et al. 2014a, b; Sudheesh et al. 2014). Consensus maps have been built in order to offer higher mapping resolution and better genome coverage. SNP markers are being preferred by researchers because of their profuse frequency, ease of scoring, and approachable to high-throughput genotyping (Desgroux et al. 2018; Gali et al. 2018; Aznar-Fernández et al. 2020). Numerous SNPs marker have been identified considering sequencing data from 4 (Hamon et al. 2013; Leonforte et al. 2013b; Duarte et al. 2014a, b), 5 (Sindhu et al. 2014), 6 (Bordat et al. 2011), 8 (Duarte et al. 2014a, b), and 12 (Tayeh et al. 2015a) populations.

Illumina GoldenGate (Deulvot et al. 2010; Leonforte et al. 2013b; Duarte et al. 2014a, b; Sindhu et al. 2014), Infinium (Tayeh et al. 2015a), and Sequenom MassARRAY (Cheng et al. 2015) platforms have been adopted for SNP genotyping. The availability of next-generation sequencing permitted detection of thousands of SNPs across the genome, as established by polymorphism studies and genetic map construction. Recently, by employing Illumina Infinium genotyping array, a consensus map was developed, which provides insights into the structure and organization of the pea genome built using 12 RIL populations (Tayeh et al. 2015a). A total of 12,802 transcript-derived SNP markers were positioned on a 15,079-marker high-density, high-resolution consensus map. So far, in total around 58 genetic maps have been constructed using different F_2 or RIL populations (Pandey et al. 2021). The developed individual and consensus genetic maps are valuable genomic tools to strengthen pea as a model for genetics and physiology and accelerate breeding.

Most recently, a new online pea marker database (PMD, www.peamarker.arriam.ru) platform has been developed to hoard/maintain valuable genomic resources including different types of pea gene based markers (Kulaeva et al. 2017). The PMD ver.1 (PMD1) included information for 2484 genic markers (Duarte et al. 2014a, b; Sindhu et al. 2014), while an updated version, PMD2, comprised information of 15,944 markers with advanced attributes (Tayeh et al. 2013, 2015a). Most recently, the whole-genome sequencing data have been produced, which facilitate detection of additional 92,457 SNPs from the pea genome (Duarte et al. 2014a, b; Boutet et al. 2016). In addition, 1000 SNP markers have been transformed to competitive allele-specific PCR (KASP) markers to develop KASP assays for ease of genotyping (Boutet et al. 2016). The SLAF-BSA and DArTseq approaches have been used and identified 12,213 and 14,880 SNP markers, respectively (Zheng et al. 2018; Aznar-Fernández et al. 2020).

The important genomic information accessible through different programs has encouraged the pea community to make speedy progress toward targeted and efficient molecular breeding. The genomics approaches, for example, fast neutron and TILLING method, have been utilized for reverse genetics studies. The fast neutron and TILLING induces deletion and point mutations, respectively, in genomic DNA targets. The pea cultivar "Cameor" (Dalmais et al. 2008) and the germplasm accession "JI 2822" (Domoney et al. 2013) have been treated to build up TILLING populations. The TILLING populations online database platform, that is, UTILLdb (http://urgv.evry.inra.fr/UTILLdb) sharing phenotypic information in

addition to gene sequence of mutant genes (Wang et al. 2008a, b; Dalmais et al. 2008; Zhuang et al. 2012; Domoney et al. 2013; Moreau et al. 2018).

In addition, the bacterial artificial chromosomes (BACs) library is normally used to produce and gather large and steady genomic clones for large genome size crops (Yu 2011). The pea cultivar "Cameor" has been used to build up a BAC library for physical mapping, genome sequencing, positional cloning, and analysis of gene structure and function (Gali et al. 2019). Another BAC library developed from a multidisease-resistant line, that is, PI 269818, which has been used for the isolation and recognition of *Fusarium wilt* resistance genes (Coyne et al. 2007). The availability of pea chloroplast genome sequence could be very crucial in transgenic and evolutionary applications (Smýkal et al. 2012). In 2019, the first chromosome-level reference genome assembly of pea variety *cv.* "Cameor" has been generated using complementary approaches (Illumina short-read sequences, PacBio RSII sequences and BAC library). The reference genome assembly representing approximately 88% of the estimated pea genome size (~ 4.45 Gb). The genome assembly holds 44,756 annotated genes with an average gene length of 2784 bp and an average exon length of 308.5 bp. The annotation also identified 2225,175 repetitive elements representing ~83% of the genome, most of which are transposable elements (Kreplak et al. 2019). This reference sequence holds great promise for accurate and in depth configuration of the pea genome and faster detection of the trait specific genes to accelerate the genomic tools development which ultimately will speed up the crop improvement program.

The transcriptome assemblies of pea are not only helpful in probing specific genes but also play a critical role in creation of high-density genetic maps. Initially, transcriptomic analysis was performed through microarray or RNA sequencing (RNA-Seq) technology during *P. pinodes* infection in pea, thereby 346 differentially regulated genes were identified, which were involved in various metabolism and processes (Fondevilla et al. 2011a, b). Another EST-based microarray analysis in pea recognized changed gene expressions related to oxidative stress and cell death during seed aging (Chen et al. 2013). NGS approach arises as a powerful tool for transcriptome analysis non-model species at reasonable cost. DeepSuperSAGE genome wide transcriptional profiling was carried out to recognize pea genes associated with resistance to *P. pinodes*. In totality, 17,561 UniTags were detected, of them, 509 UniTags expression were significantly altered in infected versus non-infected plants (Fondevilla et al. 2014). By using Massive Analysis of cDNA Ends (MACE), positional and expressional candidate genes were identified for resistance to *P. pinodes* in pea (Winter et al. 2016). Franssen et al. (2011) developed a comprehensive gene expression atlas of pea by sequencing of above-ground organs of pea cultivar "Little Marvel." Similarly, transcriptome sequencing was performed using multiple tissues of the field pea genotypes Kaspa and Parafield that vary in terms of seed and plant morphological characteristics (Sudheesh et al. 2015).

Alves-Carvalho et al. (2015) also performed transcriptome analysis using various plant tissues harvested at different growth stages planted under distinct nitrogen levels. Liu et al. (2015a, b) sequenced seed RNA libraries for one vegetable and one grain pea cultivar, from which 459 and 801 differentially expressed genes have been

discovered at the early and late seed maturation stages, respectively. By RNA sequencing of below-ground organs of pea-like nodules and root tips using an Illumina GAIIx system, followed by de novo transcriptome assembly using the Trinity program obtained about 58,000 and 37,000 contigs from "Nodules" and "Root Tips" assemblies, respectively (Zhukov et al. 2015). In further analysis, approximately 13,000 nodule-specific contigs have been found, which were known as plant-protein-coding sequences, of them, 581 sequences possessed full CDSs and considered as novel nodule-specific transcripts of pea. RNA sequencing time series analyses was performed using frost-tolerant (Cv. Champagne) and frost-sensitive (cv. Térèse) under low temperature and control condition, which led to identification of 4981 differentially expressed genes (Bahrman et al. 2019). These assemblies play instrumental role in the identification of markers and loci associated with their traits of interest (Kulaeva et al. 2017). Transcriptome analysis provides extensive information regarding genes expressions and has been uploaded to the NCBI database platform, and it facilitates users to execute BLAST searching and to study gene polymorphism.

25.15 Marker-Assisted Breeding (MAB)

Availability of closely linked molecular markers or QTLs with the target traits is the prerequisite for MAB. Remarkably, the deployment of molecular markers in plant breeding accelerates the generation of new varieties by helping plant breeders in early selection of desirable individuals using at least one pair of markers flanking a single target gene/QTL (Tayeh et al. 2015a). A number of genes/QTLs responsible for the genetic regulation of yield attributes, seed protein, and biotic and abiotic stress tolerance are available, which have been elaborated by Pandey et al. (2021). Genome-wide association (GWA) mapping has recently emerged as an important method to refine the genetic basis of polygenic resistance to plant diseases, which are being used in integrated strategies for durable crop protection (Desgroux et al. 2018). Specific markers associated with major genes have been developed for use in breeding, particularly for trypsin inhibitors (Page et al. 2002; Duc et al. 2004), flowering (Weller and Ortega 2015), and resistance powdery mildew (Reddy et al. 2015; Cobos et al. 2018), pea enation and seed-borne mosaic virus (Scegura 2017; Grimm and Porter 2020), fusarium wilt (Kwon et al. 2013; Jiang 2013) or QTLs such as lodging resistance (Zhang et al. 2006), rust (Singh et al. 2015a, b; Barilli et al. 2018), Ascochyta blight (Carrillo et al. 2014; Jha et al. 2015a, b; Jha et al. 2017), Fusarium root rot (Feng et al. 2011; Coyne et al. 2019) and common root rot (Lavaud et al. 2015; Desgroux et al. 2016) are available for MAB. Recently, marker-assisted backcrossing (MABC) has been used successfully for the introgression of QTLs for *Aphanomyces* root rot resistance (Hamon et al. 2013) into several recipient lines (Lavaud et al. 2015).

Most recently, the MABC approach has also been deployed for the introgression of three frost tolerance QTLs (Lejeune-Henaut et al. 2008). Several QTLs associated with visual quality traits, including seed coat color, seed shape, and seed dimpling,

have been detected; however, these are highly influenced by environmental conditions (Taran et al. 2003; Ubayasena et al. 2011). Some of the researchers have explained the genetic basis of the iron content in seeds and identified genetic markers and QTL to aid in breeding programmes (Kwon et al. 2012; Diapari et al. 2015; Ma et al. 2017; Gali et al. 2018). Monogenic inheritance of phytic acid-phosphorus (PA-P) concentration has been proposed, which is located with a QTL linked to iron bioavailability (Shunmugam et al. 2015), consequently allowing the development of DNA markers, which facilitates simultaneous selection for low phytate and high iron bioavailability in MAS. In recent past, QTLs for the different macro- and micronutrients, that is, boron, calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus, sulfur, and zinc as well as for seed weight, have been identified (Ma et al. 2017).

The genome-wide SNPs and the genetic linkage map permitted QTL identification for seed mineral nutrients that will serve as important resources to enable MAS for nutritional quality traits in pea-breeding programs. More recently, a high-density linkage map has been developed to map the various seed quality-related traits such as seed shape, seed dimpling, seed weight, grain yield, seed fiber, iron, selenium, and zinc concentrations (Gali et al. 2018). Likewise, Cheng et al. (2015) detected SNPs marker associated with calcium and magnesium concentration. Bangar et al. (2017) detected QTLs for iron concentration, which collectively demonstrated 51% of the phenotypic variance. Recently, Huang et al. (2017) developed a genetic linkage map and reported ten QTLs of which five were associated to flowering traits and yield component traits. Iglesias-Garcia et al. (2015) identified ten quantitative trait loci QTLs associated with the drought tolerance-related traits and accounted 9–33% of the phenotypic variation. The SSR marker associated with the drought adaptation QTLs could be useful for MAS in drought adaptation breeding programs. Lejeune-Henaut et al. (2008) reported six QTL region confirming oligogenic determinism of frost tolerance in pea with a major QTL located in the vicinity of the *Hr* locus. *Hr* is a gene controlling plant response to photoperiod (Weller et al. 2012). By using marker-trait association, out of 672 accessions, sixteen accessions were identified as frost tolerant (Liu et al. 2017). So far limited efforts have been made in MAB, but these reports have proved the usefulness of MAS and MABC in pea improvement. The availability of the reference genome will provide more opportunities to find out the genes of breeding interest and to understand the genetic background at a genome-level by deploying molecular markers amenable for high-throughput genotyping.

25.16 Coordinated System of Testing

The coordinated research program of field pea in India is being executed under the umbrella of AICRP on MULLaRP (Mungbean, Urdbean, Lentil, Lathyrus, Rajmash and Pea), which was established in 1995 and administered by the Indian Council of Agricultural Research (ICAR). The AICRP on MULLaRP has a large network of cooperating centers, covering major pulse-growing states of the country. These centers are involved in carrying out activities and strategic research in the area of

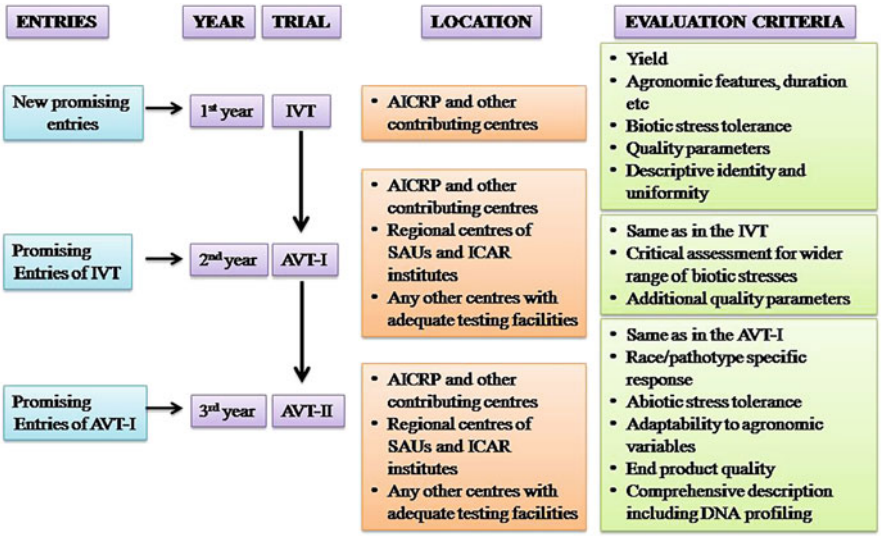

Fig. 25.3 Flow chart for testing entries under the AICRP on MULLaRP

crop improvement, production, and protection. The testing of entries for grain yield is being conducted as per revised guidelines for testing of varieties (Tandon et al. 2015) under All India Coordinated Research Project, wherein the proposed entries are tested in initial varietal trial (IVTs), advanced varietal trial-I (AVT-I), and advanced varietal trial-II (AVT-II) at multiple locations in different zones, that is, NHZ, NEPZ, NWPZ, and CZ (Fig. 25.3).

The IVTs trials are formulated with the new candidate entries submitted by cooperating breeders/institutions including zonal and national checks. The total number of test entries should be optimum according to experimental design so that trial may be implemented with more precision. At least three check varieties consisting of national, zonal, and local check shall be used continuously for three years for proper comparison with the test entries. The seeds of candidate entries and checks must be genetically pure and true-to-type and should fulfil minimum seed certification standards. The experimental design, plot size, and number of replications shall be finalized in the Annual Group Meet considering the experience gained from the precedent trials to condense experimental error. The plot size and number of replications should be harmonized at all the test locations/zone/ecology. Besides, sufficient scope for date of planting, seed rate, sowing depth, plant geometry, nutrient and water management, weed, insect-pests, and disease management shall be given in the technical program and that should be supplied to all the test locations well in time. The testing centers must be recognized in the workshop and that could be ICAR Institutes/SAUs/Main or Regional Research Centres/Zonal Research Centres/State Govt. centres, where a multidisciplinary team of scientists exists with adequate operational amenities to perform coordinated trials as per the

guidelines. All the trials shall be monitored carefully by a team of scientists deputed by the Project Director/Coordinator. The team shall visit the testing locations preferably during flowering to maturity time and record comments on the quality of the trials conductance as per the specified norms, and remark on the reliability of data likely to be produced.

Moreover, observations should be noted on the agronomic character such as days to flowering and maturity, plant height, reaction to major diseases and insect-pests, easily measurable grain quality attributes such as color, weight and appearance, etc. The details of traits on which data shall be recorded should be decided during workshop. The data received at the coordination cell shall be crucially ascertained to make a verdict on appropriateness of data for further statistical processing considering the recommendations of the monitoring team. The point of view of the zonal coordinator/concerned breeder, variation from the specified range of sowing date, specified crop management practices for the trial, for example, fertilizer doses, irrigation levels, etc., and any other serious error in conducting trial/data recording/reporting should be considered properly before any final decision. The advancement of candidate entries from IVT to AVT would be firmly on the basis of overall performance/merit of the test entries and benchmarks finalizes in workshop. AVT-I shall be constructed independently for each recognized agroecological zone for the entries promoted from the IVT on the basis of the criteria specified, the repeat entries from the previous year's AVT-I along with the check varieties as per workshop approval.

In AVT-I plot size should be larger than IVT to generate more rational estimates of the yield performance and to subsidize any possible errors of measurements inherent in small plots. The testing location shall be much more than IVT trials in a given zone. Data on disease and insect-pest resistance and other ancillary characters shall be recorded only at the locations where desirable amenities available as specified in the workshop. Data on quality parameters including biochemical and processing properties shall be produced from certain sites in specified laboratories as per workshop recommendation. The promotion of AVT-1 entries must be done primarily based on grain yield superiority with consideration of other specified characters, that is, biotic and abiotic stress tolerance/resistance, quality traits, etc. All the package and practices adopted under the IVT and AVT-I stage shall be followed at AVT-II stage also. The entries, which demonstrated desired superiority over the best check in AVT-II stage, their identification proposal is invited by project coordinator for deliberation in varietal identification committee (VIC). The VIC after detailed deliberation recommends the best entry for release and notification to central subcommittee on crop standard notification and release of varieties for cultivation in specific zones. After notification by Gazette of India, the varieties enter into seed production chain: breeder seed, foundation seed, and certified seed.

25.17 Seed Production and Seed Standards

Quality seed is the most critical agricultural input having a potential of increasing yield by about 15–20%. Quality seed production differs from commercial grain crop production in several aspects. Special operations, precautions, and operations are needed to deploy to produce seed of optimum quality. Proper selection of seed production site is important in producing quality seed of field pea. Generally, it is recommended to undertake seed production in the same climatic area or zone for which the variety has been bred, developed, tested, and recommended. It is very important to ensure that the irrigation facilities are available in seed production site. The land to be used for seed production of field pea shall be free of volunteer plants. Ensure that in the preceding season either different crop or same variety of field pea was grown to avoid mixture, and avoid taking seed production of field pea if in the preceding season different variety of field pea was grown. Quality seed production and maintenance of quality of a seed is a sequential process that starts from timely sowing, which leads to timely flowering, pollination, successful fertilization, seed development, and adequate accumulation of storage reserve in seed till maturity, harvesting at physiological maturity, threshing, drying to optimum moisture levels, seed processing, and its storage at adequate condition (Fig. 25.4).

Climatic variables like temperature, rainfall, relative humidity, carbondioxide concentration, etc., prevailing during crop growth stage and subsequently during

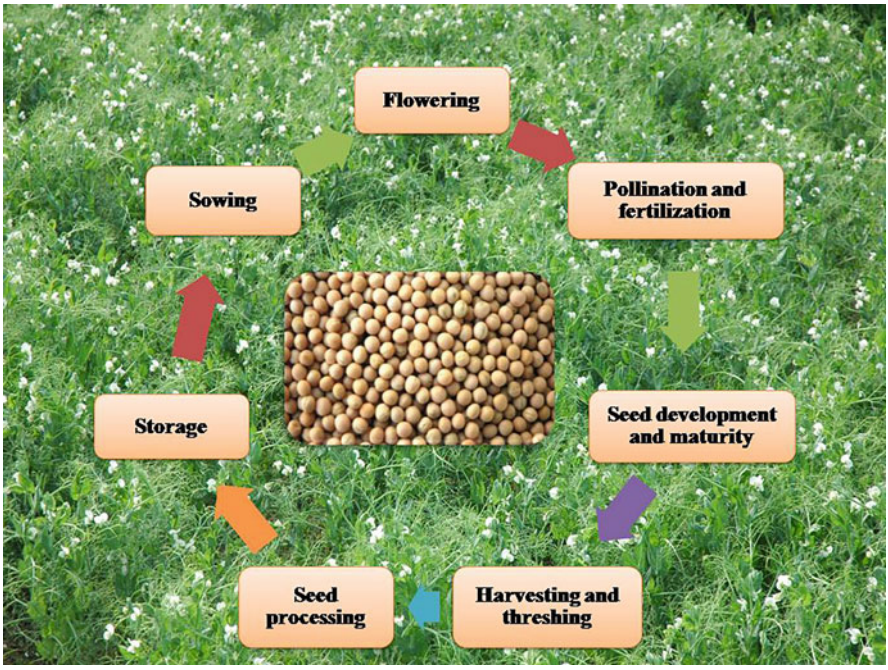

Fig. 25.4 Steps in quality seed production

storage affects the quality of seed (Maity et al. 2016; Lamichaney et al. 2018; Lamichaney and Maity 2021). Timely sowing is must to produce high-quality seed in field pea. Lamichaney et al. (2021a) have reported significant reduction in seed set, 100-seed weight and germination of field pea when planted late, which was attributed to high temperature-mediated reduction in reproductive period (forced maturity), which resulted in improper development of seed. Likewise, rainfall and elevated concentration of carbondioxide during crop growth period is reported to reduce seed quality in legumes (Lamichaney et al. 2018, 2021b, c). Therefore, precaution in each and every step is quintessential for producing and maintaining the quality of a seed. Field pea being self-pollinated, a minimum isolation distance of 5 and 10 m is essential for certified and foundation seed production, respectively, from the fields of other varieties or same variety not confirming to varietal purity requirement of Indian Minimum Seed Certification Standards (Trivedi and Gunasekaran 2013). A minimum of two inspections are required to be made, the first before flowering and the second at flowering and podding stage (Trivedi and Gunasekaran 2013). The main objective of field inspections is to verify the factors, which can affect the genetic purity of the seed and to take the corrective measures.

During preflowering inspections, the requirements on the isolation distance and land conditions are checked and to undertake roguing if any type of off-types are present. While inspections during flowering and fruiting stage are done to further check the occurrence of off-types based on flower and pod character and to remove them. During flowering, daily inspections should be done to identify the not true to type plants and to remove such off-types. A final rouging must be carried out during maturity to eliminate any chance of contamination. The field pea crop should be harvested when 90% of the pods turn brown, either by stationery threshers or manually. Bleaching, discoloration of green seed to lighter green or yellow due to loss or degradation of chlorophyll is reported to considerably reduce the quality of seed, which could occur before as well as after harvesting (McDonald et al. 2019). Bleaching before harvesting can occur due to intermittent rainfall and dry condition occurring during physiological maturity (McCallum et al. 1997; Cheng et al. 2004), and may lead to reduced germination and loss of seed vigour (Fenner 1985). Therefore, delayed harvesting should be avoided as it increases the risk of bleaching especially in green seeded cultivars (French 2016). Unfavorable storage conditions such as high temperature, high relative humidity, or light intensity could cause bleaching after harvesting (Ubayasena et al. 2013).

After threshing, the seeds should be dried to about 9% moisture before processing and storage. The maximum permitted percentage of off-types at the final inspection is 0.10% and 0.20% for foundation and certified seed production plots, respectively. Pure seed in field pea refers to any seed with a portion of seed coat attached, or a piece of seed but larger than one half the original size with a portion of the seed coat attached, seeds with cotyledon broken apart yet are held together within a seed coat (ISTA 2016). The minimum proportion of such pure seeds should be 98% for foundation as well as certified seed. However, seeds and its pieces without seed coat are considered as inert matter. Also, separated cotyledons are considered as inert matter irrespective of whether the radicle-plumule axis and/or more than half of the

Table 25.5 Field pea quality seed production standards as per Indian Minimum Seed Certification Standards

Factor	Standards for each class	
	Foundation seed	Certified seed
Pure seed (minimum)	98.0%	98.0%
Inert matter (maximum)	2.0%	2.0%
Other crop seeds (maximum)	None	5/kg
Weed seeds (maximum)	None	None
Other distinguishable varieties (maximum)	5/kg	10/kg
Germination including hard seeds (minimum)	75.0%	75.0%
Moisture (maximum)	9.0%	9.0%
For vapor proof containers (maximum)	8.0%	8.0%

seed coat are attached. Inert matter also includes the dust particles, muds, stones, and any part of seed not included as pure seeds (ISTA 2016). The maximum permissible limit of inert matter is 2% for both foundation and certified seed. In foundation as well as certified seed, not a single weed seed is permitted seed. Likewise, the maximum permissible limit of other crop seed is 0 and 5 per kg for foundation and certified seed, respectively (Table 25.5).

25.18 Conclusions and Future Perspective

Despite the substantial efforts, the dry pea productivity in many countries such as India, China, Australia, and Myanmar is still low. In addition, the production is constantly declining in countries like USA, Finland, Brazil, Ireland, Belgium, Pakistan, and Netherland, which is a serious concern and needs concentrated research and developmental efforts to reverse the trend. The yield gain in Canada is 2.0% and is superior to the yield gain in any other crops worldwide, which reveals investment made in pea breeding over the years (Rubiales et al. 2019) and it should be replicated in other countries also. Interestingly, across the countries the breeding objective is mainly focused on few traits like tendril (*afila*), dwarf plant type, and powdery mildew resistance, which led to narrow down the genetic base; hence, there is urgent need of involvement of diverse parents in regular breeding program to expand the genetic base (Dixit et al. 2014). Development of high-yielding varieties possessing multiple stresses resistance remains the main objective. Very limited usage of dry pea in terms of direct consumption prevails, therefore, proper value-addition and novel ways to use the grain or its by-products must be developed, if the popularity of this crop has to increase. Another important aspect of pea breeding is to develop multipurpose cultivars (food-feed-fodder).

There is no doubt that in addition to its use as protein source, the demand for cattle/poultry feeds and fodder will increase manifold in coming years particularly in developing nations. Noteworthy, existing abundant natural genetic variation for macro- and micronutrient and RFOs needs to be exploited for development of

biofortified cultivars. In case of genomic resources, pea has made significant progress during recent past with several major/minor genes or QTLs identified governing important traits. However, there is a need of large-scale high-throughput screening of germplasm and subsequent identification of associated genes/QTLs for various targeted traits. Besides, the introgression of these resistant sources in good agronomic background should be done by employing marker-assisted selection. The large number of biparental populations, the individual and consensus genetic maps, the dense arrays of genetic markers, the high-throughput SNP genotyping tools, the BAC libraries, the TILLING population, reference genome, proteomics, metabolomics, epigenomics, bioinformatics, transcriptomics, genomic selection, and genome editing approaches are available, which need to be embraced in regular breeding program to accelerate the genetic gain in dry pea. Most fascinatingly, the amalgamation of omics approaches with speed breeding will further speed up the breeding cycle.

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Lathyrus Breeding

26

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Abstract

Grass pea (*Lathyrus sativus* L.) is a climate-resilient nutrient-dense crop that offers food and nutritional security to many low-income communities of different underdeveloped regions of the world including South Asia, Sub-Saharan Africa and Mediterranean region. It is recognized to have a good source of protein, healthy fatty acids, vitamins and micronutrients, notwithstanding, the stigma of neurotoxin (ODAP) associated with grass pea that cause irremediable neurological disorder in humans and animals. Interestingly, it is the only known dietary source of L-homoarginine a non-proteinogenic amino acid which has admirable medicinal properties. It has better tolerance to different biotic and abiotic stresses as compared to other pulse crops. Over the years, sincere efforts are made towards the genetic improvement of grass pea to subsidize ODAP content and elevate production potential. In this book chapter, the economic importance of the crop, its origin, domestication, evolution, botanical description and floral biology have been illustrated. The accomplishment made in grass pea improvement through conventional and non-conventional breeding approaches, that is selection, hybridization, pre-breeding and distant hybridization along with application of mutation breeding have been reviewed. The current status of genomics resources

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D. K. Yadava et al. (eds.), *Fundamentals of Field Crop Breeding*,
https://doi.org/10.1007/978-981-16-9257-4_26

and marker-assisted breeding has also been deliberated. Moreover, the breeding objectives, major constraints and future thrust areas toward exploring cutting-edge tools and technique for enriching grass pea genomic resources have been outlined. Furthermore, the existing coordinated testing system for new entries and quality seed production has also been reviewed in brief. Overall, to accelerate genetic gain in grass pea along with low ODAP embracement, urgent need is being felt to explore recent advanced tools and techniques such as transcriptomics, proteomics, metabolomics, small RNAomics, epigenomics, interactomics, bioinformatics and genome editing to strengthen the grass pea breeding programme towards its transformation from orphan to main stream crop.

Keywords

Breeding objectives · Genetic resources · Grass pea · Pre-breeding · Trait genetics

26.1 Introduction

Grass pea (*Lathyrus sativus* L.) is the climate-resilient nutritious cool-season legume crop grown for food, feed and fodder (Almeida et al. 2015a, b, c; Rizvi et al. 2016; Lambein et al. 2019). It is an ancient crop which has been cultivated for more than 8000 years owing to its tolerance towards drought, flooding, salinity, problematic soils along with its ability of nitrogen fixation (Girma and Korbu 2012; Vaz Patto and Rubiales 2014a, b; Dixit et al. 2016; Sarkar et al. 2019; Gupta et al. 2021). Given traits endorsed it as an "insurance crop" that can perform exceptionally well under marginal lands with low input and ensure economic, social and nutritional security to the resource poor farmers' of developing nations in the face of changing climate (Hillocks and Maruthi 2012; Vaz Patto and Rubiales 2014a, b; Sarkar et al. 2019; Mahapatra et al. 2020). Among the *Lathyrus* species, grass pea is the most cultivated one predominantly grown for food, feed and fodder in India, Bangladesh, Nepal, Pakistan and Ethiopia (Kumar et al. 2013; Dixit et al. 2016; Lambein et al. 2019).

The grass pea has various "common" names in different countries: for example Chickling pea, Indian vetch (United Kingdom and North America), Almorta, Titos (Spain), Khesari, *Teora, Lakhdi, Lakh,* Batura (India), Alverjas (Venezuela), Gilban (Sudan), sabberi, Guaya (Ethiopia), Matri, mattra (Pakistan), Gesette (France), san lee dow (China), Saatplatterbse (Germany), chicharo (Portugal) and Pisello bretonne (Italy) (Skiba et al. 2007; Caminero Saldaña and Grajal Martín 2009; Hillocks and Maruthi 2012; Rizvi et al. 2016; Al-Snafi 2019). Broadly grass pea may be classified into two groups: one from the Indian sub-continent and second from the Mediterranean group with higher yield potential (Hanbury et al. 1999; Girma and Korbu 2012; Hillocks and Maruthi 2012). This crop is regarded as a perfect candidate crop of rice fallows of South East Asia where it holds immense potential by thriving well on residual soil moisture and provides additional income to the farming folks (Dixit et al. 2016; Maji et al. 2019).

Table 26.1 Nutritional composition of grass pea

Constituents	Amount	References
Calories	362.3 kcal/kg	Rahman et al. (1974) and Majumdar (2011)
Protein	17.7–49.3%	Sammour et al. (2007a, b), Pastor-Cavada et al. (2011) and Barpete et al. (2012a, b)
Fat	2.7%	Rahman et al. (1974)
Carbohydrates	51.8–72.91%	Tamburino et al. (2012) and Al-Snafi (2019)
Starch	32–52.3%	Urga et al. (1995) and Al-Snafi (2019)
Total lipid	1.67–2.0%	Buchanan (1904) and Tamburino et al. (2012)
Fatty acids (saturated)	16–53.69%	Grela et al. (2010) and Tamburino et al. (2012)
Fatty acids (unsaturated)	45.7–66.7%	Grela et al. (2010)
Iron (mg)	41.1–94.8 ppm	Grela et al. (2010, 2012) and Gupta et al. (2021)
Zinc (mg)	15–54.7 ppm	Hanbury et al. (2000), Urga et al. (2005), Grela et al. (2010) and Gupta et al. (2021)
Homoarginine	7.49 to 12.44 mg/g	Sacristán et al. (2015)
ODAP	0.02–2.50%	Abd El Moneim et al. (2001), Fikre et al. (2008), Xiong et al. (2015) and Barpete et al. (2021a, b)

In term of nutritious profile (Table 26.1), it is acknowledged as the cheapest source of protein in the daily diets of millions of vegetarian folks unable to have non-vegetarian product for balanced diet. The protein amount oscillated from 17.7 to 49.0% which is superior to almost other pulse crops (Sammour et al. 2007a, b; Pastor-Cavada et al. 2011). The grass pea protein is constituted of albumins (14–26%), globulins (53–66%), glutelins (15%) and prolamins (5–6%) (Duke 1981; Chandna and Matta 1994). The protein of grass pea contains 17 essential amino acids in sufficient amount especially lysine at higher level than other legumes or cereals crops (Yang and Zhang 2005). Among other essential amino acids, the most abundant are leucine, phenylalanine, threonine and valine, whereas, likely to other legumes it is deficient in sulphur-containing amino acids, that is, methionine, cysteine and tryptophan (Yan et al. 2006; Mahler-Slasky and Kislev 2010; Pastor-Cavada et al. 2011). Phosphorus and calcium content in grass pea ranges from 380.4 to 511.6 mg/100 g and 131.6 to 200.1 mg/100 g, respectively (Urga et al. 1995) which can easily meet the daily recommended intake of 500 mg/day of phosphorus and 800 mg/day of calcium for children of 4–8 years old (Yates et al. 1998; Teshager 2019). Seeds of grass pea are rich in potassium with a value ranging from 8.3 to 10.8 g/kg in dry matter.

The average copper, zinc, iron and manganese levels in grass pea are 5.1, 44.1, 62.1 and 23.7 mg/kg in dry matter, respectively. The tannin content ranges between 2.76 and 5.62 g/kg in dry matter (Grela et al. 2010). Although the lipid content in this legume is below 2% (Buchanan 1904) with nutritionally valuable well-balanced fatty acids constitution where major proportions of palmitic and linoleic acids and smaller quantities of oleic, linolenic and arachidonic acids improve its nutraceuticals

value (Hanbury et al. 2000; Chinnasamy et al. 2005; Pastor-Cavada et al. 2009; Grela et al. 2010). Therefore, grass pea is much appropriate for human consumption owing to excellent quality of fatty acid with 58% polyunsaturated fatty acid wherein linoleic acid (18:2) accounted for 51% and linolenic acid (18:3) for 6.4% (Grela et al. 2010). Grass pea is a good source of almost all essential vitamins for health viz., retinol, β-carotene, thiamine, riboflavin, niacin, pantothene, pyridoxine, folic acid and ascorbic acid (Arslan 2017). Most importantly, grass pea is much appropriate for human consumption owing to excellent quality of fatty acid with 58% polyunsaturated fatty acid wherein linoleic acid (18:2) accounted for 51% and linolenic acid (18:3) for 6.4% (Grela et al. 2010). Grela and Günther (1995) reported 40 IU and 7 mg of vitamin E and tocopherol content per kg of seeds, respectively. The measure of energy level in grass pea is similar to those for many other common feed grain legumes (Hanbury et al. 2000). Furthermore, it also showed the highest concentration of flavonoids and antioxidants (Sarmento et al. 2015).

Grass pea seed is a coffer of many compounds that can contribute to physical health and fitness of humans. For example it is the only acknowledged dietetic source of L-homoarginine amino acid which is one of the first strange non-protein amino acids (Rao et al. 1963; Bell 1962) with nutraceutical properties (Lambein 2000; Rao 2011; Singh and Rao 2013; Llorent-Martínez et al. 2017) and plays instrumental role in cardiovascular disease treatments (Rao 2011; Singh and Rao 2013; van Wyk et al. 2016). It also helps in combating the repercussion of hypoxia associated with cancer tumour development (Ke and Costa 2006; Jammulamadaka et al. 2011). Therefore, as nutraceutical, grass pea is regarded as an excellent example of a potential "functional food" (Singh and Rao 2013; Llorent-Martínez et al. 2017). Although arginine is the usual substrate for nitric oxide synthase (NOS), homoarginine also provides an alternative substrate. Thus, a daily dietary intake of L-homoarginine through small quantities of grass pea may be valuable for human health and deserves further investigation (Rao 2011). Moreover, one additional potential beneficial application of grass pea seeds is to ameliorate diabetic symptoms, as they have glycosyl phosphatidyl inositol with insulin-mimetic activity (Pañeda et al. 2001).

In addition, condensed polyphenols are present in grass pea seeds which have positive correlation with seed coat colour (Deshpande and Campbell 1992). Total phenolics in grass pea ranged from 868 to 2059 mg/kg dry matter. Condensed tannins in grass pea ranged from 0.89 to 5.18 g/kg dry matter. Grass pea seeds with darker seed coat colour contained higher levels of condensed tannins (Wang et al. 1998; Talukdar 2012a, b, c). In addition, high level of trypsin inhibitor has also been recorded in grass pea and trypsin inhibitor activity (TIA) values vary from 15.53 to 18.99 TIU/mg. Total phenolic contents ranged from 1.88 to 7.12 mg/g extract and 20.3 to 70.3 mg/100 g seeds (Rybinski et al. 2018; Al-Snafi 2019).

However, grass pea is also defamed due to neurotoxin, that is β-ODAP (β-*N*-oxalyl-l-α,β-diaminopropionic acid) which caused lathyrism, a neurodegenerative ailment in humans and domestic animals owing to its overconsumption under extreme food crisis (Lambein and Kuo 2009; Vaz Patto and Rubiales 2014a, b). The term "lathyrism" was coined by Cantani of Naples in 1873 (Barrow et al. 1974).

This disease is more prominent when grass pea was taken as core ingredient of daily diet at least 30% of the caloric ingestion for at least a period of 3–4 months (Kumar 1998). To further challenge the bad reputation of grass pea owing to its metabolite β-ODAP, a Chinese group has obtained a patent for the use of β-ODAP as a haemostatic agent during surgery (Lan et al. 2013). β-ODAP is also present in the longevity-promoting *Panax ginseng* root (Kuo et al. 2003) that under the name Dencichin is known for its haemorrhage-stopping property and thrombopoiesis treatment (Ding et al. 2018). In China, it is even supplemented in some toothpaste brands and used as an herbal medicine to avoid bleeding. Most fascinatingly, β-ODAP has also been described as a multi-functional plant metabolite which adds a new dimension to explore its potential in the treatment of Alzheimer's disease, hypoxia and long-term potential of neurons essential for memory (Singh and Rao 2013). Recent investigations have revealed that β-ODAP induces wound healing and can be considered as a natural wound curative agent (Sharma et al. 2018). An increasing number of therapeutic applications derived from grass pea may be developed in the coming years. This existing variability for different nutritional traits will be useful to the breeders for further utilization in grass pea improvement.

26.2 Common Uses of Grass Pea Worldwide

In India, Pakistan, Bangladesh and Nepal, the most common use of grass pea is as split seeds (dhal) and flour (besan) (Al-Snafi 2019). The *besan* is used to prepare many traditional food items including *pakoras*, *piazu*, *chapati*, *dhal*, *vadi*, *dhokla* and *sweets* (Pandey and Kashyap 1995; Campbell 1997; Kumar 1998; Asthana and Dixit 1998). The use of grass pea as leafy vegetable, green pods, green seeds as snacks or as cooked vegetable is also common in many countries (Peña-Chocarro and Peña 1999; Almeida et al. 2015a, b, c. In Ethiopia, grass pea is eaten in different ways such as boiled seed (*nifro*), bread (*kitta*), roasted grains (*Kollo*), flour (*Shiro*) and sauce (*wott*) (Tekle-Hainamot et al. 1995; Campbell 1997). In China, it is used as animal feed and as a supplement in food processing (Zhou and Arora 1995). Young grass pea plants are used as fodder for cattle or for grazing in many countries (Kumar 1998). As fodder, the plants can be eaten green or as hay (Duke 1981; Tekle-Haimanot et al. 1993; Ahsan et al. 2010). Notably, in Bangladesh, the grass pea seed oil is also used to cure scabies, eczema and allergy (Al-Snafi 2019).

Moreover, the other species of genus *Lathyrus,* that is *L. cicera* and *L. ochrus* are also cultivated for food and fodder purposes in Iran, Iraq, Syria, Jordan, Greece, Cyprus and Turkey (Saxena et al. 1993; Kumar et al. 2013). Some other species like *L. clymenum* and *L. hirsutus* are cultivated as minor forage crops in Greece and Southern United States (Sarker et al. 2001). A few species are valued as ornamental plants, especially *L. odoratus* (sweet pea)*, L. sylvestris* and *L. latifolius* in the modern world (Campbell 1997). Recently, the novel biological properties, that is antioxidant, enzyme inhibitory, cytotoxic effects and phytochemical profiles of selected *Lathyrus* species, that is *L. czeczottianus* and *L. nissolia*, have been noticed (Llorent-Martínez et al. 2017). Consequently, both the species hold great promise to

be utilized in designing of new phytopharmaceutical and nutraceutical formulations. Other *Lathyrus* species namely *L. cicera, L. ochrus, L. clymenum, L. latifolius L. sylvestris* and *L. tingitanus* are cultivated for both forage or grain purposes (Campbell 1997; IPGRI 2000).

26.3 Origin, Evolution, Distribution and Gene Pools of Grass Pea

Grass pea belongs to the genus *Lathyrus* consisting of 187 species (Vaz Patto and Rubiales 2014a, b; Sarkar et al. 2019) within the Fabaceae family (syn. Leguminosae), sub-family Faboideae (syn. Papilionoideae) and tribe Fabeae (syn. Vicieae) (Allkin et al. 1986; Wojciechowski et al. 2004; Kenicer et al. 2005; Smýkal et al. 2011; Schaefer et al. 2012). This genus has been grouped into 13 sections (*Orobus, Lathyrostylis, Lathyrus, Orobon, Pratensis, Aphaca, Clymenum, Orobastrum, Viciopsis, Linearicarpus, Nissolia, Neurolobus* and *Notolathyrus*) based on morphological and molecular phylogenetic studies (Kupicha 1983; Croft et al. 1999; Kenicer et al. 2005, 2009). However, studies of the chloroplast DNA of 42 *Lathyrus* species (Asmussen and Liston 1998) and AFLP analysis of 18 species (Badr et al. 2002) suggested that reclassification of some species to different sections may be required (Skiba et al. 2007). It is scattered throughout the temperate regions of the Northern Hemisphere with 52 species in Europe, 30 species in North America, 78 species in Asia and 24 species extending into tropical East Africa and 24species into temperate South America (Kupicha 1983; Allkin et al. 1985; Goyder 1986; Badr 2006). In India, *Lathyrus* species is distributed throughout the country and cultivated from 1200–3000 m altitude (Gautam et al. 1998).

The name *Lathyrus* is originated from the Greek word "la thyros" that indicates at something exhilarating, referring to the aphrodisiacal qualities credited to grass pea (Loudon et al. 1855). The most probable centre of origin is the eastern Mediterranean or Fertile Crescent, around 6000 before present (BP). This has been supported by archaeological evidences and recent phylogenetic reports (Kislev 1989; Schaefer et al. 2012) that contradicted the hypothesis of Smartt (1984) wherein the centre of origin was mentioned in Southwest or Central Asia. It is being mainly cultivated in India, Bangladesh, Pakistan, Nepal, Ethiopia, China and in many countries of Europe, the Middle East and Northern Africa (Vaz Patto et al. 2006a; Yan et al. 2006; Dixit et al. 2016). The archaeological facts witnessed that grass pea has a long history of domestication and seeds of *Lathyrus* species had been found in Turkey and Iraq in the form of collected or cultivated stuff (Lambein et al. 2019). Grass pea cultivation started around 6000 BC and might have been the first crop domesticated in Europe (Kislev 1989). Similarly, seeds from 2500 BC were identified in the oldest excavation in India (Kislev 1989) and also in the Balkan in 8000 BC. According to Vavilov (1951) *Lathyrus* has two centres of origins: one was the Central Asiatic Centre which includes northwest India, Afghanistan, the Republics of Tajikistan and Uzbekistan and western Tian-Shan, whereas the second was the Abyssinian Centre (Campbell 1997).

In general, *Lathyrus* species are classified into five groups considering taxonomy and morphology attributes namely *Aphaca*, *Nissolia*, *Clymenum*, *Cicerula* and *Lathyrus* (Asmussen and Liston 1998; Kenicer et al. 2005). Of which, first four species are annual, whereas *Lathyrus* species are mostly perennial type (Asmussen and Liston 1998; Bässler 1966; Kupicha 1983). The *Lathyrus* species has been grouped into primary, secondary and tertiary gene pools based on the cross-compatibility (Jackson and Yunus 1984; Yunus and Jackson 1991; Heywood et al. 2007; Kumar et al. 2013). The cultivated and wild races of *L. sativus* are part of primary gene pool (Heywood et al. 2007; Rizvi et al. 2016) that is classified into gene pool 1A in which white flowered and white seeded varieties are included, while gene pool 1B includes blue flowered and small speckled seeded varieties (Townsend and Guest 1974; Smartt 1984; Jackson and Yunus 1984). The primary gene pool of *Lathyrus* mainly included only one species *L. sativus* which have limited cultivars and landraces. Thus, further improvement through conventional breeding completely hinged on exploitation and utilization of other distant wild relatives (Yunus and Jackson 1991).

According to Heywood et al. (2007), a total of 10 species belongs to secondary gene pool, namely *L. cicera, L. amphicarpus, L. chrysanthus, L. gorgoni, L. marmoratus, L. pseudocicera, L. blepharicarpus, L. chloranthus, L. hierosolymitanus* and *L. Hirsutus*. However, very limited information is available regarding cross-compatibility of these species with the cultivated *L. sativus*. However, Heywood et al. (2007) further reported that some of the species such as *L. chrysanthus, L. gorgoni, L. marmoratus* and *L. pseudocicera* belonging to secondary gene pool is compatible with *L. sativus*, but only ovules were produced. Other species (*Lathyrus* sp.) which are included in the tertiary gene pool can be exploited for crop improvement activities with the help of modern biotechnological approaches, like embryo rescue techniques etc., for transferring desirable genes from tertiary gene pool to cultivated background.

Few attempts have been made to introduce novel genes through inter-specific hybridization between *Lathyrus* spp. and other crop wild relatives (CWR). Because there is huge potential in some of the species particularly ornamental *L. odoratus* for some exclusive traits like flower coloration and disease resistance that can be transferred into cultivated *L. sativus* if crosses can be made successfully (Gurung and Pang 2013). Numerous attempts were made to cross *L. sativus* with a range of *Lathyrus* spp. using embryo rescue technique (Addis and Narayan 2000). The progenitor of *L. sativus* (primary gene pool) is still unknown; however, according to the Jackson and Yunus (1984), *L. cicera* is morphologically and cytogenetically nearest to the cultivated species and it is the most probably qualifying candidate for progenitor of *L. sativus* (Hopf 1986). This small-seeded *Lathyrus* species is generally cultivated in the countries from Greece to Iran and Transcaucasia. In this area, carbonized *Lathyrus* seeds had been recovered from a number of ancient sites, going as far east as India. Recently, Kumar et al. (2013) reported that some of Mediterranean species namely *L. cicera*, *L. marmoratus*, *L. blepharicarpus* and *L. Pseudocicera* have been recognized as candidates for progenitor of *L. sativus* based on the morphological synteny with cultigens. Among the other economically

Table 26.2 Grass pea genetic resources available at different institutions/organizations worldwide

S. no.	Institute name with address	No. of accessions
1	International Center for Agricultural Research in Dry Areas (ICARDA), Morocco	4184
2	Conservatoire Botanique National des Pyrénées et de Midi-Pyrénées (CBNPMP), France	4477
3	National Bureau of Plant Genetic Resources (NBPGR) in India, New Delhi, India	2619
4	Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Bangladesh	1841
5	Royal Botanic Gardens Kew, UK	1115
6	ARS-GRIN Pullman, ARS Ft Collins, Boyce Thompson Arboretum, Arizona, ARS National Arboretum, Washington, DC, USA	949
7	National Genebank, Beijing, China	704
8	Instituto Nacional de Investigación Agraria (INIA), Chile	1424
9	Ustymivka Experimental Station of Plant Production, Ukraine	1215
10	N.I. Vavilov All-Russian Scientifc Research Institute of Plant Industry, Russian Federation	1207
11	Australian Grains Genebank Australia	1020
12	Plant Gene Resources of Canada (PGRC) Canada	840
13	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany	515
14	Centro de Recursos Fitogenéticos (CRF) Institutonacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Spain	429
15	ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India	450
16	IGKV, Raipur	1964
17	International Center for Agricultural Research in Dry Areas (ICARDA), India	400
18	Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia	586

Sources: Girma and Korbu (2012), Vaz Patto and Rubiales (2014a, b), Almeida et al. (2015a, b, c), Sarkar et al. (2019) and Anonymous (2020)

important legume crops, *Pisum sativum* is considered as the closest to grass pea, followed by lentil (*Lens culinaris*), faba bean (*Vicia faba* L.), barrel medic (*Medicago truncatula* Gaertn.), chickpea (*Cicer arietinum* L.) and *Lotus corniculatus* L. (Asmussen and Liston 1998; Ellison et al. 2006; Wojciechowski et al. 2004). CWRs are the arsenals of many important traits; therefore, they play a pivotal role towards genetic enhancement of the grass pea (Table 26.2). Nevertheless, extra-terrestrial gene transfer is rarely attempted in grass pea, notwithstanding the successful setting of viable seeds in inter-specific hybridization between *L. sativus* with *L. cicera* and *L. amphicarpus* (Khawaja 1988; Yunus 1990; Davies 1957, 1958; Addis and Narayan 2000).

On the contrary, some other CWRs can develop pods which remain devoid of viable seeds (Yamamoto et al. 1989; Yunus 1990; Kearney 1993). Interest in inter-specific hybridization in the *Lathyrus* genus was shown Burpee (1916) in sweet pea

(*L. odoratus*). Inter-specific hybridization between other species in the genus *Lathyrus* had been attempted by many researchers since the report of the successful crossing of *L. hirsutus* × *L. odoratus* by Barker (1916). Initially, distant hybridization involving *L. sativus* was successful when *L. cicera* was crossed with *L. sativus* (Lwin 1956); however, these crosses remain unsuccessful in subsequent attempts (Davies 1958; Khawaja 1988). Later on, many studies reported successful inter-specific hybridization in the genus *Lathyrus* using different species namely *L. sylvestris, L. latifolius, L. articulates, L. ochrus, L. clymenus, L. annuus, L. hierosolymilanus, L. cicera, L. marmoratus, L. blepharicarpus, L. pseudocicera*, *L. gorgoni, L. marmoratus, L. blepharicarpus, L. odoratus, L. belinenesis, L. hirsutus, L. rotundifolius* and *L. tuberosus* in specific combinations (Marsden-Jones 1919; Davies 1957, 1958; Trankovskij 1962; Khawaja 1988; Yamamoto et al. 1989; Kearney 1993; Hammett et al. 1994, 1996).

However, sincere efforts have been made to exploit secondary gene pool resources in *L. odoratus* to obtain new pigmentations and fragrance by successful crossing with *L. hirsutus*, *L. chlorantus* (Khawaja 1988) and *L. belinensis* (Hammett et al. 1994). Based on the crossing ability, chromosomal behaviour and setting of viable seed in the hybrids, novel gene transfer for crop improvement in *L. sativus* is attainable through *L. cicera* and *L. amphicarpus* which are easily crossable species with cultivated one. For other species of *Lathyrus* which are not producing viable hybrids can be utilized through biotechnology-based tools and techniques (Ochatt et al. 2001; Barpete et al. 2009, 2017, 2020a, 2021a, b).

26.4 Botanical Description

It is a herbaceous annual winter season crop which may achieve a height of about 1.5–1.7 m (Smartt 1984; Campbell 1997). It has slender, glabrous, ribbed, quadrangular, branched stem with a straggling or climbing habit, it mounts with the help of tendril instead of twining (Campbell 1997). Grass pea has noticeable leaf like, triangular to ovate shape stipules with basal attachment to petiole (Majumdar 2011). It has well-developed taproot system in which rootlets are prolonged and sheltered by small, cylindrical, branched nodules which usually bunched together in dense groups (Parihar and Gupta 2016). Grass pea can develop lateral roots and aerenchyma, which minimize oxygen use, aid oxygen transfer from shoot to root and remove harmful by-products during environmental stresses like flood (Zhou et al. 2016). The leaves are pinnate, terminates in 3–5 tendrils and consist of one or two leaflets which are cuneate at the base and acuminate at the top (Fig. 26.1a). The flowers are axillary, solitary and may be bright blue, reddish purple, red and pink with white keel (Fig. 26.1b, c).

The ten stamens are arranged in diadelphous (9 + 1) pattern with elliptoid and yellow anthers. Ovary is sessile, thin and pubescent with 5–8 ovules and style bearded below the stigma. Stigma is glandular-papillate and spatulate. The pods are oblong, flat, straight or slightly curved ending in a beak (Fig. 26.1d) and have three to five angled and wedge-shaped seeds, ranging in colour from creamish,

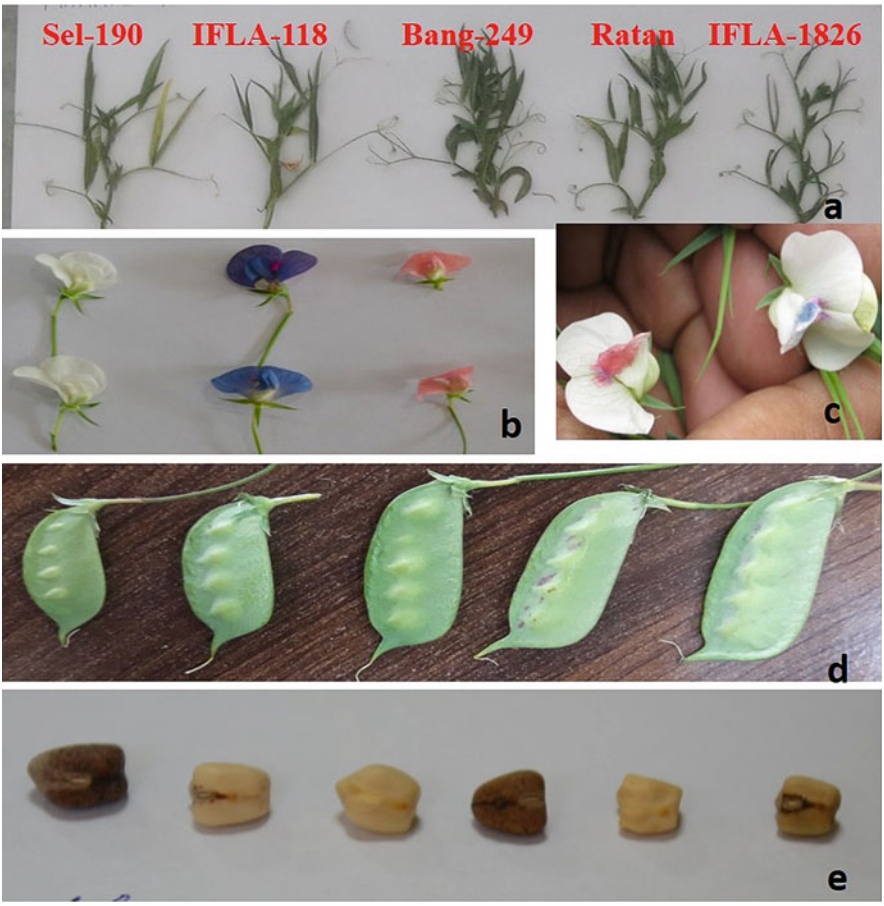

Fig. 26.1 *Lathyrus sativus* L. botanical description, (**a**) leaves and tendrils pattern of different accessions of grass pea, (**b** and **c**) flower color and keel color variation in grass pea, (**d**) grass pea pods are oblong, flat, straight or slightly curved ending in a beak, (**e**) different seed shapes with variable hilum pattern

yellow, brown grey, or light grey and occasionally spotted or mottled (Fig. 26.1e). The hilum is elliptic, and cotyledons are yellow to pinkish yellow (Fig. 26.1e). The seed germination is hypogeal type (Campbell 1997; Skiba et al. 2007; Parihar and Gupta 2016). Based on seed size, grass pea is classified into to two groups: small seeded (5.0–7.02 g/100 seed weight) and large seeded (7.15–15.92 g/100 seed weight) (Watt 1980). The small seeded is known as lakhodi or choti teora is primarily cultivated in Chhattisgarh area and large seeded known as lakh or badi teora is commonly grown in Nagpur and Bhandara district in India (Majumdar 2011).

26.5 Floral Biology, Emasculation: Pollination Techniques

Grass pea is principally self-pollinated, although some outcrossing has also been reported in this species (Mehta and Santha 1996), which is reliant on environmental or genetical factors. The average outcrossing rate ranged between 2.2% and 27.8%; however, the rate of outcrossing is varied significantly between grass pea genotypes (Rahman et al. 1995; Chowdhury and Slinkard 1997). The amount of outcrossing is 27.8% for blue, 19.4% for pink, and 9.8% for the white flowered genotypes (Rahman et al. 1995). Bees are presumed to be the predominant pollinator of grass pea (Rahman et al. 1995; Chowdhury and Slinkard 1997). According to cytological and karyotype research in *Lathyrus* genus, all known annual species and most perennial species of *Lathyrus* are diploid with $2n = 14$ chromosomes (Barpete et al. 2012b; Hao et al. 2017). Some aneuploid as well as polyploid species are also reported among the *Lathyrus* genus viz., *L. palustris* ($2n = 6x = 42$, hexaploid) and *L. venosus*, ($2n = 4x = 28$, tetraploid) (Narayan and Durrant 1983; Talukdar and Biswas 2008). Grass pea has attractive flower colour like blue, pink, red, white or various combinations. The blue-flowered ecotypes are found in South-East Asia and the South Asia with high outcrossing rate (Polignano et al. 2005a, b; Kumar et al. 2013), whereas, white flower types with less outcrossing are generally common in Mediterranean region (Smartt 1984).

Normally, in any grass-pea-breeding programmes, the crossing programme is executed under controlled condition in the greenhouse or under net house for avoiding possible outcrossing through bee or other pollinators. At the outset, the flowers are emasculated by removal of the anthers in the late bud stage. In the next morning, the styles are fertilized with pollen from desired parents plant as soon as possible following dehiscence of the anthers. Emasculation and pollination are long-lasting practices in crossing programme of grass pea which results in a sufficient number of successful cross seeds as several seeds normally develop in a single pollination attempt. The male sterility was first reported by Srivastava and Somayajulu (1981) wherein they noticed that some plants have reduced stamens and the anthers did not produce viable pollen. Quader (1987) revealed the existence of both cytoplasmic and genetic male sterility with the presence of both single and double restorer genes.

26.6 Cytogenetics and Molecular Cytogenetics

The available literature indicated Corti (1931) as the pioneer to discover chromosome number in *L. sativus* L. who illustrated the presence of $2n = 14$ chromosomes in the somatic complement. Cytological and karyotype examination demonstrated that the *L. sativus* chromosome number is $2n = 2x = 14$ throughout the genus and most of the species are diploid with rare exceptions of polyploidy (Battistin and Fernandez 1994; Campbell 1997; Schifino-Wittmann 2000; Seijo and Fernandez 2001; Talukdar 2009a, b, c, d; Barpete et al. 2012b; Hao et al. 2017). Only three perennial species contain more than 14 somatic chromosomes of which *L. pratensis*

and *L. venosus* are tetraploid with $2n = 4x = 28$ and *L. palustris* is hexaploid with $2n = 6x = 42$. However, stability in chromosome number in *L. sativus* was noticed, but substantial variations in size and morphology of chromosome have performed an important role in the evolution of diploid *Lathyrus* species (Narayan and Durrant 1983; Yamamoto et al. 1984; Klamt and Schifino-Wittmann 2000; Schifino-Wittmann 2001; Sammour 1991). From an evolutionary viewpoint, the variation in genome size is harmonious with morphological variation as well as with the life cycle (Badr 2006). The change in chromosome size is linked with 2C nuclear DNA amount variations (Ceccarelli et al. 2010). The deviation in chromosome size is often due to amplification or deletion of a chromatin section during species diversification.

Moreover, intra- and inter-specific fluctuation in chromosome size indicates noticeable difference in the quantity of DNA that affect complement size and a high percentage of DNA is moderately repetitive (Talukdar and Biswas 2008). In addition, Ali et al. (2000) exhibited that the karyotype surveillance supported well the phylogenetic relationships among *Lathyrus* species that harboured into different sections as proposed by Kupicha (1983). However, other studies demonstrated the inter-specific karyotype variations that allow species characterizations (Battistin and Fernandez 1994; Shahiquzzaman and Kabir 1994). Such type of inconsistency in karyotype (*L. odoratus L.* and *L. sativus L.*) was also identified at the intra-specific level (Murray et al. 1992b). Intra-specific variability in the position and number of secondary constrictions was observed in *L. nervosus* and *L. pubescens* (Klamt and Schifino-Wittmann 2000). Some cultivars of *L. sativus* also exhibited satellites chromosomes that varied between 1 and 2 pairs (Ghasem et al. 2011; Barpete et al. 2012b).

Several studies have been conducted for the phylogenic relationships among different *Lathyrus* species including cytological traits karyotype analysis, chromosome banding and in situ hybridization (Battistin and Fernandez 1994; Murray et al. 1992a; Schifino-Wittman et al. 1994; Unal et al. 1995; Klamt and Schifino-Wittmann 2000; Ali et al. 2000; Ali and Osman 2020;). Aneuploid and polyploid plants are also reported within *Lathyrus* species that showed the same basic chromosome number (Talukdar and Biswas 2008). In addition, a complete set of seven different primary trisomics has an extra chromosome ($2n + 1 = 15$) and has been successfully isolated and cytogenetically characterized (Talukdar and Biswas 2007). Subsequently, seven different types of tetrasomics were also isolated and characterized in the advanced selfed generations of these primary trisomics ($2n + 1$; $2n = 15$) obtained earlier in diploid ($2n = 14$) grass pea (*L sativus* L.). The extra chromosomes were noticed either in bivalent or in trivalent-univalent or in quadrivalent form at metaphase-I (Talukdar 2008). The Trisomics and tetrasomics developed tailored segregation ratios for marker genes on the extra chromosomes. These ratios can be used to place genes on chromosomes and to create linkage groups (Khush 1973). The cytological examination of the F1 hybrids attempted between *L. amphicarpus* × *L. sativus*, *L. amphicarpus* × *L. cicera* and *L. odoratus* × *L. chloranthus* was carried out by Khawaja (1988) which showed 50–70% chromosome homology and pollen fertility in consistency with the meiotic pairing (Campbell 1997).

26.7 Genetic Resources Panorama

Breeding efforts towards genetic improvement of any cultivated plant species mainly rely on the precise identification and characterization of the germplasm resources of the crop and the study of its evolution (Yunus and Jackson 1991). The comprehensive knowledge of its closest and distant relatives and geographical origins (Schaefer et al. 2012) are quintessential to accelerate any breeding process which has been already elaborated in previous sections. The most economical and significant *Lathyrus* species which is being cultivated commercially comes under the section *Lathyrus* comprising *L. sativus*, *L. cicera* and *L. odoratus*. However, in comparison to other crops, very limited efforts have been made throughout the world towards the genetic improvement of these species. The success of breeding programme relies on availability and access of suitable genetic resources. During recent years, the conservation of *Lathyrus* genetic resources engrossed more attention owing to the potential role of this climate-resilient species under current climate changing circumstances (Kumar et al. 2013, 2020). Coordinated global efforts to bring together and conserve *Lathyrus* crop species have been started in the last decades of twentieth century, with the establishment of a "Lathyrus Genetic Resources Network" (Mathur et al. 1998).

Grass pea was enlisted in conservation programme with other major food legumes in multi-lateral platforms that developed to ensure access and benefit sharing under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO 2009). On similar note, another programme was developed by the Global Crop Diversity Trust (GCDT) in the collaboration of International Centre for Agricultural Research in the Dry Areas (ICARDA) with the intention for long-term conservation strategy of *L. sativus*, *L. cicera* and *L. ochrus* (GCDT 2009). Some large collections of cultivated and wild *Lathyrus* species have already been assembled and are being maintained in ex situ (gene banks) and in situ (natural habitats) circumstances by different institutes/organizations in worldwide. The largest *Lathyrus* gene bank is available at the Conservatoire Botanique National des Pyrenees et de Midi-Pyrenees (CBNPMP) in France with a total of 4477 accessions: 53% belongs to *L. sativus* and 18% to *L. cicera*.

The second leading institute is International Center for Agricultural Research in Dry Areas (ICARDA) which holds nearly 4200 accessions at its research centre in Morocco which comprised of *L. sativus* and *L. cicera* (Vaz Patto and Rubiales 2014a, b; Sarkar et al. 2019). The third widest collection of *Lathyrus* species is also held under the European Cooperative Program on Crop Genetic Resources, hosted by Bioversity International in Rome, which hold over 4000 accessions (Hillocks and Maruthi 2012). The ICAR-National Bureau of Plant Genetic Resources (NBPGR) in India holds about 2600 accessions (3% wild types and 85% cultivated type), of them 98% are *L. sativus* and 0.04% are *L. cicera*. Another leading institute is Plant Genetic Resource Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI) having all the accessions of cultivated type (Vaz Patto and Rubiales 2014a, b). Among European countries, Germany, Hungary and

Table 26.3 Wild species with various desirable traits

Species name	Traits	References
L. tingitanus	Toxin-free gene	Zhou and Arora (1995) and Kumar et al. (2011)
L. ochrus, L. clymenum L. cicero	Resistance to broomrape	Sillero et al. (2005), Fernández-Aparicio et al. (2009, 2012), Fernández-Aparicio and Rubiales (2010) and Linke et al. (1993)
L. cicero	Low ODAP content, earliness and cold tolerance	Hanbury et al. (2000), Abd El Moneim et al. (2001), Kumar et al. (2011), Granati et al. (2003), Vioque et al. (2009) and Abd EI Moneim and Cocks (1993)
L. cicero	*Pseudomonas syringae*	Martín-Sanz et al. (2012)
L. latifolius L. sylvestris L. hirsutus	*Meloidogyne hapla* (root knot nematode)	Rumbaugh and Griffin (1992)
L. czeczottianus	Antioxidant abilities with the highest concentration of phenolics	Llorent-Martínez et al. (2017)
L. nissolia	Enzymatic inhibitory effects against cholinesterase, amylase and glucosidase	Llorent-Martínez et al. (2017)
L. cicera, L. amphicarpus, L. ochrus	Zero or low-ODAP (0.01% or less)	Campbell (1997) and Kumar et al. (2013)
L. ochrus, L. clymenum	Ascochyta blight resistance	Gurung et al. (2002) and Skiba et al. (2004a)
L. cicera, L. odoratus	Pod borer resistance	Mathur et al. (1998)

Spain hold collection with 300–500 accessions. The largest collection of *Lathyrus* in Africa is in Ethiopia with 96 accessions (Hillocks and Maruthi 2012). In addition, as a backup, a total of 2134 grass pea accessions from 44 countries have been deposited at the Svalbard Global Seed Vault (Almeida et al. 2015a, b, c). There are also other organizations at global level where small collections of grass pea germplasm are available as enlisted in Table 26.3.

Besides, the aforementioned ex situ collections, in situ preservation is also recommended for long-term conservation. In situ conservation means active and long-term conservation of genetic diversity of natural wild populations within defined areas (Maxted et al. 1997). However, there has been very limited effort invested to conserve *Lathyrus* diversity in situ; consequently, native populations are susceptible to genetic erosion or even extinction (Maxted and Bennett 2001). A multi-gene pool approach has been used by Maxted et al. (2012) for several legume genera including *Lathyrus*. This involved the collation of 61,081 unique geo-referenced *Lathyrus* records collected between 1884 and 2008. These conserved

germplasm accessions represent a huge repertoire of genetic diversity and preserve a wide range of interested agro-morphological traits such as earliness, plant architectural traits, disease and pest tolerance, as well as low ODAP content (Almeida et al. 2015a, b, c). Sincere efforts have been directed for characterization of diversity in *Lathyrus* germplasm, as for ODAP content (Fikre et al. 2008; Grela et al. 2012; Kumar et al. 2011), phenology and yield (Grela et al. 2012; Mera 2010), parasitic weed resistance (Fernández-Aparicio et al. 2012), disease resistance (Gurung et al. 2002; Vaz Patto et al. 2006a; Vaz Patto and Rubiales 2009) or quality traits (Granati et al. 2003). Characterization and judicious exploitation of existing diversity through high-throughput phenotyping and genotyping studies will uncover novel alleles that can be used to improve this underutilized crop.

26.8 Brief of Genetic Variability and Potential Donors

In spite of several advantages, limited attempts have been made towards the improvement of grass pea owing to the presence of neurotoxin (ODAP). The grass pea farmers are still cultivating traditional cultivars which contain high ODAP and susceptible to biotic and abiotic stresses, therefore ultimately gaining low yield. The basic requirement of any breeding programme is genetic diversity which ultimately plays decisive role in adaptability and survival of a species (Hamrick and Godt 1989). The estimation of genetic diversity is based on morphological (qualitative & quantitative), cytological, biochemical and molecular parameters which are helpful for the measurement of variation; however, quantitative traits are highly influenced by both environmental and genetic factors. Therefore, they might not provide actual estimation of germplasm variability (Brown and Marshall 1995). A considerable level of variability for pods per plant, grain yield and 100-seed weight was reported by Vedna Kumari and Mehra (1989) in grass pea.

A wide range of variability for days to flowering, days to maturity, pod length, seeds per pod, seed weight and grain yield has been observed (Hanbury et al. 1995; Sarwar et al. 1995a, b). In another study, Mehra et al. (1995) evaluated exotic germplasm accessions belonging to France, Bangladesh, Ethiopia, Cyprus, Afghanistan, Germany and noticed ample variability for branches per plant and pod length. Likewise, Yadav (1995) reported large range of variation for days to flowering, days to maturity, plant height, pods per plant and seeds per pod in local germplasm accessions of Nepal. Robertson and Abd El-Moneim (1996) characterized 1082 accession of 30 species for various phenological and agronomic traits and noticed plenty of variation in days to flower, seed weight and seeds per pod for *L. cicera* and days to flower for *L. ochrus*. Asthana and Dixit (1998) reported enormous variability for plant height, number of primary branches, pods per plant, seeds per pod, 100-seed weight and grain yield in a set of germplasm. Syouf (1995) stated that *Lathyrus* species were among the most promising in terms of forage yield; however, the highest-yielding forage species was *L. ochrus*.

Further, remarkable variations for morphological traits, that is leaf length, flower colour, podding structure, seed size and colour, grain yield, ODAP and protein, have

been reported in germplasm of *L. sativus* and *L. cicera* (Kaul et al. 1982; Jackson and Yunus 1984; Kumari et al. 1995; Grela et al. 2010). Number of grass pea genotypes evaluated for plant height, number of branches per plant, pods per plant, pod length, day to 50% flowering, days to maturity, seeds per pod, biological yield, 100 seed weight, flower colour, nutritional value and anti-nutritional (ODAP) concentration (Dutta et al. 1982; Deshpande and Campbell 1992; Pandey et al. 1995a, b; Campbell 1997; Tarade et al. 2007). The diversity study and cluster analysis in set of >350 germplasm reflects that ample amount of genetic variation existed for seed yield and other yield components (Parihar et al. 2013). In another study, Parihar et al. (2015) reported enormous variability for individual traits like days to flowering (60–96), days to maturity (117–142), plant height (22–89 cm), seeds per pods (2–5), primary branches (4–15) and pods per plant (13–100). Parihar et al. (2015) identified some trait-specific genotypes such as BioR 202 (higher yield/plant, pods/plant and biological yield); EC20034, JBT 29/83 and RSR/SSC-1/12 (higher yield/plant and harvest index); ET48116 (high yield/plant, no. of pods/plant). These lines may be useful in future grass pea breeding programme for developing better segregants.

Based on seed size, grass pea is grouped into three classes: large seeded (lakh type) originated from the Mediterranean region (Syria, Turkey, Italy, Spain), medium seeded from northern France and Germany and the smallest seeded (Lakhori type) belonging to the South Asian and Polish cultivars (Hanbury et al. 1995). A range of 3.45 to 22.59 g/100 seeds have been reported in Canada by Robertson and Abd El-Moneim (1995). Hammer et al. (1989) indicated that the large seeded grass pea genotypes from South Italy having a larger vegetative canopy with exceptionally high 100 seed weight. The small-seeded grass pea genotypes are prevalent in South Asian and South East Asian countries (Barpete 2015). Radwan Safaa et al. (2013) reported a reasonable genetic variability for the evaluated traits for 18 accessions of *L. inconspicuous* and found possibility of genetic improvement through simple selection for those traits. The blue-flowered ecotypes are found in South-East Asia and the South Asia (Polignano et al. 2005b; Kumar et al. 2013), whereas white flowered types are generally reported from Mediterranean region ecotypes (Smartt 1984).

High variability of ODAP content was recorded for both inter-specific and intra-specific levels in *Lathyrus* (Sammour et al. 2007a). In the beginning of last decades, Barpete et al. (2012a) observed high genetic variation in total seed protein and weight. Apart from this, some of the available *Lathyrus* germplasm having attractive yield traits such as single node double flower or pods (L900239 and L920278) and more than 30 g/100-seed weight (LS-2026, LS-8, LS-97 and Quila-blanco), these traits can be further utilized for grass pea improvement programme (Campbell and Briggs 1987; Ulloa and Mera 2010). Promising genotypes for harvest Index like Bio R-202, Bio L-203, Bio R-231 and Bio L-208 were also identified (Gautam et al. 1998). Some wild relatives namely *L. cicera*, *L. amphicarpus* and *L. ochrus* species have been identified with zero or low-ODAP (0.01% or less) gene that can be utilized for development of toxin free *Lathyrus* varieties (Campbell 1997; Kumar et al. 2013).

The variation for biotic and abiotic stresses has also been recorded in grass pea and consequently, identified genotypes (RLK-1, RLK-281, RLK-617, RPL-26,

RLK-273-1, RLK-273-3, JRL-6 and JRL-41) possessing resistance to powdery mildew (Erysiphe *pisi* syn. *E. polygoni*) (Asthana and Dixit 1998; Pandey et al. 1997; Lal et al. 1985). Some CWRs such as *L. ochrus, L. clymenum*and and *L. aphaca* also demonstrated resistance to powdery mildew (Gurung et al. 2002; Pandey et al. 1995a, b). Therefore, serious efforts need to be made to utilize these available sources to incorporate powdery mildew resistance gene into high-yielding and stable genotypes (Campbell 1997; Vaz-Patto et al. 2004; Dixit et al. 2016). Downy mildew (*Peronospora lathyri-palustris*) is another serious disease in South Asia owing to suitable climatic conditions for the development of this pathogen (Lal et al. 1985). Some resistance grass pea accessions (RLS-1, RLS-2, JRS-115, JRL-43, and JRL-16) for downey mildew have been reported in India (Lal et al. 1985; Narsinghani and Kumar 1979). Ascochyta blight (*Mycosphaerella pinodes*) is also important disease and resistance reaction for this has been recorded in ATC 80878 accession of *L. sativus* and in wild species *L. ochrus* and *L. clymenum* (Gurung et al. 2002; Skiba et al. 2004b).

Downy mildew and ascochyta blight resistance sources (Sel. 553, 555, 563, 529, 504) were also identified by Robertson and Abd El-Moneim (1998). This may be one of the most necessary and advantageous traits to transfer in cultivated legume crop. Similar to grass pea, ascochyta blight is a serious impediment in case of field pea production and complete resistance to the fungal infection has not been observed (Skiba et al. 2004a). Rust is one of the devastating diseases of grass pea in the Ethiopia and Spain (Campbell 1997; Vaz Patto and Rubiales 2009). So far, complete resistance to rust pathogens (*Uromyces pisi*) is not recorded in *L. sativus* although few grass pea lines, namely, BG-15744 and BG-23505, have been found to be partially resistant against rust (Vaz Patto and Rubiales 2009, 2014a, b). The rust-resistant genes hold great promise not only for grass pea but also for lentil, chickpea and field pea improvement. Grass pea is more susceptible to *Orobanche* or broom-rape (*Orobanche crenata*), and none of the grass pea genotypes demonstrated resistance reaction (Sillero et al. 2005).

Nevertheless, some other *Lathyrus* species, particularly *L. clymenum* and *L. ochrus*, were detected as highly resistant to *Orobanche* (Linke et al. 1993; Sillero et al. 2005). In case of insect pest, thrips (*Caliothrips indicus)* is a serious nuisance to grass pea; however, tolerance to thrips was registered in some Indian accessions (RLK-1, RLK-281, RLK-617, RPL-26, RLK-273-1, RLK-273-3, JRL-6 and JRL-41) (Asthana and Dixit 1998; Pandey et al. 1997; Lal et al. 1985). The aforementioned thrips-tolerant accessions can be further utilized for grass pea improvement program. Cyst nematodes (*Heterodera ciceri*) and root knot nematode (*Meloidogyne artiella*) also cause substantial damage in grass pea but partially resistance lines for cyst nematode (IFLA347) were identified in *L. sativus* germplasm at ICARDA (Di Vito et al. 2001) and resistance reaction for root knot nematode were noticed in *Lathyrus* wild species such as *L. latifolius, L. sylvestris*, and *L. hirsutus* (Rumbaugh and Griffin 1992).

The initial work for exploration of genetic variability for ODAP was started in 1966 in India (Lal et al. 1985). In 1970, more than 1500 samples were screened for ODAP and found few lines containing low (0.15–0.3%) ODAP (Jeswani et al.

1970). Likewise, 1000 samples were screened for ODAP content at IARI, New Delhi during 1971 wherein ODAP varied from 0.2 to 2.56% (Leakey 1979). Similarly, Asthana and Dixit (1998) reported low ODAP donors like Bio R 202, Bio L 212, Bio L 203, Bio R 231, Bio L 208, P 94–3, P 28, L5 8246 and Bio I 222. In comparison to *L. sativus*, the ODAP concentration in *L. cicera* and *L. gorgoni* is lower among the CWRs (Hanbury et al. 1999; Kumar et al. 2013). Assessment of ODAP in different species of *Lathyrus* indicated that none of the species are free from ODAP (Aletor et al. 1994; Hanbury et al. 1999; Siddique et al. 1996). It was observed that grass pea genotypes from South East Asia and Ethiopia are having high ODAP (0.7–2.4%) in comparison to the germplasm collected from Mediterranean region (0.02–1.2%) (Abd-El-Moneim et al. 2000).

Overall *L. cicera* was found to have consistently low ODAP content (0.07%) and *L. ochrus* with high ODAP content (1.35%) (Eichinger et al. 2000; Kumar et al. 2011). The ODAP and protein analyses of different populations at Ethiopia also revealed the presence of variability (Tadesse and Bekele 2004). The ample variability demonstrated for seed weight, protein and ODAP content that ranged from 40.50 to 79.23 g, 25.05% to 33.95% and 0.32% to 2.02%, respectively (Mondal and Puteh 2014). Srivastava et al. (2015) observed variation for ODAP in different genotypes of *Lathyrus* which was in the range of 0.46 to 1.14 mg/g. Gupta et al. (2018) measured neurotoxin content in a set of grass pea accessions originated in Bangladesh which ranged from 0.13 to 0.57%. The content of the anti-nutritional factor β-ODAP varied from 0.21% to 0.55% with a mean value of 0.36% in a panel of 50 grass pea accessions collected from different geographic locations of Ethiopia (Shiferaw and Porceddu 2018). Recently, a total of 702 accession of grass pea was screened for ODAP and protein content and new promising sources (ILG468, ILG1934, ILG1950 and ILG1951) were identified for low seed ODAP content and for high protein content (ILG311, ILG670, ILG688, ILG691 and ILG708) (Rajendran et al. 2019). The judicious utilization of available *Lathyrus* germplasm holds great promise toward improvement of grass pea cultivars in coming future.

26.9 Inheritance of Qualitative and Quantitative Traits

The efficiency of any breeding programme depends on the availability of information about nature and magnitude of gene action, which is utmost to accelerate its success (Shashikumar et al. 2010). The genes may display additive, dominance and/or interaction effects. In case of grass pea, very limited efforts have been made to understand the nature of gene action of complex traits such as yield and its contributing traits (Parihar et al. 2016). During 1970s, Dahiya and Jeswani (1974) found that non-additive gene action was predominant for pods/plant, 100-seed weight, seeds/pod and grain yield/plant. Kumari et al. (1993) had reported that four genes were responsible for flower pigmentation. A study was also done to understand the genetics of flower colour and its relation with ODAP content. The results revealed that both monogenic and digenic segregation was noted.

The gene action for yield and its components was studied in grass pea by Dixit (1998a, b) and revealed that additive and non-additive gene effects were involved in the expression of number of primary branches, pods per plant and grain yield per plant. Plant height was predominantly under the control of dominance gene effect. Earlier few researchers have reported high heritability for seed yield and other traits such as 100 seed weight, seeds/plant, seeds/pod and pods/plant (Kumari et al.1995; Kumar and Dubey 1996). Parihar et al. (2015) reported that the seed/pod, pod length, pod width, 100-seed weight and seed yield/plant are governed by non-additive gene action. In another study, Parihar et al. (2016) inferred that yield and its contributing traits exhibited all three types of gene actions, that is additive, dominant and epistasis. In such situation, recombination breeding could be exploited, followed by selection delayed to later generations.

There are several reports regarding the inheritance pattern of ODAP content in seed of grass pea from qualitative to quantitative inheritance (Nerkar 1972; Dahiya and Jeswani 1974; Gowda and Kaul 1982; Dahiya 1986; Quader et al. 1987; Briggs and Campbell 1990; Tiwari and Campbell 1996). Certain reports are there about the presence of modifying genes (Quader 1985) and presence of maternal inheritance (Quader et al. 1987; Tiwari and Campbell 1996). Biosynthetic pathways studies by Malathi et al. (1967) and Lambein et al. (1990) revealed that at least two enzymes were involved in the synthesis of the neurotoxin. This also suggested that more than one gene controls ODAP synthesis (Quader et al. 1987). However, Dahiya and Jeswani (1974) reported for the first time that low ODAP content is controlled by non-additive gene action. Nevertheless, later on predominance of additive and additive × additive gene effects for ODAP becomes familiar (Briggs and Campbell 1990; Mehra et al. 1993; Pandey et al. 2000; Sharma et al. 1997; Tiwari and Campbell 1996). Dahiya (1986) reported high ODAP content in white/cream seeded varieties but later on Pandey et al. (2000) contradicted with him and reported wide variation for ODAP content in white × blue and blue × red flowered F_2 populations. Hanbury et al. (1995) found positive correlation between seed size and vigour. The narrow sense heritability of the ODAP indicates effectiveness of selection in early generations for accumulation of favourable alleles in the direction of selection (Nerkar 1972; Quader et al. 1987).

The negative association of ODAP content with days to maturity and seed yield was noticed (Sharma et al. 2000; Tadesse and Bekele 2003). Interestingly, the correlation between ODAP concentration and seed weight was reported either to be positive (Hanbury et al. 1995; Siddique et al. 1996), negative (Hanbury et al. 1999) and no correlation (Kumari and Prasad 2005). Other studies also pointed out no relationship between ODAP content and other agronomic traits (Pandey et al. 1997), suggesting that selection for high yield and low ODAP can simultaneously be practiced for grass pea improvement. A recent study discovered that the biosynthesis of ODAP involves more than two genes, with dominant alleles predominating (Tripathy et al. 2015). The genotype × environment interaction plays crucial role in the inheritance of ODAP content in grass pea (Nerkar 1972; Quader 1985; Ramanujam et al. 1980; Chen et al. 1992; Dixit et al. 1997; Tadesse 2003; Jiao et al. 2011; Tripathy et al. 2015). Mondal and Puteh (2014) observed significant

positive correlation between seed size and protein content but negative association between seed size and ODAP content.

Therefore, it may be feasible to develop low neurotoxin varieties by selecting bold seed size following hybridization of bold seeded and low neurotoxin types with those having small seeds and high neurotoxin. Significantly negative correlation between protein and β-ODAP content under different cooking methods for grass pea germplasm was also recorded (Barpete et al. 2021a, b). This suggested that improvement in protein content may indirectly reduce the β-ODAP concentration in grass pea seed through breeding efforts. Although limited sincere efforts have been made till date to identify the biosynthetic pathway and discover the putative genes associated with ODAP biosynthesis. It has been noticed that ODAP is generally present in all plant parts; however, the concentration remains high in leaf and embryo during vegetative and reproductive stages, respectively (Barpete 2015). The variation in seed ODAP concentration of genotypes results due to variation in net accumulation of ODAP in leaves and pods during vegetative and early reproductive phase (Xiong et al. 2015). In a recent report, it is noticed that nutritional deficiencies of cysteine and methionine may exaggerate the neurotoxicity of ODAP which advocated that ODAP biosynthesis is allied with nitrogen and sulphur metabolism (Xu et al. 2017).

26.10 Major Constraints of Grass Pea Promotion at National and International Level

The prime stalemate in grass pea improvement both nationally and internationally is the existence of neurotoxin (β-ODAP) which is popularly known as b-N-oxalyl-amino-L-alanine (BOAA) that is responsible for irrevocable neurological mayhem in humans and animals if grass pea seeds are consumed constantly as more than 25% part of daily diet for 3–4 months (Vaz Patto et al. 2006a, b; Lambein and Kuo 2009; Vaz Patto and Rubiales 2014a, b; Dixit et al. 2016; Lambein et al. 2019). Consequently, it has been banned especially in all states of India except Chhattisgarh, Maharashtra and West Bengal (Dixit et al. 2008). However, sincere efforts have been made worldwide and a number of low ODAP and high-yielding varieties were released (Table 26.4), although the general acceptance of these varieties among the farmers is miserable due to lack of knowledge. Therefore, there is urgent need of intensive efforts for removal of this stigma from grass pea by demonstrating the potential of these technologies through on-farm trials. In addition, grass pea is being grown primarily on marginal land with minimum input under rain-fed conditions (Dixit et al. 2016) which ultimately lead to low yield potential.

However, in addition to variety of agronomic limitations, grass pea is also exposed to a number of biotic stresses to some extent: powdery mildew, rust, downy mildew, ascochyta blight, fusarium wilt, broomrape and thrips cause sizeable yield reduction under favourable environmental conditions (Campbell 1997, Robertson and Abd El-Moneim 1996; Gurung et al. 2002; Vaz Patto et al. 2006b; Barilli et al. 2011, 2012; Talukdar 2013; Abdallah et al. 2021; Sampaio et al. 2021).

Table 26.4 Improved varieties of grass pea recommended for cultivation in different countries

S. no.	Name of variety	Developing institution	Country	Brief description	References
1	Ratan (Bio L 212)	IARI, New Delhi	India	This variety was isolated from Somaclonal variation of Pusa-24 during 1997. It has yield potential of 1500–1600 kg/ha and matures in 110–115 days. The variety has low ODAP content (0.7%), large seed size and blue colour flower. This variety recommended for cultivation in north eastern plain zone and central zone	Anonymous (2020), Dixit et al. (2016), Vaz Patto and Rubiales (2014a, b), Kumar et al. (2011), ICAR (2009) and Abd El Moneim et al. (2001)
2	Prateek	IGKV, Raipur	India	This elite genotype developed by hybridization of LS-8246 × A 60 following pedigree method and released in 2006. This variety has low ODAP (0.076%) with high protein (25.83%) content. It matures in 110–115 days and has tolerance toward downy mildew, thrips and pod borers and resistance to powdery mildew. The plant height varied between 45 and 70 cm and flowers colour is blue. This variety recommended for rain-fed and *utera* cultivation and as irrigated crop in rabi late sowing of entire Chhattisgarh and MP	Dixit et al. (2016), Vaz Patto and Rubiales (2014a, b), Kumar et al. (2011), ICAR (2009) and Abd El Moneim et al. (2001) http://igau.edu.in/pdf/CropTechnologies.pdf. Accessed 19 Jun 2021
3	Mahateowda (RLS 4595)	IGKV, Raipur	India	This variety evolved through pedigree method from a cross Ratan × JRL-II and released in 2008 for cultivation in Chhattisgarh. This variety has pink coloured flowers and could be easily identified from the land races as well as earlier released varieties. The yield potential of this variety is 1000–1400 kg/ha coupled with low ODAP content (0.07–0.08%) and medium maturity duration (90–100 days). Suitable for rain-fed and utera cultivation and as irrigated crop in rabi late sowing of entire Chhattisgarh and Madhya Pradesh. Also suitable for medium to heavy soils	ICAR (2009), Abd El Moneim et al. (2001), Kumar et al. (2011), Vaz Patto and Rubiales (2014a, b) and Dixit et al. (2016); http://igau.edu.in/pdf/CropTechnologies.pdf

(continued)

Table 26.4 (continued)

S. no.	Name of variety	Developing institution	Country	Brief description	References
4	BidhanKhesari-1	BCKV, Kalyani, West Bengal	India	This elite genotypes is developed through selection and has low ODAP and high yield potential	ICAR (2009)
5	Nirmal	–	India	This genotype is also developed by selection method and released in 1980 for cultivation. It has low ODAP (0.2%) and high yield potential	Asthana and Dixit (1998) and Sarkar et al. (2003)
6	BARI Khasari-1	Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh	Bangladesh	This is tall type variety (70 cm) developed by hybridization between P-24 × local and released in 1995. It has large deep blue flower with larger seed size (1000 SW 48–52 g). It contains ODAP content 0.0137 m/g (0.04%) with average grain yield of 1720 kg/ha and matures in 125–130 days. It is tolerant to drought and salinity and also tolerant to powdery and downy mildew disease	Malek et al. (1996), Malek (1998), Abd El Moneim et al. (2001), Kumar et al. (2011) and Vaz Patto and Rubiales (2014a, b); http://dhcrop.bsmrau.net/. Accessed 26 Jun 2021
7	BARI Khasari-2	Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh	Bangladesh	This is a low ODAP variety (0.06%) developed from local materials and released in 1996. It has plant stature of 55–60 cm and blue colour flower with light grey color seed. It matures in 125–130 days and gives 1727 kg/ha grain yield. It has tolerance to drought and salinity with 24–26% protein content	http://dhcrop.bsmrau.net/. Accessed 26 Jun 2021; Malek et al. (1996), Malek (1998), Abd El Moneim et al. (2001), Kumar et al. (2011) and Vaz Patto and Rubiales (2014a, b)
8	BARI Khasari-3	Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh	Bangladesh	This variety is developed from lines developed through hybridization at ICARDA; of which one superior fixed line, i.e. Sel-190 line was released as BARI Khasari-3 in 2011. This high-yielding variety (1800–2000 kg/ha) with plant height 62–65 cm, pod no 35–38, 1000 grain weight 53–58 g, ODAP 0.04%, crop duration 120–125 days. This variety is cultivated as rely crop with Aman rice and also cultivated individual crop	http://dhcrop.bsmrau.net. Accessed 26 Jun 2021; Rizvi et al. (2016)

9	BARI Khasari-4	Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh, Pulse Research Centre, Ishurdi, Pabna	Bangladesh	This variety was developed from ICARDA low ODAP materials and released as BARI Khesari-4 in 2013. This variety has large leaflet, white flower and white colour seeds with 1000 grain weight 70 g. this variety matures in 118–117 days with yield potential of 720–1080 kg/ha. Besides, it also has tolerance to powdery mildew disease	http://dhcrop.bsmrau.net. Accessed 26 Jun 2021; Sarkar et al. (2019)
10	BARI Khasari-5	Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh, Pulse Research Centre, Ishurdi, Pabna	Bangladesh	This variety released in 2018 with high yield and low ODAP content (0.04%). It has average yield of 1480–1700 kg/ha plant height (60–70), high biomass and matures in 121–125 days. The flowers are large and dark blue. The seeds are large in size with smoothness and gray in color. This variety is also tolerant to rot and downy mildew	http://dhcrop.bsmrau.net. Accessed 26 Jun 2021; Sarkar et al. (2019)
11	BINA Khesari 1	Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh	Bangladesh	This variety was developed through mutation and released in 2001. This is high-yielding variety (1900–2400 kg/ha) having low BOAA content (less than 0.2%). It has black spotted seed coat and medium tall plant stature. This variety 100-seed weight is 7.9 g and matures in 110 days	Abd El Moneim et al. (2001), Kumar et al. (2011), Vaz Patto and Rubiales (2014a, b) and Rizvi et al. (2016)
12	Wasie	ICARDA	Ethiopia	This elite genotypes developed by hybridization using SC5 × PGRC 46071(OLAT-LS-LS-B2). It has low ODAP (0.08%) and yield potential of 1673 kg/ha with 8.6 g 100-seed weight. It is semi-erect type with blue flower colour and has resistance to powdery mildew	ICARDA (2006, 2007), Abd El Moneim et al. (2001), Kumar et al. (2011), Vaz Patto and Rubiales (2014a, b), Almeida et al. (2015a, b, c) and Rizvi et al. (2016)

(continued)

Table 26.4 (continued)

S. no.	Name of variety	Developing institution	Country	Brief description	References
13	Ali-Bar	ICARDA	Kazakhstan	It is developed by selection from germplasm line (IFLLS 554). It has yield potential of 1200 kg/ha in the dry areas with 250–300 mm precipitation. The ODAP content is 0.01% and matures in 156 days. It is a high biomass, white seeded and drought-tolerant variety	ICARDA (2006, 2007), Abd El Moneim et al. (2001), Kumar et al. (2011), Vaz Patto and Rubiales (2014a, b), Almeida et al. (2015a, b, c) and Rizvi et al. (2016)
14	Pusa 24	IARI, New Delhi	India	This variety developed through selection from germplasm and released in 1974. It has ODAP content 0.2–0.3% and yield potential of 1655 kg/ha. It has blue colour flower, grey seeds and matures in 125–130 days	Mehra et al. (1995), Abd El Moneim et al. (2001), Kumar et al. (2011), ICAR (2009), Dixit et al. (2016) and Rizvi et al. (2016)
15	LS8246	–	Canada	This variety developed through selection from Pusa 24. It has 0.03% ODAP content and high yield potential of 2050 kg/ha. It has 100-seed weight 9.3 g and maturity period ranges from 110 to 130 days. It is suitable for fodder and feed purpose	Campbell and Briggs (1987), Kumar et al. (2011) and Rizvi et al. (2016)
16	AC Greenfix	–	Canada	This is high nitrogen-fixation variety	Krause and Krause (2003)
17	Luanco-INIA	–	Chile	This elite genotype developed through selection from LS0027. It has less than 0.18% ODAP content with yield potential of more than 4000 kg/ha. It is a tall type (150 cm) and white seeded variety with large seed size	Mera et al. (2003), Kumar et al. (2011) and Rizvi et al. (2016)
18	Jumbo-INIA	–	Chile	It is a large seeded variety developed through selection	Mera et al. (2003)
19	Quila-Blanco	–	Chile	This genotype developed by direct selection and released in 1983. It has high yield potential and large white seed, uniform maturity, protein content of 24.3%	Campbell et al. (1994) and Ulloa and Mera (2010)

20	Ceora	CLIMA, WA	Australia	This is Australia's first *Lathyrus* cultivar and released in 2004. This variety was developed by hybridization between K33 × 8604. It has low ODAP content and ranges between 0.04 and 0.09% and high protein content (30%). It is a semi-erect, early to medium duration variety with 500–1800 kg/ha yields potential. It is also suitable for forage and green manure. It has tolerance to water logging, drought, disease and insect resistant. It has white flowers with dark blue flecking in the centre. Its grain is angular in shape with a greyed orange colored seed coat and a yellow cotyledon	Siddique et al. (2006), Gupta et al. (2021) and Kumar et al. (2021)
21	Chalus	CLIMA, WA	Australia	Chalus is a Syrian line released in 1998. It is slightly early maturing and has low ODAP levels of below 0.09%. This variety is developed by selection from IFLA 1279 line of *L. cicera*. It has less than 0.1% ODAP content and 6.6 g 100-seed weight. It is an early to medium duration variety with good protein (26.5%) content	Hanbury and Siddique (2000), Hanbury and Hughes (2003), Kumar et al. (2011), Vaz Patto and Rubiales (2014a, b) and Rizvi et al. (2016); https://grdc.com.au/
22	Lath-BC	–	Australia	This variety was commercialized in 1997 and the first variety available in Australia. It has red flowers, brown to green seed coat and bright yellow seed color. Seed size is comparatively small (100 seed wt of 6 g) and ODAP levels are 0.13%	https://grdc.com.au/Grain_Legume Handbook.PDF
23	CLIMA pink	CLIMA, Australia	Nepal	This variety was introduced in Nepal by CLIMA, Australia. This variety has yield potential of 1550 kg/ha with 9.0 g 100-seed weight. It has pink flowers and matures in 132 days. The ODAP content is less than 0.04%	Yadav (1996), Neupane and Tiwari (2005), Kumar et al. (2011), Rizvi et al. (2016), Neupane et al. (2017) and Sarkar et al. (2019)

(continued)

Table 26.4 (continued)

S. no.	Name of variety	Developing institution	Country	Brief description	References
24	19A	CLIMA, Australia	Nepal	This variety is developed by direct selection and introduced in Nepal by CLIMA Australia. It has average yield potential of 1075 kg/ha with 10.0 g 100-seed weight. It is blue flower colour variety and matures in 131 days	Yadav (1996), Neupane and Tiwari (2005), Kumar et al. (2011), Gharti et al. (2014), Rizvi et al. (2016), Neupane et al. (2017) and Sarkar et al. (2019)
25	20B	CLIMA, Australia	Nepal	This variety is developed using selection and introduced in Nepal by CLIMA, Australia. It has average yield potential of 750 kg/ha with 11.0 g 100-seed weight. It is blue flower colour variety and matures in 132 days	Yadav (1996), Neupane and Tiwari (2005), Kumar et al. (2011), Gharti et al. (2014), Rizvi et al. (2016), Neupane et al. (2017) and Sarkar et al. (2019)
26	BARI-2	CLIMA, Australia	Nepal	This variety introduced in Nepal by CLIMA, Australia. It has yield potential of 1000 kg/ha and 10.0 g 100-seed weight. It is blue flower colour variety mature in 135 days	Neupane and Tiwari (2005), Kumar et al. (2011), Gharti et al. (2014), Rizvi et al. (2016) and Neupane et al. (2017)
27	CLIMA-2	CLIMA, Australia	Nepal	This variety was introduced in Nepal by CLIMA Australia	Neupane and Tiwari (2005) and Neupane et al. (2017)
28	Derek	–	Poland	This variety is developed by selection frosm Der. It has average yield potential of 1920 kg/ha with 11.5 g 100-seed weight and mature in 115 days. It is semi-erect and white seeded variety	Kumar et al. (2011), Rizvi et al. (2016), Milczak et al. (2001) and Sarkar et al. (2019)
29	Krab	–	Poland	This variety is developed through selection from Kra. It has average yield potential of 2280 kg/ha with 19.3 g 100-seed weight and mature in 109 days. It is semi-erect and white seeded variety	Kumar et al. (2011), Rizvi et al. (2016), Milczak et al. (2001) and Sarkar et al. (2019)

30	Strandja	–	Bulgaria	This variety developed through local selection. It is high-yielding variety with more than 2.5 t/ha productivity and 17.0 g 100-seed weight. It has medium plant stature and early maturity duration (90 days)	Campbell and Briggs (1987), Kumar et al. (2011) and Sarkar et al. (2019)
31	Italian	–	Pakistan	Low ODAP variety	Kumar et al. (2013)
32	Poltavskaya	–	Russia	It is developed by mutation (EMS 0.01%) and has low ODAP content	Kumar et al. (2013)
33	Studenica	–	Serbia	This variety was developed by pedigree method from hybrid populations of polish cultivars and Serbian landraces. It has high grain and forage yield. The crude protein content in forage and grain dry matter is 208 g/kg and 281 g/kg, respectively	Lambein and Kuo (2009), Mikic et al. (2011) and Kumar et al. (2021)
34	Sitnica	–	Serbia	This variety was developed through pedigree method from hybrid populations of Polish cultivars and Serbian landraces. It has high grain and forage yield. The crude protein content in forage and grain dry matter is 233 g/kg and 286 g/kg, respectively	Lambein and Kuo (2009), Mikic et al. (2011) and Kumar et al. (2021)
35	Jaboulah	–	Lebanon	Jaboulah variety is derived from *Lathyrus cicera* germplasm of ICARDA and released in Lebanon during 1997. The variety is developed through ICARDA low ODAP material (IFLLC-492). This variety has broad leaflets with copper red flower color with brown and small seed size. It has high forage yield and low in ODAP content ($\leq$0.1%). The variety has high yield potential and drought tolerant	Kumar et al. (2021)
36	IFLLO 185	–	Jordan	The variety (IFLLO-185) is selected for forage purpose from ICARDA material and released during 1994 in Jorden. It has high forage yield with medium to late duration variety with good protein content. It has also high yield and resistant to Orobanche	Kumar et al. (2021)

(continued)

Table 26.4 (continued)

S. no.	Name of variety	Developing institution	Country	Brief description	References
37	Gurbuz-2001	–	Turkey	This variety was commercialized in 2002 and the first cultivated variety available in Turkey. This variety was selected and developed from ICARDA low ODAP materials (IFLVN-794) and released as Gurbuz-1 in 2002. This variety has large leaflet, blue flower and brown seed coat color with 100 grain weight 12.10 g. this variety matures in 145–155 days and yielded between 1250 and 1350 kg/ha in Mediterranean type environments	Kumar et al. (2021)

Among the abiotic factors, drought, waterlogging, salinity and temperature extremities affect quantity and quality of seeds (Campbell 1997; Herwig 2001; Kumar and Tripathi 2007; Palta et al. 2007, 2012; Polignano et al. 2009, Gusmao et al. 2012; Jiang et al. 2013; Tsegay and Andargie 2018). Scarcity of quality seed of improved and higher-yielding varieties, low acceptance rate of novel crop production technologies, minimum application of fertilizer are some of the other factors that negatively affect grass pea production (Pandey et al. 1996). Notwithstanding, it has been recommended that the application of micronutrients to deficient soils could be a lucrative approach to enhance grass pea production but farmers seldom take up such practices (Baghel et al. 1995; Mehta 1997). Furthermore, the inadequate transfer of improved technologies remains another constraint.

26.11 Breeding Progress/Varietal Development

As compared to other pulse crops, grass pea has always been ignored in terms of genetic research; however, it has been grown as a pulse crop for over 8000 years (Smartt 1984; Rahman et al. 1995). The reasons for being ignored as a pulse crop might be due to the presence of neurotoxin (ODAP) and primarily being used as a fodder. The selection criteria imposed on forage crops are opposite of grain crops in several ways. Therefore, it is recommended that the development of a more compact growth habit, coupled with some increase in seed size and eradication of the neurotoxin, could convert grass pea into one of the great value crops in the semi-arid areas of the developing nations (Campbell 1997).

Many programmes currently are addressing with several aspects of grass pea improvement simultaneously such as low ODAP content, nutritional quality, resilience to biotic and abiotic stresses, nitrogen fixation, food, fodder and forage production to ensure food security in harsh environments, low input livestock feed and a cover crop for soil conservation (Hillocks and Maruthi 2012). There are various old schools (conventional) and new schools (non-conventional) breeding approaches that have been used for genetic enhancement of grass pea over the years. In case of conventional approaches, several methods such as selection, introduction, hybridization and mutation breeding have been used (Dixit et al. 2016). In this section, we will elaborate and narrate the different breeding strategies and accomplishments made for genetic improvement of grass pea during last five decades.

26.11.1 Conventional Approaches

Albeit, the abundant reward grass pea hold but comparatively very modest attempt has been made towards its improvement due to the disgrace of ODAP (Vaz Patto et al. 2006a, b). However, most of the initial progress and around 50% varieties (Table 26.4) with low ODAP have been developed by direct selection from landraces and lines (Vaz Patto et al. 2006a, b). The traditional breeding approaches

intend primarily on hybridization of pre-selected trait specific genotypes and subsequently screening and evaluation of the ensuing progenies for targeted trait. To trim down ODAP content, low ODAP genotypes were crossed with high seed yield material with good agronomic potential in conventional breeding (Campbell 1997). The high seed yield potential has been a selection criterion for most crop improvement programmes. Conversely, on some of the yield components, that is double podding or increased seeds per pod lesser efforts were invested.

The high biomass yield of grass pea is also important and has acknowledged more attention during the recent past decades (Campbell 1997; Abd El Moneim et al. 2001; Vaz Patto et al. 2006b). This is a very important area due to the large potential of this crop for forage and straw in the North African and South Asian regions (Campbell 1997). Moreover, superfluous parameters, for instance, prostrate and indeterminate plant growth habit, prolonged duration and pod shattering (Rybinski 2003), are being addressed by numerous breeding programmes. As stated earlier, at the outset most of the grass pea varieties were developed through selection from accessible germplasm and landraces. Indeed, the grass pea advancement programme taken place in three different phases during last 80 years. The first phase (1940–1960) of grass pea improvement primarily focussed on grain yield improvement through congregation of local landraces and isolation of superior single plants progenies for high yield and delivered several high-yielding varieties (BR13, LC76, T2–12, No.91, No. 11 and B-19) pertinent for cultivation in Madhya Pradesh and West Bengal (Gautam et al. 1998; Dixit et al. 2016). The second phase (1974–1990s) was devoted for the development of high-yielding varieties along with low β-ODAP content ($\leq$0.2%) for upland conditions (Gautam et al. 1998).

Consequently, improvement programme was initiated by different countries like India in 1966 (Lal et al. 1986), Canada in 1967 (Campbell 1988), Bangladesh in 1980 (Kaul and Islam 1981) and Nepal in 1986 (Yadav and Prasad 1993). Consequently, numbers of improved varieties (Table 26.4) possessing β-ODAP content less than 0.1% were emanated as the product of different national and international breeding programmes (Abd El Moneim et al. 2001; Kumar et al. 2011, 2013; Dixit et al. 2016 ; Lambein et al. 2019). The first variety which had low ODAP content in seed (0.2%) was the Indian landmark variety "Pusa-24" and it was selected from a field in 1966 from Bihar. This variety was released in 1973–1974 particularly for upland cultivation where it was widely accepted (Dahiya and Jeswani 1974; Lal et al. 1985; Campbell and Briggs 1987; Jain et al. 1974; Mehra et al. 1995). Afterwards, "Pusa-24" variety has been used as the base parent in a number of other low ODAP varieties in India and elsewhere. For example, in Canada, LS82046 was selected from "Pusa 24," with low ODAP (0.03%) level in seed (Campbell and Briggs 1987).

However, the value/concentration of ODAP may vary in different environments, but genotype has much stronger effect (Hanbury et al. 1999). Succeeding research work led to the development of varieties suitable for upland (LSD1, LSD2) and rice fallow (LSD3, LSD6, Pusa-305, and Selection 1276) with low ODAP contents (0.15–0.20%). Especially, the LSD series was developed by selection from P-24 during 1978 (Gautam et al. 1998; Sethi et al. 1987). Notably, the intense efforts for grass pea improvement have been amplified after the development of low ODAP

genotypes (Campbell et al. 1994; Hanbury et al. 1999). Similarly, in Chile, grass pea cultivar "Quila-blanco" was developed having synchronous maturity and bold white seeds coupled with good protein concentration during 1983 by selection from the local heterogeneous population (Campbell et al. 1994). Meanwhile, various attempts have also been made to establish association between easily observable characters and ODAP for ease of selection, but it remains unsuccessful owing to polygenic inheritance of ODAP which is highly influenced by genotype, environment and their interactions (Kaul et al. 1982; Tiwari and Campbell 1996; Hanbury et al. 1999).

During past five decades (1940–1990), the prime breeding objectives were to develop high-yielding varieties with low ODAP in grass pea and inadequate efforts were made towards the development of biotic and abiotic stresses resistance varieties. Therefore, in third phase (1990s onwards), the major objectives were good yield, low ODAP and tolerance to powdery mildew and thrips. To accomplish these objectives, conventional breeding programmes of grass pea were initiated in several countries, including Australia (Hanbury et al. 1995; McCutchan 2003), Bangladesh (Malek 1998; Rahman et al. 2001), Canada (Campbell and Briggs 1987), China (Yang and Zhang 2005), Ethiopia (Tadesse and Bekele 2003; Tadesse 2003), India (Pandey et al. 1996; Sharma et al. 2000; Santha and Mehta 2001), Nepal (Yadav 1996) and Syria (Abd-El-Moneim et al. 2000, 2001; Kumar et al. 2011, 2020), Poland (Grela et al. 2010), Italy (Granati et al. 2003), USA (Krause and Krause 2003) and Chile (Mera et al. 2003).

In some of the countries mentioned above, the lathyrus breeding programmes are still functioning, though in much smaller scale as compared to other major legume crops (Vaz Patto et al. 2011). Germplasm (accessions) with low ODAP have many undesirable agronomic traits such as late flowering, low yield and susceptibility to biotic and abiotic stresses. In order to combine low ODAP with high yield, appropriate phenology and stress tolerance, breeding programs were initiated (Abd-El-Moneim et al. 2000; Addis and Narayan 2000; Crino et al. 2004; Hanbury et al. 2000; Robertson and Abd El-Moneim 1997; Vaz Patto et al. 2006a, b). Several improved varieties and lines were developed that combined low β-ODAP (<0.1%) with high-yield potential (up to 1.5 tons/ha) and resistance to a variety of biotic and abiotic stresses (Kumar et al. 2013; Dixit et al. 2016). In addition to productivity and adaptability owing to the episode of lathyrism in mankind, major breeding programmes are fundamentally intended for low ODAP content. This has now led to the development of several *L. sativus* or *L. cicera* varieties with low ODAP content and other desirable characters (Hanbury and Siddique 2000; Mera et al. 2003; Siddique et al. 2006; Kumar et al. 2011; Almeida et al. 2015a, b, c).

For example, in India, following elite lines BioR-202, BioL-203, BioL-212, BioR-231, and BioL-208 had enhanced yield and high harvest index. Of them, BioL-212 was identified and released as "Ratan" in 1997 for cultivation in the North East Plain Zone (NEPZ) and Central Zone (CZ) in India (Gautam et al. 1998; Pandey et al. 1998; Santha et al. 1998). Later, two varieties viz., "Prateek" (LS8246 × A-60) and Mahateora (BioL-212 × JRL-2) were developed using hybridization which have very low ODAP content (<0.1%) with 1.5 t/ha grain

yield (Kumar et al. 2013; Dixit et al. 2016). Similarly, in Bangladesh, two (Barikhesari-1 and Barikhesari-2) high-yielding, low ODAP (<0.29%) varieties were developed during 1995–1996 by hybridization (Malek et al. 1996; Rahman et al. 2001). Likewise, in Australia, one variety, that is Ceora with low ODAP levels (0.04–0.09%), was developed which is a derivative of a conventional cross between K33 belonging to Pakistan and 8604 (0.05% ODAP) from Bangladesh (Hanbury et al. 1995; Siddique et al. 2006). Another variety "Chalus" was developed through selection from IFLA1279 and has high protein (26.5%) and low ODAP (0.09%) content (Hanbury and Siddique 2000). On similar note, in Chile a variety namely "Luanco-INIA" with white colour bold seeded (30–35 g/100 seed weight) was developed through selection from germplasm accession "LS 0027" (Mera et al. 2003). Overall, most of varieties are developed by direct selection method followed by hybridization, mutation and tissue culture.

26.11.2 Pre-Breeding and Distant Hybridization

To broaden the genetic base of crop, the introgression of desirable alleles from outside the primary gene pool is needed through opting pre-breeding and distant hybridization. Although inter-generic hybridization is tedious, there have been several successful instances of the development of inter-specific and wide crosses with *Lathyrus* (Dixit et al. 2016). There have been successful inter-specific crosses between grass pea and other *Lathyrus* spp. particularly *L. pseudocicera*. Embryo rescue has increased the range of species in successful inter-specific crosses (Addis and Narayan 2000). The results of inter-specific hybridization in grass pea suggest that the identification and transfer of desirable traits from exotic and wild germplasm offer many opportunities for especially for development of low ODAP genotypes that particularly for crossable species including *L. cicera* and *L. amphicarpus* (Davies 1957; Khawaja 1988; Yunus 1990; Yunus and Jackson 1991).

Crosses have also been made with other CWRs such as *L. chrysanthus, L. gorgoni, L. marmoratus* and *L. pseudocicera* (Heywood et al. 2007), but only ovules were produced. Successful inter-specific hybrids between different species in specific combination were also made and pods were obtained as mentioned earlier (Davies 1958; Trankovskij 1962; Yunus 1990; Kearney 1993; Hammett et al. 1994, 1996; Kumar et al. 2011). The appraisal of wild *Lathyrus* spp. for ODAP content has clearly witnessed that the lowest ODAP amount has been noticed in *L. cicera*, followed by *L. sativus* and *Lathyrus ochrus* (Aletor et al. 1994; Siddique et al. 1996; Hanbury et al. 1999; Kumar et al. 2013). The toxin-free gene recognized in *L. tingitanus* could be used to develop varieties with low levels of toxin (Zhou and Arora 1995). In addition, wild species including *L. ochrus*, *L. clymenum* (Sillero et al. 2005) and *L. cicera* (Hanbury et al. 1999; Fernández-Aparicio et al. 2009; Fernández-Aparicio and Rubiales 2010; Robertson and Abd El-Moneim 1998) are resistant to broomrape that is not available in the cultivated gene pool.

Besides its low ODAP content, *L. cicera* may be used as a promising source of other important agronomic traits such as earliness and cold tolerance (Robertson and

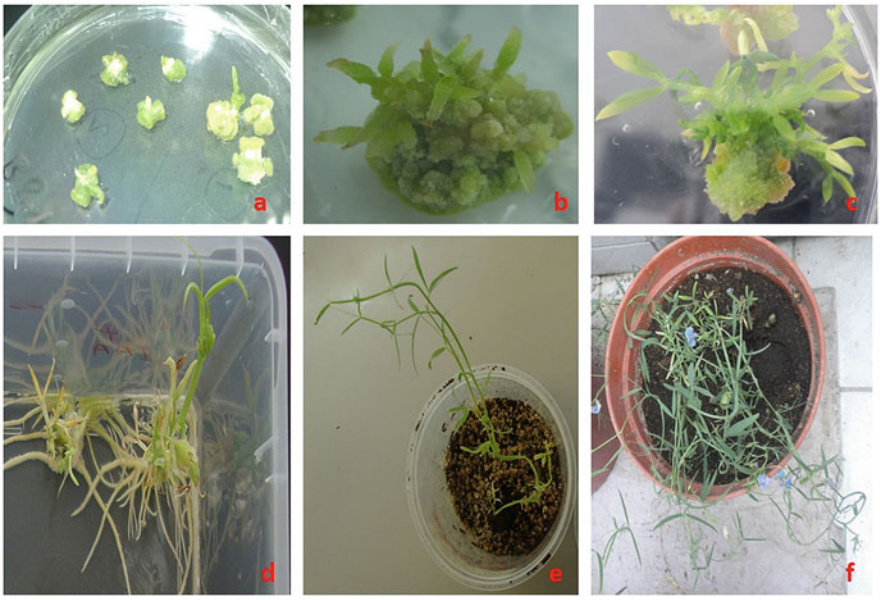

Fig. 26.2 In vitro regeneration of *Lathyrus sativus* L., (**a**) in vitro cultured of epicotyl explant, (**b** and **c**) callus-derived shoot induction from epicotyl explant, (**d**) root induction on callus-derived shoots, (**e** and **f**) acclimatization and hardening of in vitro regenerated grass pea explants

Abd El-Moneim 1998). Most importantly, the *Lathyrus* gene pool holds great promise being a source of resistance to important diseases of legumes such as *ascochyta* blight (*Mycosphaerella pinodes*), downy mildew (*Peronospora lathyri-palustris*) and powdery mildew (*Erisyphe* spp.) (Gurung et al. 2002). Therefore, the variation noticed in the *Lathyrus* wild species offers considerable potential for improvement of grass pea and other related legume species through pre-breeding. However, to overcome strong reproductive barriers among different species, various modern techniques such as biotechnological tools (Barpete et al. 2014a, 2020a, 2021b), including tissue culture (Fig. 26.2), somaclonal variation (Barpete et al. 2014b, 2020b) and protoplast fusion, may also need to be utilized (Ochatt et al. 2001; Piwowarczyk and Pindel 2015; Tripathy et al. 2016). Therefore, reliable and reproducible protocols are prerequisite for successful genetic transformation (Barik et al. 2005; Barpete et al. 2016, 2017).

26.11.3 Mutation Breeding

During recent years, being a climate resilience crop, the demand and importance of this crop has increased. The grass pea improvement is impeded owing to its narrow genetic base which resulted due to self-pollination and inter-specific incompatibility

(Nerkar 1976; Singh and Chaturvedi 1997). Consequently, mutation breeding has been embraced as improvement strategy, since it has been regarded as an ultimate way of creating new genetic variation. It can be a valuable supplement to conventional plant breeding to create additional genetic variability that may be utilized by the plant breeder in the development of desired cultivars for specific purposes (Campbell et al. 1994). In case of grass pea, the chemical mutagens such as EMS (ethyl methane sulphonate) and NMU (N-nitroso-N-methyl urea) are more efficient than radiation in the production of chlorophyll mutations (Nerkar 1976). Nevertheless, different genotypic responses have been noticed when exposed to gamma irradiation (Prasad and Das 1980b). Likewise, the mutagenic effectiveness is in the order of NMU, EMS and gamma rays and efficiency in vice versa (Singh and Chaturvedi 1997). A wide range of morphological mutations have been noticed which affects growth habit, maturity, branching, stem shape, leaf size, stipule shape, flower color and structure, pod size, seed size and seed coat colour (Nerkar 1976; Prasad and Das 1980a; Waghmare et al. 2001; Rybinski 2003; Talukdar and Biswas 2006; Biswas 2007; Talukdar 2009a, b).

Corresponding to morphological changes, chromosomal changes including translocations were induced in grass pea by mutagenesis (Biswas and Biswas 1997; Talukdar 2009a). Mutants with enhanced salt tolerance have been developed which led to an increase in the activities of antioxidants such as superoxide dismutase and ascorbate peroxidase (Talukdar 2011a, b). Mutagenesis approach has also been utilized to create additional genetic variability to develop zero/low ODAP varieties (Talukdar 2009a). Mutagenic treatments have also created noticeable biochemical changes in grass pea, most of them have been reported to contribute directly or indirectly to plant defence system. For instance, a glutathione (GSH)-deficient mutant (*gshl-1*) was isolated from gamma-ray-treated M_2 progeny of the genotype BioL-212 and this mutant demonstrated greater sensitivity to cadmium (Talukdar 2012c). Conversely, improved tolerance to salt and metal toxicity was obtained through induced mutagenesis (Talukdar 2011b, 2013).

One such mutant, *dwf1*, registered an increase in foliar GSH content and normal growth under cadmium stress (Talukdar et al. 2001; Talukdar 2010). Likewise, an EMS-induced mutant, *rlfL*-1, was characterized that portrayed increased rate of cell division and cell growth. Some other mutants like *gshl*-1, an ascorbate (AsA)-deficient mutant (*asfL*-1) and a GSH-overproducing mutant have been examined to ascertain the role of arsenic on wilt tolerance (Talukdar 2013). On the similar note, physical mutagenesis developed an *asfL*-1 mutant having only 42% leaf and 20% root ascorbate content compared to the base material. The results pointed towards the possible incidence of a reorganization event involving antioxidant defence machinery in *asfL*-1 that efficiently mitigated the adverse effect of ascorbate deficiency and permitted survival under salt-stress conditions (Talukdar 2012a). Furthermore, two flavonoid-deficient mutants, namely, *fldL*-1 and *fldL*-2, were produced by EMS mutagenesis wherein leaf flavonoid content was reduced up to 20% relative to CWRs (Talukdar 2012b).

By mutation breeding using EMS (0.01%) and gamma rays (250 Gy), two varieties such as “Poltavskaya” in Russia and “Bina Khesari 1” in Bangladesh

were developed, respectively (Kumar et al. 2011, 2013). In vitro culture has also been used to induce somaclonal variation (Ochatt et al. 2002; Roy et al. 1993; Zambre et al. 2002; Barpete et al. 2014a, 2020b). Induced mutagenesis and somaclonal variation created new variability that offers great opportunities to breeders for the selection of lines with traits of interest. Recently, Tripathy et al. (2014) observed somaclones with variation in flower colour, seed colour, leaflet length and breadth, foliage and pod pigmentation which may be used as genetic markers in breeding. Besides, variants with broad leaf, dwarf height, long pod, large seed, short duration and synchronous maturity are agronomically desirable. Notably, a large seeded somaclone NGOG 5 recovered with high seed yield and low neurotoxin content (ODAP) that can be used as a desirable candidate for future breeding programmes. Notably, dwarf mutants with erect and determinate growth habit, and various leaflet and tendril mutants (Talukdar 2009a, b, c, d), provide an opportunity to restructure the present plant type of grass pea, which is dominated by traits such as prostrate and indeterminate growth habit, weak and tall stature and lodging type (Rybinski 2003). In field pea, the dwarf leafless plant type has better standing ability and is amenable to machine harvesting owing to conversion of leaflets into tendrils, thus development of similar plant type in lathyrus could aid in escalating its productivity (Ali and Kumar 2009; Snoad 1974).

26.12 Breeding Objectives

The prime objectives of any crop breeding programme are to accelerate production potential with sustainability of this potential by developing resistance against disease, pest and unfavourable environments. For grass pea, major breeding objectives can be summarized as follows:

26.12.1 Low ODAP Content (<0.1%)

Sincere efforts have been made across the globe and numbers of varieties have been developed as mentioned in Table 26.4. Although the ODAP content is still an important goal of most of the current grass pea breeding programmes and needs continuous efforts to accelerated development of low- or near-zero-level ODAP genotypes.

26.12.2 High Grain Yield

The mean yield potential of most of the released varieties is in the range of 1.0–2.0 t/ha, except for some of the varieties (Table 26.4). Hence, yield increment up to 2.5–3.0 t/ha should be the selection criterion for most of the crop improvement programmes. In addition, some of the other yield attributes that affect yield such as double podding or increased seeds per pod need sufficient attention. While

improving yield and adaptation to the environment, emphasis is also given to ensure that the palatability, intake and other nutritive values of grain are acceptable.

26.12.3 High Biomass

In many parts of the world, there is a shortage of feed and fodder for livestock and this is especially true for many arid regions where grass pea is being grown. In many cases, the value of the fodder equals or exceeds that of the grain produced (Campbell 1997). The high biomass yield of grass pea is also important and has received more attention during the recent decades (Abd El Moneim et al. 2001; Campbell 1997; Vaz Patto et al. 2006b). This is a very important area due to the large potential of this crop for forage and straw in the North African and South Asian regions (Campbell 1997). Moreover, negative breeding for undesirable traits such as prostrate plant habit, indeterminate growth, late maturity and pod shattering should be handled by several breeding programmes (Rybinski 2003). At the same time as improving biomass and adaptation to the environment, emphasis also should be given to ensure that the palatability, intake and other nutritive values of herbage, hay and straw are acceptable.

26.12.4 Resistance to Biotic Stresses

Grass pea is mainly exposed to following biotic stresses like powdery mildew (*Erysiphe pisi*), rust (*Uromyces fabae*), downy mildew (*Peronospora lathyri-palustris*), ascochyta blight (*Mycosphaerella pinodes*), fusarium wilt, broomrape (*Orobanche crenata* Forsk.) and thrips (*Caliothrips indicus*) which causes considerable yield penalty under congenial environments (Campbell 1997; Robertson and Abd El-Moneim 1996; Gurung et al. 2002; Vaz Patto et al. 2006b; Barilli et al. 2011, 2012; Talukdar 2013; Abdallah et al. 2021; Sampaio et al. 2021). However, efforts have been made and some of the improved varieties developed and genotypes identified for tolerance to prevailing biotic stresses. Actually, this crop is being cultivated mainly by resources poor farmers under poor management with no chemical control for diseases and pests. Hence, continuous efforts with more intensity are required to develop varieties resistant to prevalent biotic stresses.

26.12.5 Resistance to Abiotic Stresses

Grass pea has comparatively better tolerance to drought, flooding, salinity, temperature extremities (high and low) and problematic soils than other pulse crops (Dixit et al. 2016; Sarkar et al. 2019). In case of abiotic stress resistance screening, the scarcity of methodologies to identify resistant genotypes has hampered the proper exploitation in breeding of grass pea and subsequent understanding of the mechanisms underlying resistance to environmental injuries is also lacking.

However, the effects of drought and salt stress on different morphological and physiological traits have been ascertained and several *L. sativus* salt- and drought-resistant genotypes have been reported (Talukdar 2011a, b; Silvestre et al. 2014).

26.12.6 Bio-Fortified Genotypes

In addition to low ODAP to combat the hidden hunger among the resource poor vegetarian populations of developing nation's particularly Asia and Africa where grass pea is prominently grown, cultivars with high iron, zinc and methionine need to be developed. Henceforth, there is an urgent need to develop grass pea cultivars having high iron and zinc with lesser amount of anti-nutritional factors that may promote this underutilized and neglected crop to mitigate micronutrient malnutrition of the underprivileged communities.

26.12.7 Mechanical Harvesting Amenable Genotypes

In future, scarcity of farm/agricultural labour is anticipated that will lead to increase in the cost of cultivation which creates the necessities of developing grass pea varieties suitable for mechanical harvesting. For mechanical harvesting, genotype should have more ground clearance with lodging resistance.

26.13 Genomics-Enabled Improvement

Genomics provides various tools and techniques to tackle the emerging challenge of escalating grain yield, quality and stability of production in the face of anticipated climate changes (Kole et al. 2015). The application of DNA markers has proved successful to facilitate marker-aided selection (MAS) for crop improvement. The further advancement in plant genomics by developing functional DNA marker at large scale offers additional resources to get better understandings of crop diversity at species and gene levels that ultimately assists in acceleration of the pace of genetic improvement (Muthamilarasan et al. 2013, 2014). The following section summarizes the current scenario of genomic resources in grass pea and their possible utilization in marker-assisted/genomic-assisted breeding.

26.13.1 Genomics Resources Panorama

This crop could not make much advancement using conventional breeding in the past, and very fewer attempts have been made in molecular biology due to scarcity of reliable molecular markers representing the entire genome (Yang et al. 2014). Indeed, *Lathyrus* is lagging behind in case of genomic resources as compared to other pulses crops. To date, three linkage maps have been developed for any

Lathyrus species using molecular markers (Chowdhury and Slinkard 1999; Skiba et al. 2004a; Santos et al. 2018). One of them was developed by using 11 RAPD markers, 1 isozyme marker and flower colour (Chowdhury and Slinkard 1999). The another maps were constructed using 47 RAPDs, 7 cross-amplified pea microsatellite simple sequence repeats (SSR) markers and 13 cleaved amplified polymorphic sequence (CAPS) markers (Skiba et al. 2004a). This map was used to conduct quantitative trait loci QTL analysis to evaluate a backcross population for resistance to ascochyta blight. However, no candidate genes were identified at that time for these resistance QTLs that hamper their use in precision breeding (Vaz Patto et al. 2006b). However, these two linkage maps have not been sufficiently saturated with markers and offered many gaps and short linkage groups; therefore, these could not be aligned and compared with linkage maps of other legume species (Vaz Patto et al. 2006b; Almeida et al. 2015a, b, c).

During recent decades, genetic diversity in grass pea has been delineated by various molecular markers such as RFLP (restriction fragment length polymorphism), RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) (Croft et al. 1999; Hanada and Hirai 2000; Chtourou-Ghorbel et al. 2001; Barik et al. 2007; Tavoletti et al. 2007; Nosrati et al. 2012). The existing taxonomic categorization of *Lathyrus* has supported using internal transcribed spacer (ITS), nuclear ribosomal and chloroplast (cp) sequence-specific DNA markers (Kenicer et al. 2005). Generally EST-SSR marker system has a high degree of conservation and can be transferred among species, but the numbers of ESTs for *L. sativus* (178) and *L. cicera* (126) are very limited as compared to ESTs (8702) available for *L. odoratus* (Dixit et al. 2016; Lambein et al. 2019). A set of SSR markers including 20 SSRs was developed using an in silico survey (Lioi et al. 2011). The cross-species and cross-genus amplification of molecular markers system facilitates comparative genomic mapping by providing an alternative for the development of new molecular markers for orphan species (Gutierrez et al. 2005). For instance a large number of molecular markers from *Medicago truncatula*, garden pea, lentil, lupine, and faba bean were shown to be transferable to *L. cicera* and *L. sativus* for their future applications in mapping and diversity studies (Zhu et al. 2005; Chandra 2011; Almeida et al. 2014a).

Genomic and EST microsatellites were the most commonly attempted cross-species amplification marker systems in *Lathyrus*. Some of these marker systems, like microsatellites being co-dominant markers, have an additional advantage for linkage map development (Vaz Patto et al. 2011). In four diverse accessions of *Lathyrus* which belongs to different species, that is *L. sativus*, *L. cicera*, *L. ochrus*, *L. tingitanus*) and *P. sativum* seven SSRs were validated (Lioi and Galasso 2013). The genotyping of 176 accessions with EST-SSRs developed two subpopulations using a model-based population structure analysis wherein authors predicted gene flow among the accessions across the geographical regions in India (Soren et al. 2015). During last decades, different groups used EST-SSR for diversity analysis in different set of genotypes (Shiferaw et al. 2012; Sun et al. 2012; Gupta et al. 2018; Arslan et al. 2020). Ponnaiah et al. (2011) have developed seven *Lathyrus*-specific EST–SSR markers. Ghorbel et al. (2014) employed an Inter-Simple Sequence

Repeats (ISSRs) technique to assess genetic diversity and relationships of seven Mediterranean species of the *Lathyrus* belonging to different sections: *Lathyrus, Clymenum, Nissolia and Aphaca*.

Cleaved amplified polymorphic sequence (CAPS) and derived-CAPS (dCAPS) marker were also developed for utilization in Lathyrus (Almeida et al. 2014a, b). Most recently, in silico mining of nucleotide sequences recognized 203 SSRs, of which 150 markers were screened and only 60 markers were amplified 75 alleles with polymorphic information content (PIC) of 0.45 (Soren et al. 2020). The genetic diversity of three *Lathyrus* species (*L. sativus*, *L cicera* and *L. ochrus*) was assessed using ten SSR markers (Aci et al. 2020) and subsequent population structure demonstrated that *Lathyrus* accessions were divided into three populations regardless of their geographic origin. These results are mostly in conformity with the morphological classification of these species (Kupicha 1983) as well as other DNA-marker-based classifications such as RAPD (Croft et al. 1999), RFLP (Chtourou-Ghorbel et al. 2002a, b), ISSR (Belaid et al. 2006) and SSR (Wang et al. 2015).

SSRs and EST-SSRs have specifically been selected by breeders because of their polymorphic character and co-dominant inheritance, as well as the large number of alleles per locus and abundant in distribution throughout the genome (Gupta et al. 2018; Varshney et al. 2005). These markers allowed the use of *Lathyrus* as a source of interesting traits for other related species and vice versa; the availability of number of molecular markers for *Lathyrus* species, in particular for *L. cicera* and *L. sativus*, has been increased during recent decade. These markers will be useful for molecular plant breeding in the future.

Genetic mapping and QTLs analysis, by means of bi-parental or association mapping (AM) populations, have accelerated the untangling of genetic control of targeted traits which eventually led to MAS, QTL, and AM studies or direct calculation and further genomic selection (GS) (Kulwal et al. 2011). Earlier, AM and GS were hampered due to restricted coverage of genome by available markers. But during recent decade, the next-generation sequencing (NGS) technologies have become popular on its success of sequencing DNA at exceptional speed, thus enabling remarkable scientific achievements and novel biological applications (Mardis 2008; Schuster 2008; Kole et al. 2015). The application of NGS platforms in *Lathyrus* to generate large-scale SSR-enriched sequence data and to develop SSR markers will facilitate the construction of high-resolution maps for positional cloning and QTL mapping (Yang et al. 2014; Wang et al. 2015).

In recent past, Yang et al. (2014) developed 50,144 non-redundant SSR primers, of which 288 were randomly selected for validation among 24 accessions comprised of 23 *L. sativus* and one *L. cicera* accession. Of them only 74 primers showed polymorphic pattern and remaining were either monomorphic or could not be amplified. The large number of SSR markers developed in this study would make a significant contribution to genomics-enabled improvement of grass pea. Of them 30 high-throughput SSRs were further employed to analyse 266 *Lathyrus* accessions and 17 relatives from Africa, Europe, Asia and ICARDA (Wang et al. 2015). The population structure analysis delineated the possibility of gene flow between the European and African accessions, which was further supported by unweighted pair

group method with arithmetic mean (UPGMA)-based cluster analysis and principal component analysis (PCA).

The RNA-Seq technology has been used to design 200 EST-SSR markers, of which 40 markers were validated and only 62.5% marker registered polymorphism between two accessions. Furthermore, they identified 2634 contigs containing SNP (Almeida et al. 2014a, b). The first high-throughput transcriptome assemblies were generated by the RNA-sequencing technology in grass pea genotypes to unravel the molecular mechanisms underlying pre-haustorial rust resistance (Almeida et al. 2014b). This study generated a large number of new gene-based molecular tools: ESTs, EST-SSRs, and single nucleotide polymorphism (SNP) based markers. These markers will be instrumental for future work on high-throughput mapping for uncovering the genetic basis of disease resistance in *L. sativus* and, eventually, comparative mapping with other legume species.

Later, by coupling high-throughput sequencing (Illumina) technology with serial analysis of gene expression (SAGE) analyses, a set of differentially expressed genes was identified in the leaves of *L. sativus* in response to *ascochyta lathyri* inoculation (Almeida et al. 2015a, b, c). Likewise, Hao et al. (2017) conducted RNA-sequencing based transcriptome analysis using Illumina NextSeqTM500 platform and obtained 570 million quality-filtered and trimmed cDNA sequence reads with total length of over 82 billion bp. Approximately two million contigs and 142,053 transcripts were assembled from RNA-Seq data, which resulted in 27,431 unigenes; of these unigenes, 3204 EST-SSR primers were designed, 284 of which were randomly chosen for validation. Of these validated unigenes, 87 EST-SSR primers produced polymorphic amplicons among 43 grass pea accessions selected from different geographical locations. Meanwhile, 146,406 SNPs were screened and 50 SNP loci were randomly chosen for the competitive allele-specific PCR (KASP) validation. Finally, 42 SNP loci were successfully transformed to KASP markers. However, the detection of sufficient number of molecular markers and construction of highly statured genetic linkage map in grass pea is lagging as compared to the other legume crop, which is the prerequisite for localizing the position of the genes/QTLs in the genome that will certainly facilitate MAS programme.

26.13.2 Marker-Assisted Breeding

To perform MAS, the fundamental requirement is availability of closely linked molecular markers with the trait of interest. The DNA markers in addition to assessing the level of genetic diversity in phylogenetic studies have also been used in plant breeding (Vaz Patto et al. 2006a, b). Markers are being used as necessary tools to find out the number, position and individual effects of genes/QTLs controlling traits of interest by linkage mapping and QTL analysis (Campbell et al. 1994). Most importantly, the application of molecular markers in plant breeding hastens the generation of new varieties by helping plant breeders in early selection of desirable individuals based on genetic architecture rather than external appearance (Tanksley et al. 1989; Almeida et al. 2015a, b, c). In addition, markers could

establish the association of phenotypic characters with the genomic loci accountable for them, which could make easy gene transfer to appropriate agronomic background. So far, different types of molecular markers as given in previous section such as RFLP, RAPD, SCAR, AFLP, SRAP and EST-SSR have been used for diversity study in grass pea (Chtourou-Ghorbel et al. 2001; Hanada and Hirai 2003; Marghali et al. 2016; Nosrati et al. 2012; Tavoletti and Iommarini 2007; Lioi et al. 2011; Ponnaiah et al. 2011; Shiferaw et al. 2012; Sun et al. 2012; Lioi and Galasso 2013; Soren et al. 2015; Gupta et al. 2018; Arslan et al. 2020).

The number of molecular markers available for *Lathyrus* species, in particular for *L. cicera* and *L. sativus*, has increased in recent decade (Chandra 2011; Lioi et al. 2011; Shiferaw et al. 2012; Almeida et al. 2014a; Soren et al. 2020). So far limited efforts have been made for establishment of marker trait association in grass pea. In first decades of twenty-first century, only one attempt was made for QTL analysis in a backcross population for ascochyta blight resistance. However, none of the candidate genes were found associated with these resistance QTLs at that time which hampered their use in precision breeding (Vaz Patto et al. 2006b). Later, with the development of high-throughput and dense genotyping, association mapping has taken advantage over bi-parental population by generations of more recombination in short time span (Morrell et al. 2012; Cobb 2013). In addition, NGS platforms generated large-scale SSR-enriched sequence data and enabling mining of SSR markers (Yang et al. 2014; Wang et al. 2015) which will facilitate the construction of high-resolution maps for positional cloning and QTL mapping. Most recently, in grass pea, significant marker–trait association was developed with six markers (Soren et al. 2020).

Although first linkage map of *L. cicera* has been constructed using part of the developed markers in a RIL population. This map covered 724.2 cM (mapping interval of 2.4 cM) with 7 major and 2 minor linkage groups. This study provides a large new set of genic polymorphic molecular markers with potential for mapping rust resistances in this robust species and its most closely related species *L. sativus* (Santos et al. 2018). It also represents the first step towards genomics-assisted precision breeding in *L. cicera*. Overall, there is an urgent need to develop a more comprehensive genetic map for grass pea, with identification of valuable genes and QTLs for MAS and with the possibility of alignment with other species in a comparative mapping approach. Further development of this approach will facilitate the transfer of resistance to diseases such as downy mildew and rusts and selection of superior plants at the seedling stage, through MAS. Linkage maps, gene cloning and MAS will hasten the introgression of novel genes for such traits as disease resistance, low ODAP and increased methionine, to develop germplasm which can be used to improve locally adapted cultivars.

26.14 Modernization of Crop Improvement Programme

To accelerate genetic gain in grass pea, there is urgent need of modernization of ongoing improvement programme by further embracement with advanced tools and techniques. As briefed in previous section substantial number of molecular markers have been used for deciphering genetic diversity in grass pea (Chtourou-Ghorbel et al. 2001; Marghali et al. 2016; Nosrati et al. 2012; Tavoletti and Iommarini 2007; Lioi et al. 2011; Ponnaiah et al. 2011; Shiferaw et al. 2012; Sun et al. 2012; Lioi and Galasso 2013; Soren et al. 2015; Gupta et al. 2018; Arslan et al. 2020). The recent adoption of NGS has facilitated the development of considerable numbers of SSRs, SNPs and transcriptome assemblies in grass pea (Yang et al. 2014; Almeida et al. 2014a, b; Hao et al. 2017; Santos et al. 2018). Discovery of novel genes/alleles for trait of interest needs to be executed via genotyping-by-sequencing (GBS) approaches (Kole et al. 2015). Correspondingly, genome-wide association studies (GWAS) could be used to recognize the genomic regions controlling traits of interest by performing marker trait association's analysis in diverse collection of germplasms that are genotyped and phenotyped for traits of interest.

NGS coupled with GWAS increases the mapping resolution for precise positioning of genes/alleles/QTL linked to the targeted traits (Ma et al. 2012; Liu et al. 2013; Varshney et al. 2014) which is still lacking in grass pea. The genome sequencing of crop is essential for understanding biochemical and physiological processes that discover plant traits and their behaviour towards external environmental extremities (Gedil et al. 2015). The rapid advancement in genome sequencing technologies (Barba et al. 2014) has resulted in unprecedented increase of genomic information which eventually increased opportunities to apply this resources into crop improvement programme, for example through the development of genome-wide marker assays (Rius et al. 2015; Nybom et al. 2014). The draft genome sequencing of grass pea has been recently available, but unfortunately, so far, no significant development has been made on grass pea genome editing to tackle any abiotic or biotic stresses and quality aspects (Emmrich et al. 2020; Kumar et al. 2020). In the rapidly changing scenery of life science technologies, a number of new tools have emerged, particularly for deciphering gene function and metabolic pathways including transcriptomics, proteomics, metabolomics, small RNAomics, epigenomics, interactomics and bioinformatics (Gedil et al. 2015).

It is important to adopt the above-mentioned tools and techniques for the better understanding of biological processes which are the key factors for enhancing productivity and quality of grass pea. Recently with the advancement of NGS-based technologies, transcriptomic-based studies have also been initiated in grass pea (Yang et al. 2014; Almeida et al. 2015a, b, c; Chapman 2015; Tan et al. 2017; Xu et al. 2018; Rathi et al. 2019). Transcriptome profiling has been employed for extrication of grass pea–*U. pisi* interaction to discover the putative biochemical pathways and transcripts governing resistance (Almeida et al. 2014a). Similar use of transcriptomic studies has been made in grass pea differing in aschochyta blight resistance reaction for identification of putative proteins/pathways controlling its resistance (Almeida et al. 2015a, b, c). The biosynthesis pathway of β-ODAP is still

in ambiguity in grass pea; therefore, a transcriptome analysis was conducted to ascertain the genes and pathways controlling β-ODAP synthesis during different growth stages and concluded that the cysteine synthase genes influenced β-ODAP accumulation and were coregulated with primary metabolism (Xu et al. 2018). So, far only one transcriptome study has been done in grass pea pertaining to abiotic stress tolerance and some transcripts concerning drought tolerance have been detected (Rathi et al. 2019).

A recent study on grass pea identified several miRNAs associated with drought tolerance. Of which, 8 miRNAs were upregulated under drought stress while 12 known miRNAs were down-regulated under drought condition (Bhat et al. 2020). In this study, a number of novel miRNAs have also been identified. Further studies are needed to functionally characterize different miRNAs available in grass pea involved in various stress signalling and development of transgenic plants to combat abiotic stresses through functional genomics approach. In conjunction with the genomic and transcriptomic progress, proteomic research has also been exploited in grass pea. By using proteomic, 100 protein spots through two-dimensional gel electrophoresis were identified, which were at least two times differentially expressed when exposed to independent treatment of salt stress, cold stress and abscisic acid treatment for 36 h compared to control plants (Chattopadhyay et al. 2011). The small interfering RNA (siRNA)-mediated gene silencing and virus-induced gene silencing (VIGS) also dictate the gene expression solitary at post-transcriptional level (Unver and Budak 2009; Kasai et al. 2011; Banerjee et al. 2017; Lee et al. 2017). However, in *L. odorata,* Phytoene desaturase (PDS) gene was silenced using VIGS approach, but this has not been replicated in other *Lathyrus sp.* (Grønlund et al. 2008).

Gene regulation through siRNA or miRNA often leads to other effects also, hence, to get rid of that genome-editing technologies are acquiring noteworthy attention to specifically deregulate a targeted gene (Zhang et al. 2015). Generally, three approaches of genome editing are popular among the researchers, namely, zinc finger nuclease (ZFN), transcription activator like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR-cas9) mediated approaches having potential merits and demerits of each one of them (Zhang et al. 2020). Some other reverse genetic approaches such as TILLING (Targeting Induced Local Lesions IN Genomes) and Eco-TILLING which detects induced mutation and natural mutation, respectively, need to be explored properly (McCallum et al. 2000; Comai et al. 2004). However, sincere efforts have been initiated at John Innes Centre, Norwich, Norfolk, England, for studying EMS mutagenized populations for searching low ODAP mutant (Emmrich 2017).

Grass pea hold great promise as a good candidate for both TILLING and Eco-TILLING as the genomic resources are meagre as well as it is recalcitrant in nature during genetic transformation. The development of transgenic in grass pea through *Agrobacterium*-mediated transformation and particle bombardment showed great promise after regeneration of complete plants via tissue culture (Gharyal and Maheshwari 1980; Malik et al. 1993; Roy et al. 1991, 1992; Zambre et al. 2002; Kumar et al. 2011; Barpete et al. 2016, 2021a, b). To replicate the success of other

major crops into grass pea, suitable regeneration and transformation protocol need to be optimized (Barpete et al. 2017, 2020a, b). Sincere efforts have been devoted for optimization of regeneration protocol in tissues culture by using shoot tips, stem, leaf as well as root, seed and epicotyl explants and meristematic tissue (Zambre et al. 2002; Barik et al. 2005; Barpete et al. 2014a, b, 2021a, b).

The transformation was attempted through *Agrobacterium*-mediated and biolistic gene gun (Barna and Mehta 1995; Barik et al. 2005). Moreover, in *L. maritimus Agrobacterium rhizogenes*-mediated transformation and subsequently somatic embryogenesis was also reported (Jiangbo and Jingfen 2002). So far, based on available literature very few attempts were made towards grass pea transformation. Hence, urgently intensive efforts are needed for the optimization of genetic transformation protocol in grass pea and successful generation of transgenic lines of reduced ODAP content as well as improved abiotic stress tolerance. Recent advances in molecular biology, biochemical pathways and metabolic change offer scope to produce genetically bio-fortified grass pea to increase its nutritional value (Kumar et al. 2011; Gupta et al. 2021).

26.15 Coordinated System of Testing

Before the establishment of All India Co-ordinated Pulses Improvement Project (AICPIP) in 1967, the research efforts toward grass pea improvement have remained isolated at individual level. The AICPIP provided an opportunity to access the improved materials to pulse breeders and to test their improved breeding lines over multi-locations across the country. Later on this crop was brought under the compass of separate All India Coordinated Research Project (AICRP) on MULLaRP (Mungbean, Urdbean, Lentil, Lathyrus, Rajmash and Pea) which got operational in November 1995. The coordinated research programme of grass pea in India is carried out through the aegis of AICRP on MULLaRP administered by the Indian Council of Agricultural Research (ICAR). The AICRP on MULLaRP has a large network of 28 AICRP centres covering major pulse-growing states of the country. These centres pursue activities and strategic research in the area of crop improvement, production and protection. Besides, AICRP on MULLaRP also coordinates the nucleus and breeder seed production to meet out the demand of quality seed in the country.

Recently, Tandon et al. (2015) revised the guidelines for testing of varieties under All India Coordinated Research Project, in which the proposed entries are being tested consecutively for 3 years in three trials, that is Initial varietal trials (IVTs), advanced varietal trials-I (AVT-I) and advanced varietal trials-II (AVT-II) at multi-location in different zones (Figs. 26.3 and 26.4).

The IVTs are constituted with the new entries proposed by cooperating breeders/institutions along with the specified number of check varieties. The total number of test entries including checks should not be more so that appropriate experimental design may be implemented without any compromise. A minimum of three check varieties, comprising of national, zonal and local check, shall be used and remain

Fig. 26.3 Grass pea AICRP field trials in central zone (**a**) and north-eastern plain zone (**b**)

unchanged for a minimum period of 3 years to enable comparison with the same test entries. The seed of candidate entries and checks must be genetically pure, true-to-type and meet the requirements of minimum seed certification standards. The experimental design, plot size and number of replications shall be decided in the Annual Group Meet on the basis of the experience gained from the past trials over years, to reduce experimental error. The plot size and number of replications should be homogeneous at all the test locations/zone/ecology. In addition, proper scope for date of sowing, seed rate, depth of sowing, plant geometry, fertilizer, irrigation, weed, insect-pests and disease management, etc. shall be mentioned in the technical programme and supplied at all the test locations.

The test centres shall be identified in the workshop and that could be ICAR Institutes/SAUs/Main or Regional Research Centres/Zonal Research Centres/State Govt. centres, where a multi-disciplinary team of scientists is available with enough operational facilities to carry out coordinated trials as per the instructions. All the trials shall be monitored meticulously by a team of scientists constituted by the Project Director/Coordinator. The team shall visit the testing locations during flowering to maturity time and record observations on the quality of the trials conducted and on the management as per the specified norms, and comment on the reliability of data likely to be generated. Additionally, observations should be recorded on the agronomic character like days to flowering and maturity, plant height, lodging, thresh ability; response to important diseases and insect-pests; easily measurable grain quality attributes such as colour, weight, appearance etc. The details of characters on which data shall be recorded should be specified by the workshop. All the data that is received at the coordination cell shall be crucially examined to make a decision on suitability of data for further statistical processing based on the recommendations of the monitoring team. Suggestions by the zonal coordinator/concerned breeder, deviation from the specified range of sowing date, specified crop management practices for the trial such as fertilizer doses, irrigation

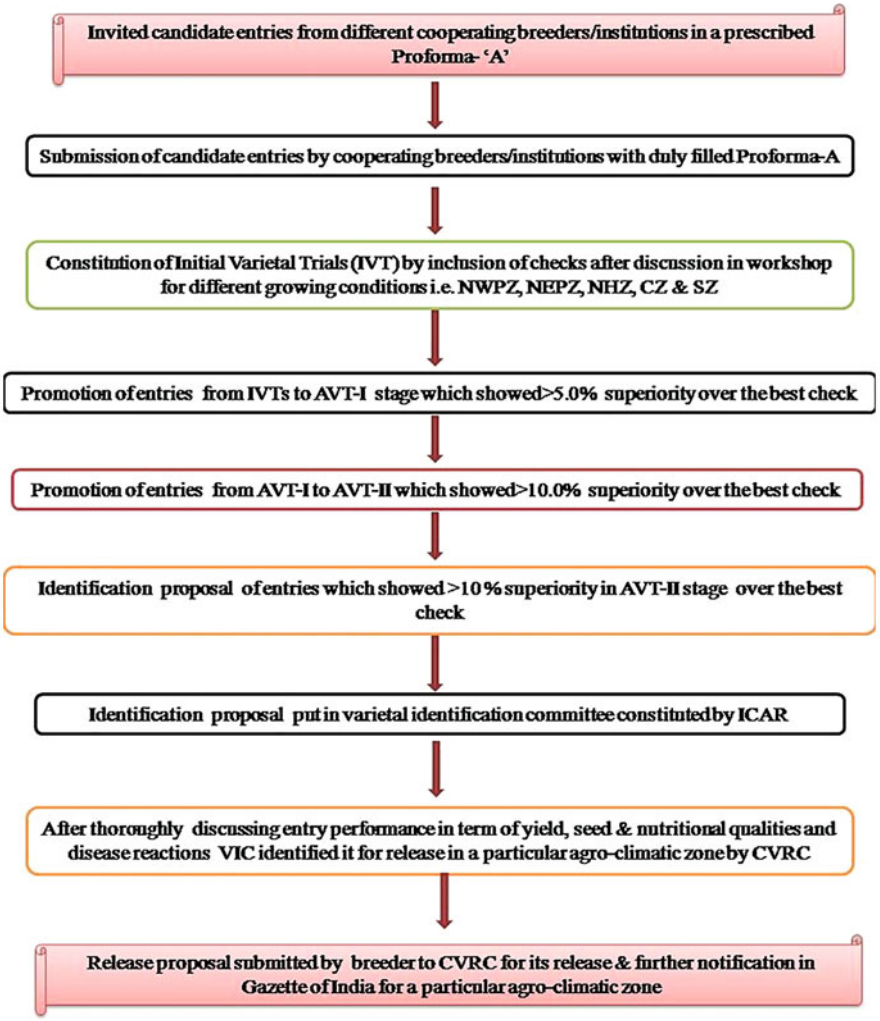

Fig. 26.4 Coordinates system for testing of new entries of grass pea in India

levels and any other severe error in conducting of trial/data recording/reporting should be considered aptly.

The promotion of entries from IVT to AVT would be strictly based on the overall performance/merit of the test entries and criterias finalized in workshop. AVT-I shall be constituted separately for each recognized agro-ecological zone by the entries promoted from the IVT on the basis of the criteria specified, the repeat entries from the previous year's AVT-I and the check varieties as per workshop recommendation. The number of entries in AVT-1 should normally not exceed 20. Higher number may be considered under exceptional cases with the approval of house (committee)

during workshop. In AVT-I, plot size should be larger than IVT to make more realistic estimates of the yield performance and to reduce inadequacies/errors of measurements inherent in small plots. The number of trial sites shall be much more than IVT trials in a given zone. Data on disease and insect-pest resistance and other ancillary characters shall be recorded only at the centres where facilities exist as specified in the workshop. Data on quality parameters including biochemical and processing properties shall be generated from selected sites in specified laboratories as per workshop recommendation.

The promotion of AVT-1 entries may be done mainly based on grain yield superiority with consideration of other specified characters. All the practices specified under the IVT and AVT-I stage shall be followed at AVT-II stage also. The entries which showed desired superiority over the best check in AVT-II stage will be selected and the identification proposal of that genotype is invited by project coordinator to put in varietal identification committee (VIC). The VIC after thorough discussions recommends the best entry for release and notification to central sub-committee on crop standard notification and release of varieties. After notification, the varieties enter into seed production chain: breeder seed, foundation seed and certified seed.

26.16 Seed Production and Seed Standards

A number of grass pea improved varieties have been released from various breeding programmes as already described in the previous section. In any plant species if outcrossing frequency rate is up to 30%, exceptional efforts are required for maintaining the purity of cultivars (Rahman et al. 1995; Chowdhury and Slinkard 1999; Almeida et al. 2015a, b, c). The outcrossing percentage is less in white flowered cultivars in comparison to blue, pink and crimson colour (Kiyoshi et al. 1985; Rahman et al. 1995). The proper isolation distance during seed production is essential to maintain genetic purity and phenological features of the developed cultivars. Land to be used for seed production of grass pea shall be free of volunteer plants. To maintain the genetic purity of the seed considering reported out-crossing, the existing minimum isolation distance, that is 5 m and 10 m for certified and foundation seed production, respectively, from the fields of other varieties for certification, need to be revisited. A minimum of two inspections are required to be made, the first before flowering and the second at flowering and fruit stage (Trivedi and Gunasekaran 2013).

The main objective of field inspections is to verify the factors which can affect the genetic purity of the seed and to take the corrective measures. During pre-flowering inspections, the requirements on the isolation distance and land conditions are checked and to undertake roguing if any off-types plants are present. At flowering and fruiting stage, inspection must be done to further check the occurrence of off-types based on flower colour and pod character and to subsequently remove them. During flowering, daily inspections should be done to identify the not true to type plants and to remove such off-types. A final rouging must be carried out during

Table 26.5 Grass pea seed standards as per Indian Minimum Seed Certification Standards

Factor	Standards for each class	
	Foundation seed	Certified seed
Pure seed (minimum)	98.0%	98.0%
Inert matter (maximum)	2.0%	2.0%
Other crop seeds (maximum)	5/kg	10/kg
Weed seeds (maximum)	5/kg	10/kg
Other distinguishable varieties (maximum)	10/kg	20/kg
Germination including hard seeds (minimum)	75.0%	75.0%
Moisture (maximum)	9.0%	9.0%
For vapour proof containers (maximum)	8.0%	8.0%

Source: ISTA (2016)

maturity to eliminate any chance of contamination. The maximum permitted percentage of off-types at the final inspection is 0.10% and 0.20% for foundation and certified seed production plots, respectively (ISTA 2016).

The minimum proportion of such pure seeds should be 98% for foundation as well as certified seed. However, seeds and its pieces without seed coat are considered as inert matter. Also, separated cotyledons are considered as inert matter irrespective of whether the radicle-plumule axis and/or more than half of the seed coat are attached. Inert matter also includes the dust particles, muds, stones and any part of seed not included as pure seeds (ISTA 2016). The maximum permissible limit of inert matter is 2% for both foundation and certified seed. Likewise, the maximum permissible limit of other crop seed and weed seed is 5 and 10 per kg for foundation and certified seed, respectively (Table 26.5).

26.17 Future Thrust Areas

To promote or exploit the potential of grass pea to ensure nutritional security of resource poor people of developing countries and sustainable pulses production, the quintessential step in coming years is the development of grass pea as a safe crop for human and animal consumption with low level ODAP (<0.1%) content. Further, for judicious utilization of available genetic resources in international and national gene banks, a comprehensive characterization needs to be undertaken using high-throughput phenotyping. In case of genomic resources, grass pea is still under-researched as compared to other pulses crops; therefore, intensive efforts should be made using recent molecular tools and techniques to create ample genomic resources. The delineation of inheritance pattern of economically important traits and a dense linkage map of *Lathyrus* species needs to be developed. To speed up functional genomics studies, development of various mapping populations including RILs, NILs, TILLING and MAGIC populations is considered necessary for trait–marker association and gene inactivation/deletion studies.

To accelerate genetic gain, further research on different *Lathyrus* species using genetic, cytogenetic techniques, inter-specific hybridization and advanced molecular approaches needs to be undertaken. Overall, there is urgent need to develop low-toxin and high-yielding varieties with resistance to various biotic and abiotic stresses and rapid delivery of that to farmers. For horizontal extension of grass pea particularly in *Utera* system of India, the development of high-yielding genotypes with earliness, deep root system, small seed size, erect plant habit and medium plant height is urgently required. In addition, other *Lathyrus* species also need to be explored for their suitability in different cropping systems. Intensive efforts may be placed to develop genotypes with medium maturity, higher biomass, bold seed and input responsiveness for irrigated areas with high yield potential that can be used as dual purpose.

The draft genome sequence of grass pea has been recently published and further progress in that will pave the way toward genomics-enabled breeding. Advanced tools and techniques, such as transcriptomics, proteomics, metabolomics, small RNAomics, epigenomics, interactomics, bioinformatics and genome editing, may be embraced to strengthen the grass pea improvement programme. Till date, the exact physiological and molecular mechanisms controlling ODAP content in grass pea remains uncharted that need urgent attention. National and International level collaborative research efforts are imperative to bring the grass pea crop in mainstream legume crop.

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