Satbir Singh Gosal
Shabir Hussain Wani *Editors*

Accelerated Plant Breeding, Volume 3

Food Legumes

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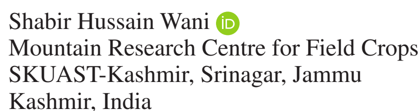
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Professor Neil Clifford Turner

Prof. Neil C. Turner is an Adjunct Professor at the University of Western Australia and Visiting Professor at Lanzhou University, Lanzhou, China, and the North West Agricultural and Forestry University, Yangling, China. Born in Preston in the United Kingdom, he was educated at Balshaw's Grammar School, Leyland, UK, 1951–1958, was awarded a BSc in Agricultural Science from Reading University, Reading, UK, in 1962, a PhD in Agronomy from Adelaide University, Adelaide, Australia in 1968, and a DSc in Agriculture from Reading University in 1983.

Prof. Turner's research career began at the Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA, where he was an Assistant Plant Physiologist from 1967 to 1974. In 1974 he joined CSIRO Plant Industry in Canberra, Australia, as a Senior Research Scientist and remained with CSIRO for 31 years until retirement in 2005. In 1984, Prof. Turner was appointed Research Leader of the newly-established CSIRO Dryland Crops and Soils Research Unit in Perth, Australia, and remained with CSIRO in Perth as a Chief Research Scientist until 2005. Subsequent to retiring from CSIRO, he was appointed as the Director of the Centre for Legumes in Mediterranean Agriculture at the University of Western Australia, Perth, Australia, 2006–2007; Winthrop Research Professor in the UWA Institute of Agriculture and Centre for Plant Genetics and Breeding, 2008–2015; and Adjunct Professor in the UWA Institute of Agriculture and UWA School of Agriculture and Environment from 2016 to the present. Prof. Turner was a Visiting Scholar in the Department of Botany at the University of Aberdeen, Scotland, UK,

in 1973; an Alexander von Humboldt Research Fellow in the Department of Plant Ecology at the University of Bayreuth, Germany, in 1982; a Visiting Scientist at the International Rice Research Institute, Los Baños, The Philippines, in 1983; and an Alexander von Humboldt Research Fellow in the Department of Botany at the University of Würzburg, Germany, in 1993.

Prof. Turner has been recognized for his contributions to agricultural science and the environment in Australia and internationally. He was made a Fellow of the American Society of Agronomy in 1982, a Fellow of the Crop Science Society of America in 1985, a Fellow of the Australian Academy of Technological Sciences and Engineering in 1992, a Fellow of the Australian Institute of Agricultural Science and Technology in 1995, a Foreign Fellow of the National Academy of Agricultural Sciences (India) in 2003, and was awarded a medal by the Australian Institute of Agricultural Science and Technology in 1993, an Australian Commonwealth Centenary Medal in 2003, the Chancellor's Medal by the University of

Western Australia in 2012, the Dunhuang Award by the Gansu Provincial Government in China in 2011, and a Friendship Award by the Government of the People's Republic of China in 2012 for excellence in research and outstanding contributions to dryland agriculture in Australia, India, and China. Prof. Turner's research has focused on the agronomic and physiological responses of crops and trees to water deficits, the adaptation of crops to water-limited environments, and the optimization of productivity and sustainability of cropping systems in the Mediterranean climatic regions of Australia through both management and breeding. This led more recently to a research focus on the implications of climate change for crop production in Australia, East Timor, and China. Prof. Turner has edited 12 books or special issues of journals and his research and that of his students and collaborators has been published in almost 400 peer-reviewed journal papers and book chapters. Prof. Turner was a contributor to the establishment of course development and

research training to PhD level at Haryana Agricultural University in the late 1970s, and for nearly 10 years he was the Research Coordinator for a project that successfully incorporated capacity building and the development of drought-resistant chickpea germplasm for India and Australia funded by the Australian Centre for International Agricultural Research.

This book is dedicated to Professor Neil Clifford Turner for his lifetime contribution to the betterment of sustainable agricultural production in a changing environment and for his training of many students and scientists in developing and developed countries.

Foreword

Food legumes are amongst some of the earliest food crops to be domesticated and cultivated by man and still constitute important sources of dietary protein (20–45%) for populations living in Asia, Latin America and Africa. Sometimes referred to as the 'poor man's meat', they are an important source of lysine, iron, phosphorous, calcium, zinc and magnesium, folate (vitamin B9), riboflavin (vitamin B2), and vitamin K andantioxidants, making them an ideal supplement for cereals. In different countries they are consumed as immature seeds, dry seeds, roasted seeds, condiments, ground for flour for baking or roasting, and as fermented products. With the ability to fix atmospheric nitrogen, they can be grown without added fertiliser nitrogen, help to improve the fertility of the soil for subsequent crops and improve soil health. A large number of improved cultivars possessing higher pod/seed yield, resistance to biotic and abiotic stresses, better nutritional and cooking qualities, and early maturity have been developed. However, for food legumes in general, seed yields are not yet comparable to the cereal crops, even when the differences in energy requirements for proteins versus carbohydrates are taken into account. The harvest index of food legumes is generally 15–20% compared to 45–50% for cereals such as wheat and rice. Nevertheless, several food legumes flourish in poor soils and adverse weather conditions and are preferred by farmers, especially for rainfed conditions and for crop rotations to maintain soil health. An efficient genetic improvement program is necessary in food legumes to meet the growing demand for food and nutritional security. There is a need to accelerate the breeding process for germplasm enhancement and development of new cultivars. In this situation, innovative techniques/technologies such as embryo culture, speed breeding, marker-assisted selection, transgenic breeding and genome editing can be exploited to supplement/complement conventional breeding approaches. *Accelerated Plant Breeding: Food Legumes*, edited by Dr. Satbir Singh Gosal and Dr. Shabir Hussain Wani, includes chapters on the important food legumes: chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* (L.) Millsp.), lentil (*Lens culinaris* Medik.), green gram (*Vigna radiata* (L.) R. Wilczek), urdbean (*Vigna mungo* (L.) Hepper), common bean (*Phaseolus vulgaris* L.), field pea (*Pisum sativum*), soybean (*Glycine max* (L.) Merr.), and horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.)**.** This book

provides current information to assist in the acceleration of plant breeding. I thank the editors, Dr. S.S. Gosal and Dr. Shabir Hussain Wani for bringing together valuable information on breeding for food legumes. I am confident that this book will be of great help to research workers, teachers and students.

Adjunct Professor Neil C. Turner, BSc, PhD, DSc
Former Winthrop Professor and Director,
Centre for Legumes in Mediterranean Agriculture,
University of Western Australia, Perth, Australia.
Former Chief Research Scientist, CSIRO Plant Industry.

Preface

Food legumes constitute an important element of daily diet particularly in Asia, Latin America, and Africa. These are important source of proteins especially for vegetarians and also a good source of lysine, phosphorous, iron, zinc, calcium, magnesium, folate, riboflavin, and antioxidants, which make them a best supplement to cereals. Besides, legumes play an important role in fixing atmospheric nitrogen and hence improve the soil health. Many of these crops are being grown by farmers in low-input food production systems as catch crops and also for crop rotations. Plant breeding aims at creation of new varieties, superior to the old varieties/parents by combining valuable traits. Using different breeding strategies, a series of high yielding, disease resistant varieties with improved nutritional quality have been developed the world over. Although, the Harvest Index (15–20%), particularly in pulses, has been achieved, yet it is not comparable to cereals (45–50%) like wheat and rice. Thus, the development of climate-resilient varieties with improved traits pertaining to seed yield and resistance to biotic/abiotic stresses remains a high priority in food legume breeding. The low inherent genetic potential and greater variance due to environment (E) and genotype × environment (GE) interactions have been the major limiting factors in the improvement of these crops. The quest for efficient breeding of pulse crops can benefit greatly from powerful new technologies which can be used in conjunction with conventional approaches to accelerate the varietal development. Therefore, this volume deals with conventional and modern tools/techniques such as embryo culture, marker-assisted selection, genomic selection, transgenic breeding, speed breeding, genome editing, high-throughput phenotyping/genotyping, experimental design, indirect selection, and data-driven decision tools for rapid improvement of pulse crops. This volume includes chapters prepared by specialists and subject experts on different crops/aspects in relation to accelerated breeding. In addition to the general chapters, separate chapters have been included on chickpea, lentil, pigeon pea, green gram, urdbean, dry pea, soybean, horsegram, and common bean. We earnestly feel that this volume will be highly useful for students, research scholars, and scientists working in the in the area of plant breeding, genomics, cellular/molecular biology and biotechnology at universities, research institutes, R&Ds

of agricultural MNCs for conducting research, and various funding agencies for planning future strategies.

We are highly grateful to all learned contributors, each of whom has attempted to update scientific information of their respective area and expertize and has kindly spared valuable time and knowledge.

We apologize whole heartedly for any mistakes, omissions, or failure to acknowledge fully.

We would like to thank our families (Dr. Satwant Kaur Gosal (wife of SSG), Sana Ullah Wani, Taja Begum, Sheikh Shazia, Yasir Wani, and Muhammad Saad Wani (father, mother, wife, brother, and son of SHW)) for their continuous support and encouragement throughout the completion of this book.

We highly appreciate the all-round cooperation and support of Springer Nature Switzerland AG, Gewerbestrasse 11, 6330 Cham, Switzerland, for their careful and speedy publication of this book.

Ludhiana, Punjab, India Satbir Singh Gosal
Kashmir, India Shabir Hussain Wani

Contents

1 Efficient Breeding of Pulse Crops 1
Shiv Kumar, Priyanka Gupta, Hasnae Choukri, and Kadambot H. M. Siddique

2 Advances in Chickpea Breeding and Genomics for Varietal Development and Trait Improvement in India 31
Ashutosh Kushwah, Shayla Bindra, Inderjit Singh, G. P. Dixit, Pankaj Sharma, S. Srinivasan, P. M. Gaur, and Sarvjeet Singh

3 Conventional and Biotechnological Approaches for Targeted Trait Improvement in Lentil 67
Dorin Gupta, Rama Harinath Dadu, Prabhakaran Sambasivam, Ido Bar, Mahsa Azad, Navya Beera, Rebecca Ford, and Sajitha Biju

4 Updates of Pigeonpea Breeding and Genomics for Yield Improvement in India 109
Pankaj Sharma, Inderjit Singh, Abhishek Bohra, Indra Prakash Singh, Abha Tiwari, Mehak Sethi, Ashutosh Kushwah, and Sarvjeet Singh

5 Genomics-Assisted Breeding Green Gram (*Vigna radiata* (L.) Wilczek) for Accelerating Genetic Gain 143
J. Shanthala, D. L. Savithramma, P. Gazala, Bharath Kumar Jambagi, and Shri Krishna P. Desai

6 Breeding for High-Yielding and Disease-Resistant Urdbean Cultivars. 173
Debjyoti Sen Gupta, Jitendra Kumar, Ashok Kumar Parihar, and Sanjeev Gupta

7 Lentil Breeding in Genomic Era: Present Status and Future Prospects 193
Jitendra Kumar, Debjyoti Sen Gupta, and Pravin Tiwari

8 Chickpea Breeding for Abiotic Stress: Breeding Tools and 'Omics' Approaches for Enhancing Genetic Gain 211
Uday Chand Jha, Harsh Nayyar, Rintu Jha, Chaitanya Prasad Nath, and Dibendu Datta

9 Recent Advances in Mungbean Breeding: A Perspective 235
Suma C. Mogali and Gurudatt M. Hegde

10 Genetic Advancement in Dry Pea (*Pisum sativum* L.): Retrospect and Prospect 283
A. K. Parihar, G. P. Dixit, A. Bohra, D. Sen Gupta, Anil K. Singh, Nitin Kumar, D. Singh, and N. P. Singh

11 Translational Genomics and Breeding in Soybean 343
Anita Rani and Vineet Kumar

12 Efficient Improvement in an Orphan Legume: Horsegram, *Macrotyloma uniflorum* (Lam.) Verdi, Using Conventional and Molecular Approaches 369
Rakesh Kumar Chahota, Nisha Thakur, and Reecha Sharma

13 Molecular and Conventional Breeding Strategies for Improving Biotic Stress Resistance in Common Bean 389
T. Basavaraja, Aditya Pratap, Vikas Dubey, S. Gurumurthy, Sanjeev Gupta, and N. P. Singh

Correction to: Genomics-Assisted Breeding Green Gram (*Vigna radiata* (L.) Wilczek) for Accelerating Genetic Gain C1

Index 423

About the Editors

Satbir Singh Gosal has been a Bursary holder of The Royal Society, London, is a former Director at the School of Agricultural Biotechnology and Ex-Director of Research at Punjab Agricultural University, Ludhiana, India. He was an Honorary Member of the Board of Assessors (Australian Research Council, Canberra), Biotechnology Career Fellow, The Rockefeller Foundation, USA, and President of the Punjab Academy of Sciences. He has published more than 200 research papers in refereed journals and 35 book chapters. He has coauthored one textbook and has coedited five books including three with Springer.

Shabir Hussain Wani received his PhD in Genetics and Plant Breeding from Punjab Agricultural University. He has published over 100 peer-reviewed papers and has edited 13 books on plant stress physiology, including 7 with Springer. He also served as a Review Editor for *Frontiers in Plant Science* from 2015 to 2018. He is currently an Assistant Professor at the Mountain Research Centre for Field Crops of the Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir in India.

Chapter 1
Efficient Breeding of Pulse Crops

**Shiv Kumar, Priyanka Gupta, Hasnae Choukri,
and Kadambot H. M. Siddique**

1.1 Introduction

Pulse crops, known for their high protein content and biological nitrogen-fixing ability, belong to the family *Fabaceae* and offer many health benefits for people and the planet. About a dozen pulse crops are grown for human food and animal feed in various crop–livestock systems, mainly in rainfed dry areas across the continents, including major pulse crops—dry beans (*Phaseolus vulgaris*), dry peas (*Pisum sativum*), chickpea (*Cicer arietinum*), cowpea (*Vigna unguiculata*), lentil (*Lens culinaris*), pigeonpea (*Cajanus cajan*), and faba bean (*Vicia faba*)—and several minor pulses, such as mung bean (*V. radiata*), black gram (*V. mungo*), and grass pea (*Lathyrus sativus*) that are grown under specific agro-ecosystems to meet specific local demands. The inclusion of pulses in rotation with cereals helps to improve system yields, enhance net carbon sequestration, and lower the carbon footprint. In a recent study, the lentil–wheat system produced the lowest carbon footprint at −552 kg CO_2 eq/ha (Gan et al. 2014). Pulse crops are well-suited to low-moisture conditions due to their low protein yield-based water footprint (6.58 m^3/kg pulse vs 9.25 m^3/kg cereal) (Ding et al. 2018). Pulse cultivation potentially reduces greenhouse gas emissions and supports biodiversity (Watson et al. 2017). Such factors make pulses a key rotation crop in the sustainable intensification and diversification of cereal-based cropping systems. A recent study identified economic advantages of pulse–cereal rotations over cereal mono cropping, including higher yields, gross margins, and consumption (Yigezu et al. 2019b).

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Pulses provide more protein, micronutrients, and other bioactive compounds than cereals (Champ 2002) and generally contain more prebiotic carbohydrates than processed cereals (Johnson et al. 2015; Siva et al. 2019), thus changing gut microbial composition and regulating intestinal movement (Manning and Gibson 2004). The role of pulses in alleviating the hidden hunger caused by micronutrient deficiencies, which are widely prevalent among two billion people, mainly in South Asia and sub-Saharan Africa, has been recognized (Thavarajah et al. 2011). Current evidence shows that dietary pulses are associated with reduced cardiovascular disease, hypertension, and obesity incidence (Viguiliouk et al. 2019). The current consumption of pulses remains low in the diets of millions (Mitchell et al. 2009; Mudryj et al. 2012), and there is a drive to incorporate them as part of a healthy diet to improve cardiometabolic health, as pulses may reduce cholesterol, support weight management via glycemic responses, and aid digestive health (Mudryj et al. 2014; Tilman and Clark 2015). Pulse protein complements the amino acid profile of cereal protein with consequent improvements in the biological value of cereal–pulse diets. To improve rumen digestion and animal growth, crop residues are needed that contain substantially higher N concentrations than those provided by cereal residue (0.6–0.8%); adding pulse residue improves fodder N concentration (Blümmel et al. 2012).

Despite their role in sustainable agri-food systems, pulses have not attracted enough research investment to increase production and productivity commensurate with the demands of the rising population (Murrell 2016). Past breeding efforts have made modest gains in developing short-duration disease-resistant varieties of pulse crops through classical breeding approaches. A recent study reported that India, a major pulse-producing country, has the capacity to increase chickpea productivity by 40% using current varieties and standard crop management practices (Hajjarpoor et al. 2012). This suggests that the adoption of current varieties is limited and/or management practices are incorrectly implemented to translate the genetic potential in farmers' fields. In Africa, the yield gap in pulse crops ranges from 40% to 85% (Tittonell and Giller 2013). The same holds for pulse crops in other developing countries where many improved varieties have been released for cultivation, but their adoption remains limited due to various reasons, including the lack of availability of quality seeds and the perception of unstable productivity. Recent findings have demonstrated that the grain yields of pulse crops are as reliable as those of other spring-sown crops in the major production systems of northern Europe (Reckling et al. 2018).

Given the track record of productivity growth through genetic and management innovations, recent breakthroughs in science and technologies, and the enhanced carrying capacity of national and international programs, researchers are optimistic that the desired pulse supply is achievable provided we integrate appropriate experimental design, modern phenotyping and genotyping tools and techniques, and data-driven decision tools throughout the breeding and testing pipelines. This chapter deals with how to integrate these modern tools and techniques in current pulse breeding programs to effectively and efficiently develop climate-smart varieties.

1.2 Production Trends

Globally, the current production of pulses is estimated at 85.66 million tons from 88 million ha with an average yield of ~1000 kg/ha (Table 1.1). Of the major pulses, faba bean has the highest average yield (1914 kg/ha) followed by dry peas (1897 kg/ha), lentil (1178 kg/ha), chickpea (946 kg/ha), dry beans (865 kg/ha), pigeonpea (863 kg/ha), and cowpea (549 kg/ha). These yields are very low relative to those of major cereal crops, such as rice, wheat, and maize. Studies on pulse crops have been instrumental in defining the basic principles of genetics and plant breeding and contributed to the remarkable progress in agricultural production. However, science-led increases in production have varied between crops. Figure 1.1 shows the comparative increases in the average yield of cereals and pulses from 1961 to 2017. The increase in average pulse yields (from 637 to 1009 kg/ha) has been modest compared to the dramatic increases in cereal productivity (from 1353 to 4074 kg/ha). As a result, the cultivation of pulses has been relegated to areas where cereals are either not profitable or constrained by harsh agro-climatic conditions or the lack of availability of production inputs. Recently, yield increases even in major cereal crops have slowed, reportedly to less than 2.4% per year, which will be insufficient to meet global food demands by 2050 (Ray et al. 2013).

Among pulses, dry peas recorded the highest productivity gains from 1961 to 2017, followed by lentil, chickpea, dry beans, and pigeonpea. Within this period, the maximum yield gains of major pulse crops occurred from 1999 to 2017, followed by 1981 to 1999 and 1961 to 1981 (Fig. 1.2). The recent yield gains were due to the expansion of pulse cultivation in developed countries, including Canada, Australia, and the USA, to not only capture the ecological benefits of pulse cultivation but also market opportunities in global trade. However, not all pulse crops had productivity boosts at the same time. For example, maximum yield gains for lentil, chickpea, and pigeonpea occurred from 1999 to 2017 and pea from 1981 to 1999. During the Green Revolution (1961–1981), pulse productivity growth was relatively stagnant and even negative for some crops such as pigeonpea and chickpea; this was due to the adoption of short-duration varieties of pigeonpea and chickpea in place of land

Table 1.1 Average global area, production, and yield of major pulse crops (2015–2017)

Crop	Area (million ha)	Production (million tons)	Grain yield (kg/ha)
Dry beans	33.82	29.27	865
Dry peas	7.51	14.25	1897
Chickpea	13.05	12.35	946
Cowpea	12.25	6.72	549
Lentil	5.56	6.55	1178
Pigeonpea	5.96	5.14	863
Faba bean	2.41	4.60	1914
Others	7.46	6.78	909
Total pulses	**88.02**	**85.66**	**973**

Source: FAOSTAT, retrieved on November 2019

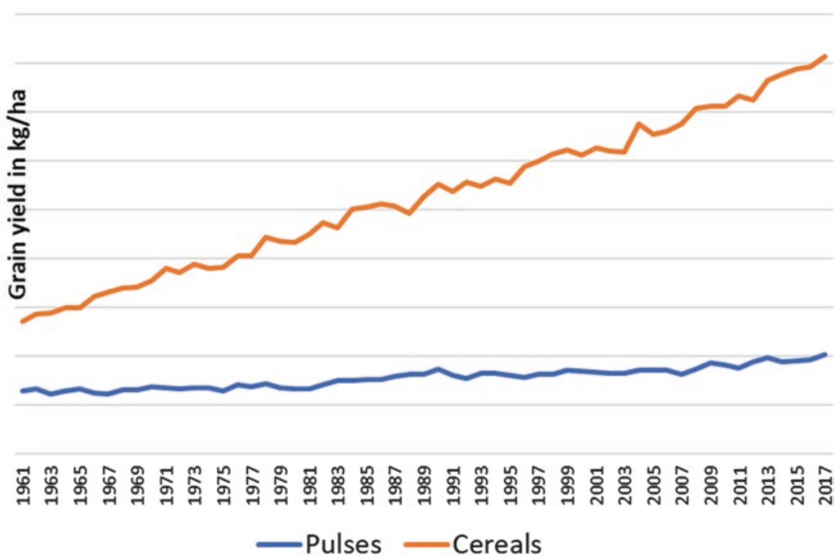

Fig. 1.1 Trends in grain yields of pulses and cereals from 1961 to 2017(FAO data)

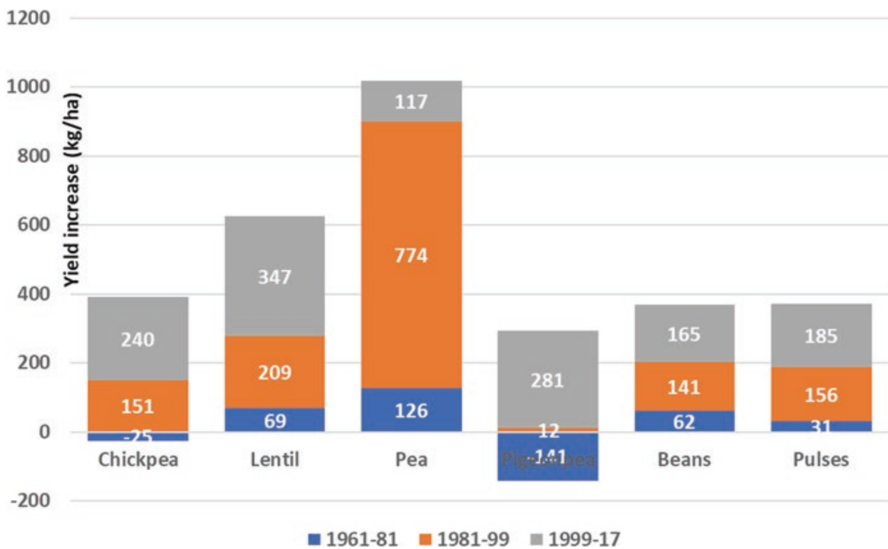

Fig. 1.2 Periodic changes in grain yield of major pulse crops

races and the geographical shift in pulses production to short-season windows. In contrast, rice, wheat, and maize production increased due to technological advancements, government policies, emphasis on food security, research investment, and need of the time.

Current pulse production does not meet the current demands of the ever-increasing population. In recent years, more people have been substituting animal protein with plant protein, putting further pressure on the demand for pulses. With rising incomes and preferences for vegetarian diets in response to the food impact on climate change, this gap is expected to be 10.9 million tons by 2025 (Cluff 2016; Joshi and Rao 2017). Indeed, grain yields are estimated to fall by 5–10% per degree increase in climate warming (Challinor et al. 2014), with more significant losses likely for pulse crops in some areas. Most pulses are grown in fragile agro-ecosystems that will be the most susceptible to climate change. For example, chickpea production in India has moved to central and south India; any changes in temperature or rainfall pattern could seriously impact its production and productivity.

1.3 Overview of Past Breeding Efforts

Science-based breeding of pulse crops started with the principles of heredity proposed by Mendel. Since then, various classical breeding methods—including introduction; pure line selection; mass selection; recombination breeding, pedigree, bulk, bulk-pedigree, and single seed descent selection methods; introgression through stepwise and simultaneous backcrossing; recurrent selection; mutation breeding; hybrid breeding; and synthetics—have been used to develop improved pulse varieties that not only are high yielding but also can withstand disease and insect pests and perform well under a range of environmental conditions. Improved plant types, appropriate phenology, photo-thermo insensitivity, resistance to key diseases and insect pests, tolerance to abiotic stresses (mainly drought, heat, and cold), mechanical harvesting, herbicide tolerance, large seeds, and improved nutritional quality are some of the important traits that have been targeted during pulse breeding. The breeding method used varies according to the crop, the capacity of breeding programs, available germplasm, and knowledge of the trait under selection and its association with other traits. The disease and insect pest spectrum can vary between regions, seasons, and crops. Grain quality and nutritional value can vary between markets and industry. While good progress has been made in incorporating disease resistance under the control of major genes, imparting resistance to insect pests remains a challenge in pulse breeding programs.

1.3.1 Genetic Resources

Genetic diversity is crucial for meeting the basic food and nutritional demands of the rising population and serves as the base for the response to selection. Information on the genetic resources of crop species, including pulse crops conserved in various national and international gene banks, is available online at global portals—Genesys

(https://www.genesys-pgr.org/) and GRIN (https://www.ars-grin.gov/)—and in a 1996 FAO publication "The second report on the state of the world's plant genetic resources for food and agriculture" (http://www.fao.org/3/i1500e/i1500e00.htm). The FAO report stated that CG centers hold in trust 35,891 accessions of common bean, 33,359 of chickpea, 15,588 of cowpea, 13,289 of pigeonpea, 10,864 of lentil, 9186 of faba bean, 6129 of field pea, and 3225 of grass pea (Table 1.2). Since then, these numbers have increased, with the ICARDA gene bank holding 50,968 accessions of pulse crops, including 15,749 of chickpea, 14,597 of lentil, 10,034 of faba bean, 6131 of pea, and 4457 of grass pea. Similarly, ICRISAT holds 20,764 and 13,783 accessions of chickpea and pigeonpea, respectively. CIAT, IITA, and AVRDC hold 37,938, 16,460, and 10,946 accessions of *Phaseolus* beans, cowpea, and *Vigna* species, respectively. These collections also include a sizable number of crop wild relatives (CWRs), which are excellent sources of novel traits/alleles due to their historical record of adaptation to a diverse range of habitats. In addition, many national gene banks hold sizable repositories of genetic resources. For example, the national gene bank in India holds >73,000 accessions of different pulse crops. The International Treaty on Plant Genetic Resources for Food and Agriculture, adopted by the 31st Session of the Conference of FAO in 2001, provided an institutional framework for international collaboration to use these genetic resources.

While it is important to collect and conserve genetic resources in gene banks, it is more important to use them to widen the genetic base of crop varieties. In the past, dependence on limited variability has resulted in a narrow genetic base for many important traits. It is estimated that 80–95% of the accessions in world collections lack proper characterization and evaluation data (http://www.fao.org/3/i1500e/i1500e00.htm). To facilitate accessibility and better use of the germplasm available with gene banks, various sets of core (Frankel 1984; Brown 1989), mini-core (Upadhyaya and Ortiz 2001), focused identification of germplasm strategy (FIGS) (Mackay and Street 2004), and reference (Odong et al. 2011) germplasm have been developed. The FIGS strategy is being pursued at ICARDA using robust

Table 1.2 Global status of germplasm accessions of pulse crops

Crop	Global status	CG center	NBPGR, India
Chickpea	98,285	33,359	14,704
Lentil	58,405	10,864	9989
Vigna species	–	–	5549
Common bean	261,963	35,891	1514
Grass pea	26,066	3225	2797
Field pea	94,001	6129	3070
Cowpea	65,323	15,588	3317
Pigeonpea	40,820	13,289	12,859
Faba bean	43,695	9186	–
Others	183,078	13,690	19,579
Total	**1,069,897**	**141,221**	**73,378**

Source: http://www.fao.org/3/i1500e/i1500e00.htm

geographical data sets, which has proven successful for various adaptive traits such as tolerance to heat, drought, cold, and salt and resistance to insect pests and diseases (https://www.icarda.org/research/innovations/focused-identification-germplasm-strategy-figs). There are now FIGS sets for chickpea, lentil, grass pea, and faba bean that can be used to discover and deploy useful genes into desired agronomic backgrounds. Similarly, mini-core sets of chickpea, pigeonpea, and groundnut developed at ICRISAT are a rich source of variability for desired traits in breeding programs (Upadhyaya et al. 2013). Except for a few traits, there is adequate variability for important economic traits in the existing germplasm of pulse crops. The assembly of structured and representative sets of germplasm from the global collection brings efficiencies for identifying and using germplasm with favorable alleles/traits.

1.3.2 Variety Development

From a breeding perspective, the genetic potential of a crop is determined by its genetic makeup, inter and intra-allelic interactions, and interactions of genotypes with environmental factors. A conventional breeding program generally uses parents of diverse origins to combine traits that contribute directly or indirectly to the yield and economic value of the crop. Following a selection–hybridization–selection–testing cycle, a breeder constructs new breeding lines based on phenological adaptation, agronomically desirable traits, resistance to prevailing stresses, quality aspects, and consumer preferences. Classical breeding schemes under the aegis of CGIAR centers and national agricultural research systems (NARS) have developed ~3700 improved varieties that are grown in diverse agroecology across the world (based on published country records and personal communication with major breeding programs). South Asia alone accounts for 1001 varieties of pulse crops (Kumar et al. 2016). Similarly, 1106 varieties of pulse crops have been released in sub-Saharan Africa (https://www.asti.cgiar.org/diiva). A decade-long effort under the Tropical Legumes project developed 266 improved varieties (Varshney et al. 2019). The first phase of the CGIAR Research Program on Grain Legumes (2012–2016) delivered 252 varieties and 4 hybrids of pulse crops in target countries. ICARDA developed more than 405 varieties of lentil (158), Kabuli chickpea (165), faba bean (75), and grass pea (7) using classical breeding methods. Being partially cross-pollinated, various breeding methods have been used to develop improved varieties of faba bean such as Outlook, Encore, Giza 843, Misr3, and Sakha1 using recurrent selection, Hama2 and Hama3 from the hybrid bulk population (synthetics), and Basabeer, Shambat, and Eddamer using the bulk selection method. About 460 varieties of pulse crops have been bred in various countries using a mutation approach (http://mvd.iaea.org/). Among pulses, pigeonpea has benefited from hybrid breeding, resulting in the development of GMS-based hybrids (GTH1, ICPH8, PPH4, CoH1, CoH2, AKPH4104, AKPH2022) in India (Saxena et al. 2006). Despite yield advantages of 25–40%, these hybrids were unfavorable with

growers due to seed production difficulties. Later, three CGMS-based hybrids—ICPH2671, ICPH3762, and ICPH2740—with 40–50% yield advantages over local checks were developed and commercialized in different states of India (Saxena et al. 2015). Some improved varieties released in a country have been introduced and accepted in neighboring countries.

With the development of molecular markers, breeding programs previously based on phenotypic selection have started to use marker-assisted selection (MAS). While still in its infancy, MAS holds great promise because it increases selection accuracy and reduces the selection process time. Recently, a marker-assisted backcrossing method was used to develop two drought-tolerant chickpea varieties (Geletu in Ethiopia and Pusa Chickpea 10216 in India) and a wilt-resistant chickpea variety (Super Annigeri 1 in India) (Mannur et al. 2019). Several varieties of soybean have been released by the private sector using MAS as a routine breeding method. Similar MAS efforts are underway in common bean, cowpea, pigeonpea, and lentil.

Past breeding efforts have had a positive impact on the production and productivity of some pulse crops. Productivity gains have increased when considered with the markedly reduced crop duration of these varieties. This has increased their per day productivity relative to other rainfed crops, and they can be introduced into new niches and incorporated into existing cropping systems. For example, the adoption of short-duration lentil varieties, such as BARImasur-3, BARImasur-4, BARImasur-5, BARImasur-6, BARImasur-7, and BARImasur-8, has improved productivity in Bangladesh. A recent fingerprinting study showed that 99% of the lentil area in Bangladesh is now under improved varieties (Yigezu et al. 2019a), delivering an additional 55,000 tons of lentil, valued at US$ 38 million annually. Similarly, chickpea production in Myanmar has increased by 19% with the introduction of five improved varieties (Yezin3, Yezin4, Yezin6, Yezin8, and Yezin11) covering 96% of the chickpea area (Win et al. 2014). Similar success stories are available for chickpea and lentil in Ethiopia (Verkaart et al. 2016), lentil in Canada (https://www150.statcan.gc.ca/n1/pub/96-325-x/2014001/article/14041-eng.pdf), chickpea in Australia (Siddique et al. 2013), and mung bean in South Asia (Chadha 2010). A scaling-up study in Ethiopia indicated that the production output of common bean, chickpea, and lentil increased by 89%, 85%, and 97%, respectively, with 53–59% of output growth attributed to varietal yield increases and the remainder due to area expansion (Abate et al. 2011).

1.3.3 Breeding Progress

Breeding progress is a measure of the increase in the average genetic value of progenies for a specific trait, relative to the base population. A widely used method for estimating breeding progress is "Era genetic gain," which is a regression of the average performance of varieties improved for a specific trait over their release years (Duvick 1984). The genetic trend or realized rate of genetic gain from a breeding

program is a useful indicator of a breeding program's success (Ustun et al. 2001). Annual yield improvements for major crops currently range between 0.8% and 1.2%, which is about half that needed to meet the future demands for plant-based products (Li et al. 2018). A genetic trend study on lentil in Morocco reported yield gains of 35 kg/ha/year from 1989 to 2018 (Idrissi et al. 2019), with the yield advantage of improved varieties over the local check increasing from 16 to 67%. In Ethiopia, 11 lentil varieties released between 1980 and 2010 had an estimated yield gain of 18–28 kg/ha/year in a two-location study (Bogale et al. 2015). Similarly, a yield gain of 0.55% was reported in Kabuli chickpea in Ethiopia (Belete et al. 2017). The current annual genetic gains of <0.7%, reported for various pulse crops, will not meet the growing demands of these crops.

Despite many improved varieties, the narrow genetic base of pulses is a significant constraint to increasing genetic gains, as well as poor selection accuracy and selection efficiency, which are due to bottlenecks in the processes of evolution and domestication and further compounded by breeding programs undertaken at various research stations. Pedigree analysis of pulse varieties released in India showed extensive and repetitive use of superior genotypes in hybridization (Kumar et al. 2004). Selection accuracy is very poor in pulses as a result of a high proportion of variance due to environment (E) and genotype × environment (GE) interactions on the expression of quantitative traits (Kumar and Ali 2006). On average, 70–80% of the variation in MET (multilocation environment trials) is attributed to E, 17–27% to GE, and <3% to genotypes (Imrie and Shanmugasundaram 1987; Malhotra and Singh 1991; Kumar et al. 1996). This indicates an urgency to not only widen the genetic base of different pulse crops but also to improve selection accuracy and efficiency through the integration of modern tools and techniques.

1.4 Accelerating Breeding Efficiency in Pulses

Developing high-yielding nutritious pulse varieties that withstand climate variability and virulent pathogens and their evolving races and biotypes is challenging. The breeding cycle generally takes 7–10 years, depending on the reproduction system, genotype, heritability, GE interactions, ploidy level, and domestication events (Fig. 1.3).Conventional breeding suffers from genetic drift and the loss of desirable genes. To create successful varieties, breeders hybridize plants with desired traits and select the best offspring over multiple generations. Generally, high-yielding parental lines are crossed to secure a high mean performance of the progeny. Major limitations that plant breeders encounter relate to identifying adequate germplasm, efficiently incorporating the germplasm, efficient selection procedures, and improved evaluation procedures (Baenziger and Peterson 1992). To identify superior progeny, ensure genetic gain in the next selection cycle, and maintain long-term selection gain, it is important that the cross also generates high genetic variance. The development of product profiles for new varieties that respond to the requirements of farmers, processors, and traders and consumer preferences from the outset

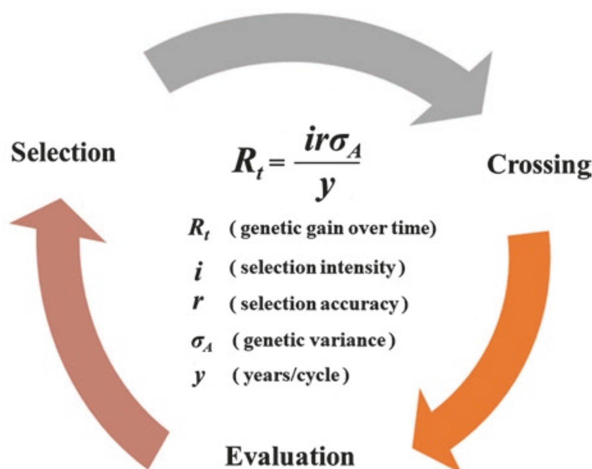

Fig. 1.3 Breeding cycle and breeder's equation

is warranted for successful breeding of pulse crops. This requires foresight and market analyses to align product profiles with market demands involving different stakeholders and prioritization of the value-added traits based on product profiles for final breeding decisions. Foresight analysis helps to ensure the release of varieties that are adapted to expected changing conditions, including pests and diseases.

Plant breeding programs have not taken full advantage of the principles of quantitative genetics advocated by Wright (1920) and applied successfully by Lush (1937) and others in animal breeding to increase the rate of genetic improvement; indeed, quantitative traits such as grain yield require gradual improvements over numerous selection cycles to accumulate favorable alleles (Bernardo 2008, 2010). The main obstacle to effective breeding for quantitative traits in pulses is the lack of knowledge about the concept of genetic gain from selection. Outcomes of selection experiments have confirmed that the rate of genetic gain per cycle depends on selection accuracy, selection intensity, and additive genetic variance, as expected from the theory of quantitative genetics (Hallauer and Darrah 1985). These selection experiments also demonstrated the impressive ability of populations to respond to selection over the long term. Improvements in traditional tools and techniques can increase the effectiveness of breeding programs, but they cannot significantly reduce costs, as conventional breeding remains a "numbers game," i.e., growing bigger populations to increase the probability of selecting desired recombinants (Choi and Kronstad 1986; Slinkard et al. 2000).While conventional breeding has done well in the past, with plants evaluated and selected by their phenotype under field conditions, the current challenge to break yield barriers in pulse crops is more complicated under the climate change and variability scenario and requires innovative approaches to change the "numbers game" perception to well-defined precision breeding.

Knowledge and careful selection of parental germplasm, appropriate mating design, accuracy of phenotyping, and effective and responsive use of genomics information are keys for maximizing the breeding process. Applying modern tools and techniques requires access to controlled environment facilities (greenhouses, growth chambers) for rapid generation advancement of breeding material, phenotyping platforms for high-throughput screening of breeding material, mechanization of field operations, digitalization of data recording, and data management systems to increase selection and operational efficiency. Low-cost genotyping platforms and genomic resources are increasingly available with draft genome sequences and resequencing data for most pulse crops (Varshney et al. 2018). As a result, pulse breeders now have access to high-throughput phenotyping and genotyping tools and services, efficient experimental designs, data management tools, statistical models, and bioinformatics to accelerate the transfer of genes/QTLs and their combinations, accurately and efficiently, into popular varieties. Given the recent advances in understanding the genetic control of agronomic traits and the plethora of tools and techniques available, the development of climate-smart varieties should be expedited in coming years. The authors suggest the following steps to ensure this occurs:

1.4.1 Defining the Target Population of Environments (TPE)

The presence of GE interactions has an important bearing on breeding pulse crops, which are generally grown in areas with a high frequency and intensity of stresses. Most varieties are developed through repeated selection cycles under high-yielding conditions at experimental stations, which translates into lower yields in farmers' fields. The approach of using low-yielding sites for selection has been largely neglected or even discouraged in pulse breeding programs. This explains why pulse breeding programs have largely missed the opportunity to significantly impact productivity compared with cereals. Such failures have been used to argue that productivity management through good agronomic practices needs to be prioritized to improve pulse productivity. If crops are grown in both high- and low-yielding environments, as is the case with pulse crops, different varieties might be needed to maximize yields in each environment (Atkin and Frey 1990). Improving the efficacy of breeding programs to cater for diverse environmental conditions requires these environments to be divided into relatively homogeneous TPEs that satisfy the specific varietal needs of each region.

Defining a TPE requires relevant data collection and sound methodology to segregate the major pulse production areas into geo–bio–physically distinct units for the development of effective crop improvement programs for each pulse crop. Such information is valuable for the strategic allocation of representative evaluation sites for the TPE (Chauhan and Rachaputi 2014). The identification of crop-specific key locations for evaluation would significantly improve the efficiency and effectiveness of pulse breeding. In recent years, crop simulation and modeling approaches have brought a new paradigm to breeding for target environments. Using modeling

to characterize environments in the TPE can assist in unraveling GE interactions to aid selection decisions and improve the rate of yield gain in crop improvement programs. Chapman et al. (2000) suggested that weighting genotype performance by the representativeness of the selection environment in each MET with respect to the TPE would be advantageous in breeding programs in variable environments.

1.4.2 High-Throughput Phenotyping

The selection of suitable parents for hybridization and useful recombinant progenies is key to the success of a plant breeding program and depends on the phenotyping precision of the traits under selection. Current methods of phenotyping are slow and labor-intensive. Breeders still use traditional tools and empirical methods to assess yield and quality. As a result, precision phenotyping is now recognized as a major bottleneck in the current breeding pipelines of pulse crops. To increase efficiency, reliable, precise, cost-effective, less labor-intensive, and easily applied phenotyping methods and tools for target traits are needed. Screening germplasm in phytotrons, growth chambers, and fully controlled greenhouses provides ideal conditions to investigate physiological and molecular mechanisms of stress tolerance, while germplasm screening in the open field requires detailed characterization of the prevailing stress in the target environment.

In recent years, efforts have been directed at developing high-throughput phenotyping devices, including hyper-spectral, multispectral, and thermal sensors and fluorescence (Romano et al. 2011). Different recording techniques are being used, ranging from affordable visible-light color cameras to expensive magnetic resonance imaging devices. New smart machines and sensor-based technologies can automate the measurement of large plant numbers. High-throughput field phenotyping (HTFP) can accurately characterize crop traits in populations containing thousands of individuals in a nondestructive and remotely sensed manner (Araus and Cairns 2014) and has promise for the reliable phenotyping of increased numbers of breeding lines using smaller plot sizes and at earlier stages of population development to reduce the duration of breeding cycles and the loss of potentially important alleles with linkage drag (Rebetzke et al. 2019). The identification of novel phenotypes and retention of unique alleles will enable their recycling in subsequent crossing and population development. Beyond technology, the identification of traits to be measured and screening conditions are equally important. It has also been difficult to define how stresses should be imposed experimentally, which has led to high variability in stress treatments and a lack of standardized protocols for assessing stress tolerance. However, the development of standard protocols has improved the reproducibility and reliability in identifying stress-tolerant genotypes.

1.4.3 Simulation Models for Appropriate Breeding Scheme

Plant breeders have traditionally relied on selection experiments to guide their decisions in a breeding scheme (Rutkoski et al. 2015), but these experiments are time-consuming and expensive. In addition, breeding strategies involve many interacting components; the compatibility of these components is critical for efficiency. Thus, a system that helps breeders to seek new breeding strategies could be beneficial; in this regard, simulation tools would be useful for breeders to choose an optimal breeding scheme. Yabe et al. (2017) presented a simple and flexible simulation platform for breeders to evaluate breeding schemes and choose an optimal breeding strategy among several possibilities. Simulation models can be used to predict appropriate trait phenotypes and selection protocols in breeding programs to achieve ideotypes (Boote et al. 1996; Yin et al. 2003). A recent simulation study of wheat breeding showed those rapid cycles of recurrent selection with optimal contribution selection and moderate selection intensity are the best strategy in the long run to improve all traits in the economic index (Cowling et al. 2018). van Eeuwijk et al. (2019) discussed several genotype-to-phenotype (G2P) models for predicting phenotypes across environments to obtain a larger response to selection.

Simulation studies are useful for resource allocation and the detection of unexpected outcomes before conducting a field trial. Recent simulation studies have been used to compare various breeding schemes considering genetic improvements and cost efficiencies and the impact of genetic architecture (Wang et al. 2003). Lorenz (2013) highlighted the importance of simulation studies for resource allocation in training populations in genomic selection (GS) while evaluating different numbers of replications and population sizes. Hickey et al. (2014) conducted simulations to determine training population designs and suggested that the best training population design depended on marker density. Bernardo and Yu (2007) compared a simulation study with field breeding to reveal that GS had 18 to 43% larger gains than marker-assisted recurrent selection (MARS) when the target traits were controlled by 20, 40, and 100 QTLs under heritabilities of 0.2, 0.5, and 0.8, respectively. The simulation results were consistent with the field trials where GS showed 14 to 50% larger gains than MARS (Massman et al. 2013).

1.4.4 Enhancing Genetic Variability

Many useful alleles have remained in landraces and CWRs (crop wild relatives) after processes of domestication and breeding, resulting in a narrow genetic base of the cultivated gene pool (Tanksley and McCouch 1997; Smýkal et al. 2015). Breeders have traditionally been reluctant to use CWRs due to the complex, long-term, and unpredictable outcomes as a result of linkage drag, sterility of progeny, and self-incompatibility. However, useful alleles with improved fitness to climate change scenarios could be incorporated with a more systematic and targeted use of

CWRs in crop improvement programs (Vincent et al. 2013; Dempewolf et al. 2017). There are now genetic procedures to identify CWRs with adaptation to abiotic stresses (Sanderson et al. 2019). Composite sets, diversity panels, and reference sets offer scope for mining allelic diversity, dissection of population structure, and association mapping for detection of QTLs that can be used in pulse breeding programs. Marker-based diversity analyses have enabled gene banks to define core germplasm, providing a user-friendly entry point for breeders to access extensive and varied collections. Markers that are tightly linked to a gene of interest can be used on the core set to identify germplasm that bear different alleles at the locus of interest. This will enable breeders to identify new traits or novel alleles for introgression into agronomic backgrounds. Several QTLs have been mapped for traits related to morphology, phenology, grain yield and its components, disease resistance, vigor, etc. using bi-parental populations (Varshney et al. 2014; Roorkiwal et al. 2018; Sivashakthi et al. 2018). Advanced backcross QTL populations would be valuable for understanding the genetic architecture of traits of interest and to develop improved varieties. Multi-parental populations, such as nested association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) populations, would further enhance allelic diversity and novel recombinants. Targeting Induced Local Lesions in Genomes (TILLING) populations, which combine traditional chemical mutagenesis with high-throughput genome-wide screening for point mutations in desired genes, are powerful for creating novel mutant alleles for both functional genomics and crop improvements (McCallum et al. 2000). TILLING has been incorporated into breeding programs to improve the mutagenesis method.

Plant breeding programs rely on meiotic crossovers (COs) that allows the stacking of desired traits into elite lines. However, the number of COs is generally low, with some regions virtually devoid of them, such as those flanking centromeres (Nambiar and Smith 2016), and the null effect of double crossovers. This limits the genetic diversity that can be incorporated in breeding programs; thus increasing recombination is a desirable trait in plant breeding (Wijnker and de Jong 2008; Crismani et al. 2013; Blary and Jenczewski 2019). A recent study showed that meiotic recombination could be increased in hybrid plants, up to almost eightfold (Fernandes et al. 2018). The next necessary step will be to assess, through detailed case studies and simulations, at which stage in a breeding program tinkering with CO formation can maximize genetic gain and how well this approach integrates with other breeding tools. This opens up the possibility of manipulating recombination to enhance the efficiency of plant breeding programs.

1.4.5 Selection Indices

Breeders strive to develop superior varieties with many traits in chorus. As conventional breeding methods attempt to simultaneously select for many traits, there is an overall loss of genetic gain and an increase in the length of breeding cycles required to generate the final variety. This is further complicated by undesirable

between-trait relations. Selection-based indices (simultaneous multi-trait selection) can be used to avoid the limits of single-trait selection. These indices allow the use of a single value in the selection process since the analysis is carried out using linear combinations of phenotypic data of different traits of agronomic interest with the genetic properties of a population. The objective is to guarantee improvements in the population's genotypic values and consequently the efficiency of the selection process. Many selection indices have been used as an effective selection criterion in pulse breeding programs. To obtain selection indices, families from the base population are evaluated to estimate genetic and phenotypic parameters, such as heritability and genetic correlation coefficients for the set of traits being considered. Selection criteria are traits used in the estimation of breeding values. Decisions on which traits should be included in the selection criteria should be based solely on economics and not whether they are difficult or easy to measure or change genetically. An economic selection index is understood as a combination of economic weights and genetic information from more than one trait. This requires a bioeconomic model to calculate economic values for traits in the selection and propose selection indices and estimate genetic gain, based on the selection criteria used in a breeding program. Improvement in the selection index is not accompanied by an improvement in each trait included under selection, but its use may balance different traits (Massman et al. 2013).

1.4.6 Indirect Selection

Direct selection based on grain yield is commonly practiced in pulse breeding programs. Being a quantitative trait, environmental effects, GE interactions, low heritability, negative trait linkages, and nonadditive gene action reduce the efficiency of using grain yield as the sole selection criterion and, thus, complicate the efforts of selection. To overcome these difficulties, breeders focus on indirect selection for secondary traits that are easy to measure, highly heritable, and highly correlated with grain yield to make better selection gains. Therefore, dissection of complex traits, such as yield and stress tolerance, into component traits offers an option for breeding successful varieties with gradual genetic gain over many cycles of selection. For example, grain yield in pulses is determined by traits, such as plant type, phenology, number of branches and nodes, number of pods and seeds, seed size, etc. Understanding the nature and magnitude of gene actions of component traits and their associations is critical for the selection of appropriate breeding strategy and parental lines. For example, seed weight being governed by mostly additive genes is the most stable and heritable trait and hence an important selection criterion for improving grain yield in pulses. Thus, selecting complex traits by indirect selection can bring efficiencies in breeding programs. In the past, the potential value of indirect selection for breeding such complex traits has been realized with morphological markers. One of the best examples of indirect selection is the dwarf plant type in wheat and rice, which has been used for selecting nitrogen-responsive, non-lodging,

high-yielding varieties. This has created opportunities for plant breeders to use certain morphological, physiological, and biochemical traits when selecting for grain yield.

1.4.7 Marker-Assisted Selection

Molecular marker-assisted selection (MAS) overcomes the problems associated with phenotype-based selections. The use of molecular markers has not only improved the accuracy of crosses but also allowed breeders to produce varieties with previously difficult traits to incorporate (Xu and Crouch 2008). Early-generation MAS means the selection and elimination of unwanted plants at an early stage of the breeding process. This helps breeders to focus on fewer promising plants, resulting in a higher efficiency and accuracy of selection (Collard and Mackill 2008). Many MAS techniques have been developed, including marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genomic selection (GS), which have been successfully used to breed soybean with cyst nematode resistance (Concibido et al. 1996), common bean with anthracnose resistance (Miklas et al. 2003), lupin with phomopsis stem blight and anthracnose resistance, and chickpea with fusarium wilt resistance (Mannur et al. 2019).

Introgression and pyramiding of multiple genes affecting the same trait is a challenge for breeding programs. MAS offers the potential to more precisely assemble target alleles in the same genotype, with less unintentional losses and fewer selection cycles. For most crops, >90% of the recurrent parental genotype can be recovered within two generations when a suitable number of markers and adequate number of progenies are used for background selection (Tanksley et al. 1989). This represents a substantial time saving compared to conventional backcross breeding. The loss of minor QTL is generally observed when a round of MAS is replaced by a round of phenotypic selection. Simulation studies have shown marker-assisted approaches more efficient for QTL even with very low heritability (Moreau et al. 2000). To realize the full potential of MAS in pulse breeding programs, there is a need to develop high-throughput phenotyping systems for QTL mapping, improved understanding of GE interactions and epistasis, and computational tools tailored to the needs of molecular breeding programs (Herzog and Frisch 2011). Using MAS approaches, commercial breeding programs have reported twice the rate of genetic gain over phenotypic selection (Eathington 2005; Crosbie et al. 2006; Ragot et al. 2007). Current MAS strategies fit the breeding programs for the traits that are highly heritable and governed by a single gene or one major QTL that explains a large portion of the phenotypic variability. Many pulses programs have started using markers to increase the effectiveness of selection in breeding and to significantly shorten varietal development time (Kellya et al. 2003). Though there are reports of higher selection gain from MAS (Brumlop and Finckh 2011), there is a need to prioritize target traits for MAS considering the value of the trait, the precision of phenotype

assessment required, and the costs associated with direct selection methods compared with the costs of indirect MAS.

1.4.8 Genomic Selection

With the availability of high-density genome-wide molecular markers like SNPs and DArT markers, GS has emerged as a promising tool in crop breeding (Meuwissen et al. 2001), which uses predictive computational models that are developed from a training population to make genome-wide predictions of the genetic value of breeding progenies for selection (Bernardo and Yu 2007; Jannink et al. 2010). High marker density is required to capture most of the linkage information between QTLs and markers. The comprehensive information on all possible loci, haplotypes, and marker effects across the entire genome is then used to calculate the genomic estimated breeding value (GEBV) of a genotype in the breeding population. For successful GS, the experimental population must be established. GS eliminates the need to collect phenotypic data in all breeding cycles.

One downside of GS is the level of GEBV prediction accuracy, which in turn is influenced by the training population size (Daetwyler et al. 2008), marker density (Poland and Rife 2012), heritability, statistical models (Heslot et al. 2012), linkage disequilibrium (Habier et al. 2007), and population structure (Windhausen et al. 2012). Simulation studies demonstrated that GEBV accuracy is within 0.62–0.85. Heffner et al. (2009) used a previously reported GEBV accuracy of 0.53 and reported three- and twofold annual gain in maize and winter barley, respectively. However, many studies have shown that improvements in prediction accuracies have reached a plateau despite increased marker density. Therefore, finding an optimal balance between the number of probes/markers and predictive ability is important for cost-effective GS implementation. Recent work in GS for chickpea improvement showed prediction accuracies from 0.138 for seed yield to 0.912 for 100-seed weight (Roorkiwal et al. 2016). Several studies confirmed the time saving and precision of GS in plant breeding programs over conventional breeding and MAS when traits are controlled by a large number of QTL (Heffner et al. 2009). Keeping that in mind, investing time and resources to obtain a high-quality reference genome is worthwhile given the benefits it confers to downstream genetic analyses and the decision-making process for breeding programs.

1.4.9 Rapid Generation Advancement

Breeding cycles are lengthy with 7–10 years to fix the breeding value and test in METs. Being the denominator in the classical “Genetic Gain” equation, time reductions can have a profound effect on annual genetic gain. Important options for hastening the breeding cycle include off-season nursery, doubled haploid, and speed

breeding. Off-season nursery has been used for a long time but is limited to two generations per year and the burden of identifying an appropriate location. The doubled haploid method rapidly shortens the process of creating homozygous genotypes by using colchicine and has been successfully applied in breeding programs for asparagus, barley, eggplant, melon, pepper, rapeseed, rice, tobacco, triticale, wheat, and maize. Brennan and Martin (2007) suggested its use in a breeding program to reduce the release time of a new variety, thereby increasing its economic value by 20–30%. However, pulses have not benefited from this technology because of their recalcitrant nature, requiring tissue culture laboratory and skills to carry out the process. Recently, speed breeding has been suggested for rapid breeding cycles through the manipulation of light and temperature, high-density planting, and the single seed descent method. Speed breeding protocols have been developed and refined for many pulse crops including chickpea, lentil, grass pea, field pea, and pigeonpea that enable 4–6 generations per year (Ghosh et al. 2018; Lulsdorf and Banniza 2018; Samineni et al. 2019; Saxena et al. 2019).

1.4.10 Recombinant DNA Technology

Genetic engineering techniques can directly transfer genes of interest from any source into a plant. Consistent efforts in refining gene-transfer methods and tissue culture techniques have led to the production of transgenics in a variety of crop plants. Various gene-transfer methods have been developed, including *Agrobacterium*, physicochemical uptake of DNA, liposome encapsulation, electroporation of protoplasts, microinjection, DNA injection into intact plants, incubation of seeds with DNA, pollen tube pathway, use of laser microbeam, electroporation into tissues/embryos, silicon carbide fiber method, particle bombardment, and "in planta" transformation. Among these, *Agrobacterium* and "particle gun" methods are widely used for genetic transformation. Transgenic crops hold the potential to solve unsolved issues, such as *Helicoverpa* pod borer in chickpea and pigeonpea and ODAP in grass pea. The first reports on transgenic pulses were reported in adzuki bean (Eapen et al. 1987; Köhler et al. 1987) and soybean (Hinchee et al. 1988; McCabe et al. 1988). Since then, several studies have been conducted to improve agronomic traits in pulses, including insect resistance (Ignacimuthu and Prakash 2006; Mehrotra et al. 2011; Sawardekar et al. 2012; Ganguly et al. 2014), drought tolerance (Bhatnagar-Mathur et al. 2009), and salt tolerance (Suripeddi et al. 2011) in chickpea, drought resistance in cowpea (Sadhukhan et al. 2014), and disease resistance in pea (Amian et al. 2011). Methionine content has been increased through the expression of methionine-rich storage albumin from Brazil nut in common bean (Aragão et al. 1999; Rachel et al. 2019) and the sunflower seed albumin gene in lupins (Molvig et al. 1997). In 2019, GMO cowpea with resistance to *Maruca vitrata* was released for cultivation in Nigeria (https://www.seedquest.com/news.php?id_crop=2093).Transgenic crops are needed for traits that cannot be

easily improved genetically through conventional approaches due to the lack of satisfactory sources of desirable gene(s) in the crossable gene pool.

1.4.11 Genome Editing Technologies

Targeted mutagenesis technologies are now widely used in plants to swiftly and conveniently generate desirable mutations. Genome editing allows changes in targeted DNA sequences, involving the deletion, substitution, or addition of one or more bases. Genome editing requires prior information on gene identity and function, leading to targeted mutations. This can be done through new approaches that rely on specific and programmable nucleases (Zhang et al. 2019). Programmable nucleases can produce specific changes at a desired location within the genome, such as zinc finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats/CRISPR-associated system (CRISPR/CAS). Each of these technologies seeks to induce a precise change in the genome, creating new desirable alleles to speed up the development and release of new varieties and/or broaden the genetic pool of desirable alleles. There are two basic requirements for the delivery of editing components into the genome: availability of the genome sequence and effective transformation methods. The genomes of many pulse crops have been sequenced and annotated and are publicly available along with re-sequenced accessions (Varshney et al. 2018). Several transformation methods have also been developed and used for the transformation of pulse crops, with *Agrobacterium tumefaciens* the most common (Eapen 2008). CRISPR-Cas9 is the most commonly used system for genome editing in model crops (Wang et al. 2014). Among pulse crops, CRISPR-Cas9 was first used for soybean genome modifications via hairy root transformation mediated by *A. rhizogenes* (Jacobs et al. 2015; Michno et al. 2015) and has since been used to edit symbiotic nitrogen fixation related genes in *Lotus japonicus* (Wang et al. 2016) and symbiosis receptor-like kinase gene inactivation in cowpea (Ji et al. 2019). The high precision and flexibility afforded by CRISPR-Cas9 have widened the scope of genome editing possibilities to revolutionize future crop improvement research. Recently, the first genome-edited variety, high oleic oil soybean, was successfully commercialized (Kim and Kim 2019), confirming the potential of the genome editing technology to improve pulse crops. For plant breeding, this means that scientists can edit the genomes of elite varieties to produce new varieties in a single generation, unconstrained by existing variation or the need to select for favorable combinations of alleles in large populations (Scheben and Edward 2017). Potential genes that could be edited, however, need to be prioritized.

1.4.12 Experimental Design

To control micro- and macro-environmental variability, efficient field designs are important at all stages of a plant breeding program to ensure efficient, cost-effective operations (Sarker and Singh 2015; Lado et al. 2016). The choice of experimental design and spatial correction determines the capacity to find superior genotypes. Designs can range from an un-replicated trial at a single location to replicated METs that could involve many locations over several years. In most cases, genotypes are generally arranged to minimize the variance of differences between them to maximize selection gains. Larger populations allow greater selection intensity and the probability of identifying superior recombinants, whereas increased replication improves heritability, especially for highly complex traits prone to measurement error and random environmental deviations. In the early stages of a plant breeding program, expected genetic gains could increase by screening many genotypes, rather than having more precise comparisons of a few genotypes (Bos 1983; Gauch and Zobel 1996). Minimizing the variance of differences between genotypes and using multi-environment variance components for different crops, Talbot (1984) found that 12 sites with two replicates across 2 years was reasonable for most crops, with more years increasing the precision than more locations.

Early-stage evaluation in pulse breeding is commonly practiced on large numbers of experimental lines that have been derived from multiple crosses and field-tested without replication. Precision in estimating the genetic potential of these genotypes at this stage is crucial for pulse breeders to avoid Type II errors and the loss of potentially valuable cultivars. The challenge is to accurately select superior lines to maximize genetic gains. However, heterogeneity among experimental plots and field trends—common in agricultural field experiments—are likely to affect yield and its components (Becker 1995). Proper design and analyses are necessary to further reduce experimental error variance (Cullis and Gleeson 1991). Stringer and Cullis (2002) recommended introducing spatial analyses to account for fertility trends and plot competition in non-replicated early-stage trials. The spatial method is suited to selection in early-stage trials with the potential to maximize genetic gains, as indicated by improvements in phenotypic correlations among traits (Cullis et al. 1998; Edmé et al. 2007). An augmented or modified augmented design or any spatial analysis should be used for selection in the early stages of crop breeding programs with no replication.

Several approaches have been proposed to control spatial variability, such as nearest-neighbor adjustment (Katsileros et al. 2015), smoothing techniques including penalized spline analysis (Piepho and Williams 2010; Velazco et al. 2017), modeling the variance–covariance matrix of spatial correlations using geostatistical components (Piepho and Williams 2010) or mixed models (Smith et al. 2005). Experimental designs such as alpha designs or partially replicated (P-REP) have been recommended for early-stage breeding trials (Piepho et al. 2015). In general, full replications at the early stages of a breeding cycle may not be optimal, and P-REP designs offer a more efficient and practical option for improving selection

efficiency (Paget et al. 2017). In some studies, early-stage evaluation over several locations using a P-REP design is more efficient than increasing replications within a single location (McCann et al. 2012; Paget et al. 2017). González-Barrios et al. (2019) compared six experimental designs combined with four spatial correction models and found that the moderate mega-environmental design (MED) strategy, which accounts for spatial variability and GE interactions, had the largest response to selection. It had the best resource allocation strategy and could potentially increase the selection response by up to 43% in breeding programs when genotypes are evaluated in METs. This experimental design is especially suited for the screening of many genotypes each year for genetic gain. Therefore, modeling spatial correlations might improve the estimation of genotypic effects, even after a good experimental design (Borges et al. 2019). In lentil, the spatial analysis model was better than the commonly used RCB design model at enhancing the precision of genotypic means and heritability and breeding progress (Sarker and Singh 2015).

For late-stage yield trials of elite lines, alpha designs are used extensively in plant breeding due to their flexibility in the number of entries that can be evaluated, the size of the incomplete block, and the reasonable error control (Borges et al. 2019). While incomplete block designs significantly improve the efficiency of plant breeding field trials, allowing for block differences in two directions can further improve precision (Kempton et al. 1994). One useful approach is to start with an alpha design arranged with rows as incomplete blocks and then rearrange the order of the entries in each row to balance as best as possible across the columns. These efficient row–column designs are available in CycDesign (Whitaker et al. 2001).

For an efficient breeding program, statistical methods used for data analysis need to be accurate, efficient, and informative. The yield data generated from a breeding program arises from a series of MET trials. Early methods focused on analysis of variance techniques with no insight into the nature of GE interactions, thus hindering selection decisions. Recently, mixed model approaches have become popular for analyzing a series of variety trials, as summarized by Kempton (1984). Despite clear benefits of the general mixed model approach, adoption within plant breeding programs has been very slow (Smith et al. 2005). In particular, the use of more complex models and the assumption of random rather than fixed variety effects are not widespread. In contrast, animal breeding programs have used REML and BLUP for many years as the basis for selection and estimation of breeding values and genetic parameters. Despite the availability of multiyear, multicycle, and multiphase data in plant breeding programs, selection is often based on single-year, single-cycle, and single-phase data. MET data can be analyzed using linear mixed models in either one- or two-stage analysis. A one-stage analysis is usually more efficient than a two-stage analysis (Smith et al. 2005) but computationally expensive (Möhring and Piepho 2009) when used to analyze many environments (Piepho et al. 2012). Moreover, in the early stages of such analyses, multiyear analysis was impractical due to the lack of computing power to handle extensive unbalanced data. With recent advances in analytic power, a two-stage analysis is computationally efficient (Möhring and Piepho 2009) and can handle large amounts of data and more complex models. A two-stage analysis can be used to model various

randomization layouts and within environment error in the first stage (Piepho et al. 2012), with adjusted genotype means for the across-environment second-stage analysis. A spatial model can be fitted for each field and the effects of genotype and genotype × year interactions separated in the estimation of variance components across fields (Arief et al. 2015). Thus, in general, the combined analysis of data across years and cycles is better for making selection decisions to advance test entries but is not commonly used in plant breeding programs (Arief et al. 2019).

Digitalization, automation, and mechanization in breeding procedures need to be adopted during the breeding process. More emphasis is required on uniform ontology, digitalized pedigree information, barcoding of breeding material, digital data recording in the field, data management system, and quality control. An Integrated Breeding Platform (http://www.integratedbreeding.net) is one such initiative that is helping breeding programs to modernize and improve their breeding efficiencies. For instance, the Breeding Management System of Integrated Breeding Platform is being used extensively at ICRISAT and ICARDA breeding programs. Further, the recent Excellence in Breeding (http://excellenceinbreeding.org/) platform brings the components of CGIAR crop improvement to a single platform to focus on better integration and modernization of breeding programs in developing countries.

1.5 Conclusions

Breeding pulse crops for high yield and superior adaptability to new and variable climates is imperative to ensure food and nutritional security and ecosystem services. Past breeding efforts have made modest progress in developing improved varieties with limited genetic gains in pulse crops. Rapid advances in genotyping and phenotyping and their decreasing costs have enabled the widespread application of these tools to unravel the genetic basis of important and complex traits, thus accelerating the progress of breeding programs. Several other resources, including mathematical models, data analysis skills, field experiment design, barcode labeling, and databases for storing genotyping and phenotyping data, are essential for the successful execution of breeding efforts. Large-scale meta-analysis for genetic mapping in pulse crops will be crucial for better marker-trait association. Even though genomic-assisted crop breeding began nearly 30 years ago, the use of genomics in pulse improvement is far from widespread. Transgenics and genome editing could be valuable for pulse crops that do not have must-have traits available in the gene pool, or backcrossing is impractical due to a long generation interval or infeasible due to a heterozygous recurrent parent. Recently, incredible progress has been made in the technologies that support data analytics and artificial intelligence, which will significantly impact breeding operations. Foremost among those tools is cloud computing, which has recently become more available and affordable. This means that staggering amounts of data can be stored, cleaned, organized, and studied with relative ease. Machine learning with enhanced processing power will improve predictive modeling efficiency. High-powered algorithms are being

developed to help plant breeders make more precise predictions from hyperspectral data to identify high-yielding crop traits. These innovative technologies will help to shape the future of pulse breeding, amid a growing world population and changing climate.

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Chapter 2
Advances in Chickpea Breeding and Genomics for Varietal Development and Trait Improvement in India

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2.1 Introduction

Chickpea (*Cicer arietinum* L.) is a temperate self-pollinated legume crop, originated from southeastern Turkey (Ladizinsky 1975). It is an annual species having chromosome number $2n = 2x = 16$ and haploid genome size of 738 Mb (Varshney et al. 2013a). India, Pakistan, Australia, Canada, Turkey and the USA are the major chickpea-producing countries. India ranks first in chickpea area as well as production with 11.38 million tonnes produced from 10.56 million ha during 2017–2018 (Dixit 2018). The wild progenitor of chickpea is believed to be *C. reticulatum* L., while *C. arietinum* L. is the only cultivated species of genus *Cicer*. Broadly chickpea has been divided in two distinct types based on seed morphology, *desi* type with small seed having brown coat seed colour and *kabuli* type with large seed having cream or beige seed coat colour.

Chickpea grains are rich in proteins (20–22%), carbohydrates (~40%), vitamins and several minerals such as phosphorus, calcium, manganese, potassium, magnesium, iron and zinc (Jukanti et al. 2013). It also contains significant amount of essential amino acids, viz., leucine, isoleucine, lysine, valine and phenylalanine. Consumption of chickpea helps in reducing diabetes due to lower glycemic index. Chickpea seed oil

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contains unsaturated fatty acids such as oleic acid and linoleic acid which are good for the heart. It also contains various phytosterols such as tocopherols, β-sitosterol, sterols and tocotrienols which exhibit anti-bacterial, anti-fungal, anti-tumoric and anti-inflammatory properties. It also contains several bioactive compounds like isoflavones, phytates and phenolic compounds, which are associated with potential health benefits and helps in prevention of cardiovascular diseases, blood pressure, cancer and obesity. Chickpea is consumed as *dal* prepared from split cotyledons and snacks prepared from *besan* (chickpea flour) in Indian subcontinent while as soups, stews and salads in African regions. It is also consumed as roasted, salted, boiled, raw vegetable and fermented forms. In addition to its nutritive benefits in human diet, chickpea also fixes atmospheric nitrogen efficiently and helps in improving soil health and fertility.

Molecular markers help in accelerating the process of trait improvement by understanding the genetic basis of the traits (Varshney et al. 2007). Selection of traits having low heritability which are highly influenced by the environment can be easily performed by molecular markers. The molecular markers are also helpful in the transfer and pyramiding of multiple genes simultaneously, introgression of genes from wild species into cultivated one with minimum linkage drag, description of any germplasm, assessment of genetic relatedness amongst accessions and mapping of several quantitative trait loci (QTLs) governing economically important traits. Thus, the molecular tools help in speeding up the conventional breeding approaches efficiently and offer the rapid and precise alternative for improvement of quantitative traits like yield and resistance/tolerance to various biotic and abiotic stresses.

During the past 10 years, large-scale genomic resources have been developed for chickpea improvement. Molecular marker technologies have made it feasible to locate genomic regions of various quantitative traits for use in marker-assisted selection (MAS). This further prompted to use molecular breeding approaches, namely, marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), advanced backcross quantitative trait loci (AB-QTL) analysis and genomics-assisted breeding (GAB) in chickpea breeding programmes. Next-generation sequencing technologies led to rapid development of molecular markers in chickpea on a large scale. These advanced resources and technologies have been utilized for construction of dense linkage maps and identification of several molecular markers associated with agronomically important traits. The chapter describes progress in varietal development, availability of genetic and genomic resources and their deployment for multiple trait breeding and genomics-assisted chickpea breeding.

2.2 Germplasm and Genetic Resources

Chickpea genetic resource comprises of 99,877 accessions including 1476 wild *Cicer* accessions at global level. These accessions are safeguarded and maintained amongst 120 national and international gene banks located across 64 countries (Upadhyaya et al. 2018). The National Bureau of Plant Genetic Resources, India, holds 14,704 chickpea accessions including cultivated and wild species. The International Crop

Research Institute for the Semi-Arid Tropics (ICRISAT) holds largest chickpea germplasm collection of 20,764 accessions representing 59 countries of origin.

The wild *Cicer* species consist of useful variation for many desired traits including resistance/tolerance to various biotic and abiotic stresses (Croser et al. 2003; Gaur et al. 2010; Kaur et al. 2013; Singh et al. 2013), productivity traits (Singh and Ocampo 1997; Singh et al. 2005) and biochemical traits (Kaur et al. 2010). Availability of passport information on agronomic and nutrition traits and resistance to biotic and abiotic stresses have been the major challenge for utilization of germplasm in breeding programmes for developing trait-specific genotypes. As many as 16,990 chickpea accessions were evaluated at ICRISAT for 13 traits to form a core collection comprising 1956 accessions so as to promote significance of global chickpea genetic resources in genomics and breeding (Upadhyaya et al. 2001). Further, a mini-core collection of 211 chickpea accessions has also been developed (Upadhyaya and Ortiz 2001). The ICRISAT and ICARDA with their joint efforts have developed a reference set of 300 lines under the Generation Challenge Program (GCP) of the Consortium of International Agricultural Research Centres depicting genetic variability available in the germplasms maintained at the aforementioned institutions (Upadhyaya et al. 2008). These manageable numbers of accessions representing mini-core, core and reference sets of germplasm contribute ideal resource for association genetics, gene mapping and cloning, allele mining and applied breeding for the development of elite cultivars. Long history of breeding efforts made towards few domesticated traits has inflated the crop yields but narrowed the genetic base.

Conventional breeding approaches have made a significant improvement in chickpea and contributed towards bringing pulses self-sufficiency in India. The pedigree analysis tracing parents back to 120 in *desi* and 53 in *kabuli* of 138 varieties (103 *desi* and 33 *kabuli*) developed through hybridization revealed that IP 58 (27), C 1234 (26), JG 62 (18), S 26 (18) and Chaffa (15) were the frequently utilized parents in *desi* (Fig. 2.1a) while Rabat (26), Pb 7 (24), Banda Local (14), Etah Bold (14), Guamchil 2 (14), P 458 (14) and GW 5/6 (14) were involved in development of *kabuli* varieties (Fig. 2.1b). This clearly indicated that very few genotypes have been used to develop chickpea varieties released in India as earlier reported by Kumar et al. (2004). Thus, there is need to involve more and diverse germplasm, primitive landraces and wild *Cicer* species in hybridization for cultivar development (Verma et al. 1990; van Rheenen et al. 1993; Nadarajan and Chaturvedi 2010; Mishra et al. 2013a, b; Singh et al. 2014). A large number of donors identified through multi-location screening have been listed in Table 2.1.

2.3 Varietal Development

A systematic breeding work on chickpea started in 1905 at Imperial Agricultural Research Institute, Pusa (Bihar), and subsequently at other centres by mainly concentrating towards collection of landraces. In the initial phase of varietal development in the 1970s, major emphasis was laid on increasing yield potential over

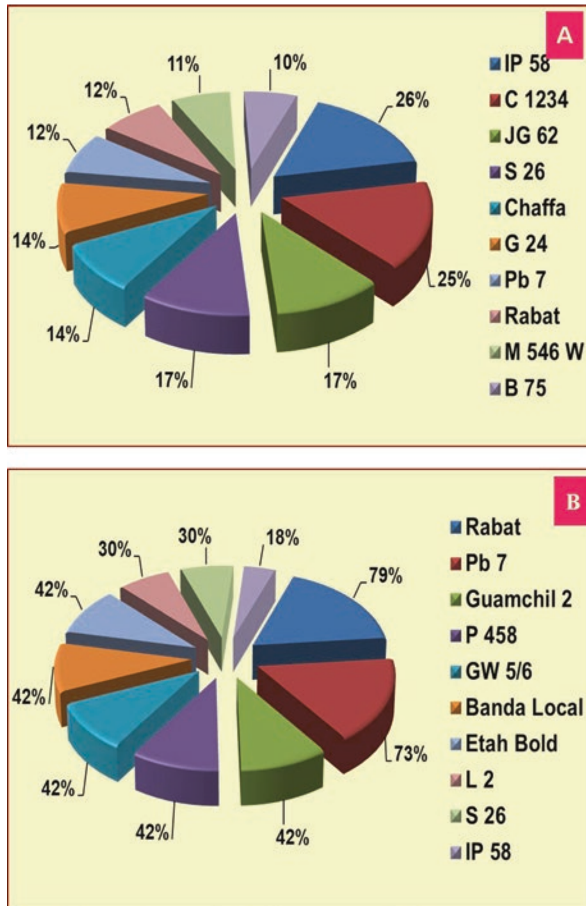

Fig. 2.1 (**a**) Per cent utilization of major parents in development of *desi* chickpea varieties. (**b**) Per cent utilization of major parents in development of *kabuli* chickpea varieties

landraces; hence most of the varieties were developed via selection and purification of existing landraces. Varieties like Dahod yellow, Chaffa, Annegri-1, Ujjain 21, BR 78 and Gwalior 2 are selection from the local germplasm/landraces. During the 1980s, major emphasis was laid on breeding for disease resistance. Systematic breeding programme led to the identification and development of disease-resistant/disease-tolerant donors/varieties against major diseases particularity *Fusarium* wilt and *Ascochyta* blight. As a result, varieties like 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. were evolved exhibiting potential in minimizing the wilt incidence. In the early 1980s, *Ascochyta* blight outbreak caused substantial damage to chickpea crop in northern states like Punjab, Haryana, north-west Rajasthan and Jammu region. Hence, the emphasis was laid to develop

Table 2.1 Donors identified for major biotic and abiotic stresses

Trait	Donors identified
Fusarium wilt	AKG 1303, RLBG 2, WR315, Avrodhi, RLBG 3, BDNG 2017-1, RKG18-1, NBeG 857, ICCV 171105, NBeG 798, PBC 546-18, JG 2017-50, RKG 13-515-1, H 12-22, GL 14015, Bidhan Chola 1, GAG 1620, H 15-25, PG 221, JG 2018-53, GNG 2418, BG 4007, RG 2016-133, GNG 2438, JG 2018-54, BDN 9-3, BCP 4, GL 88341, GL 87079, Phule G 5, Phule G 81-1-1 (Vijay), Phule G 12, Phule 87,207, ICCV 10, ICCV 2, ICCC 37, ICCC 42, KPG 59, H 86-72, IPC 92-37, DCP 92-3, SAKI 8516 (JG 16), BGM 443, JCP 27, BDNG 88, GL 83119, GL 84038, HC 1, GNG 663, KPG 259-4, GL 86123, KPG 143-1, H 86-18, GPF 2, JG 12, JG 24, HK05-169, JSC 40, JG 2000-04, GJG 0919, GJG 0904, GJG 0814, CSJK 54, Phule G16111, GJG1603. NBeG 776, RKG 13-55, GNG 2325, PG 209, JG 74315-2, IPC 08-11, PG 211, JG 2017-50, Phule G 0819, JG 2017-49, GJG 0922, GNG 2391, GL 13037, IPC 07-28, NBeG 779, H 12-63, SCGP-WR 28, BCP 60, GJG 0814, IPCK 10-134, IPC 17-28, GJG 0921, GJG 1010, SCGP-WR 32, GJG 904, IPC 08-69, CSJK 96
Ascochyta blight	DKG 964, PBG1, PBG 7, GNG 2207, GNG 2171, E 100Ym, E100Y, PG 82-1, EC 26446, BRG8, ICC7002, GL84038, GL 84099, GL 90169, GL 23094, GLK 24092, GLK 24096, BG 276, H 82-5, H 86-18, H 75-35, Gaurav, GL 88016, ICC 1069, BG 267, GNG 469, BG 362, GNG 1581, IPC 79, IPC 129, H03-45, ILC 3279
Botrytis grey mould	IPC 15-95, IPC 15-202, IPC 15-183, IPC 15-48, IPC 15-113, IPC 16-48, GCP 101, RVG 202, CSJ 556, GNG 1581, IPC 15-185, ICC 1069, IC 12483, Dhanush, ICCW 92, ICCV 41, HK 94-134, CSJK 72, GL 10006, GLW 69, GLW 91
Dry root rot	H14-14, RLBG 3, BDNG 2017-1, ICCV 171117, CSJ 902, BG4001, DBGC-2, GJG 1607, NBeG 798, PBC 546-181, BG 3091, BG 372, IPC11-30, GJG 1603, Phule G 15109, RKG 18-4, BDNG 21-1, RKGK 13-499, GNG 2453, MABT 66-266, IG 2018-110, NBeG 786, CSJ 867, IPC 2013-74, RKG 13-223, RKGK 13-223, RKGK 13-159, JH 13-09, BG 3062
Herbicide tolerance	ICC 1205, ICC 1161, ICC 07110, ICC 1164, ICC 1381, GL 22044, GLK 10103, NDG 11-24
Wilt + dry root rot	ICC 8383, ICC 10466, ICC 12237, ICC 12269, GNG 2226, IPC 2007-28, IPC 2010-134, H 86-84, H 86-18
Wilt + gram pod borer	ICCL 86102, ICCL 86111, ICCX 730020
Wilt + *Ascochyta* blight	GL 83119, GL 84038, GL 84096, GL 84107, H 83-84, H 83-60, FLIP 82-78-C, FLIP 83-7-C, FLIP 82-74-C, FLIP 84-43-C, FLIP 84-130-C, ILC 171, GL 91058, GL 91060, GL 88341, FLIP 96-41, ICCV 89445, ICC 1272, ICC 3137, IC 4074, IPC 97-1, DKG 964
Drought tolerance	ICC 4958, ICC 8261
Heat tolerance	ICCV 92944 (JG 14), ICC 15614, JSC 55, JSC 56, ILWC 115, ILWC 21, EC 556270
Cold tolerance	GL 26018, GL 28202
Salinity tolerance	CSG 8962, ICCV 10, JG 62

Ascochyta blight-resistant varieties, thereby resulting in the release of landmark varieties like PBG1, PBG 5, GNG 469, Gaurav, PBG 7 (Fig. 2.2) and GNG 2171 for cultivation in blight-prone areas.

Under All India Coordinated Pulses Improvement Project (AICPIP), the evaluation of genotypes in two separate trials (*kabuli* and *desi*) started in 1981–1982. Later in 1982–1983, *desi* chickpea trials were bifurcated in two categories – normal sown and late sown. Subsequently, JG 74 was identified for central and northern India. 'Bold Seeded' trial was constituted in 1983–1984 to facilitate the release of high-yielding and large-seeded *desi* chickpea varieties. A special trial to screen breeding lines against *Ascochyta* blight started in 1982–1983. During the 1990s, major thrust was given to breed for short-duration, multiple-resistance, drought-tolerant and high-input responsive varieties. Breeding for short duration (90–110 days) was directed in the environment where the growing season is short to escape from terminal drought and heat for successfully raising a crop. Development of short-duration varieties like JG 16, JG 11, Vijay, Vikas, Vishal, JGK 1, KAK 2, ICCV 2, ICCV 10, etc. helped in expanding chickpea area in southern and central part of the country. In spite of reduction in duration, the yield potential of these early varieties remained almost similar to long-duration varieties. Similarly, in states like Uttar Pradesh, Bihar, parts of Chhattisgarh, Jharkhand, Haryana and Punjab where rice fields are vacated quite late after the harvest of rice, early-maturing varieties amenable to late planting like Pusa 372, Udai, RSG 963, BGM 547 and Rajas were developed. In 1991–1992, two special trials for evaluation of genotypes under high input

Fig. 2.2 A high-yielding *Ascochyta* blight-resistant variety PBG 7

conditions and for salinity tolerance were constituted. Later in 1995–1996, a trial to evaluate breeding lines under drought was constituted.

In order to evolve large-seeded *desi* (>20 g/100 seeds) and *kabuli* (>25 g/100 seeds) varieties, coordinated trials were implemented since 1983–1984 and 1995–1996, respectively, and as a result, varieties like Pusa 256, JG 11, Samrat, Phule G 5, Vishal and BGM 547 were developed in *desi* group. Similarly, *kabuli* varieties such as BG 1003, BG 1053, Haryana Kabuli Chana 1, Haryana Kabuli Chana 2, KAK 2, JGK 1, Vihar and Virat were developed after considering the consumer's preference for large-seeded *kabuli* types (Chaturvedi et al. 2010). A wilt-resistant variety, DCP 92-3, was released for the areas where high soil moisture or frequent winter rains or high fertility causes more vegetative growth and subsequently causes lodging of the crop. Later, varieties for specific conditions like CSG 8962 for mild salinity conditions of north west plain zone, JG 14 for heat tolerance for central India and RSG 888 for cultivation in moisture stress or rainfed conditions of Rajasthan, Haryana and Punjab were developed. In recent years, *kabuli* varieties like HK 05-169, L 555 (GLK 26155), GNG 1969, and L 556 (GLK 28127) were released for north Indian conditions. For north hill region, cold-tolerant *kabuli* varieties like CSJK 6 and Phule G 0027 were released, whereas varieties like JSC 55 and JSC 56 were released for late sown conditions of central India. Now, emphasis is being laid on development of extra-large-seeded *kabuli* chickpea varieties with seed size more than 50 g/100 seeds. Several promising entries are in advance varietal trails, and few varieties like Phule G 0517, PKV 4–1 and MNK-1 have been developed with seed size more than 50 g/100 seeds which fetch premium price in market. These varieties are being popularized amongst farmers through FLDs and State Agricultural Department. The farmers of India are now gradually adopting mechanization of farm operations for improving efficiency and reducing cost of cultivation. The farmers are demanding chickpea cultivars which can be directly harvested by combine harvesters. Most of the present-day chickpea cultivars are not well suited to machine harvesting because the plant height and plant architecture are not suitable for mechanized harvesting. Development of chickpea cultivars with tall (>55 cm.) and erect growth habit is required. In the recent years, few machine-harvestable varieties such as NBeG 47, Phule Vikram, RVG 204 and BG 3062 have been released in India for southern and central India. So far, more than 210 chickpea varieties have been developed for cultivation in different parts of the country since the inception of All India Coordinated Research Project on Chickpea (Singh 2014; Dixit 2015). The milestones in chickpea varietal development during the past 100 years are given in Table 2.2.

At present, the major emphasis of AICRP on chickpea is on collection, evaluation, characterization, and utilization of germplasm for developing improved varieties. Linkages are being established with national and international institutions to make use of new knowledge in frontier areas like biotechnology, information technology, etc. There is a need to have dedicated research efforts on development of cultivars responsive to irrigation and high fertility conditions for rehabilitating chickpea in northern India. Drought tolerance would continue to be the most important trait for two-third of the chickpea area that is rainfed. The programmes need to

Table 2.2 Milestones in chickpea improvement research during the past 100 years

Year	Product developed
1926	Varieties developed through selection: NP 17, NP 25, NP 28 and NP 58
1940s	Varieties developed through hybridization: C12/34 and type 87
1948	Variety with wide adaptability released: Chaffa
1960s	First variety for south India released: Annegiri 1
1960	First wilt-resistant variety released: C 104 First widely adaptable variety for north India C 235 developed
1969	First release through All India Coordinated Pulse Improvement Project (AICPIP): GNG 114
1970	Bold (large)-seeded variety for central India released: Radhey
1970	Spontaneous Mutant of RS 10 released as RS 11
1976	First *kabuli* variety released: L 144
1979	First green seeded variety developed: Hare Chhole
1982	First *Ascochyta* blight-resistant variety released: GL 769
1984	First variety developed through *desi* x *kabuli* introgression – Pusa 256
1985	Varieties released through mutation breeding: Pusa 408, Pusa 413, Pusa 417
1985	Russian tall donors used and tall variety developed: Pusa 261
1992	First variety released for late sown condition through AICRP – KPG 59 (Uday)
1993	First short-duration *kabuli* variety developed – ICCV 2 (Sweta)
1994	First drought-tolerant variety release for rainfed condition – Vijay
1998	For high input condition, first lodging-resistant variety developed: DCP 92–3
1998	First salinity-tolerant variety released: CSG 8962
1999	First officially released Gulabi gram variety: JGG 1
1999	First variety developed through polygon breeding: JG 11
1999	First large-seeded *kabuli* variety released: KAK 2
2002	First drought-tolerant variety developed: RSG 888
2003	First large-seeded *kabuli* variety for south India: Vihar
2005	First variety through inter-specific hybridization: Pusa 1088
2008	Large-seeded *kabuli* variety (IPCK 2002–29) for central India developed
2009	Extra-large-seeded (>50 g/100 seed wt.) *kabuli* varieties MNK 1, Phule G 0517, IPCK02, PKV 4-1 developed
2011	Heat-tolerant variety JG 14 released
2017	Chickpea varieties amenable to machine harvesting developed for Andhra Pradesh (NBeG 47), Karnataka (GBM 2) and Maharashtra (PhuleVikram)
2019	Chickpea varieties amenable to machine harvesting developed central India (Phule G 08108, JG 20016-24, BG 3062)
2019	Release and notification of chickpea varieties evolved through marker-assisted selection backcrossing (MABC) developed for drought tolerance (BGM 10216) and *Fusarium* wilt resistance (MABC WR SA 1)

continue efforts on enhancing resistance/tolerance to abiotic and biotic stresses for improving yield stability (Malhotra and Saxsena 1993). There is a need to enhance precision and efficiency of breeding programmes. This would include novel approaches for enhancing genetic base of the breeding populations,

Fig. 2.3 GL 13042 – a high-yielding variety having moderate level of resistance to *Botrytis* grey mould derived from an inter-specific cross (GPF 2 x *Cicer judaicum* acc. 185)

genomics-assisted breeding, precision phenotyping, rapid generation turnover and efficient breeding data management system. Efforts are being made to introgress desirable traits from wild *Cicer* species at different institutes. In this endeavour, PAU, Ludhiana, successfully crossed an elite cultivar GPF 2 with *C. judaicum* acc. 185 to introgress resistance against *Botrytis* grey mould. A high-yielding inter-specific derivative line, GL 13042 (Fig. 2.3), possessing moderate level of resistance to *Botrytis* grey mould has been identified for released in Punjab state. It will be the first variety developed from inter-specific cross with *C. judaicum*.

2.4 Major Constraints

Chickpea is prone to a large number of biotic (diseases, insect pests, nematodes, weeds) and abiotic (drought, heat, cold, salinity, alkalinity, etc.) stresses. Abrupt rise or drop in temperature, terminal soil moisture stress or excess rains during crop growth result in low productivity. These biotic and abiotic constraints limiting chickpea yields in different states are listed in Table 2.3.

Table 2.3 Biotic and abiotic stresses to chickpea production in different states of India

States/area	Biotic stresses	Abiotic stresses
Jammu and Kashmir, Himachal Pradesh, Uttarakhand	*Ascochyta* blight, *Fusarium* wilt, dry root rot	Drought, cold, fog, frost
Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, Uttaranchal, North Rajasthan and western Uttar Pradesh	*Fusarium* wilt, dry and wet root rot, *Ascochyta* blight, *Botrytis* grey mould, stem rot	Drought, heat, cold, fog/frost, salinity, excess vegetative growth, poor partitioning of photosynthates
Eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal, Assam	*Fusarium* wilt, dry and wet root rot, collar rot, *Botrytis* grey mould	Drought, temperature extremities, fog, salinity
Gujarat, Maharashtra, Madhya Pradesh, Chhattisgarh, part of Rajasthan	*Fusarium* wilt, dry root rot, collar rot, stunt	Drought, heat, salinity, frost in parts of Madhya Pradesh, less biomass accumulation (short growing period)
Andhra Pradesh, Karnataka, Tamil Nadu	*Fusarium* wilt, dry root rot, collar rot, stunt	Drought, heat, less biomass accumulation (short growing period)

2.4.1 Biotic Stresses

2.4.1.1 *Fusarium* Wilt

In 32 countries across 6 continents in the world, chickpea wilt (Nene et al. 1991; Singh and Sharma 2002) was reported as a major problem causing losses varying from 10 to 90% (Jimenez-Diaz et al. 1989; Singh and Reddy 1991). Chickpea genotypes vary in the progress of initial symptoms of wilt, indicating different degrees of resistance controlled by a few major genes. Such individual genes that are part of oligogenic resistance mechanism delay the onset of disease symptoms leading to late wilting. Resistance has been reported against *Fusarium* wilt in the indigenous chickpea germplasm (Singh et al. 2012). Reliable and efficient screening methods have been established for evaluating a large number of genotypes under field conditions at several AICRP centres.

2.4.1.2 *Ascochyta* Blight

It is the most important foliar disease of chickpea in many parts of the world including India. It is caused by *Ascochyta rabiei* resulting in yield losses ranging from 10% to 100% (Nene and Reddy 1987; Singh 1990). *Ascochyta rabiei* isolates have been classified into either a two- or three-pathotype system (I, II and III) according to their levels of virulence (Udupa et al. 1998; Chen et al. 2004; Jayakumar et al. 2005). Under new breeding approach, plant breeders have shifted to gene pyramiding in elite lines instead of incorporating vertical resistance. An alternative strategy to deploy different lines possessing resistance against different races of the

pathogen prevalent in different regions can also be effective in order to minimize yield losses caused by *Ascochyta* blight.

2.4.1.3 *Botrytis* Grey Mould

It is the second major foliar disease of chickpea prevalent in 15 countries including India, Bangladesh, Nepal, Pakistan, Australia, Argentina, Myanmar, Canada, Columbia, Hungary, Mexico, Spain, Turkey, the USA and Vietnam. Earlier there was no reliable source known for resistance to BGM in India (Singh and Reddy 1991), but derivative lines from the inter-specific crosses of *C. arietinum* and *C. pinnatifidum*, developed at PAU, Ludhiana, exhibited moderate to high level of genetic resistance against BGM (Kaur et al. 2013) and can be incorporated into elite lines to develop high-yielding chickpea cultivars with durable resistance.

2.4.1.4 Pod Borer

Pod borer (*Helicoverpa armigera*) is the major insect pest infesting chickpea crop, predominantly causing damages across Asia, Africa, Australia and some other chickpea-growing regions. Being a polyphagous insect, pod borer is known to cause damage to more than 182 plant species. The development of cultivars resistant or tolerant to *H. armigera* could be integrated in the pest management strategy particularly in the developing countries (Fitt 1989; Sharma and Ortiz 2002). More than 14,000 chickpea germplasm accessions screened under field conditions at ICRISAT for resistance towards *H. armigera* (Lateef and Sachan 1990) led to the identification and release of moderately resistant/tolerant chickpea cultivars (Gowda et al. 1983; Lateef 1985; Lateef and Pimbert 1990). Still complete resistance against pod borer is far from reach, as 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).

2.4.1.5 Bruchids

Significant level of storage losses occurs in the Mediterranean region and in India by storage pest bruchids (*Callosobruchus chinensis*) where infestation levels approach 13% (Mookherjee et al. 1970; Dias and Yadav 1988) to total loss (Weigand and Tahhan 1990). Till date there is no report of resistance in the cultivated chickpea, though wild chickpea accessions have shown some 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).

2.4.1.6 Weeds

In addition to other biotic factors, seasonal weeds associated with chickpea crop such as *Phalaris minor* (L. Retz), *Avena fatua*, *Lolium temulentun* (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), *Cirsium arvense* (L. Scop), *Cyperus rotundus* (L), *Cynodon dactylon* (L. *Pers*) etc. are posing serious threat to chickpea productivity. It is specifically observed to be major problem of concern during winter rains when the weeds become major yield-limiting factor. Farm labour days are becoming expensive gradually; thus there is a need of herbicide-tolerant varieties (Sandhu et al. 2010; Gaur et al. 2012a). Systematic screening of reference set and elite breeding lines exhibited large genetic variations against post-emergence herbicide (imazethapyr) tolerance in chickpea (Gaur et al. 2013a; Chaturvedi et al. 2014a; Gupta et al. 2018). These have paved a way to develop post-emergence herbicide-tolerant varieties of chickpea.

2.4.2 Abiotic Stresses

2.4.2.1 Drought

Drought is the most important abiotic stress globally, contributing immensely to the yield losses in chickpea. Generally, it is terminal drought that has an adverse effect on the crop productivity (Khanna-Chopra and Sinha 1987). In order to counter drought stress, cultivation of early maturing cultivars for areas frequently affected by drought was found promising, as it would help in judicious utilization of the available soil moisture efficiently, thereby leading to relatively higher yields. In addition, root traits have gained more importance in recent years as genotypes with longer root systems have revealed better drought tolerance by extracting moisture from deeper soil regimes. Apart from this, wild *Cicer* species have been screened, and a few accessions of *C. pinnatifidum* and *C. reticulatum* were found to be resistant against drought (Toker et al. 2007). In the case of cultivated chickpea, ICC 4958 has been used extensively as a potential donor for drought tolerance. Chickpea introgression lines with improved drought tolerance (ICC 4958, used as donor) were found promising in India and Kenya (Gaur et al. 2012a). However, the introgression lines with improved root traits showed high G x E interaction when tested at several locations in central and southern India.

2.4.2.2 Heat Stress

Chickpea is adapted to cool climatic conditions. In the scenario of climate change and changing cropping pattern, the crop is being exposed to high temperature (>35 °C) during the reproductive phase, causing severe yield penalty. Reproductive

period was found to be sensitive to heat stress conditions; if temperature rises above the threshold level, it would affect the pod formation and seed set causing reduced grain yield (Summerfield et al. 1984; Wery et al. 1993; Wang et al. 2006; Basu et al. 2009; Kumar et al. 2013). Moreover, high temperature has been observed to cause adverse effects on seed germination, respiration, membrane stability, photosynthesis, hormone level, nutrient absorption, protoplasmic movement, quality of seeds, fruit maturation, fertilization, materials transport, withering, burning of lower leaves, desiccation of poorly developed plants, stunting flower and pod abortion, reduced root nodulation, nitrogen fixation and seed yield (Chen et al. 1982; Saxena et al. 1988; Kurdali 1996; Wahid and Close 2007). Although chickpea is 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), acute heat stress could lead to high-yield losses and crop failure (Devasirvatham et al. 2012). Large genetic variations have been observed for heat tolerance in chickpea as revealed in multi-location screening of reference set against heat stress in India (Krishnamurthy et al. 2010). A field screening technique for heat tolerance has been standardized, and several sources of heat tolerance were identified (Gaur et al. 2014). A heat-tolerant variety JG 14 was released in India and found promising under both normal and late planting conditions in central, southern and eastern states.

2.4.2.3 Cold Stress

Typically chickpea grown during winter season is more productive than the traditionally grown spring season in the Mediterranean region (Singh and Hawtin 1979). This is particularly due to long growing season and better moisture availability. But winter season crop experiences problems such as flower drop and pod abortion leading to major yield loss as soon as mean day temperature falls below 15 °C (Savithri et al. 1980; Srinivasan et al. 1999; Clarke and Siddique 2004; Nayyar et al. 2005). Studies in Australia have highlighted the complete lack of cold/chilling tolerance in the domesticated gene pool and demonstrated greater tolerance potential in the annual wild relatives (Berger and Turner 2007; Berger et al. 2012). Preliminary studies in Australia demonstrating that the wild relatives that readily cross with chickpea (*C. reticulatum*, *C. echinospermum*) appear to have considerably more vegetative cold and reproductive chilling tolerance than domestic chickpea. More efforts are needed for identifying novel sources of cold tolerance and to develop the breeding population for identifying cold-tolerant genotypes.

2.5 Genomic and Transcriptomic Resources

Genomic studies aim towards the direction of gene/QTL mapping and identification of metabolic pathways affecting chickpea productivity which accelerates the genetic advance under selection and enhanced genetic gain. Thus, several international

platforms have been initiated for developing and further exploiting the chickpea genomic resources in genomics-assisted breeding. Initially isozymes as biochemical markers have been utilized in chickpea. Isozymes catalysed the same chemical reaction but differ in their electrophoretic mobility. Segregation pattern of isozyme markers was reported in the F_2 generation developed from inter-specific crosses of *Cicer arietinum* with *C. reticulatum* and *C. echinospermum* (Gaur and Slinkard 1990a; b). Based on the isozyme profiling of annual and perennial chickpea accessions, the *Cicer* species were classified into four categories (Kazan and Muehlbauer 1991) and were confirmed in some later studies (Ahmad et al. 1992; Labdi et al. 1996; Tayyar and Waines 1996).

After the development of molecular markers, RFLP markers have been extensively exploited in *kabuli* and *desi* type of chickpea for diversity analysis (Udupa et al. 1993), for identification of centre of genetic diversity (Serret et al. 1997) and for construction of linkage map (Simon and Muehlbauer 1997). The RAPD markers have also been employed for polymorphism assessment (Banerjee et al. 1999), trait mapping (Tullu et al. 1998) and genetic diversity analysis and to identify the phylogenetic relationship amongst accessions (Sant et al. 1999; Iruela et al. 2002; Singh et al. 2003). With the discovery of AFLP markers, they have also been used in genetic diversity analysis, to find out the phylogenetic relationship of germplasm lines (Nguyen et al. 2004; Shan et al. 2005; Talebi et al. 2008) and linkage map construction (Winter et al. 2000). Microsatellite markers are the highly efficient markers in chickpea which were developed from sequencing of probe genomic libraries (Winter et al. 1999; Hüttel et al. 1999), microsatellite-enriched libraries and bacterial artificial chromosome (BAC) clones (Nayak et al. 2010; Thudi et al. 2011). These microsatellite markers have also been utilized in construction of linkage maps and gene/QTL mapping. DArT (Diversity Arrays Technology) markers are also used in chickpea excessively for diversity analysis and constructing linkage maps. ICRISAT has developed the DArT arrays in chickpea with 15,360 clones in association with DArT Pty Ltd. (Thudi et al. 2011). Similar trend of narrow genetic diversity has been observed using DArT markers in gene pool of cultivated *Cicer* species than in wild *Cicer* species (Roorkiwal et al. 2014b).

Single-nucleotide polymorphism (SNP) markers are the highly efficient molecular markers which are profoundly used in chickpea. Facilities for analysis of genetic diversity, fine mapping of genes, genome-wide association studies, genomic selection and evolutionary studies are being provided by SNP genotyping platforms. Ample amount of sequencing data has been generated with the advancement of next-generation sequencing (NGS) technologies. By using Sanger sequencing technology, over 20,000 expressed sequence tags (ESTs) have been developed from drought and salinity stress-challenged tissues at specific stage in chickpea (Varshney et al. 2009b). Further, extra sequencing data from more than 20 tissues representing different varietal developmental stages were generated (Hiremath et al. 2011). By analysing the pooled sequencing data with the help of NGS transcripts and Sanger ESTs, first transcript assembly has been generated with 103,215 tentative unique sequences (TUSs), which further employed for identification of thousands of SNPs. Several thousand of SNPs were also identified through several sequencing platforms like Illumina sequencing platform (Varshney et al. 2013b), allele-specific

sequencing technique (Gujaria et al. 2011; Roorkiwal et al. 2014a) and 454 transcriptome sequencing platform (Deokar et al. 2014). A high-resolution linkage map of genomic and transcriptomic SNPs has been constructed containing 6698 SNPs which were mapped on 8 linkage groups having size of 1083.93 cM from an interspecific RIL mapping population (Gaur et al. 2015). A high-throughput SNP genotyping platform (Axiom *Cicer* SNP Array) has been developed and used for constructing high-density linkage maps by using two RIL mapping populations (Roorkiwal et al. 2017). A total of 13,679 SNPs spanning 1033.67 cM and 7769 SNPs spanning 1076.35 cM have been used for constructing linkage map.

Sequence-based trait mapping has been successfully enabled due to advancement of NGS technologies as it is time- and cost-effective. Several techniques such as skim sequencing, genotyping by sequencing (GBS) and whole genome re-sequencing provide large-scale marker data useful for high-resolution sequence-based trait mapping (Pandey et al. 2016). GBS approach has been employed for refinement of *QTL-hotspot* (Jaganathan et al. 2015) identified from an intra-specific RIL mapping population developed from the cross between ICC 4958 and ICC 1882, whereas skim sequencing approach has identified 84,963 SNPs by employing the same parental cross, out of which 76.01% were distributed over eight pseudo-molecules (Kale et al. 2015). Through integrated reference genome-based GBS approach, >40,000 genome-wide SNPs (Kujur et al. 2015) and through de novo-based GBS approach >80,000 genome-wide SNPs have been identified (Bajaj et al. 2015) using 93 wild and cultivated chickpea accessions. These SNP markers are being used in genomics-assisted breeding programmes at large scale. Various SNP genotyping platforms such as KASP markers (Hiremath et al. 2012) and VeraCode and GoldenGate (Roorkiwal et al. 2013) were generated for exploiting the genome-wide large-scale SNP marker information in chickpea improvement breeding programmes.

The gene/QTLs can also be identified through transcriptomics approach. Transcriptome profiling of various biotic and abiotic stresses challenged specific plant tissues, and expressed sequence tags (ESTs) have played an instrumental role for development of functional markers which can be further utilized in chickpea improvement breeding programmes. Several functional markers have been developed from ESTs for various biotic and abiotic stresses in chickpea (Buhariwalla et al. 2005). A total of 177 new EST-SSRs functional markers have been developed from salinity and drought stress-responsive ESTs (Varshney et al. 2009b). Development of NGS technologies has played a major role in large-scale transcriptome and genome sequencing. Transcriptome sequencing has led to ample amount of information about the gene candidate in chickpea. A transcriptome assembly has been constructed by using a number of 103,215 tentative unique sequences (TUSs) based on several FLX/454 reads and Sanger ESTs (Hiremath et al. 2011). An array of 34,760 contigs of transcriptome sequence representing ~35.5 Mb through Illumina and FLX/454 sequencing and 53,409 contigs of transcriptome sequence which represents ~28 Mb through Illumina sequencing were assembled (Garg et al. 2011a, b). A hybrid assembly has also been constructed using 46,369 contigs of transcriptome sequence from different developmental stages of plant tissues exposed to various stresses (Kudapa et al. 2014).

2.6 Linkage Maps, Physical Maps and Functional Maps

In the process of various genomics-assisted breeding approaches, discovery of the specific markers tightly linked with gene/QTL of interest appears as the initial step (Kumar and van Rheenen 2000). Before the identification of tightly linked markers, constructions of linkage/genetic maps are prerequisite which allowed the gene mapping and gene tagging in molecular breeding as well as characterization of the specific genomic regions and deciphering the gene action involved in phenotypic expression of these traits of interest (Tanksley 1993). The closely linked markers thus obtained would serve as final genomic sequence for positional cloning of the respective trait of interest (Varshney et al. 2009a). Construction of linkage maps in chickpea significantly developed from morphological markers to sequence-based markers like SNPs, InDels and DArT (Roorkiwal et al. 2018; Kushwah et al. 2020). Adopting the next-generation sequencing platforms enabled the large-scale genome-wide SNP discovery which leads to construction of high-resolution saturated linkage maps in chickpea (Deokar et al. 2014; Jaganathan et al. 2015; Kujur et al. 2015) which facilitates fine mapping of genes/QTLs as well as positional cloning of these genes/QTLs to know the underlying candidate genes involved in phenotypic expression of the trait of interest.

Utilization of large-scale transcriptomic resources as EST-SSRs and EST-SNPs helps to construct transcript maps in chickpea. These transcript maps have immense target-specific gene/QTL mapping, positional cloning and identifying the candidate genes responsible for economically important traits in chickpea. First large-scale transcript map employing EST-SSRs, EST-SNPs and intron spanning region has been developed in an inter-specific mapping population of chickpea spanning about 767 cM of the total genome size with inter-marker distance of 2.5 cM (Gujaria et al. 2011). Another transcript map has been constructed with a different set of EST-derived genic molecular markers spanning 1498 cM of the total genome size having inter-marker distance of 3.7 cM by using the same inter-specific mapping population of chickpea (Choudhary et al. 2012). Further, by using TOGs (tentative orthologous genes)-SNPs, a highly saturated large-scale transcript map was constructed spanning about 788.6 cM of the total genome size (Hiremath et al. 2012). Now, this high-resolution inter-specific transcript map was exploited to develop the first draft version of whole genome sequences of chickpea variety CDC Frontier (Varshney et al. 2013a). Further improvement has been done for construction of highly saturated inter-specific genetic/linkage map spanning map length of 949 cM of the total genome size using SSRs and SNPs markers developed from various transcription factors of specific candidate genes (Saxena et al. 2014). Now, these SSRs and SNPs markers derived from transcription factors of specific candidate genes responsible for phenotypic expression of targeted traits can play an instrumental role in genomics-assisted chickpea improvement breeding programmes.

2.7 Trait Mapping for Various Biotic and Abiotic Stress Tolerance and Yield-Related Traits

The exploitation of DNA-based genetic markers including sequence-based molecular markers tightly linked to trait of interest helps to define the genotypic constitution of crop plants as well as to overcome the confounding effects of genotype x environment interactions, problems of stage dependency and several operational difficulties. Mapping of several economically important traits responsible for various abiotic and biotic stress tolerance and yield improvement traits paves the way for efficient exploitation of molecular breeding in chickpea. Application of these molecular markers tightly linked to complex traits has been successfully applied in various genomics-assisted breeding approaches. Recently, genome-wide association study (GWAS) approach is being significantly utilized for identification of several sequence-based molecular markers related to yield and yield-related traits against various abiotic and biotic stress conditions.

A genomic region on LG4 has been identified as *QTL-hotspot* for several major QTLs responsible for drought stress tolerance which explains up to 58% of phenotypic expression for various root-related traits under rainfed conditions, and the estimated size of this *QTL-hotspot* was 29 cM on the linkage/genetic map and 7.74 Mb on the physical map of chickpea genome (Varshney et al. 2014a). Now, this *QTL-hotspot* genomic region was further refined by genotyping-by-sequencing (GBS) approach to 14 cM on genetic map from 29 cM as well as ~4 Mb on the physical map from 7.74 Mb of chickpea genome and incorporated 49 new SNPs in this genomic region (Jaganathan et al. 2015). Now this genomic region was again refined by using a combination of GWAS-based gene enrichment analysis of skim sequenced data approach and sliding window-based bin mapping approach, and this *QTL-hotspot* was split into two sub-genomic regions, i.e., *QTL-hotspot-a* of size of 139.22 Kb and *QTL-hotspot-b* of size of 153.36 Kb (Kale et al. 2015).

A comprehensive GWAS approach using whole genome sequencing and candidate gene-based approach has been exploited for discovery of 312 molecular markers responsible for drought and heat stress tolerance-related traits in chickpea (Thudi et al. 2014). Likewise, a total of 25 putative candidate genes harbouring two genomic regions having four QTLs were identified on LG5 and LG6 which were responsible for heat tolerance-related traits in chickpea (Paul et al. 2018). Several major QTLs responsible for salinity tolerance-related traits have also been identified in chickpea. Several molecular markers closely associated for salinity tolerance-related traits have been identified on LG1, LG2, LG3 and LG7 using RIL mapping population developed from the cross between ICC6263 (salinity sensitive) and ICC1431 (salinity tolerance) under salinity conditions (Samineni 2010). In another study, major QTLs for yield and yield-related traits responsible for salinity tolerance were identified on LG3 and LG6 by using RIL mapping population derived from a cross between ICCV2 (salinity sensitive) and JG62 (salinity tolerant) under salinity conditions (Vadez et al. 2012). Further, a total of 46 major QTLs including 19 QTLs for several phonological traits and 27 QTLs for yield and yield-related traits

responsible for salinity stress tolerance have been identified using a RIL mapping population developed from the cross between ICCV 2 (salinity sensitive) and JG 11 (salinity tolerant) which was clustered on LG5, LG7 and LG8 (Pushpavalli et al. 2015).

Major QTLs responsible for *Ascochyta* blight (AB) resistance were found to be located on LG2, LG3, LG4 and LG8 on the chickpea linkage map and validated the min different genetic backgrounds of chickpea by utilizing different mapping populations (Kottapalli et al. 2009; Millán et al. 2013). Another major QTL for *Ascochyta* blight resistance has been mapped which was located on LG6 on the chickpea genetic map using the CDC Frontier as a source of AB resistance (Anbessa et al. 2009). In another study, one major QTL for seedling resistance and one minor QTL for adult plant resistance against *Ascochyta* blight were identified using RIL mapping population (Garg et al. 2018). Recently, Deokar et al. (2019) identified a total of 11 major QTLs and 6 major QTLs responsible for AB resistance on LG1, LG2, LG4, LG6 and LG7 using two different RIL mapping populations respectively through NGS-based bulked segregant analysis (BSA) approach.

The first gene mapped for *Fusarium* wilt resistance was H1 (*foc* 1) providing resistance to race 1 which was tagged by the RAPD markers (Mayer et al. 1997). Another group have also found other RAPD markers (UBC-170550, CS-27700) closely linked with *Fusarium* wilt resistance gene to race 4 (Tullu et al. 1999). In another study, ISSR markers (UBC-855500 and CS-27700) have been utilized for tagging of *Fusarium* wilt resistance gene to race 4 (Ratnaparkhe et al. 1998). Several SSR markers, like TR59 and $OPJ20_{600}$ which were tightly linked to the *Fusarium* wilt resistance gene *foc* 0 (Cobos et al. 2005), TA110 and H3A12 linked to *Fusarium* wilt resistance gene *foc* 1, H3A12 and TA96 linked to *Fusarium* wilt resistance gene *foc* 2 (Gowda et al. 2009), TA96 and TA194 linked to *Fusarium* wilt resistance gene *foc* 3 (Sharma et al. 2004; Gowda et al. 2009), TA96 and CS27 linked to *Fusarium* wilt resistance gene *foc* 4 (Winter et al. 2000; Sharma et al. 2004) and TA59 and TA96 linked to *Fusarium* wilt resistance gene *foc* 5 (Sharma et al. 2005; Cobos et al. 2009), have been successfully mapped which are responsible for providing resistance against *Fusarium* race 0, 1, 2, 3, 4 and 5, respectively. Recently, a total of five major QTLs tightly linked to *Fusarium* wilt resistance gene were detected which were located on LG2, LG4 and LG6 providing resistance against race 1 of *Fusarium* wilt (Garg et al. 2018).

2.8 Genomics-Assisted Breeding (GAB) for Trait Improvement

GAB involves the integration of genomic tools for enhancing selection efficiency and accuracy in the breeding process. Major strategies which come under the category of GAB are genomics, proteomics and transcriptomics for discovery of tightly linked molecular markers associated with economically important traits that help in

prediction of phenotype from the genotype. Advancement of NGS technologies for high-throughput genotyping has made possible to develop large-scale genome-wide markers. Marker-assisted backcrossing (MABC) approach is helpful for requisite gene pyramiding of several QTLs together in a specific genetic background and generally used for significant improvement of breeding traits governed by major genes/QTLs. Although several economically important traits are polygenic in nature, MABC has limited applications. Thus for improvement of polygenic characters, marker-assisted recurrent selection (MARS) has been considered as a better option. Genome-wide selection or genomic selection (GS) approach has emerged as a powerful approach for selection of desirable progenies obtained from the favourable crosses (Jannink et al. 2010). Advanced backcross QTL (AB-QTL) approach has been exploited for simultaneous identification as well as transfer of desirable alleles from wild species or wild relatives into elite ones for the development of improved lines as the wild species accumulates several superior alleles which are responsible for tolerance to several biotic and abiotic stresses (Tanksley and Nelson 1996). AB-QTL approach has been efficiently utilized for introgression of productivity enhancing traits and resistance traits to diseases from *C. reticulatum* in chickpea (Singh et al. 2005).

AB resistance in chickpea has recessive phenotype in terms of genetics which shows complex inheritance pattern. MABC approach has been successfully exploited for introgression QTLs responsible for double podding and QTLs responsible for resistance to AB simultaneously in elite chickpea cultivars through continuous backcrossing of donors moderately resistant to AB and adapted cultivars (Tar’an et al. 2013). A stepwise MABC approach has been exploited by Varshney et al. (2014b) for the development of *Fusarium* wilt (FW) and AB-resistant lines by incorporating two QTLs for AB and *foc* 1 locus for FW into an elite chickpea cultivar, C 214. Three rounds of backcrosses and three rounds of selfing (Varshney et al. 2014b) result into the development of three resistant lines for FW and seven resistant lines for AB. This approach has also been utilized for introgression of resistance against two races (*foc* 2 and *foc* 4) individually and gene pyramiding of resistance to two races (*foc* 1 and *foc* 3) for FW and two different QTLs providing resistance to AB in chickpea (Varshney et al. 2014b). Recently, five germplasm lines showing resistance against fw race *foc 2* have been introgressed in the genetic background of Pusa 256, an elite chickpea cultivar, using SSR markers (Pratap et al. 2017). Several efforts are in pipeline for introgression of resistance to FW and AB in several highly promising cultivars in various research institutes like ICAR-Indian Agricultural Research Institute (New Delhi), Punjab Agricultural University (Ludhiana) and ICAR-Indian Institute of Pulse Research (Kanpur). Apart from this, introgression of genomic regions has also been performed for yield. Similarly, for enhancing drought tolerance in chickpea, QTLs/genomic regions on LG04 labelled as *QTL-hotspot* (up to 58% phenotypic variability) for root-related traits were introgressed into JG 11 (Varshney et al. 2013b). A set of 20 BC_3F_4 lines was evaluated at three locations in India, and several location-specific lines giving significantly higher yield than JG 11 were identified (Gaur et al. 2013b). The introgression lines showed high level of GxE interaction when evaluated at different locations in India.

Efficiency of MARS depends on the total genetic gain achieved by selection accuracy, marker-trait associations, selection efficiency and distribution of desirable alleles across the parents. In chickpea, MARS has been exploited for accumulation of desirable set of alleles against drought stress by using crosses ICCV 04112 × ICCV 93954 and ICCV 05107 × ICCV 94954 (Samineni et al. 2017). The crosses JG 11 x ICCV 04112 and JG 130 x ICCV 05107 were carried out in chickpea to combine the desirable alleles for QTLs governing yield using the MARS approach. A total of 188 F_3 plants each from two crosses were genotyped using SSR markers, and $F_{3:5}$ progenies were evaluated at multi-locations. Few major and several minor QTLs relating to yield and yield component traits have been identified. On the basis of QTL information on several yield and yield-related parameters in different F_5 progenies, four lines from the cross JG 11 x ICCV 04112 and three lines from the cross JG 130 x ICCV 05107 were selected having several combinations of favourable alleles for recombination cycle. Now these shortlisted lines were subjected to further two recombination cycles, and F_1 plants having favourable alleles for yield and yield-related traits were identified from both the crosses, and those having favourable alleles in homozygous condition were grown. Now these shortlisted homozygous F_1 plants were advanced to F_4 generation for further evaluation. In this way, numerous recombination cycles in MARS approach help in accumulation of the frequency of favourable alleles related with economically important traits.

GS approach can be a deployable approach for chickpea yield improvement in near future due to availability of precise phenotyping of several chickpea breeding lines, presence of large linkage disequilibrium (LD) blocks in chickpea breeding populations as well as the availability of large-scale genome-wide marker genotyping system like DArT and SNP markers. Moving in this direction, ICRISAT has started efforts for exploitation of this approach in chickpea breeding programme by using a set of 320 elite chickpea lines which were genotyped by DArT markers. Precise phenotyping has been carried out at two locations, i.e., Patancheru and New Delhi, for yield and yield-related traits. Six different statistical GS models have been employed by utilizing phenotyping and genotyping data which provides promising results with higher prediction accuracies (up to 0.91) for yield and yield-related traits (Roorkiwal et al. 2016). Based on the lessons learned from the study, a new set of training populations is being developed separately for *desi* and *kabuli* types for achieving higher prediction accuracy for yield and yield-related traits. The selected training populations include promising breeding lines and well-characterized germplasm lines that have been used in crossing programme in the past 10 years for developing high-yielding chickpea varieties. Higher prediction accuracies can be obtained through inclusion of G × E effects by GS approach considering multiple variables simultaneously in chickpea breeding programmes (Roorkiwal et al. 2018). Pre-breeding programmes in GS models will be highly favourable since that will help in screening the accessions for subsequent introgression (Crossa et al. 2017). Varshney et al. (2018) has been proposed a tentative outline of sequence-based breeding using GS approach. According to this, all possible parental lines of a specific breeding programme have to be sequenced at higher depth. These founder genotypes can be sequenced to develop GWAS approach or to

develop HapMap that can be further used for selection of suitable parental combinations having higher frequency of favourable alleles. By using higher number of lines, large number of crosses has to be made followed by early generation selection with existing ten SNP panels. Now GS approach can be performed on selected lines from such crosses by using the training model developed from the germplasm set representing the segregating populations. Best genotyping platform for GS approach can fix SNP array, although this may not be feasible for large-scale breeding programmes. Thus, segregating populations (F_6/F_7 generations) can be sequenced at lower coverage using skim sequencing or 384-plex-based genotyping platform. High-throughput genotyping data of parental lines and other available germplasm lines can be used for developing practical haplotype graph (PHG) which will help in identification of SNP markers. By using sequence-based approaches, these SNP markers can be evaluated using rhAmp-SNP genotyping technology or DArT-seq SNP genotyping technology. By this way, GS approach-based breeding programme can be exploited using these segregating populations, and elite lines and lines having higher genomic estimated breeding values (GEBVs) can be selected.

2.9 Rapid Generation Advancement/Speed Breeding

Global food security for ever-growing human population necessitates accelerated breeding and research programmes so as to meet future food demands. Pace between time required and development of improved varieties has to be optimum to meet breeding challenges. Longer generation time required by crops slows down the progression towards fast-track research and development of improved varieties. Rapid generation advancement (RGA) or speed breeding methods have been used in chickpea for advancing three generations per year under field and greenhouse conditions (Gaur et al. 2007). In 2018, a group of researchers were able to reduce generation cycle to 5.6 per year in wheat, 5.3 in barley, 3.7 in canola and 4.5 in chickpea under specially modified glasshouses with sodium vapour lamps (Watson et al. 2018). These protocols involve a sequence of steps such as drying of seeds (5 days at 35 ^{O}C), imbibitions of seeds (1 day) and chilling treatment (4 ^{O}C) to advance a single generation in chickpea. Further in 2019, a new cost-effective and less cumbersome method of RGA/speed breeding has been proposed in chickpea by manipulating photoperiod and temperature (Samineni et al. 2019). The study was conducted over 2 years using six cultivated chickpea varieties belonging to early, medium and late maturity groups. Results showed that the mean total number of generations produced per year was, respectively, 7, 6.2, and 6 in early-, medium-, and late-maturing genotypes (Samineni et al. 2019). Further, RGA will fit well with the GS model of breeding where no phenotyping is required to select candidate genotypes in the early generation. Hence, RGA technology has huge scope to implement new breeding tools to improve the efficiency and accuracy of selection in developing improved varieties.

2.10 Future Research Priorities

Chickpea being a winter season (*rabi*) crop does not cope well to warm climate. With increasing temperature and associated weather fluctuations due to climate change and shift in major chickpea cultivable area from cooler regions of northern India to warmer region of central and southern India, imparting drought and heat stress resistance in chickpea has become indispensable. Developing early to extra-early varieties of chickpea with drought and heat tolerance is an important objective of AICRP on chickpea. Genomic resources were found promising for enhancing the efficiency of selection in breeding programmes and identification of genomic regions for several complex traits. Utilizing the molecular markers, researchers have developed wilt-resistant (Super Annegeri 1) and drought-tolerant (Pusa 1088) chickpea varieties under ICAR-ICRISAT collaboration. Currently, the crop improvement focuses on using integrated breeding approaches for the accelerated development of improved breeding materials with diverse desired traits such as high yield potential, improved resistance/ tolerance to biotic and abiotic stresses, resilience to climate change, labour saving, market-preferred grain traits and improved quality of produce; deployment of genomic selection for accelerate genetic gains; bioinformatics; digital data capture, data management and breeding management system for modernization of breeding programmes (Chaturvedi et al. 2014b). The major focus areas are presented below.

2.10.1 Germplasm Characterization

Evaluation of wild species had resulted in identification of genes for resistance to several biotic stresses such as *Botrytis* grey mould (*C. judaicum* and *C. pinnatifidum*), *Ascochyta* blight (*C. bijugum*, *C. pinnatifidum* and *C. yamashitae*) and *Fusarium* wilt (*C. bijugum*) (Infantino et al. 1996; Kaur et al. 2013). Two wild species, *C. reticulatum* and *C. echinospermum*, are cross compatible with the cultivated *C. arietinum* and are reported to be resistant to several pests (cyst nematodes, leaf minor and bruchids) and diseases (*Fusarium* wilt, *Ascochyta* blight and phytophthora) and tolerant to cold (Berger et al. 2012). The earlier studies indicated that *C. pinnatifidum*, a valuable source for several biotic and abiotic stresses, can be crossed successfully with cultivated chickpea (Fig. 2.4) for the transfer of resistance to *Botrytis* grey mould and *Ascochyta* blight (Sandhu et al. 2005; Kaur et al. 2013). The ICRISAT, Patancheru, has developed core/ mini-core sets of chickpea germplasm. In recent past, more than 14,000 accessions of chickpea have been evaluated and characterized through ICAR-IIPR and NBPGR collaboration at Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri, to add value. Recently, three accessions (ILWC 115, ILWC 21 and EC 556270) of *C. reticulatum* have been identified as heat tolerant and are being utilized in

hybridization (IIPR Annual Report 2014–15). Currently, new diseases such as dry root rot and collar rot became prominent in several chickpea-growing areas where high level of resistance was not found in cultivated species. Efforts should be made for screening of germplasm for these important diseases to develop resistant varieties.

2.10.2 Trait Identification and Germplasm Enhancement

To reduce vulnerability against environmental fluctuations and biotic stresses, there is need to broaden the genetic base of future chickpea varieties through pre-breeding efforts. A large number of diverse germplasm lines, primitive landraces and accessions of wild *Cicer* species are available in gene banks at NBPGR, ICRISAT and ICARDA which are being supplied from time to time to breeders for use in breeding programmes.

Fig. 2.4 An inter-specific F_1 hybrid between cultivated chickpea and wild *Cicer pinnatifidum* with prostrate growth habit

2.10.3 Regaining Chickpea Area in Northern India

Tailoring suitable plant types possessing lodging tolerance, *Ascochyta* blight and pod borer resistance and response to high inputs are likely to enhance chickpea yields in northern India. Further, combining tall and erect growth habit will help in reducing humidity inside crop canopy facilitating better solar light interception. The erectness of the varieties will make them suitable for mechanical harvesting.

2.10.4 Varieties for Vegetable Purpose

Appropriate strategies are required to be adopted to develop high-yielding chickpea (*desi*) varieties for green immature grain for vegetable purpose as it has high demand in the market (Sandhu et al. 2007). This will also help in expanding chickpea cultivation in many parts of the country including Punjab, Haryana, western Uttar Pradesh, Jharkhand, Odisha and parts of West Bengal ensuring supply of protein through this nutritious pulse. More efforts are required to pyramid genes responsible for earliness in chickpea so that super early varieties can be developed (Gaur et al. 2015). In addition of earliness, there is need to incorporate cold tolerance, greenness in seeds at the time of physiological maturity, large/medium large seed size and resistance to diseases in high-yielding background for mid-October sowing so that green pods can be harvested by end of December. However, besides all mentioned traits, high temperature tolerance will be required for staggered sowing (north India) or delayed sowing so that green grains can be supplied for longer duration as per demand. Development of super early maturing varieties will help in minimizing losses due to gram pod borer and other stresses as well.

2.10.5 Kabuli Chickpea Varieties for Export and Domestic Consumption

Extra-large-seeded (>50 g 100-seed weight) *kabuli* genotypes with high resistance to *Fusarium* wilt have been identified (Gaur et al. 2006), and several varieties (Phule G 0517, MNK 1, JGK 5, PKV 4–1) have been developed for cultivation in central and southern India. The systematic quality seed production of extra-large-seeded varieties has provided much needed stability in productivity. There is a huge demand for high-yielding extra-large *kabuli* varieties having semi-erect/erect growth habit along with combined resistance to soil-borne diseases in central and southern India. Similarly, for northern India, ample scope exists to regain area under chickpea through development and popularization of extra-large seed varieties. Further, large-seeded *kabuli* types fetch high premium to farmers in domestic and international markets; therefore efforts should continue to improve large-seeded varieties of *kabuli* chickpea.

2.10.6 Machine-Harvestable Chickpea for Reducing Cost of Cultivation

Mechanization of farm operations is essential for improving efficiency of agriculture and reducing cost of cultivation. In many countries such as Australia, Canada, the USA, Turkey, Syria, etc., chickpea harvesting is fully mechanized. In India, all pulse crops are largely harvested by hand because the available cultivars are bushy types which are difficult to harvest using machines. Manual harvesting has become an expensive field operation due to labour scarcity and increasing labour costs; hence Indian farmers are increasingly demanding varieties suitable for machine harvesting. Since chickpea is grown over ~10 million ha area, development of varieties amenable to mechanical harvesting will attract farmers for chickpea cultivation as cost of cultivation will also get reduced with the adoption of machine harvesting. The traditional cultivars are generally having semi-spreading growth habit, and pods at lower nodes are close to the ground, thus not very much suitable for mechanical harvesting. Chickpea varieties possessing tall (>55 cm crop height) and erect/semi-erect growth (>60^0 branch angle from soil surface) and at least with 25 cm ground clearance (no pods up to 25 cm crop height) are needed for mechanical harvesting. Such tall and erect varieties can very well be grown with higher population density in central and southern India ensuring higher yields. In northern India, where fog and humidity are major limiting factors to sunlight, tall and erect plant type will have more solar light penetration which will help in minimizing humidity buildup in chickpea canopy ensuring minimum damage due to foliar diseases. The release of cultivars suited to mechanical harvesting will benefit farmers by reducing cost of cultivation and increasing net profit from cultivation of winter season pulse crops. Recently machine-harvestable varieties (NBeG 47, GBM 2, RVG 204, Phule Vikram, BG 3062) of chickpea have been released for cultivation in central and southern India.

2.10.7 Herbicide-Tolerant Varieties

Chickpea fields are infested by different types of seasonal weeds causing significant yield losses. At present there is no chickpea cultivar possessing tolerance to post-emergence herbicides, and the manual weeding is a major weed control strategy which is time-consuming and expensive. Multi-location testing of several germplasm lines identified large genetic variations for post-emergence herbicide (imazethapyr) tolerance in chickpea (Gaur et al. 2013a; Chaturvedi et al. 2014a). A good number of chickpea genotypes were screened at PAU, Ludhiana, against two post-emergence herbicides, imazethapyr and carfentrazone-ethyl, to identify tolerant genotypes. A large genetic variation was observed for tolerance against both the herbicides (Fig. 2.5). In general, genotypes showed more sensitivity to carfentrazone-ethyl at early growth stage, but at late growth stage, they showed more sensitivity to imazethapyr. Three genotypes, viz., GLK 10103, NDG 11-24 and GL 22044, were

Fig. 2.5 Genetic variation for tolerance to post-emergence herbicide carfentrazone-ethyl in chickpea

found to be tolerant to both the herbicides, imazethapyr and carfentrazone-ethyl, and can be used in the chickpea improvement programme (Gupta et al. 2018).

2.10.8 Varieties with Better Nutrient Acquisition Efficiency

Chickpea responds well to application of fertilizers though farmers seldom apply nutrients. Phosphorus (P) is required for proper growth and development of plants, and low phosphorus availability in soil affects nodulation adversely. It is also an established fact that phosphoric fertilizers applied in previous crop get fixed in soil and can be made available to next crop, if varieties with better P acquisition and use efficiency are developed. Development of chickpea varieties having better P acquisition efficiency and ability to grow well on P-deficient soils will ensure stable yields. Cultivation of P acquisition efficient (PAE) varieties in low-input production systems will help in reducing cost of cultivation by bringing down requirement of 'P', thus saving huge foreign currency as large amount of phosphoric fertilizers are imported from elsewhere. At ICAR-IIPR, a large number of germplasm lines and elite breeding lines were screened for PAE which revealed large genetic variations. There is need to have systematic research for identification of gene(s) or QTLs responsible for PAE and their subsequent transfer to develop better PAE chickpea varieties.

2.10.9 Nutritionally Rich Varieties

Large variations have been observed in seed protein content of chickpea opening doors to enhance protein content in future varieties, though trait is governed by multiple genes. The adoption of high-protein chickpea varieties will ensure higher order of availability of protein from per gram consumption of chickpea. Further, there is need to develop chickpea varieties with higher β-carotene (precursor of vitamin A) levels and micronutrient contents. Limited studies conducted so far on assessing genetic variability for nutritional quality traits in chickpea germplasm suggest large genetic variation for β-carotene (0.4–0.1 μg per g seed weight), Fe (35–150 ppm) and Zn (25–50 ppm). Thus, opportunities exist for developing varieties with enhanced contents of β-carotene, iron and zinc. The only anti-nutritional factor associated with chickpea is raffinose family of oligosaccharides (RFOs) which are responsible for causing flatulence on consumption. A recent study indicates wide range of RFOs (1.58 to 5.83 mmol/100 g seed) in chickpea germplasm. Thus, ample scope exists to develop chickpea varieties with higher contents of protein, β-carotene, iron and zinc and lower contents of RFOs.

In a preliminary study, 19 popular commercial cultivars of India were analysed for their Fe and Zn contents in four locations representing different agro-climatic zone of the country to study the genotypic (G) and genotype X environment (G X E) interactions on these two mineral micronutrients. In addition, distribution of phytic acid (PA), an important anti-nutrient that chelates and reduces the mineral bioavailability, was also analysed. Influence of other agronomic traits such as days to flowering (DF), plant height (PH) and 100 seed weight (SW) was also analysed on Fe and Zn content. Fe and Zn content showed positive correlation indicating a possibility of their co-selection in breeding. RSG44, JG315, Virat and Vihar had higher Fe (>70 ppm) and Zn (>40 ppm) at all locations. Such genotypes will be useful in breeding programmes for enhancing the mineral micronutrient content (Personal Communication Archana Joshi).

2.10.10 Integrated Breeding

Isozyme markers were used in developing the first linkage map of chickpea (Gaur and Slinkard 1990b) and establishing phylogenetic relationships amongst annual *Cicer* species (Kazan and Muehlbauer 1991; Ahmad et al. 1992). Recently, a large number of genomic resources have been developed for deployment to improve targeted traits. The year 2013 began by adding a milestone in chickpea genomics as the draft genome sequence of chickpea genome was reported jointly by the scientists working at ICRISAT and ICAR institutes (Varshney et al. 2013a). The information revealed by the draft genome sequence will further boost efforts on development of genomic resources and their applications in chickpea improvement. Integrated breeding approaches utilizing conventional and genomics would improve precision

and efficiency of selection in breeding efforts for developing cultivars better adapted to diverse growing environments (Gaur et al. 2012b; Varshney et al. 2013b). Considering the importance of accelerated breeding, ICAR-IIPR has established Regional Station Cum Off-season Nursery Centre at Dharwad (Karnataka) for rapid generation turnover to reduce time required to attain homozygosity to develop mapping populations and pure line varieties.

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Chapter 3
Conventional and Biotechnological Approaches for Targeted Trait Improvement in Lentil

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Abbreviations

AFLP	Amplified fragment length polymorphism
APX	Ascorbate peroxidase
ASAP	Allele specific amplified primer
AUD	Australian dollar
BA	Benzyladenine
BAC	Bacterial active chromosome
BAC	Bacterial artificial chromosome
BARI	Bangladesh Agricultural Research Institute
BC	Backcross
BIBAC	Binary bacterial artificial chromosome
BLAST	Basic local alignment search tool
CAPS	Cleaved amplified polymorphic sequences
cDNA	Complimentary DNA

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Cl^-	Chloride ion
CMS	Cell membrane stability
CS	Climate smart
CTD	Canopy temperature depression
CWSI	Crop water stress index
DArT	Diverse array technology
dCAPS	Derived CAPS
DH	Double haploid
DNA	Deoxyribonucleic acid
DS	Dormant seeding
DTI	Drought tolerance index
DUS	Distinctiveness, uniformity and stability test
EC	Electrical conductivity
EMBL-EBI	European Molecular Biology Laboratory
eQTL	Expression QTL
EST-SSR	Expressed sequence tag-derived simple sequence repeats
Fe	Iron
FISH	Fluorescence in situ hybridization
GA3	Gibberellic acid
GABA	γ-Aminobutyric acid
GBS	Genotyping by sequencing
GEO	Gene Expression Omnibus
GMP	Geometric mean productivity
GSI	Germination stress index
GUS	Transient β-glucuronidase
HI	Harvest index
HM	Harmonic mean
HMM	Hidden Markov model
ICARDA	International Center for Agriculture Research in the Dry Areas
IIPR	Indian Institute of Pulses Research
IPCC	Intergovernmental Panel on Climate Change
IRLC	Inverted repeat-lacking clade
ISSR	Inter-simple sequence repeats
ITAP	Intron targeted amplified polymorphism
ITS	Internal transcribed spacer
K	Potassium
K^+	Potassium ions
KEGG	Kyoto Encyclopedia of Genes and Genomes
LIS	Legume Information System
MAB	Marker-assisted breeding
MABC	Marker-assisted backcrossing
MABCB	Marker-assisted backcross breeding
MARS	Marker-assisted recurrent selection
MAS	Marker-assisted selection

Mg	Magnesium
Mha	Million hectare
miRNA	MicroRNAs
MP	Mean productivity
Mt	Million tons
Na+	Sodium ions
Na+/K+	Sodium to potassium ratio
NBPGR	National Bureau of Plant Genetic Resources
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
NILs	Near-isogenic lines
NUE	Nutrient use efficiency
PAR	Photosynthetically active radiation
PBA	Pulse Breeding Australia
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PLANEX	Plant co-expression database
QTL	Quantitative trait loci
R/FR	Red/far red
RAPD	Random amplified polymorphic DNA
RGA	Resistance gene analogues
RIL	Recombinant inbred line
RNA	Ribonucleic acid
RNAi	RNA interference
RS ratio	Root-shoot ratio
RWC	Relative water content
SCAR	Sequence characterized amplified region
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide *gel* electrophoresis
SNP	Single-nucleotide polymorphism
SPLAT	Specific polymorphic locus amplification test
SRAP	Sequence-related amplified polymorphism
SSD	Single seed descent
SSI	Stress susceptibility index
SSR	Simple sequence repeats
STI	Stress tolerance index
STS	Sequence tagged site
Super-SAGE	Serial analysis of gene expression
Tc	Canopy temperature
TI	Heat tolerance index
VIGS	Virus-induced gene silencing
VNTR	Microsatellite variable number tandem repeats
VPD	Water pressure deficits
WUE	Water use efficiency
YAC	Yeast active chromosome
Zn	Zinc

3.1 Introduction

Lentil is a true diploid ($2n = 2x = 14$) annual plant with 4 Gbp genome size (Arumuganathan and Earle 1991). Lentil is an important legume crop which offers paddock to plate health benefits by enriching soil through N-fixing symbiotic relations with rhizobium (Jarpa-Parra 2018) and is embedded in cereal legume-based farming system as a high-value cash crop. It is one of the nutritious grain legumes being rich in dietary protein (20.6–31.4%), vitamins, minerals and many essential amino acids (lysine and tryptophan) (Erskine et al. 1990; Faris et al. 2013; Johnson et al. 2013; Ray et al. 2014; Jarpa-Parra 2018) and also having other benefits such as high fibre and low glycaemic index (Srivastava and Vasishtha 2012; Moravek et al. 2018). Among various food legume crops, the lentil has not seen tremendous adoption primarily in developing countries despite crop's ability to thrive under limited water conditions. Its high protein content makes it the best alternative to animal-based protein for vegetarian people. Pulses are grown over 95.2 Mha area and lentil covers 6.6 Mha area worldwide. Lentil among less privileged crops has not seen immense improvement in its productivity over the past few decades – 0.8 (1997) to 1.2 ton/ha (2017) – at the world level (FAO 2020).

To meet the food demand of increasing human population, by 2050 we need to produce double the amount of food from half of the available resources by facing the vagaries of climate change. Crop yields around the world will significantly be affected due to climate pressures and narrow genetic base of staple crops. Biotic and abiotic stresses will not only affect quantity but also the quality of the produce. Legumes which play a significant role in crop cycle not only fall second to cereals but have been neglected and grown mostly on marginal lands especially in developing countries and have lost genes of importance (Bejiga and Degago 2000). Along with cultivation on marginal lands which generally have low soil fertility, lentil crop is mostly grown as rainfed and is subjected to mainly terminal drought and heat stress (abiotic) and various fungal and bacterial diseases – ascochyta blight, rust, stemphylium blight, collar rot, root rot, white mould, fusarium wilt and anthracnose (Kumar et al. 2013; Sharpe et al. 2013). Currently, cultivated lentil cultivars do possess tolerance/resistance to some abiotic and biotic stresses; still, the breeding focus of these cultivars primarily has been for higher yields. Therefore, changing climate has threatened scumming of most of the cultivars to various biotic and abiotic stresses over a short period of time after their release. Looking at the significance of legumes including lentil in current crop production system around the world, targeted trait improvements for resistance to various stresses, improved quality and higher yields will ascertain sustained quality production over the years to meet the growing demand for healthy food alongside facing the challenges of drastic climatic events. The narrow genetic base of lentil cultivars due to their reliance on few improved cultivated germplasm is certainly a great concern (Singh et al. 2014), though there is a hidden wealth of wild and distant lentil relatives which possess untapped genes of interest to be targeted for further desired improvements of existing and development of new lentil cultivars (Ford et al. 1997; Duran et al. 2004;

Gupta and Sharma 2007; Gupta et al. 2019). Therefore, this chapter will focus on targeted trait improvements accomplished using conventional and biotechnological approaches in lentil as well as genetic resources explored for traits from close and distant wild and cultivated sources not included in existing cultivars around the world.

3.2 Pre-breeding for Targeted Trait Improvement in Lentil

Cultivated lentil has been categorized in two major groups based on its seed size – small-seeded, 'microsperma' (2–6 mm), and large-seeded, 'macrosperma' (6–9 mm). Wild species *L. orientalis* is considered the wild progenitor of cultivated lentil (Zohary 1972) as ascertained by higher percentage of crossing ability of these two which mostly leads to fertile hybrids. Wong et al. in 2015 classified genus *Lens* into primary (cultivated lentil, *L. culinaris*; wild lentil species, *L. orientalis* and *L. tomentosus*), secondary (*L. lamotte* and *L. odomensis*), tertiary (*L. ervoides*) and quaternary (*L. nigricans*) distinct gene pools and related species mentioned in brackets. Various researchers have demonstrated that primary and secondary gene pools harbour compatible species and majority of the genotypes can be crossed through conventional breeding techniques and with or without any exogenous application of growth hormones or assist via tissue culture techniques (Ahmad et al. 1995; Fratini et al. 2004; Gupta and Sharma 2005). However, pre- and postfertilization barriers hamper successful introgression of genes of interest primarily from tertiary and quaternary gene pools into cultivated lentil (Gupta and Sharma 2007; Singh et al. 2013).

Long-term sustainability of lentil cultivars to mitigate stresses and sustain higher-quality yields will hugely depend upon their ability to harbour many genes of agronomical importance as well as biotic and abiotic stress-resistant/tolerant genes, which are mostly of quantitative nature. As without stable resistance/tolerance in existing cultivars, huge yield penalties are experienced by growers around the world. Because of low or nil resistance to biotic stresses in most of the existing cultivars, reliance on chemical control has increased so does the crop production cost.

Therefore, the trait targeted approach in breeding programmes needs a focus on tapping germplasm with multiple traits/genes of interests or common defence mechanisms (Gupta et al. 2019). Agronomical traits of importance, resistance to biotic stresses and tolerance to abiotic stresses have been identified among wild and distant lentil genotypes with superior expression to the popular cultivated lentil cultivars in various studies (Table 3.1).

In-depth understanding of underlying mechanisms of stress tolerance/resistance and ability of wild genotypes to thrive under such stresses provides insight into tapping right species and desirable traits of interest for introgression into the cultivated background. Among various stresses, drought management operates either through drought avoidance or tolerance. Presence of dense leaf hairiness, closure of stomata

Table 3.1 Pre-breeding identification of various genes of interest from cultivated and wild lentil germplasm

Trait of interest	Germplasm	References
Agronomical traits		
Early flowering and maturity, leaf area, higher number of leaves, pods and seeds per plant	Wild and cultivated germplasm	Hamdi et al. (1991), Ferguson and Robertson (1999), Gupta and Sharma (2006), Singh et al. (2014)
Abiotic stresses		
Drought avoidance		
Early vigour, root traits, rapid root growth, root-shoot ratio, nodulation, flowering and maturity, desired canopy structure, leaf surface, stem length, stomatal traits, high yield	Wild, cultivated and mutant germplasm	Erskine and Saxena (1993), Silim et al. (1993a, b), Salam and Islam (1994), Erskine et al. (1994), Shrestha et al. (2005), Idrissi et al. (2016), Biju et al. (2017), Gorim and Vandenberg (2017a, b)
Drought tolerance		
Seedling survival and vigour, root traits (root length, lateral roots number, root weight), root-shoot ratio, plant height, pod and seed number, grain yield and harvest index, early flowering and maturity, germination stress index, cell membrane stability, electrolyte leakage, water use efficiency, relative water content, osmotic regulation, drought susceptibility index, crop water stress index, canopy temperature depression, drought tolerance efficiency	Wild and cultivated germplasm	Hamdi and Erskine (1996), Mia et al. (1996), Sarker et al. (2005), Gupta and Sharma (2006), Shrestha et al. (2006), Stoddard et al. (2006), Salehi et al. (2008a, b), Chakherchaman et al. (2009), Aswaf and Blair (2012), Kumar et al. (2012a), Idrissi et al. (2015), Mishra et al. (2014, 2016, 2018), Singh et al. (2017), Biju et al. (2018)
Heat tolerance		
Higher antioxidant activities, pollen germination and viability, nodulation, heat tolerance index (TI) and cell membrane thermostability, number of filled pods, seed weight and yield	Cultivated germplasm	Chakraborty and Pradhan (2010, 2011), Choudhury et al. (2012), Barghi et al. (2013), Delahunty et al. (2015), Gaur et al. (2015), Kumar et al. (2016, 2017), Bhandari et al. (2016), Sita et al. (2017)
Cold and frost tolerance		
Early vigour, controlled freezing test, winter hardiness and survival rate	Wild and cultivated germplasm	Hamdi et al. (1996), Ali et al. (1999), Sarker et al. (2002), Kahraman et al. (2004)
Salinity tolerance		

(continued)

Table 3.1 (continued)

Trait of interest	Germplasm	References
Seed germination, nodulation, root-shoot length and weight, water use efficiency, sodium-potassium ratio, soluble sugars, proline, antioxidant activity, salt tolerance percentage, salinity scores, stress indices, biomass yield	Wild and cultivated germplasm	Rai and Singh (1999), Hamdi et al. (2000), Yasin et al. (2002), Maher et al. (2003), Cicerali (2004), Sidari et al. (2007), Kokten et al. (2010), Siddique et al. (2013), Ouji et al. (2015), AL-Quraan and AL-Omari (2017), Kumawat et al. (2017), Aslam et al. (2017)
Biotic stresses		
Ascochyta blight	Wild and cultivated germplasm	Gurdip et al. (1982), Cromey et al. (1987), Iqbal et al. (1990), Abi-Antoun et al. (1990), Sugha et al. (1991), Ahmed and Beniwal (1991), Andrahennadi (1994), Bayaa et al. (1994), Erskine et al. (1996), Ahmad et al. (1997), Nasir and Bretag (1998), Tullu et al. (2006, 2010a, b), Iqbal et al. (2010), Dadu et al. (2017, 2018)
Anthracnose	Wild and cultivated germplasm	Buchwaldt et al. (2004), Tullu et al. (2006), Fiala et al. (2009), Shaikh et al. (2012), Vail et al. (2012)
Botrytis grey mould	Cultivated germplasm	Karki et al. (1993), Bretag and Materne (1999), Kuchuran et al. (2003), Lindbeck et al. (2008)
Fusarium wilt	Wild and cultivated germplasm	Bayaa et al. (1995), Erskine et al. (1996), Nasir (1998), Gupta and Sharma (2006)
Powdery mildew	Wild germplasm	Gupta and Sharma (2006)
Rust	Wild and cultivated germplasm	Singh et al. (1994), Negussie et al. (1998), Sarker et al. (1999, 2004), Gupta and Sharma (2006), Fikru et al. (2007), Peñaloza et al. (2007), Sadiq et al. (2008)
Stemphylium blight	Cultivated germplasm	Kant et al. (2017)
Viral disease resistance		Makkouk and Kumari (1990), Kumari and Makkouk (1995), Makkouk et al. (2001), Latham and Jones (2001), Rana et al. (2016)
Insect resistance		
Aphids	Cultivated germplasm	Kumari et al. (2007)
Sitona weevils	Wild germplasm	El-Bouhssini et al. (2008)

Partially adapted from Gupta et al. (2019)

in a regulated manner, enhanced antioxidant levels, osmotic adjustment, and yield are related to drought tolerance in lentil. Recent studies by Gorim and Vandenberg (2017a, b) suggested that wild lentils and climatic conditions of their place of origin must have evolved them to cope with drought stress through different mechanisms of escape, avoidance or tolerance. These mechanisms operate through the expression of various traits such as late onset of flowering, less water loss through transpiration, putting less biomass through reduced plant height and letting roots grow deeper. As expected, some genotypes did express more than one drought stress management strategies.

So far various researchers have identified many useful genes from cultivated and wild germplasm (Table 3.1) which either have been transferred into existing lentil cultivars or are part of current breeding programmes or still need to be considered for their inclusion in lentil breeding programmes.

3.3 Important Traits of Interest for Breeding Strategy

The first challenge in breeding for multiple traits is to determine and prioritize traits which are most important for the target environment and market. Several traits of importance can simultaneously be targeted for genetic improvement of lentil cultivars. However, prioritization of traits is very important, as there is a cost for every trait the plant expresses in the final phenotype. Breeders should focus on the identification of genotypes with desired adaptation to biotic and abiotic stresses, superior grain quality, nutritional attributes and appropriate phenology to match with the environment. An overview of region-wise targeted traits across the globe and traits that can be used for genetic improvement of lentil is summarized in Table 3.2 and Fig. 3.1, respectively. For multiple trait selections and integration, a breeding programme must focus on the traits that are associated genetically.

3.4 Conventional Breeding Approaches for Targeted Trait Improvement in Lentil

Lentil is the oldest domesticated self-pollinating crop (Erskine 1997) with less than 0.8% of natural cross-pollination (Wilson and Law 1972). Unlike the other major oldest domesticated cereals or pulses, the history of lentil spread indicates that the crop improvement in lentil has largely been achieved through natural and artificial selection within landraces (Erskine 1997). A survey in 1979 reported that landraces occupied greater than 80% of the area under cultivation in the major countries (Solh and Erskine 1981). Later, with the commencement of the International Center for Agriculture Research in the Dry Areas (ICARDA) in 1977, lentil improvement programmes received valuable assistance, and subsequently, different breeding

Table 3.2 List of region-wise targeted traits in lentils for improvement across the globe

Region	Targeted trait
Africa	Yield and related traits, nutritional enhancement, heat and drought tolerance, resistance to ascochyta blight, anthracnose and rust
Eastern Europe	Yield and related traits; nutritional enhancement; tolerance to heat, cold and drought; resistance to ascochyta blight, anthracnose and fusarium wilt
North Africa	Yield and related traits; nutritional enhancement; tolerance to salinity, heat, cold and drought; resistance to ascochyta blight, anthracnose, fusarium wilt, botrytis grey mould, rust and stemphylium blight
North America	Yield and related traits; nutritional enhancement; tolerance to salinity, heat, cold and drought; resistance to ascochyta blight, anthracnose, botrytis grey mould, rust and stemphylium blight
Oceania	Yield and related traits; nutritional enhancement; tolerance to heat, drought and boron; resistance to ascochyta blight, botrytis grey mould
Russia	Yield and related traits, nutritional enhancement, tolerance to heat and drought, resistance to ascochyta blight
South America	Yield and yield-related traits; nutritional enhancement; tolerance to heat, drought and cold; resistance to ascochyta blight, fusarium wilt, botrytis grey mould and rust
South Asia	Yield and related traits; nutritional enhancement; tolerance to salinity, boron, heat, drought and cold; resistance to ascochyta blight, fusarium wilt, anthracnose, botrytis grey mould, rust and stemphylium blight
Western Asia	Yield and related traits; nutritional enhancement; tolerance to heat, drought, cold, salinity, boron; resistance to ascochyta blight and fusarium wilt
Western Europe	Yield and related traits, nutritional enhancement, tolerance to heat and drought, resistance to ascochyta blight, fusarium wilt, botrytis grey mould and rust

Adapted and modified from Rana et al. (2019)

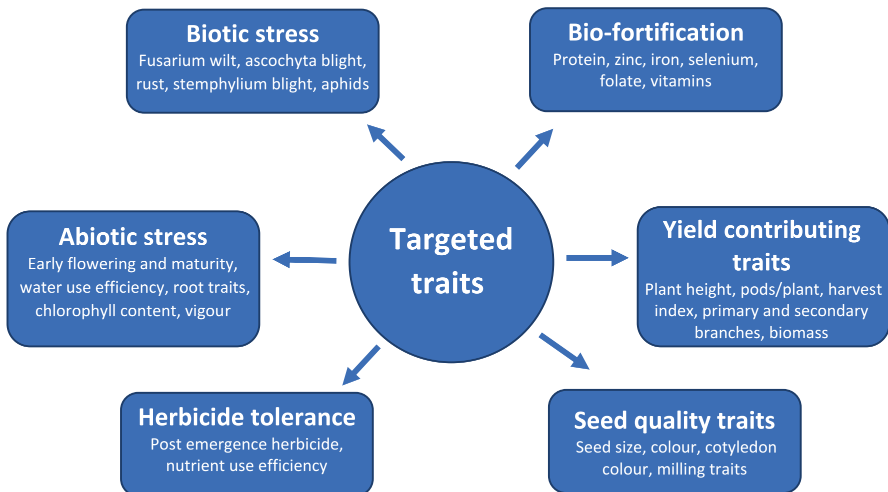

Fig. 3.1 Targeted traits used in lentil breeding strategies

strategies were laid out. Soon after its inception, as one of its mandate, ICARDA in collaboration with other national institutes emphasized collection of genetic resources in the view of unanticipated future needs. Successive efforts resulted in the accumulation of large collection of germplasm at various gene banks around the world including ICARDA, which hosts around 10,800 wild and cultivated lentil genotypes (Global Crop Diversity Trust 2008). The variability for various economically important traits within these conserved germplasms has been characterized to some extent as given in Sect. 3.2 and is revealed useful in breeding and selection programmes (Tullu et al. 2010a, b; Singh et al. 2018).

Like any other self-pollinating crop, breeding methods for incorporation of target traits employed in lentil majorly included pure line selection, hybridization, backcross, bulk, pedigree and single seed descent (SSD) method (Rahman et al. 2009). As a result of these methodologies, a total of 146 cultivars have been released until 2017 across major lentil-producing countries with targeted traits (Table 3.3). During early adaptation of lentil, pure line selection was extensively used to release cultivars with adaptability to wider areas and superior performance in terms of yield and disease resistance for ascochyta blight, rust and fusarium wilt. A few popular cultivars to release through pure line selection include Pant L 406, Pant L 639, L 830, L 4076, B 77, etc. in India, Barimasur 1 in Bangladesh, Shital in Nepal and Masoor 85 in Pakistan (Rahman et al. 2009). Cultivars that performed well in a country were often introduced in another country with similar climatic conditions.

Table 3.3 Lentil cultivars released from national programmes using ICARDA-supplied genetic material during 1977–2017

Region	Country	Number of cultivars	Targeted traits
Asia	Bangladesh, India, Nepal, Pakistan, China, Afghanistan, Iran, Iraq, Syria, Lebanon, Jordan, Yemen, Turkey	80	High yield; seed traits; micronutrient enrichment; short duration; suitability to machine harvesting; resistance to ascochyta blight, rust, stemphylium and fusarium wilt; tolerance to drought, frost and cold
Africa	Ethiopia, Egypt, Morocco, Libya, Tunisia, Algeria, Lesotho, Sudan, Eritrea	39	High yield and seed quality, early maturity, seed traits, adaptation to new environments, suitability to machine harvesting, resistance to wilt, rust and powdery mildew
The Americas	Argentina, Chile, Canada, Ecuador, USA	7	High yield and biomass, erect, resistance to ascochyta and rust, drought tolerance
Oceania	Australia, New Zealand	12	High yield, early maturing, resistance to ascochyta blight and botrytis grey mould
Europe	Portugal	2	High yield, large-seeded, tall
Central Asia and the Caucasus	Georgia, Uzbekistan, Azerbaijan	6	High yield, tall and erect, suitability for machine harvesting, high protein content, lodging resistance, rust resistance

Adapted from Sarker et al. (2009) and the data is sourced from ICARDA's website https://indms.icarda.org/pages/12

Some of the successful introductions include Vipasha and VL 507 in India and Mansehra 89 and Shiraz 96 in Pakistan (Rahman et al. 2009). Likewise, several other introductions have enriched local gene pools and led to the development of cultivars with greater yield stability in major lentil-producing countries (Laskar et al. 2019).

Cross-breeding is the widely chosen method in the recent past by breeders particularly to introgress special traits from exotic or other popular germplasm to the locally adapted cultivars (Laskar et al. 2019). The crosses have not been just limited to single crosses between two parents but involved double, three-way and multiple crosses. In a successful hybridization, selection of parents, as well as selection post crossing in resultant generations, is crucial to produce cultivars with desirable traits (Sarker et al. 2009). Selection procedures often varied with the objective of the breeding programme though the aim is to retain the best lines towards the end of selection cycle. Some of the methods used in the lentil breeding programme included pedigree, bulk population, recombinant-derived family and SSD. Furthermore, few modifications existed to bulk method with single pod selection being employed at F2 and F3 and single plant selections at F4 (Muehlbauer et al. 2009). These populations were screened for various traits including ascochyta blight resistance, seed shape, seed colour, pod drop, shattering and biomass. Selected lines were then evaluated for yield and quality in target environments. Popular cultivars in Australia such as PBA Ace, PBA Bolt and Nipper were developed using this method (Pulse Australia 2019).

SSD in lentil has often been used to produce recombinant inbred line populations (RILs) for use in constructing linkage maps (Eujayl et al. 1998; Tullu et al. 2008; Saha et al. 2010; De la Puente et al. 2012; Gupta et al. 2012a, b; Fedoruk et al. 2013; Kaur et al. 2014; Temel et al. 2015; Ates et al. 2016, 2018; Sudheesh et al. 2016a, b; Aldemir et al. 2017; Polanco et al. 2019) and identification of quantitative trait loci (QTL) controlling traits of interest such as resistance to ascochyta blight, anthracnose and fusarium wilt, tolerance to frost and winter hardiness and several other economically important traits (Ford et al. 1999; Rubeena et al. 2006; Tullu et al. 2008; Gupta et al. 2012a, b; Ates et al. 2016, 2018; Sudheesh et al. 2016a, b; Aldemir et al. 2017; Bhadauria et al. 2017; Polanco et al. 2019). To further accelerate the generation of a new cultivar, speed breeding integrated with SSD (Watson et al. 2017) has been employed in lentil, and an F_7 RIL population of cross *L. culinaris* × *L. ervoides* targeting aphanomyces root rot resistance has been developed in less than 300 days (Lulsdorf and Banniza 2018).

Mutation techniques have been tested in lentil as a complementary breeding strategy to introduce a desirable trait which is absent in the available germplasm (Muehlbauer et al. 2009). Some popular cultivars with different traits of interest have been developed and released worldwide using irradiation and ethyl methanesulfonate (EMS) as a source of mutagens. Majority of cultivars developed through mutation breeding registered in the Indian subcontinent have a variety of improved attributes such as high yield, resistance to rust and blight, tolerance to cold and early maturity (Laskar et al. 2019). Cultivars bred through mutation breeding outside the Indian subcontinent possessed some useful traits such as high yield, high protein

content, suitability to machine harvesting, resistance to fusarium wilt, blight, botrytis and anthracnose, tolerance to drought and herbicide resistance (Laskar et al. 2019). In Canada and Australia, EMS treatment was used to produce lentil mutants tolerant to imidazolinone herbicides (Slinkard et al. 2007; Mao et al. 2016). This trait is now integrated into all the lentil cultivars that are released in these countries, and some popular cultivars include CDC Impala, CDC Imperial, CDC Imigreen, CDC Peridot, etc. in Canada and PBA Herald XT and PBA Hurricane XT in Australia.

The upgraded cultivars produced using the above methodologies provided better stability, wide adaptation and extended yielding capacity as an outcome of the collective effect of genes transferred from close and distant germplasm. However, improvement in yields has been only marginal but not significant (Singh et al. 2013). This phenomenon in lentil as suggested before is largely attributed to the loss of valuable alleles for high productivity and low genetic variation within the cultivated species (Muench et al. 1991; Alveraz et al. 1997; Ford et al. 1997; Ferguson et al. 2000; Duran et al. 2004). To potentially recreate the genetic variability and maximize the lentil productivity, several attempts (discussed below) have been made to domesticate wild lentils that are known to house several desirable genes (Cohen et al. 1984; Ladizinsky et al. 1988; Muehlbauer et al. 1989; Vandenberg and Slinkard 1989; Fratini et al. 2004; Fratini and Ruiz 2006; Gupta and Sharma 2007; Fiala et al. 2009; Tullu et al. 2013; Kumari et al. 2018; Polanco et al. 2019).

The gene pool structure suggested by Wong et al. (2015) and as discussed previously in this chapter also reflects the crossability between cultivated and wild lentils, which varies with the percentage of chromosomal similarities between the species (Ladizinsky et al. 1988; Fratini et al. 2004; Gupta and Sharma 2007). Conventional crossing techniques have been used to produce hybrids between cultivated and wild lentils. Although sufficient success has been realized for the crosses between *L. culinaris* × *L. orientalis/L. odemensis* (Muehlbauer et al. 1989; Vandenberg and Slinkard 1989; Fratini et al. 2004; Gupta and Sharma 2007; Singh et al. 2013), the efforts were futile in obtaining fertile interspecific hybrids between *L. culinaris* and *L. ervoides* and *L. nigricans* species (Abbo and Ladizinsky 1991, 1994; Gupta and Sharma 2007). Pre- and postfertilization barriers owing to pollen-stigma incompatibility and embryo abortion, respectively, are identified as the key reasons for such unsuccessful hybridizations in lentil (Gupta and Sharma 2005).

Nonetheless, to overcome these pre- and postfertilization barriers and unlock the breeding potential of wild lentils, various methods termed as wide hybridization protocols have been developed (Cohen et al. 1984; Liu et al. 2005; Van de Wiel et al. 2010). A few of these methods including tissue culture techniques such as embryo and ovule rescue, exogenous use of growth hormones (gibberellic acid-GA_3) and micrografting have been tested in lentil to produce viable hybrid plants (Cohen et al. 1984; Ahmad et al. 1995; Gupta and Sharma 2005; Yuan et al. 2011; Saha and Muehlbauer 2014). Utilizing the success of the wide hybridization protocols, interspecific hybrids derived from crosses between *L. culinaris* and *L. orientalis*, *L. odemensis*, *L. ervoides* and *L. lamottei*, have been advanced to produce several RIL populations to detect lines with useful traits. Evaluation of these populations

revealed useful variations for various traits including agronomical traits (plant height, days to flowering and maturity), yield and yield-related traits (number of branches/plant, number of pods/plant, seed yield/plant, biological yield/plant and harvest index) and resistance to anthracnose, rust and ascochyta blight (Ye et al. 2000; Gupta and Sharma 2007; Fiala et al. 2009; Vail et al. 2012; Singh et al. 2013; Tullu et al. 2013; Kumari et al. 2018; Dadu et al. 2019; Polanco et al. 2019).

Although promising variations have been reported through wide crosses, cultivars developed with wild species in the pedigree are yet to be registered worldwide. One of the major reasons for avoidance of wild lentils in breeding programmes is the linkage drag effect, which may result in deriving unwanted/lethal genes along with desired genes (Singh et al. 2018). However, with the availability of high-throughput phenotyping and genotyping methods, utilization of wild lentils in breeding programmes is expected to increase circumventing the linkage drag effect (Wang et al. 2017).

3.5 Biotechnological Approaches

3.5.1 Tissue Culture for Targeted Trait Improvement in Lentil

The main purpose of employing tissue culture techniques in lentil was to reduce the genetic distance between wild and cultivated germplasm as described in Sect. 3.4. Upon the success achieved in wide crosses, the objective of the tissue culture in lentil has been upgraded to transfer desirable traits. This needed construction of large F_1 populations to produce large numbers of F_2 seeds useful for assessing the trait introgression. Through consistent efforts, several fertile plants from wide crosses have been produced using tissue culture methods with different explants (Table 3.4).

Cohen et al. (1984) produced viable hybrids from interspecific crosses between *L. culinaris* × *L. ervoides* and *L. Nigricans* using embryo rescue protocol. Following Cohen et al.'s (1984) embryo rescue protocols and some minor modifications, several other successful interspecific crosses for traits such as anthracnose resistance have been produced between cultivated lentil and wild lentil species (Fratini and Ruiz 2006; Fiala et al. 2009). Ovule rescue method was applied to obtain interspecific hybrids from crosses between *L. culinaris* and *L. Tomentosus* (Suvorova 2014). Although these methods helped to rescue embryos from aborting, difficulties existed in transforming the embryos into a viable plant. Gupta and Sharma (2005) were successful in rescuing ovules of interspecific crosses involving cultivated lentil and *L. ervoides* and *L. nigricans*, but the embryos did not differentiate to form root organ. To overcome this research gap, Yuan et al. (2011) proposed a fusion of embryo rescue and micrografting methods. They used shoot regenerations of six wild lentil species as scions and grafted them onto the rootstocks of faba bean which helped the lentil shoots to establish and develop into functional plants. The method

Table 3.4 Explants chosen for tissue culture experiments in lentils

Explant of choice	Reference
Cotyledonary tissue	Chhabra et al. (2008)
Cotyledonary node	Sevimay et al. (2005), Chhabra et al. (2008), Bermejo et al. (2012), Özdemir and Türker (2014)
Cotyledons with a small part of the embryo axis	Tavallaie et al. (2011)
Decapitated embryo	Omran et al. (2008), Bagheri et al. (2012), Das et al. (2012), Sarker et al. (2003b)
Ovule-embryo	Cohen et al. (1984), Ahmad et al. (1995), Gupta and Sharma (2005), Fratini and Ruiz (2006), Fiala et al. (2009), Galina (2014)
Hypo- and epicotyl-derived callus	Williams and McHugen (1986)
Embryonic axis	Saxena and King (1987)
Meristem tip	Bajaj and Dhanju (1979)
Shoot	Khentry et al. (2014)
Seed	Chopra et al. (2011a, b)

Adapted and modified from Laskar et al. (2019)

given by Yuan et al. (2011) showed a remarkable survival rate. Saha and Muehlbauer 2014 following a similar approach produced large numbers of F_2 seed from interspecific crosses between *L. culinaris* and *L. tomentosus*, *L. odemensis* and *L. lamottei*. Readers are further encouraged to read the detailed review by Gupta et al. (2018) on cytogenetic manipulations in lentil using tissue culture methods.

3.5.2 Embryo Rescue Assisted Breeding

The embryo rescue method has been used for shortening the breeding cycle in lentil to produce elite cultivars in short span of time. Ochatt et al. (2002) also described shortening of the life cycle for lentil based on in vitro culture of embryos. Bermejo et al. (2016) have studied an efficient in vitro assisted single seed descent technique where seeds were obtained in about 78–80 days for macrosperma and 107–110 days for microsperma genotypes. This method significantly reduced the days to flowering up to 13–15 days in macrosperma and 42–45 days in microsperma with a possibility to have four generations in a year.

Various researchers have attempted double haploid (DH) technology (Croser et al. 2006; Wędzony et al. 2008; Germanà 2011) which has the potential to simplify crop breeding through the production of haploid plants in a single generation. Legumes, being recalcitrant, have not seen much success through in vitro techniques; however efforts were made in lentil for DH production to obtain pure homozygous plants, though it couldn't produce plantlets (Keller and Ferrie 2002). Croser and Lulsdorf (2004) also reported the same difficulty with the regeneration of

embryos through in vitro techniques. Later abiotic stress pretreatments, such as centrifugation, electroporation and osmotic shock, were shown to have a positive effect on induction of androgenesis in a number of species including legumes (Hosp et al. 2007; Ribalta et al. 2012). Deswal (2018) stated that various compositions of hormones and different stress treatments also became effortless in lentil to get homozygous plants. Till today, none of the attempts for lentil DH protocols are successful.

Somatic embryogenesis, adventitious shoot production comprising de novo meristem formation (organogenesis) and axillary shoot production using pre-existing axillary buds and meristems are the techniques under micropropagation to provide large materials in less time (Ahmed et al. 2001). Cotyledonary nodes have been used to obtain multiple shoots (Mallick and Rashid 1989; Polanco and Ruiz 2001). Polanco and Ruiz 2001 achieved 5–20 shoots per immature seed of 4 lentil genotypes on media supplemented with BAP. They obtained a higher frequency of shoot regeneration from the cotyledonary node of wild lentil explants using thidiazuron (TDZ). In order to conserve wild resources of lentil, Sevimay et al. (2005) used the micropropagation technique to provide disease-free material for lentil improvement with TDZ in culture medium and cotyledonary node as an explant. Recalcitrant nature of lentil limits many in vitro approaches by affecting the root initiation process. To overcome this limitation, slight modifications in culture medium such as replacing IAA with chlorinated IAA or adjusting the concentration of NAA from 1 to 1.5 mgL^{-1} reported higher rooting efficiency in lentil (Polanco and Ruiz 2001; Ye et al. 2002; Saha et al. 2015). Sarker et al. (2012) approached a new way of in vitro flowering with cotyledonary nodes. They decapitated macrosperma cultivar embryos with one cotyledon attached as explants. While attempting gene transformation in lentil microsperma cultivars, Das et al. (2012) developed a protocol for and witnessed in vitro flowering and pod formation. Although there is still a huge gap to ascertain successful in vitro regeneration protocols in lentil, successful transfer of traits particularly resistance to diseases (anthracnose, ascochyta blight, stemphylium blight) from distant cultivars using tissue culture techniques has been achieved (Fiala et al. 2009; Tullu et al. 2013; Saha et al. 2015; Polanco et al. 2019; Dadu et al. 2019).

3.5.3 Transgenic Approaches for Targeted Trait Improvement

The methodological developments in lentil tissue culture made the elementary way for genetic transformation. Transgenic approaches have been evolved as a reassuring methodology to work with elite traits, which are not transferable through conventional breeding (Gardner 1993). *Agrobacterium*-mediated gene transfer has gained commercial importance when it succeeded in transferring insect resistance and herbicide tolerance traits. Horizontal gene transfer mediated by *Agrobacterium tumefaciens* has been limited in legumes being non-hosts. Different *Agrobacterium tumefaciens* strains such as *C58*, *Achh5*, *GV3111* and *A281* have been evaluated to ascertain lentil explant's susceptibility for the transformation process (Warkentin

and McHughen 1991). All the strains are capable of inducing tumours with high frequency, which highlights the possibility of horizontal gene transformation in lentil. Another strain – *A281* – has shown the capability to introduce heavy tumours on different explants of 21 lentil genotypes, where transgene GUS expression was low with such tumours (Khawar and Ozcan 2002). Successful herbicide resistance gene *acetolactate synthase (ALS)* transfer was achieved through vertical gene transformation via biolistic method using highly regenerable lentil cotyledonary node meristems. This led to successful transgene expression in putative transformants (Gulati et al. 2002). Transgenic lentil shoots were produced with an overall frequency of 1.01%. To develop an efficient, rapid, reproducible and genotype-neutral in vitro regeneration system for lentil, SAAT (sonication-assisted *Agrobacterium tumefaciens* transformation) method was used with a super virulent *Agrobacterium tumefaciens* strain *EHA105* to transfer T-DNA containing *nptII* and *uidA* genes into whole lentil seeds. Further transfected shoots could differentiate into roots and shoots on a medium with IBA and kanamycin (Chopra et al. 2011a, b). Many explants, including shoot and root apices, epicotyls, cotyledonary nodes, nodal segments and embryonic axes, were used as explants for *Agrobacterium*-mediated genetic transformation (Warkentin and McHughen 1991, 1992, 1993; Lurquin et al. 1998; Öktem et al. 1999; Mahmoudian et al. 2002; Akcay et al. 2009). Multiple explants were studied by Sarker et al. (2003a) for their regeneration ability followed by gene transformation through *Agrobacterium tumefaciens*. Histochemical staining experiments showed that epicotyl explants exhibited highest transgene expression followed by decapitated embryos, which were found to be more effective in the formation of multiple shoots and were thus suggested as suitable explants for lentil transformation.

Among abiotic stresses, drought and salinity are two important stresses; to improve tolerance to these stresses, *DREB1A* gene with *rd29A* promoter has been introduced into lentils via decapitated embryo with *Agrobacterium*-mediated transformation (Khatib et al. 2011). Expression analysis proved gene function in putative transformants by RT-PCR analysis. This was the first reported abiotic tolerance transformant in lentil. Hashem (2007) developed the first marker free transformation system in legumes and improved fungal resistance in lentil by transferring *Ripgip* gene through decapitated embryos with one cotyledon and achieved 35% of transformation efficiency; further rooting was achieved by micrografting. Though there are successful reports for rooting, still, its application is limited as the substantial successful protocols are not available; therefore, there is still an immense need to develop alternative stable protocols for root regeneration in lentil.

3.5.4 High-Throughput Sequencing for Targeted Trait Improvement in Lentil

Improvement of desired traits in plants is based on breeding and selection for individuals that harbour the genetic components that will consistently produce the expected crop qualities. Conventionally this process had been performed by selecting the best performing individuals in each generation and expecting that their performance would be indicative of their genetic potential or introducing new genetic material to gain the advantages of hybrid heterosis. However, these methods have relied on the plant's phenotype alone, often influenced by environmental effects and interactions with its genotype and were not indicative of its true genetic potential to inherit its performance to the next generation.

Current biotechnological approaches for breeding and selection for the trait improvement in lentil rely on comprehensive knowledge of the lentil genome and the genetic variations within different landraces and cultivars. Molecular markers, such as microsatellites or simple sequence repeats (SSRs) and single-nucleotide polymorphism (SNP), provide an accurate way to track down the presence and transfer of specific genetic alleles between individuals and generations. Traits that are strongly linked to these markers could be improved by selection for individuals that possess the high-performing alleles in these markers in an approach that was named marker-assisted selection (MAS) (Nadeem et al. 2018). For example, identification of quantitative trait loci (QTL) that are linked to desired traits requires high-density linkage maps developed from molecular markers, such as microsatellites or SSR and SNP, so they can be incorporated in marker-assisted selection programmes (Kumar et al. 2015; Nadeem et al. 2018). Furthermore, functional annotation of transcripts and expressed sequence tags (ESTs) to identify target genes that are involved in the molecular pathways governing the trait of interest depends on existing gene databases of lentil and other closely related legume and plant species.

During the last decade of the twenty-first century, the discovery of gene sequences and molecular markers for lentil and other crops were based on a low-throughput, labour-intensive and time-consuming workflow consisting of cloning sheared DNA fragments, followed by polymerase chain reaction (PCR) amplification and Sanger 'shotgun' sequencing (Sharpe et al. 2013). This resulted in a modest number of molecular markers available for QTL studies and genetic map construction in lentil, limiting the analysis resolution to wide genomic regions and posing a major difficulty to accurately identify and annotate the responsible genes in the loci of interest. For example, two consecutive studies aiming at developing new SSR markers for lentil to construct the genetic map and determine genetic variation in wild and cultivated lines resulted in just over 40 microsatellite markers (Hamwieh et al. 2005, 2009). Furthermore, the large size of the lentil genome, approximately 4 billion base pairs (Gb) long, made it impractical to fully sequence using the shotgun sequencing method that was available at the time (Kumar et al. 2015).

High-throughput sequencing (HTS), or next-generation sequencing (NGS) as it is often referred to, was introduced in the mid-2000s and revolutionized genomic

research by offering massively parallel sequencing of short nucleic acid molecules at high accuracy, affordable prices and within a short timeframe. Early HTS technologies included Solexa's (now Illumina) sequencing-by-synthesis, Roche pyrosequencing and Ion Torrent's Ion Proton which differed by their method of library preparation protocols, amplification substrate (silica flowcell in Solexa vs. emulsion of microbeads in the latter two), signal detection mode (fluorescence signal, light emission and pH change, respectively) and read lengths produced (Varshney et al. 2009). These technologies were rapidly adopted and applied to produce large volumes of sequence data from a wide range of model and non-model species, as well as commercially important crops, including legumes.

Once introduced, NGS was employed for whole-genome sequencing (WGS) of lentil cultivars in an effort to sequence, assemble and annotate the entire genome. These efforts led to the release of draft *L. culinaris* genomes, covering roughly two-thirds of its length (2.7–2.9 Gb out of the expected 4 Gb). These drafts enabled the discovery of thousands of SNPs to be used by MAS breeding programmes and annotation of genes and functional markers, but despite substantial resources invested in these sequencing projects, the full-length genome is yet to be assembled (Bett et al. 2014, 2016).

Though assembling the entire lentil genome proved extremely challenging with short-read NGS technologies, they were found to be well suited for RNA sequencing (RNA-seq), capable of capturing the entire transcriptome of multiple samples in a single run. Transcriptome sequencing in lentil tissues was then used to identify genes that are functionally associated with a trait of interest, such as resistance to fungal diseases (Khorramdelazad et al. 2018) and drought tolerance (Singh et al. 2017). RNA-seq was also utilized in lentil to identify SNP markers within the transcribed regions of the genome, focusing on variants that are more likely to be associated with phenotypic changes (Kaur et al. 2011; Temel et al. 2015).

Another strategy to utilize NGS-derived short reads to accurately identify thousands of SNPs throughout the genome is by using a combination of restriction enzymes to fragment the DNA and subsequently sequence just the DNA fragments that are flanked by the enzymes' cut sites. By doing so, it is possible to call SNP variants from a partial, reduced-complexity representation of the genome, enabling genotyping of multiple samples at a fraction of the cost of whole-genome sequencing (WGS). The methods, like genotyping-by-sequencing (GBS) and its variants, restriction site-associated DNA sequencing (RAD-seq) and diversity array technology sequencing (DArT-seq) have been applied recently as cost-effective methods to call SNP variants and assign genotypes in a large number of samples such as RIL segregating families (Elshire et al. 2011; He et al. 2014). The acquired SNPs are then applied to construct genetic maps and identify QTL for traits, such as iron content in seed (Aldemir et al. 2017), fungal disease resistance (Bhadauria et al. 2017) and root and shoot drought tolerance (Idrissi et al. 2016), and assess genetic diversity and population structure in a diverse collection of global genotypes (Pavan et al. 2019).

Despite the continuous development of NGS platforms, led by Illumina, and major improvements in the bioinformatics software used in the analysis of the

produced data, these short-read technologies proved limited in certain tasks, such as assembling genomes with large repetitive regions and transposable elements. To fill that need, new third-generation sequencing platforms were developed by Pacific Biosciences (PacBio) and Oxford Nanopore that perform single-molecule real-time sequencing and produce reads up to 1 Mbp long (van Dijk et al. 2018). The long reads produced by these technologies are currently more expensive to sequence and inferior in their base calling accuracy in comparison with Illumina's established short-read platforms, which makes them less suitable for SNP calling applications in large sample experiments. However, a hybrid approach, using PacBio or Oxford Nanopore long reads to allow better scaffolding across gaps and repetitive regions and high coverage of Illumina's proven cost-efficient accurate short reads to settle inaccuracies, is a promising strategy in genome assembly applications (Madoui et al. 2015; Jung et al. 2019).

The unprecedented wealth of sequences produced by NGS technologies introduced new challenges for data storage, annotation, access and sharing. Online databases, such as the American National Center for Biotechnology Information (NCBI), GenBank and Short Read Archive (SRA) collections, provide public access to annotated and raw sequences (Cochrane et al. 2016). In addition to species-specific genes and sequences, NCBI offers homology search tools, mainly within the BLAST suite, for annotation of unknown sequences and other comparative genomics applications, that are particularly useful in non-model crops such as lentil, which lack the genomic resources available for other well-studied model organisms (Camacho et al. 2009; Bhat et al. 2018).

The Cool Season Food Legume Crop Database (https://www.coolseasonfoodlegume.org/, Washington State University) is an online portal that provides comparative genomics and genetics tools for legumes such as chickpea, pea, lentil and faba bean, though it only includes the full genome of chickpea. KnowPulse (http://knowpulse.usask.ca/portal/), from the University of Saskatchewan Pulse Crop Research Group, currently hosts the only annotated draft genome of lentil, as well as established genetic maps and a large collection of SNP markers from known cultivars, though the access to these resources is restricted and requires preapproval (Sanderson et al. 2019). These resources are under continuous development to follow advancements in sequencing technologies and equip lentil researchers and breeders with tools and genomic resources required for molecular-based breeding and trait improvement.

3.5.5 Transcriptomics for Targeted Trait Improvement in Lentil

RNA sequencing has recently been applied to transcriptome profiling in order to enable profound insight into the genome functions that occur in response to different conditions simultaneously (Wang et al. 2009). Lentil possesses a very large

nuclear genome with non-coding and repetitive DNA components which contribute the majority of nuclear DNA content (Ford et al. 1999). Transcriptome profiling is a powerful and the most popular tool providing a cost-effective in-depth analysis of the transcribed portions of the lentil genome (Kaur et al. 2011). This method has been applied widely to investigate the genes responsible to improve lentil production including crop productivity and quality, defence to biotic stresses and tolerance to abiotic stresses. In January 2020, there are about 10,352 ESTs available for lentil (NCBI, 2020, https://www.ncbi.nlm.nih.gov/nuccore).

Second-generation transcriptome profiling of six lentil genotypes (Northfield, ILL2024, Indianhead, Digger, ILL6788 and ILL7537) using Roche 454 GS-FLX Titanium enabled large-scale unigene assembly and SSR marker discovery (Kaur et al. 2011). Of 3470 SNPs and EST-SSRs, a set of 2393 EST-SSR markers have been developed and validated in lentil (Kaur et al. 2011). In 2013, using 454 pyrosequencing technology, transcriptome sequencing of lentil could develop 3′-cDNA reads from 9 *L. culinaris* and 2 *L. ervoides* genotypes (Sharpe et al. 2013). A 1536 SNP Illumina GG array was developed and used to construct an SNP-based genetic map of *L. culinaris* (Sharpe et al. 2013). Illumina GAII technology and de novo transcriptome assemblies identified lentil SSR markers to utilize in diversity analysis (Verma et al. 2013). Further success has been achieved to identify 50,960 putative SNP markers with transcriptome profiling of 2 lentil cultivars (Precoz and WA8649041) and 101 F_7 RILs (Temel et al. 2015). These SNP markers were successfully utilized to generate an SNP-based linkage map using Illumina CASAVA (Temel et al. 2015). The following year, seven RNA-seq libraries were generated and sequenced from a variety of tissue types of lentil cultivar *Cassab* (Sudheesh et al. 2016a, b). A unigene set comprising 58,986 contigs and scaffolds was developed for further genomic exploration (Sudheesh et al. 2016a, b). Several transcriptomic studies were conducted for targeted trait improvement in lentil such as crop quality, defence response and stress tolerance, and the results added more details to the genomic resources of lentil.

Lentil sensitivity to the water scarcity and drought period could highly affect lentil growth and productivity (Morgil et al. 2019). Thus, to address this issue of lentil drought tolerance at the seedling stage, Illumina HiSeq 2500 platform for transcriptome profiling of drought-tolerant (PDL2) and drought-sensitive (JL3) lentil cultivars was performed among different physiological and biochemical parameters (Singh et al. 2017). A recent study focused on a drought-sensitive lentil cultivar (Sultan from Turkey) and specifically on its root, leaf and stem under short- and long-term water deficits, utilizing Illumina HiSeq 4000 sequencing platform with de novo RNA-seq-based transcriptome analysis (Morgil et al. 2019). Results led to the detection of the root as the most sensitive plant organ to the period of drought stress in lentil as transcriptional changes during a long-term drought stress have been over six times more than short-term stress in the root system (18,327 compared to 2915 differentially expressed genes (DEGs), respectively (Morgil et al. 2019)). Moreover, gene ontology analysis depicted the differences in transcriptional regulation of biological processes such as protein phosphorylation, embryo development, seed dormancy, DNA replication and maintenance of root meristem as a response to

long-term drought stress (Morgil et al. 2019). Heat-responsive genes and their role in regulatory mechanisms of lentil through genome-wide transcriptomic study on PDL2 (tolerant) and JL3 (sensitive) mutants revealed many high-quality SNPs, microsatellites and insertion-deletions (indels) (194,178, 141,050 and 7388) (Singh et al. 2019). Furthermore, DEGs analysis revealed that PDL-2 has higher membrane stability index (MSI) and pollen germination under heat shock compared to JL3 and that the cell wall and secondary metabolite pathways are mostly exposed to heat stress effects (Singh et al. 2019).

Transcriptomic studies also helped with understanding of disease defence mechanisms in lentil. Transcriptional changes during early stages of *A. lentis* infection causing devastating ascochyta blight disease were comprehensively profiled in a resistant (ILL 7537) and a susceptible (ILL 6002) lentil genotype using Ion Proton sequencing system along with de novo RNA-seq-based transcriptome analysis (Khorramdelazad et al. 2018). Differential expression analysis helped with identification of 24 genes involved in the 3 main defence-response stages within the first 24 hours of pathogen attack (Khorramdelazad et al. 2018). Some detected protein kinases are involved in pathogen recognition and defence signalling pathways. Also, PR2, PR4 and PR10 from pathogenesis-related (PR) protein families have been characterized with a role in biochemical defence against the pathogen, and many structural and hypersensitive-response (HR) related genes which play a part in systemic acquired resistance (SAR) and cell death were detected (Khorramdelazad et al. 2018).

Application of high-throughput sequencing approaches facilitates generation and characterization of reference transcriptome datasets that leads to gene-based marker discovery, which in turn can be useful in genetic map construction among other purposes.

3.5.6 Linkage Mapping and QTLs for Targeted Trait Improvement in Lentil

Prior to the determination of the chromosomal location of a desirable gene, it is pivotal to develop linkage map that may be thought of as a 'road map' of the chromosomes (Collard et al. 2005). Identification of quantitative trait loci for important agronomic traits has been made possible in several plant species with the availability of polymorphic markers and linkage maps (Verma et al. 2015). In the recent past years, molecular markers have helped to know the gene networks underlying the quantitatively inherited traits and linked to genomic regions (QTLs/genes) controlling such traits have been identified in several crops including lentil (Kumar et al. 2017).

In lentil, the availability of molecular markers associated with agronomically important traits is limiting the use of the biotechnological tool in breeding programmes. However, the use of molecular markers has been accelerated due to the

enrichment of genomic resources in the recent years (Kumar et al. 2015), and molecular markers including SNP, SSR, inter-simple sequence repeat (ISSR) and direct amplification of minisatellite DNA (DAMD) have been developed (Hamwieh et al. 2005, 2009; Kaur et al. 2011; Temel et al. 2015; Verma et al. 2015; Khazaei et al. 2016).

3.5.6.1 Linkage Mapping from Single Mapping Populations

Linkage mapping based on single mapping populations was mainly constructed based on F_2 populations. Havey and Muehlbauer (1989) developed the first DNA-based marker genomic map of lentil from 20 restriction fragment length polymorphisms (RFLP), 8 isozymes and 6 morphological markers segregating in a single interspecific cross (*L. culinaris* × *L. orientalis*). Later, several interspecific crosses were utilized to create linkage maps in lentil (Weeden et al. 1992; Tahir et al. 1993; Vaillancourt and Slinkard 1993; Tahir and Muehlbauer 1994). Linkage mapping across the *Lens* genome became very popular with the introduction of PCR-based markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and RFLP (Kumar et al. 2012b, 2014, 2015). Subsequently, the first extensive linkage map of lentil was constructed in the late 1990s from RAPD, AFLP, RFLP and morphological markers using $F_{6:8}$ of RIL population from *L. culinaris* and *L. orientalis* (Eujayl et al. 1998). The map covered 1073 cM of the lentil genome with an average distance of 6.0 cM between adjacent markers.

Intraspecific mapping populations found to be more informative for QTL identification and to tag desirable genes than interspecific mapping populations (Kumar et al. 2015). Thus, in the early 2000s, the first intraspecific lentil map was developed using 100 RAPD, 11 ISSR and 3 resistance gene analogue (RGA) markers using F_2 population of lentil cultivars ILL5588 and ILL7537 with different resistance backgrounds against *A. lentis* causing ascochyta blight (Rubeena et al. 2003). The resulted intraspecific map spanned a total length of 784.1 cM comprising nine linkage groups. Another intraspecific linkage map, comprising 38 RAPD, 30 AFLP, 3 ISSR and 1 morphological marker, was constructed using a F_2 population from a cross between ILL6002 (ascochyta blight-susceptible) and ILL7537 (ascochyta blight-resistant) cultivars (Rubeena et al. 2006).

The first lentil map with short sequence repeat (SSR) markers was developed based on the segregation analysis of 5 different types of molecular and morphological genetic markers in 113 F_2 plants obtained from a cross of *L. culinaris* and *L. orientalis* (Duran et al. 2004). This map contained a total of 200 markers including 71 RAPDs, 39 ISSRs, 83 AFLPs, 2 SSRs and 5 morphological loci. Markers (161) were grouped into ten linkage groups covering 2172.4 cM of the genome, with an average distance between markers of 15.87 cM at a LOD score of 3.0. Phan et al. (2007) constructed another linkage map (928.4 cM) containing 18 SSR markers and 79 intron-targeted amplified polymorphic (ITAP) gene-based markers, using a F_5 RIL population from a cross between ILL5588 and ILL5722. This map was

constructed to develop a gene-based genetic map of lentil and to characterize syntenic relationships with *Medicago truncatula* as well as to integrate the resulting genic and comparative map with the other comprehensive genetic map of lentil. This map contained seven linkage groups comprised of 5–25 markers that varied in length from 80.2 to 274.6 cM. Gupta et al. (2012b) constructed a genetic linkage map using 114 F_2s derived from the interspecific cross of *L. culinaris* and *L. orientalis*. F_2 population used for this linkage map exhibited sufficient polymorphism for DNA markers, including variation for rust resistance and other agro-morphological traits.

Abiotic obstacles like water deficit and boron toxicity could also affect lentil growth and productivity to a large extent (Idrissi et al. 2016; Rodda et al. 2018). Kaur et al. (2014) performed large-scale SNP discovery and dense genetic mapping in a lentil intraspecific cross and identified a single chromosomal region controlling tolerance to boron toxicity. Another intraspecific linkage map constructed using a RIL population derived from a cross of Precoz × WA8649041 identified QTLs for flowering time in lentil (Kahriman et al. 2015). In order to detect the QTLs conferring drought tolerance in lentil, a total of 252 codominant and dominant markers were used to create a genome map from a population of 132 RILs developed from a cross between two contrasting parents, ILL6002 (drought tolerant) and ILL5888 (drought sensitive) (Idrissi et al. 2016). Markers were mapped on 9 linkage groups, and 18 QTLs regulating a total of 14 root and shoot traits were identified. A population of F_6 178 RILs (boron-tolerant line ILL2024 × susceptible line ILL6788) was studied to characterize genomic sources of tolerance to elevated soil boron toxicity in lentil (Rodda et al. 2018). A high-quality genetic linkage map was established with 758 markers that cover 1057 cM of lentil genome, and a single boron tolerance genomic region was identified which accounted for up to 76% of phenotypic variation. Another intraspecific linkage map was constructed containing 12 LGs with a total length of 1868 cM and identified genome regions associated with earliness and plant height using RILs derived from a cross between Eston × PI 320937 (Tullu et al. 2008).

Polanco et al. (2019) constructed a high-density interspecific (*L. culinaris* × *L. odemensis*) genetic map based on functional markers for mapping morphological and agronomical traits and resistance to ascochyta blight in lentil. SNPs and short indels were used to construct this map from a F_7 RIL population derived from the interspecific cross between *L. culinaris* and *L. odemensis*. The genome regions corresponding to a QTL governing time of flowering (chromosome 6), three QTLs controlling seed size (chromosomes 1 and 5) and three QTLs for *Ascochyta blight* resistance (chromosome 6) were identified.

3.5.6.2 Linkage Mapping from Multiple Mapping Populations (Consensus Maps)

While the conventional genetic linkage maps were created from a single mapping population, 'consensus maps' were created from multiple mapping populations. Consensus map offers various advantages including (a) higher marker density in single map and better genome coverage, (b) detection of the position of common markers across different mapping populations, (c) better assignment of linkage groups to chromosomes, (d) detection of conserved marker locus position, (e) identification of chromosomal rearrangements and degree of gene duplication, (f) comparison of genes of interest or QTLs across the maps and (g) creation of a basis for comparing genomes between related species (Ford et al. 2007).

Hamwieh et al. (2005) reported a comprehensive *Lens* map covering 715 cM, comprising 283 genetic markers by reconstructing the linkage map created by Eujayl et al. (1998). This map was based on microsatellite and AFLP markers. A total of 41 microsatellite and 45 AFLP markers were mapped using 86 RILs of ILL5588 × L692-16-1(s) cross. The map contained 283 markers spanning over 751 cM, with an average marker distance of 2.6 cM. Furthermore, resistance to the fungal disease, fusarium vascular wilt, was localized on the linkage group. Rubeena et al. (2006) constructed a consensus map by anchoring seven linkage groups with those of a previously constructed map (Rubeena et al. 2003) for tagging ascochyta blight resistance from two F_2 populations, viz. ILL5588 × ILL7537 and ILL7537 × ILL6002. This study demonstrated the transferability of QTLs among populations as markers were closely linked to the major QTL with a potential to future marker-assisted selection for disease resistance. Phan et al. (2006) used 126 cross-species markers from *Medicago truncatula* to generate comparative genetic maps of lentil and white lupin. Eventually, they used 18 common SSR markers to connect the new map with another already constructed comprehensive map in lentil by Hamwieh et al. (2005). They compared ESTs from the phylogenetically distant species, *M. truncatula*, *Lupinus albus* and *Glycine max*, and produced 500 ITAPs. The study reported 90%, 80% and 70% of the ITAP markers amplified genomic DNA in *M. truncatula*, *L. albus* and *L. culinaris*, respectively. The comparative map of *L. culinaris* was constructed based on 79 ITAP markers. The *L. albus* comparative map was developed from 105 gene-based markers together with 223 AFLP markers. Moderate chromosomal rearrangement was observed between *M. truncatula* and *L. culinaris* genomes, although a direct and simple syntenic relationship existed between the genomes. A population of 94 RILs at F_5 generation from a cross between ILL5588 × ILL5722 was used to construct a linkage map. The map clustered into 11 linkage groups covering 1156.4 cM of the genome, and 3 QTL regions were detected separately for each seedling and pod resistance that mapped to LG1 and LG9 and LG1, LG4 and LG5 linkage groups, respectively (Gupta et al. 2012a).

In another study on *L. ervoides* defence to few fungal pathogens, a population of 94 RILs at F_9 generation of a cross between two *L. ervoides* genotypes was used (Bhadauria et al. 2017). This high-density genetic linkage map developed from the comparative mapping between the genetic map of *L. ervoides* with *L. culinaris*

spanned 740.94 cM, and composite interval mapping detected five, six and three QTLs on chromosomes 1 and 5 controlling resistance to *Colletotrichum lentis* race 0, *C. lentis* race 1 and *Stemphylium botryosum*, respectively.

Recently, Ates et al. (2018) employed diversity array technology (DArT) markers to construct a consensus linkage map of lentil using three different lentil RIL populations (CDC Redberry × ILL7502, ILL8006 × CDC Milestone and PI320937 × Eston). The map had 9793 markers, covering a total of 977.47 cM distance with an average distance of 0.10 cM between adjacent markers and contained 7 linkage groups representing 7 chromosomes of the lentil genome. The mentioned studies are examples of genomic research within the last two decades. The available genomic datasets for lentil provide a powerful tool for crop improvement in lentil. Partial *L. culinaris* reference genome (v1.2, KnowPulse, Bett and Cook 2006) is available, and the whole reference genome of lentil can speed up and facilitate the genomic studies in this crop.

Until now, classical plant breeding approaches utilizing selection-recombination and selection cycles have contributed successfully to improve lentil crops. However, these approaches are inaccurate and time-consuming, particularly for improving complex quantitative traits. As we understand, the recent developments in molecular marker technologies have made it possible to localize genomic regions and assess their phenotypic effects on various quantitative traits. In lentil, several agronomic traits such as plant height, days to flowering, winter hardiness, pod dehiscence, growth habit and yield have been genetically dissected using both inter- and intraspecific populations (Taran et al. 2003; Kahraman et al. 2004; Fratini et al. 2007; Tullu et al. 2008). Similarly, QTLs for resistance to diseases like ascochyta blight, rust, anthracnose and stemphylium blight have also been mapped (Ford et al. 1999; Rubeena et al. 2006; Tullu et al. 2006; Gupta et al. 2012a, b; Sudheesh et al. 2016a, b; Bhadauria et al. 2017). Seed weight (Verma et al. 2015) and seed-related morphological and quality traits have been genetically mapped in lentil (Fratini et al. 2007; Fedoruk et al. 2013; Saha et al. 2013; Khazaei et al. 2017, 2018). Molecular markers linked to the QTLs identified in lentil are presented in Table 3.5, and these markers can be aid in targeted trait selection and improvement.

As explained earlier, quantitative traits have been mapped in lentil for the purpose of associating molecular markers with phenotypic traits. However, very few molecular markers are used in lentil breeding because many of the molecular markers are not reproducible in multiple populations (Ford et al. 2009). QTLs affecting earliness and plant height were identified on LG1, LG2, LG4, LG5, LG9 and LG12 at Saskatoon and Floral evaluation locations (Tullu et al. 2008). For days to flowering, a QTL (DTF1-d) explained phenotypic variation of 56.9%. Plant height QTLs explained a gross phenotypic variation of 95%, and a compact genomic region consisting of six QTLs for plant height and early flowering was detected within a map distance of 17.10 cM (Pote 2013). Similarly, Saha et al. (2013) identified map positions of some important agro-morphological traits including days to 50% flowering, plant height, seed diameter, 100 seed weight, cotyledon colour and growth habit in lentil. Three major QTLs governing seed diameter were mapped in lentil by applying random amplified polymorphic DNA markers by Fratini et al. (2007). Fedoruk

Table 3.5 Marker-trait association studies conducted in lentil

Trait	Marker(s) linked with associated QTL(s)	Phenotypic variation reaction explained by the QTL (%)	Reference
Ascochyta blight resistance	RAPD	90	Ford et al. (1999)
	RAPD, AFLP, ISSR	Up to 50	Rubeena et al. (2006)
	RFLP, AFLP	41	Tullu et al. (2006)
	ITAP, SSR, ISSR	Up to 61	Gupta et al. (2012b)
Earliness	RAPD, AFLP, SSR	37–46	Tullu et al. (2008)
Plant height	RAPD, AFLP, SSR	31–40	Tullu et al. (2008)
	RAPD, ISSR, AFLP, SSR, morphological markers	38.2	Fratini et al. (2007)
Branches at the first node	RAPD, ISSR, AFLP, SSR, morphological markers	91.7	Fratini et al. (2007)
Total number of branches	RAPD, ISSR, AFLP, SSR, morphological markers	54	Fratini et al. (2007)
Height at the first node	RAPD, ISSR, AFLP, SSR, morphological markers	33.3	Fratini et al. (2007)
Flowering time	RAPD, ISSR, AFLP, SSR, morphological markers	90.4	Fratini et al. (2007)
Pod dehiscence	RAPD, ISSR, AFLP, SSR, morphological markers	81.3	Fratini et al. (2007)
Seed weight	RAPD, ISSR, AFLP, SSR, morphological markers	18.2	Fratini et al. (2007)
Seed diameter	RAPD, ISSR, AFLP, SSR, morphological markers	37	Fratini et al. (2007)
Winter hardiness	RAPD, ISSR, RFLP	20.45	Kahraman et al. (2010)
Cotyledon colour class (cy)	SNP, SSR, colour loci	23	Fedoruk et al. (2013)
Seed thickness	SNP, SSR, colour loci	8.4	Fedoruk et al. (2013)
Seed diameter	SNP, SSR, colour loci	Up to 60	Fedoruk et al. (2013)
Seed plumpness	SNP, SSR, colour loci	Up to 50	Fedoruk et al. (2013)
Days to 50% flowering	SNP, SSR, colour loci	Up to 34	Saha et al. (2013)
Hundred seed weight	SSR, SRAP, RAPD	17.5	Saha et al. (2013)

(continued)

Table 3.5 (continued)

Trait	Marker(s) linked with associated QTL(s)	Phenotypic variation reaction explained by the QTL (%)	Reference
Plant height	SSR, SRAP, RAPD	15.3	Saha et al. (2013)
Seed diameter	SSR, SRAP, RAPD	32.6	Saha et al. (2013)
Stemphylium blight resistance	SSR, SRAP, RAPD	46	Saha et al. (2010)
Boron tolerance	SNP	71	Kaur et al. (2014)
Flowering time	SSR	57	Kahriman et al. (2015)
Seed weight and size	SNP	27.5–48.4	Verma et al. (2015)
Selenium content	SNP	6.3–16.9	Ates et al. (2016)
Drought tolerance	SSR	69.7	Singh et al. (2016)
Root and shoot traits	SNP, SRAP	27.6–28.9	Idrissi et al. (2016)
Days to 50% flowering	SSR	58–97	Kumar et al. (2018)
Plant height	SSR	24.0–47.0	Kumar et al. (2018)
100 seed weight	SSR	1.6–5.5	Kumar et al. (2018)
Days to maturity	SSR	99–133	Kumar et al. (2018)
Seed coat spotting	SNP, short indels	85.07	Polanco et al. (2019)
Stem pigmentation	SNP, short indels	33.96	Polanco et al. (2019)
Seed size	SNP, short indels	28.26	Polanco et al. (2019)
Flower colour	SNP, short indels	84.20	Polanco et al. (2019)
Flowering time	SNP, short indels	55.73	Polanco et al. (2019)
Ascochyta blight resistance	SNP, short indels	27.14	Polanco et al. (2019)

Adapted and modified from Kumar et al. (2015) and Kumar et al. (2018)

et al. (2013) observed that loci 16 for seed coat colour and pattern mapped to linkage groups 2 (Ggc), 3 (Tgc) and 6 (Scp) while the cotyledon colour locus (Yc) mapped to linkage group 1 in lentil. Verma et al. (2015) identified QTLs for the seed weight and size traits by single marker analysis (SMA) followed by composite

interval mapping (CIM) which resulted in one QTL each for the two traits (qSW and qSS) that were co-localized on LG4 and explained 48.4% and 27.5% of the phenotypic variance, respectively. Multiple QTLs for lentil seed diameter, thickness and plumpness were mapped in lentil via single-nucleotide polymorphism (SNP) markers (Fedoruk et al. 2013). The most stable and significant QTLs for seed diameter and plumpness were detected near the cotyledon colour locus (Yc), which explained 60% and 50% of the phenotypic variation for these traits, respectively, in that population (Fedoruk et al. 2013). Recently, these genomic regions were validated with a cultivated lentil association mapping panel (Khazaei et al. 2018).

The created genomic tools with mapping technology are the keys to MAS breeding strategies resulting in crop productivity and quality improvement for any crop. Despite the huge success in identifying QTLs controlling a wide variety of traits in lentil and the identification of the functional variants underlying these QTLs, the success of marker-assisted selection (MAS) for major genes in large public breeding programmes requires more efforts following examples from other major crops.

3.6 Conclusion

Towards improvement in lentil breeding programme, it is necessary to breed for multiple traits including seed yield and quality as well as resistance to abiotic and biotic stresses in order to develop more durable cultivars with superior grain quality to meet market demands under challenging environmental conditions. In the last two decades, significant efforts have been made to understand the genetics and genomics of lentil from wild and cultivated sources. Genomics-assisted breeding is relatively a powerful and fast approach to develop high-yielding cultivars adapted to different environmental conditions. Recent developments in the molecular tools including marker-assisted -selection, backcrossing, gene pyramiding and recurrent selection, and genome-wide selection have the potential for accelerated improvement in the effectiveness of breeding strategies.

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Chapter 4
Updates of Pigeonpea Breeding and Genomics for Yield Improvement in India

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4.1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], a resilient legume crop, belongs to subtribe Cajaninae which includes 12 genera. Genus *Cajanus* contains 32 species distributed in India and Australia (van der Maesen 1986). Pigeonpea is a perennial shrub, majorly cultivated as an annual crop in semi-arid tropical areas because of its buffering capacity against various non-favourable environmental conditions (Sharma et al. 2019). It provides sufficient quantity of proteins in the diet of poor people especially living in the Asian countries. In addition to nutritional food security, pigeonpea has good potential for sustainable agriculture to fulfil the demands of rapidly growing human population globally. Worldwide, it is grown on 7.03 million hectares with annual production of 4.89 million tonnes and productivity of 695 kg ha^{-1} (FAOSTAT 2017). India being a major contributor of pigeonpea produces nearly 64% of global production. Pigeonpea was domesticated nearly 3500 years ago from the wild ancestor *Cajanus cajanifolius*, and India is viewed as primary centre of origin of pigeonpea (Vavilov 1951; van der Maesen 1990; Varshney et al. 2017a). Recent evidences based on whole-genome re-sequencing (WGRS) data of 292 pigeonpea genotypes including landraces and wild relatives confirmed that pigeonpea spread out from India to sub-Saharan Africa and finally South America and Meso-America (Varshney et al. 2017b).

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Traditionally pigeonpea cultivars are grown for multiple usage, containing food (de-hulled split peas), fodder (fresh green leaves and silage), feed (powder of crushed dry seeds) and fuel wood (dry stems). Besides, pigeonpea being a member of leguminous family restores soil fertility by adding atmospheric nitrogen, organic matter and nutrients through leaf litter (Kumar Rao et al. 1983). Its deep root system has ability to withstand drought conditions and also enhances soil infiltration through breaking hard soil pan (Ae et al. 1990). Its ability to yield more with limited inputs/resources, pigeonpea remains the most suited crop of poor farmers in sustainable agricultural systems. In the early twentieth century, genetic improvement program in pigeonpea has been started through small-scale evaluation of field collections. These efforts led to identification of various disease-resistant landraces which were further utilized for improvement in productivity, but these attempts did not yield expected results (Mahta and Dave 1931; Shaw et al. 1933). Different research organizations, viz. Indian Council of Agricultural Research (ICAR), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Indian state agricultural universities, have initiated pigeonpea improvement during the second half of the twentieth century (Ramanujam and Singh 1981). Nearly 100 varieties of pigeonpea were released from different pigeonpea improvement programs which lead to increase in cropped area (+54%) and total production (+56%). Despite release of more than 100 pigeonpea varieties, crop productivity remains stagnated at around 700–800 kg/ha.

The present chapter offers recent updates on research in pigeonpea breeding programs, viz. history, constraints against yield improvement, available resources, recent approaches and genomic tools for pigeonpea improvement.

4.2 Pigeonpea Breeding and Improvement: A Retrospect

Pigeonpea breeding in India was initiated by parallel inputs of Shaw, Mahata and Dave in 1933 (Shaw et al. 1933). Shaw defined four types of plant habits in pigeonpea, whereas Mahata and Dave conferred early and late maturing lines with high-yielding capacity. After this, several initiatives were undertaken towards germplasm collection and evaluation for various beneficial traits. Pigeonpea germplasm has rich diversity, and a total of 13,771 lines are deposited at the ICRISAT gene bank, India (Gowda et al. 2013; Pazhamala et al. 2015); 11,221 entries are retained at National Bureau of Plant Genetic Resources (NBPGR), India (Singh et al. 2014; Pazhamala et al. 2015), whereas US Department of Agriculture (USDA), Kenya Agricultural Research Institute's National Gene Bank of Kenya (KARI-NGBK) and National Plant Genetic Resources Laboratory, Philippines, have secured around 4116, 1288 and 433 accessions, respectively (Singh et al. 2013; Pazhamala et al. 2015; Upadhyaya et al. 2016). To capture the entire genetic variation available in gene pool, concepts of core collection (1290 accessions) and mini-core collection of 146 accessions (Gowda et al. 2013; Pazhamala et al. 2015) and reference set (Upadhyaya et al. 2016) were adopted in pigeonpea. Nearly 80% of diversity in

gene pool was covered by representative of core collections (Reddy et al. 2005; Upadhyaya et al. 2006; Gowda et al. 2013).

Although pigeonpea has rich germplasm, its utilization in the breeding programs has remained limited (Pazhamala et al. 2015). In India, pigeonpea breeding gained momentum with the establishment of All India Coordinated Pulses Improvement Project (AICPIP) in 1967 by ICAR. This was followed by the creation of improved organizational structure in the form of All India Coordinated Research Project on Pigeonpea in 1996. More than 100 pigeonpea varieties have been released for cultivation in different agroclimatic zones of India since inception of AICPIP in 1967 (Singh et al. 2016).

4.2.1 Breeding Methods Followed for Pigeonpea Improvement

Under the umbrella of AICRP-Pigeonpea, various national research centres work together for the common goal at varied agroclimatic zones of India. Till date a total of 157 varieties including hybrids have been developed in pigeonpea through the network of AICPIP and AICRP following the breeding methods like selection, mutation, hybridization and heterosis breeding. Breeding efforts have met with significant success with the development of various high-yielding varieties with marked level of resistance to major biotic stresses such as disease like Fusarium wilt (FW) and sterility mosaic disease (SMD). The majority of pigeonpea-based cropping systems in India belong to medium (160–180 days) duration. The early maturing varieties (<140 days) are mainly cultivated in north-western plain zone (NWPZ) and parts of south and central India. In case of short duration pigeonpea, several varieties were released over the last decade for different zones, viz. CORG 9701 (south), GAUT 001E (central), TT 401 (central), TJT 501 (central), PA 291 (north-western plain zone: NWPZ), VLA 1 (NWPZ), Phule T 0012 (central), PRG 176 (Ujjwala) (Telangana state), VL Arhar 1 (ICPL 88039) (north hill region) and PAU 881 (NWPZ). The most widely cultivated varieties in the medium maturity group include ICPL 8863/Maruti (south), ICPL 87119 (Asha) (south and central), JKM 189 (central), BSMR 736 (Maharashtra), BSMR 853 (central) and BDN 711 (central). Bahar, NA1 and IPA 203 are the popular pigeonpea varieties in the late maturing group that are cultivated primarily in the north-eastern plain zone (NEPZ) of India. List of important pigeonpea varieties developed and released is given in Table 4.1.

4.2.1.1 Mutation Breeding

Mutation breeding was employed to generate variability since mid-1950s. Initial experiments were mainly aimed at observing morphological variations induced by mutagenesis. Further experiments were expanded to study effectiveness and efficiency of various physical and chemical mutagens. A wide array of economically

Table 4.1 Important varieties of pigeonpea developed and released in India

S. No.	Variety	Pedigree	Release year	Recommended area	Salient features
1.	TS 3 R	TS-3 × ICP8863	2011	Karnataka	Indeterminate, matures in about 150–155 days, grams, yield 12–16q/ha
2.	PKV, Tara (TAT-9629)	ICPL84008 × TT6	2011	Maharashtra	Indeterminate, about 180 days maturity, yield 18–20q/ha
3.	Rajeev Lochan	Sel. from ICPL 92060	2011	Chhattisgarh	Indeterminate, yields about 18q/ha
4.	VLA 1	ICPL 6 × pant 2	2011	Uttarakhand and NWPZ	Indeterminate
5.	WRG-65	WRG-13 × ICPL-87051	2012	Andhra Pradesh	Indeterminate, matures in about 160–165 days
6.	BDN 711	Sel. From BPG 111	2012	Maharashtra	Indeterminate, matures in about 155–160 days, white seeded, yields about 16q/ha
7.	RGT 1	Allapurlocal×SMR853	2012	Andhra Pradesh	Semi-determinate, yields about 16–20q/ha
8.	Phule T 0012	ICP 332 × BSMR 736	2013	Central zone	Indeterminate, matures in about 150–155 days
9.	BRG 4	BRG 2× ICPL 99046	2014	Karnataka	Indeterminate, yields about 15–16q/ha
10.	IPA 203	(Bahar× AC 314) × AC314	2015	NEPZ	Indeterminate, matures in about 250 days, yields about 25q/ha
11.	GRG811 (Dharmaraj)	ICP 8863 × ICPL 96058	2015	Karnataka	Indeterminate, matures in about 165 days, yield 13–16q/ha
12.	TDRG-4	ICPL332 × ICP8863	2015	Telangana	Indeterminate, matures in170–180 days
13.	LRG52	LRG41 × ICP8863	2015	Andhra Pradesh	Indeterminate, 170 days to mature

(continued)

Table 4.1 (continued)

S. No.	Variety	Pedigree	Release year	Recommended area	Salient features
14.	Rajendra Arhar-1	Pusa-9 × ICP8863	2015	Bihar	Indeterminate, matures in about 260–270 days, yields about28–30q/ha
15.	GJP-1	LRG 41 × BDN 2	2016	Gujarat	Indeterminate, yields about 20q/ha
16.	PRG 176	ICPL88039 × ICPL88034	2016	Telangana	Indeterminate, matures in about 130–135 days
17.	GT103	MS Pusa 33 × UPAS 120	2016	Gujarat	Indeterminate, yields about 15–16q/ha
18.	BDN716	BSMR 736 × BSMR 198	2016	Maharashtra	Indeterminate, maturity 160–165 days
19.	PAU 881	H 89–5 × ICPL 85024	2007/ 2017	Punjab/NWPZ	Indeterminate, early maturing in about 132 days, yields about 13–14q/ha
20.	AL 882	Pusa 992 × ICPL 85024	2018	Punjab	Determinate, early maturing in about 132 days, yields about 13–15q/ha
21.	IPH15–3	PA163A × AK250189R	2019	NWPZ	Indeterminate, 150 days maturity, yields about 15q/ha
22.	WRGE93	PRG100 × ICPL84031	2019	SZ	Indeterminate, maturing in 150–165 days, yields about 16–17q/ha
23.	GRG152	JamadarLocal2 × TS-3R	2019	CZ	Indeterminate, 160–165 days maturity, yields about 20.0q/ha
24.	PA6	ICPL84023 × ICPL88039	2019	NWPZ	Indeterminate, 150 days maturity, yields about 16q/ha
25.	MPV106	MPV10 × MPV15	2019	SZ	Indeterminate, 170 days maturity, yields about 15 q/ha

useful mutants, both induced and spontaneous, such as resistant to key diseases, male sterility, short duration, plant type, seed size and plant height, have been reported. Some of them have been either released directly as variety or used as donor for improving traits. Only four varieties, viz. CO3 (mutant of CO1) round seeded in 1977, TT 5 (mutant of T 21) bold seeded in 1983, TT 6 (mutant of T 21) bold seeded in 1984 and CO6 (mutant of SA1) bold seeded in 1991, could be developed through mutation breeding.

4.2.1.2 Varietal Improvement Through Selection

Pigeonpea is an often cross-pollinated crop, and cross-pollination varies from 5% to 70% which depends upon the location of field, population of honey bees, temperature and other climatic factors. This cross-pollination creates a lot of variability with respect to different qualitative as well as quantitative traits which provides ample opportunity for selection with in a population. In the beginning of pigeonpea improvement program, most of the varieties were developed by employing the selection method.

4.2.1.3 Varietal Improvement Through Hybridization

After a long period of following the selection method of breeding, the need of hybridization was felt by breeders because of low variability within particular maturity group, incorporation of specific traits like disease resistance in good agronomic bases, combining yield components and exploitation of combining ability of parental lines of interest. List of high-yielding varieties developed and recommended for different states of India is given in Table 4.2.

4.2.1.4 Pigeonpea Improvement Through Heterosis Breeding

Availability of male sterile system was required for the large-scale production of hybrid seeds. So, the efforts started for the identification of male sterility systems in pigeonpea at ICRISAT. The main search for male sterility systems in pigeonpea is done at ICRISAT by screening germplasm to identify male sterile plants. Concerning hybrid breeding in pigeonpea, genic male sterility (GMS) and cytoplasmic male sterility (CMS) have been discovered to support hybrid development. GMS has been reported to be controlled by nuclear genes of recessive nature. In pigeonpea, Deshmukh (1959) reported the first instance of male sterility as a spontaneous mutant. These efforts helped to identify the accession with genetic male sterility having translucent anthers, and sterility was controlled by single recessive gene ms_1 (Reddy et al. 1978). One more source of genetic male sterility associated with brown anthers and regulated by non-allelic single recessive gene ms_2 was identified (Saxena et al. 1983).

Table 4.2 Pigeonpea varieties recommended for different states

State	Recommended varieties
Andhra Pradesh	LRG 38, LRG 41, LRG 52, CORG 2012-25, WRGE 93, MPV 106
Bihar	Bahar, DA 11, Pusa 9, MA 6, MAL 13, Rajendra Arhar 1, IPA 203
Chhattisgarh	ICPL 87119, Rajeev Lochan, Rajeshwari, GRG 152
Delhi	Pant A 291, Pusa 992, PA 16, PAU 881
Gujarat	AGT 2, GT 102, GT 103, GT 104, GJP 1, GNP 2, Rajeshwari, GRG 152
Haryana	Pant A 291, Pusa992, PAU 881, PA 6, IPH 15-3
Karnataka	ICP 8863, ICPL 87119, WRP 1, TS 3 R, GRG 811, CORG 2012-25, BRG 2, BRG 4, BRG 5, BRG 3, Co 2012-25, WRGE 93, MPV 106
Madhya Pradesh	ICPL 87119, TT 401, Gwalior 3, MA 3, JKM 189, TJT 501, Rajeshwari, GRG 152
Maharashtra	ICPL 87119, BDN 708, BDN 711, BDN 716, TT 401, Rajeshwari, Vipula, AKT 881, PKV Tara, GRG 152
Punjab	Al 15, AL 201, Pusa 992, PAU 881, AL 882, PA 6, IPH 15-3
Rajasthan	UPAS 120, VLA 1, PAU 881, PA 6
Tamil Nadu	CO 6, Co RG 9701, CoRG 2012-25, WRGE 93, MPV 106
Telangana	WRG 65, RGT 1, PRG 176, TDRG 4, CoRG 2012-25, WRGE 93, MPV 106
Uttar Pradesh	PA 291, Pusa992, UPAS 120, Bahar, MA6, MAL 13, IPA 203, IPA 206, IPH 15-3, PA 6
West Bengal	WB 20,B 7, B 517, Pusa 9, IPA 203

Similarly, Saxena and Kumar (2003) found a genetic male sterile mutant that was selected from an inbred population of cultivar ICPL 85010. Three genes, namely, *ms1*, *ms2* and *ms3*, have been reported in pigeonpea for imparting genic male sterility (Saxena et al. 2010). The first hybrid using GMS system was ICPH 8 (ICPH 82008), which was developed and released in 1991 for commercial cultivation. Hybrid ICPH 8 was developed by crossing ms Pabhat DT line with ICPL 161. The male sterile line ms Pabhat DT was developed by introgressing *ms1* gene from the pigeonpea genotype MS 3A to the pigeonpea genotype Prabhat as a result of backcrossing. Having semi-spreading and indeterminate growth habit, the hybrid ICPH 8 matured in 142 days and offered 41% yield advantage over the pigeonpea cultivar UPAS 120 (http://oar.icrisat.org/561/1/ PMD_40.pdf). The Punjab Agricultural University, Ludhiana, also developed GMS-based hybrid PPH 4 in 1994. Later, some more GMS-based hybrids, namely, AKPH 4101, AKPH 2022 and CO PH 1, were released, respectively, in 1997, 1998 and 1997 in India (Table 4.3). But even with high yield potential, these could not become popular at farmer's field due to difficulty in seed production. Through extensive studies in this area, cytoplasmic genetic nuclear male sterility (CGMS) system was identified in pigeonpea which helped to overcome the constraints of GMS system. CGMS systems have played a significant role to improve the productivity of many economical crops through the use of hybrid vigour. So, to break the yield plateau, CMS-based hybrid technology has been successfully developed in pigeonpea (Saxena et al. 2010b). Recent discovery of stable cytoplasmic nuclear male sterility (CMS) and fertility restoration

Table 4.3 Pigeonpea hybrids based on genetic male sterility

Hybrid	Days to maturity	Plant type	Yield (q/ha)	Adaptability	Year of release
ICPH8	125	Indeterminate	17.8	Central zone	1991
PPH4	137	Indeterminate	19.3	Punjab	1994
COPH1	117	Indeterminate	12.1	Tamil Nadu	1994
COPH2	125	Indeterminate	10.5	Tamil Nadu	1997
AKPH4101	135	Indeterminate	20.0	Central zone	1997
AKPH2022	180	Indeterminate	18.0	Maharashtra	1998

system has caused a shift towards development of CMS hybrids in pigeonpea (Bohra et al. 2017a).

In pigeonpea, out of nine CMS systems, only A_2 CMS from *C. scarabaeoides* and A_4 CMS from *C. cajanifolius* have been utilized in hybrid development for the exploitation of heterosis in hybrid breeding programs. A_4 CMS is not stable under Punjab conditions, while A_2 CMS showed great promise because its stable expression and early maturing CMS lines have been developed which were found stable for male sterility across the locations under different growing environments as well as under different temperatures and photoperiods (Sandhu et al. 2015). The frequency of fertility restorers for both the CMS systems is higher than the other CMS systems. The availability of CMS systems in pigeonpea has helped to solve the problem of manual emasculation and crossing, which increases the efficiency and production of commercial hybrid seeds.

4.3 Constraints for Yield Improvement

Breeding in pigeonpea has always been the biggest bet for breeders. The inherent crop-specific constraints are detailed below:

4.3.1 Lack of Genetic Diversity

The polymorphic studies on *Cajanus* accessions revealed that the limited scope of genetic diversity within the primary gene pool shifted the research interests towards exploitation of wild relatives from secondary, tertiary and quaternary gene pools through suitable gene transfer techniques. Although wild relatives were genetically diverse, yet the lack of required information about their gene pool was the biggest obstacle for direct use in breeding program, and prolonged research inputs are required for the implementation in breeding program (Goodman 1990). The involvement of wild relatives in breeding program has many lacunas including lagging genetic enhancement, poor agronomic traits, incomplete characterization and limited collections (Saxena et al. 2014).

4.3.2 Photoperiod Sensitivity

Flower induction in pigeonpea depends on shorter light period and long hours of darkness (Silim et al. 2007; Vales et al. 2012). Along with photoperiod, initiation of flowering also depends on relative day and night temperature. This limits pigeonpea cultivation zone beyond 30° northern and southern latitudes (Saxena 2008). Various studies revealed the inverse correlation between photosensitivity and earliness (Wallis et al. 1981). Pigeonpea cultivation at regions of higher altitude and latitude is restricted by its photoperiod and low-temperature sensitivity (Turnbull et al. 1981) which limits its utilization in alternative cropping system (Vales et al. 2012).

4.3.3 Linkage with Undesirable Traits

The target gene transfer into cultivated varieties/elite lines is generally hindered by linkage of desirable traits with undesirable traits, for example, it took 12–14 generations to transfer the high protein genes from *C. scarabaeoides* and *C. albicans* to the cultivated background while maintaining productive phenology and high yield (Saxena and Sawargaonkar 2015).

4.3.4 The Issue of Yield Plateau

The major reason for low acceptability of pigeonpea in food cultivation program is lack of stable lines with high yield in varied environmental conditions. More than 100 varieties of pigeonpea have been released; however the productivity is dwindling below 1000 kg/ha (Singh et al. 2016). Presently many long-term and short-term schemes including breeding new-generation inbred and hybrid cultivars are released under the National Food Security Mission of India to overcome the yield threshold. This would perhaps help in shattering the bar of yield plateau in pigeonpea.

4.3.5 Harvest Index

To attain the expected harvest index, breeding efforts were diverted towards determinate cultivars as traditional pigeonpea cultivars had low harvest index. Inefficiency in terms of increased harvest index was observed by implementing both determinate and indeterminate cultivars as observed by Sheldrake and Naraynan (1979). The indeterminate lines show different performance with respect to the environmental condition, as these overpower the harvest index of determinate lines in climatic zones where growing season was long but its reversal was observed in environment with shorter

growing seasons (Chauhan et al. 1998). Although when yield is measured along with harvest index, a negative correlation is observed between the two which makes it quite difficult to focus separately on harvest index. So, there is need to focus on both, harvest index and yield, without compromising either of it (Chauhan et al. 1995).

4.3.6 Genetic Control of Stresses

Pigeonpea growth and sustainable yield is affected by multiple stress conditions prevailing in the natural environment. These stress conditions include both biotic and abiotic factors. Biotic stress including insect (mainly pod borers and pod fly) and diseases (mainly wilt and sterility mosaic) influenced the pigeonpea breeding and development. Success of the breeding program is dependent on the genetic resistance against insects which is reported to be either lacking or is of very low level. Overall there is lack of information for genetic control against insect resistance, whereas inheritance pattern of wilt resistance is well established. Although certain genes are identified which control wilt resistance, further research is needed to identify different biotypes of *Fusarium udum* and inheritance pattern of its resistance gene(s) to facilitate the wide range breeding program for development of resistant cultivars (Saxena et al. 2014). Next to wilt, sterility mosaic virus (SMV) is another threat in pigeonpea breeding. Resistance for SMV is governed by a recessive gene, but information regarding its mode of inheritance is obscure (Saxena and Sharma 1990). Cultivars for wilt and sterility mosaic have already been incorporated in various breeding programs covering 60–70% cropped area, especially Maruti and Asha which are two successful pigeonpea cultivars with exquisite role in eradicating wilt and sterility mosaic diseases in certain parts of India. Among the abiotic stresses, drought and waterlogging are two major challenges in pigeonpea breeding. Very scanty information is available regarding drought tolerance, and physiological parameters related to drought tolerance are not well understood in pigeonpea. Waterlogging is another abiotic stress which retards the yield in pigeonpea. Waterlogging tolerance involves metabolic adaptations which vary with species, plants and tissues. Physiological and morphological traits combating the stress induced by waterlogging are well known which include parenchyma cells, lenticels and adventitious root. These traits and gene regulating their inheritance can be tracked down to develop waterlogging tolerant varieties.

4.3.7 Genetic Contamination of Seed Purity

Seed purity proves out to be the major limitation in pigeonpea breeding among all other obstacles discussed so far. Pigeonpea is known for insect-mediated outcrossing which deteriorates seed purity with every generation, and it is a resource-exhaustive process to maintain genetic purity of seed for a particular trait. Chances of trait dilution due to outcrossing are more if target trait is governed by recessive gene (Saxena

et al. 2016a). Bahar, a high-yielding and SMV-resistant variety, is a good example which lost its desired trait over generations due to lack of proper seed maintenance. There are examples where the outstanding disease-resistant cultivars have become highly susceptible over a period of a few years in the absence of good seed management system. Another important trait is maturity which needs proper seed management. The cultivar UPAS 120 was released as an early maturing (120 days), but due to unrestricted breeding, its maturity is augmented by almost 30 days which is hampering the perspective of pigeonpea – wheat rotation. Therefore, out of all constraints described so far, prior need is to establish controlled breeding strategies and maintain seed purity to sustain the breeding outputs in pigeonpea.

4.4 Genetic Resources Available

Genetic diversity is boon in any breeding program, but the collection of germplasm, its maintenance and consistent up gradation is mandatory for applied research outcomes. Pigeonpea accessions are contributed by almost 52 African, Asian and Latin American countries. Besides these, approximately 47 wild relatives of pigeonpea are identified and restored for further utilization as per research thrust in coming era. The ICRISAT gene bank has maintained 555 accessions of 67 wild species belonging to 6 genera (Kumar et al. 2011; Upadhyaya et al. 2011; Sharma et al. 2013; Pazhamala et al. 2015). India came forward as a primary centre for collection of primary pigeonpea germplasm which offered a wide range of scope in terms of morphological, nutritional and physiological traits Upadhyaya et al. 2016). The major trait around which pigeonpea germplasm classification revolves is maturity period which ranges from <90 to >250 days, and this trait is important for adaptation in accordance to different agroclimatic conditions. For exploiting the variability in maturity period, the diverse accessions are classified into 11 maturity groups which can be used in varied permutation and combination as per target of the respective breeding program for this trait (Saxena 2008). Along with maturity, these primary genetic resources consist of lines with diversity in several other traits including cytoplasmic nuclear male sterility, floral variants, genetic male sterility and biotic and abiotic resistance. The aim of collection and further classification of the collected accessions is to provide genetic resources which best suit to the required research for which ICRISAT divided germplasm into subsets of core, minicore and reference sets (Upadhyaya et al. 2016). The mini-core collection came up with entries resistant to different stress conditions including waterlogging (23), pod borer (10), salinity (16), wilt (6), sterility mosaic (24) and pod borer (14). Along with resistance, sub-groups were formed for yield increase (54) and biofortification such as high zinc (15) and high iron (15). NARS has done parallel efforts in identification and restoration of trait-specific germplasm such as wilt resistance (39), SMD and wilt resistance (24), early maturity (8) and high yield (2) (Upadhyaya et al. 2016). Overall these genetic resources act as reference point for targeted research in pigeonpea.

4.4.1 Genetic Information

Genetic information is relevant in predicting the performance of an introgressed trait in response to different environmental conditions. Several traits which are important for pigeonpea breeding have direct or indirect interactions with biological events, other traits and environmental factors. The complex mode of inheritance patterns and associated pleiotropic effects of target trait generates the need of proper genetic information which can be implemented by breeders to design breeding programs for development of new varieties. Pigeonpea genome is diverse, but there is sparse knowledge regarding cumulative inheritance of quantitative traits and their interaction with environment and the natural outcrossing that leads to heterozygosity of desired traits, adversely affecting the targeted breeding approaches. It is well defined that agronomic characters in pigeonpea are controlled by genes with additive and nonadditive effects, whereas yield and yield-related traits are affected by major genes with additional pleiotropic effects (Green et al. 1981; Saxena and Sharma 1990).

4.4.2 Screening Technologies for Key Stresses

There is a strong need to have screening techniques for biotic and abiotic stresses as the pigeonpea cultivation is adversely affected by various stresses. In this endeavour ICRISAT has developed efficient field screening methods to identify lines susceptible to wilt and sterility mosaic virus (Nene et al. 1981). For screening against phytophthora blight, a screening technique using pot culture has also been developed (Mamta and Ghosh 2016). Techniques for screening against abiotic stresses like waterlogging and salinity were also developed, especially for screening against salinity by giving salt treatment at early stages (Subbarao et al. 1991; Chauhan et al. 1997; Srivastava et al. 2006; Sultana et al. 2013; Singh et al. 2016). Screening for drought tolerance is not reliable as several traits are governing drought tolerance, so there is a need to refine screening techniques for such traits.

4.4.3 Cytoplasmic Nuclear Male Sterility Systems

Cytoplasmic-nuclear male sterility (CMS) is the result of interaction between cultivated nuclear genome and wild cytoplasm. CMS system is already used in many crops to exploit the hybrid vigour (Bohra et al. 2016). Initial attempts by Reddy and Faris (1981) led to the development of CMS lines in pigeonpea through utilizing cytoplasm of *C. scarabaeoides*. A number of CMS lines have been identified after intensive selections and backcrossing (Saxena 1996). Nine CMS systems (A_1–A_9) have been developed till date in pigeonpea (Table 4.4). These systems have

Table 4.4 List of CMS sources derived from different *Cajanus* species

Sr. No.	Wild relative	Designation	Source
1	*Cajanus sericeus*	A_1	Ariyanayagam et al. (1995)
2	*Cajanus scarabaeoides*	A_2	Tikka et al. (1997)
3	*Cajanus volubilis*	A_3	Wanjari et al. (2001)
4	*Cajanus cajanifolius*	A_4	Saxena et al. (2005)
5	*Cajanus cajan*	A_5	Mallikarjuna and Saxena (2005)
6	*Cajanus lineatus*	A_6	Saxena et al. (2010a)
7	*Cajanus platycarpus*	A_7	Mallikarjuna et al. (2006)
8	*Cajanus reticulatus*	A_8	Saxena (2013)
9	*Cajanus lanceolatus*	A_9	Srikanth et al. (2014)

differences in stability of sterility and fertility restoration (Singh et al. 2017). Out of these, A_2 and A_4 CMS systems are well studied and commercially utilized for the development of pigeonpea hybrids in India (Saxena 2013).

4.4.4 Natural Cross-Pollination

Outcrossing is a natural constrain while maintaining seed purity in pigeonpea breeding, but it has a positive role in hybrid seed production. In pigeonpea the extent of outcrossing varies from place to place as it ranges from 0–48.0% in India, 13.0–70.0% in Kenya and 14.0–19.6% in Sri Lanka (Saxena et al. 2016a). Overall cross-pollination can act as boon when used for hybrid seed production, but its interference with maintenance of the seed purity should be monitored regularly.

4.5 Approaches and Accomplishments of Pigeonpea Breeding

4.5.1 Hybrid Breeding

In the past, pedigree selections were exploited to breed high-yielding cultivars, but unfortunately, the released varieties did not show significant gains in productivity. To break the yield plateau, much focus is devoted to hybrid pigeonpea breeding technology (Saxena et al. 2015) through the development of number of CMS lines along with their maintainers and fertility restorers. Initially, a set of hybrids was generated through natural outcrossing, and further these were evaluated in multilocation trials. Gujarat Agricultural University was the first to develop the first early maturing CMS-based hybrid (GTH 1) with the yield advantage of >50% over the control and 25.3% standard heterosis in the on-farm demonstrations. As mentioned in the earlier section, several hybrids were developed in India by using GMS

system; however despite high yield, these could not become popular at farmer's field due to difficulty in seed production.

The CMS system was identified in pigeonpea through extensive research that has helped to overcome the limitations of GMS system. Following release of the first CMS hybrid GTH 1 in 2007, some superior hybrids (ICPH 3491, ICPH 3497 and ICPH 3481) were developed with >40% standard heterosis and evaluated in diverse environments. The CMS-based pigeonpea hybrid ICPH 2671 was developed at ICRISAT with the 35% yield advantage over the control cultivar, i.e. Maruti and released for Madhya Pradesh (Saxena et al. 2016a). Later on, with this accomplishment, two more hybrid cultivars (ICPH 3762 and ICPH 2740) were developed and released for Odisha and Telangana with 40% yield advantage over the check cultivars (Saxena et al. 2016b). More recently, the CMS hybrid IPH 15-03 has been developed by ICAR-Indian Institute of Pulses Research (IIPR) for cultivation in the NWPZ region. This hybrid has shown 28.3%, 55.2% and 31.91% superiority over the checks Pusa 992, PAU 881 and ICPL 88039, respectively.

4.5.2 Breeding for Biotic Stresses

The biotic stresses in pigeonpea include fusarium wilt, sterility mosaic and phytophthora blight diseases and pod borers. Research efforts made towards breeding for these stresses were discussed below.

4.5.2.1 Fusarium Wilt

Fusarium wilt is a potent disease which usually appears at seedling stage but spreads during flowering and podding stage ultimately causing 30–100% yield loss in pigeonpea. The disease symptoms for wilt are similar to that of drought even when enough moisture is present in soil. In pigeonpea a single dominant gene is reported to govern resistance against fusarium wilt. Breeding strategies involving landraces in hybridization followed by pedigree selection have given exquisite results for disease resistance. In India, Maruti was one of the wilt-resistant varieties which were well accepted by farmers of north Karnataka and southern Maharashtra, with adoption rate of approximately 60%. Besides Maruti, Asha (ICPL 87119) is another disease-resistant variety cultivated in India. Conventional breeding approaches are continuously implemented to identify more such varieties with combination of other important traits by screening of 976 breeding and germplasm lines in wilt-sick plot. The output of this work carried out in ICRISAT for the identification of several promising wilt-resistant genotypes, which lead to identification of ICPL 20109, ICPL 20096, ICPL 20115, ICPL 20116, ICPL 20102 and ICPL 20094 as resistant genotypes (Sharma et al. 2016). Fusarium wilt is known to inflict pigeonpea production in eastern and southern parts of Africa, but the pattern of virulence was much different from that of Asia, and the most adaptable pigeonpea variety which has

revolutionized breeding is “Nandolo Wanswana”, a wilt-resistant selection from a Tanzanian landrace (ICP 9145). It occupies 60% areas in Malawi region. The virulence pattern existing in Eastern South Africa (ESA) is entirely different from that of Asia, and a greater range of resistance against fusarium wilt is offered by cultivars from ESA. This diverse range of germplasm for wilt resistance is obtained by continuous screening in wilt-sick plot at different regions including Kenya, Malawi and Tanzania, and these genotypes are supposed to be used as donors in breeding programs in different agroclimatic zones.

4.5.2.2 Sterility Mosaic Disease

Sterility mosaic disease (SMD) in pigeonpea can cause up to 100% yield loss at severe conditions. A single eriophyid mite (*Aceria cajani*) is vector which carries and transmits pigeonpea sterility mosaic virus (PPSMV). A major identification feature of disease is the presence of patches of pale green plants without reproductive growth. Inheritance of resistance for SMD is governed by two duplicate dominant genes (Sv1 and Sv2) and two duplicate recessive gene (sv3 and sv4) present at four independent loci (Saxena 2008). SMD resistance is obtained when either of two dominant gene and both recessive genes are present at their respective locus (Saxena 2008). The spray of acaricides has proven to be beneficial to control the mite population, but detailed information regarding genomic segments attributing disease resistance will be further beneficial if implemented in genomic-assisted breeding (Saxena et al. 2017a).

4.5.2.3 Phytophthora Stem Blight Resistance

It is a soil borne fungus *Phytophthora drechsleri* f.sp. *cajani* that can survive as dormant mycelium in soil. There is 98% yield loss reported by phytophthora as the diseased plant dries up rapidly (Pal et al. 1970). Phytophthora is usually sporadic, but in places of high moisture in soil due to frequent rainfall, it attains epidemic properties (Bisht et al. 1988). Resistance for this fungal disease is governed by single dominant gene Pd1 (Saxena 2008). Sick plot screening method proved out to be best for screening large pigeonpea germplasm (Singh and Chauhan 1992).

4.5.2.4 Pod Borers

Pigeonpea cultivation is vulnerable to almost 150 insect species which include legume pod borer, *Helicoverpa armigera* Hübner, spotted pod borer, *Maruca vitrata* Geyer, pod fly and pod sucking bug, *Clavigralla gibbosa* Spin. These insects are known to infest pigeonpea crop standing in field, whereas the bruchids, *Callosobruchus chinensis* L., cause extensive losses in storage. In India average production loss is 30% due to insect infestation on pigeonpea. Among all the insects,

Helicoverpa armigera is the most catastrophic pest negatively affecting pigeonpea cultivation from ages. There is a need to establish a reliable resistant source for increasing productivity. However, there is very less genetic diversity for *Helicoverpa* resistance among cultivated gene pool of pigeonpea. To tackle this problem, conventional screening methods are used to identify insect-resistant varieties. Although screening process is hindered by various factors including variation in flowering time of different pigeonpea cultivars and variation in insect population with space and time. Different techniques are used to overcome these problems for effective screening of resistant pigeonpea cultivars including planting times and use of hotspot locations, grouping the test material according to maturity and height, augmenting insect populations in the field, tagging the plants/inflorescences, artificial infestation in the field, caging the plants with insects in the field, detached leaf assay, etc. Many morphological (trichomes, cell wall lignification, branching and podding habit, and pod wall hairs and trichomes) and biochemical factors (secondary metabolites) are reported to enhance resistance, which can be used as additional screening parameter for rapid detection of resistant plant material with benefit of overpowering variation due to environmental factors and insect density. Implementing all these factors, ICRISAT has screened >7000 germplasm for insect tolerance especially against *Helicoverpa* and identified only a few promising accessions, but their stability over different agroclimatic conditions was a major concern. In spite of all these issues, Abhaya (ICPL 332) was released as first medium maturing *Helicoverpa*-resistant genotypes by joint efforts of ICRISAT and ICAR. ICPL 332 has shown about 30% decrease in pod damage as compared to normal cultivars in insecticidal free trials for 3 years, which effectively increased the yield. The major approach for developing insect-resistant varieties was to shift towards wild relatives for sources of resistance to insects. Wild relatives of pigeonpea such as *C. scarabaeoides* (L.) Thouars, *C. sericeus* (Benth. ex Baker) Maesen and *C. acutifolius* (F. Muell.) Maesen are highly resistant to *H. armigera* (Mallikarjuna et al. 1997; Jadhav et al. 2012). Attempts have also been made to transfer pod borer resistance from the wild relatives into the cultivated background at PAU, Ludhiana. In addition to productivity enhancing traits, resistance to *Maruca vitrata* was also introgressed from wild *C. scarabaeoides* (ICP 15683) into cultivated pigeonpea (ICPL 20329). A good number of interspecific derivatives having higher level of resistance to pod borer (*Maruca vitrata*) were identified. Derivative having higher number of fruiting branches and pods per plant showing variation for foliage colour was also identified (Figs. 4.1 and 4.2). These derivatives are being utilized in breeding program to develop high-yielding pod borer-resistant cultivars (Singh et al. 2018).

Gene pyramiding for two or more insect-resistant genes is one of the options for insect resistance breeding program. Along with these breeding strategies, transgenic approach was also exploited for insect resistance, and target genes for this approach include cry1Ab (Ramu et al. 2012). Gene pyramiding with two different insecticidal genes and tissue-specific expression to reduce the risk of developing insect resistance is another attractive option to combat this pest for durable resistance. Expression of a chimeric cry1AcF (encoding cry1Ac and cry1F domains) gene in transgenic pigeonpea has been demonstrated towards resistance to *H. armigera*.

Fig. 4.1 Interspecific derivative between *Cajanus scarabaeoides* and *C. cajan* with light foliage, higher fruiting branches and pods per plant

Apart from this, an advanced generation population derived from a cross with secondary gene pool wild relative, *C. acutifolius*, as the pollen parent has shown considerable resistance against for pod borer.

4.5.3 Breeding Strategies to Combat Abiotic Stresses

Abiotic stresses including various environmental conditions which are under consistent changes warrant the need of designing varieties which can adapt to the broad range agroclimatic conditions. In pigeonpea abiotic stresses have severely reduce productivity. The target of breeding program is to develop varieties which are well suited to varied intensity and periodicity of stresses and have sustained productivity for coming years. To screen the germplasm for any stress tolerance, various parameters must be measured including stress susceptibility index, stress tolerance index and productivity under stress conditions. Along with these, superficial genetic factors which are regulating abiotic stress tolerance are important as they lay down the key strategies for developing varieties against stress. The abiotic stresses which mainly affect pigeonpea include drought, temperature, salinity and waterlogging (Araujo et al. 2015).

Fig. 4.2 Interspecific derivative between *Cajanus scarabaeoides* and *C. cajan* with dark foliage, higher fruiting branches and pods per plant

4.5.3.1 Drought Tolerance

Pigeonpea has deep root system which makes it less vulnerable to drought stress as compared to other pulses; however in light soils, terminal and intermittent stress adversely affects pigeonpea yield which can be combat by irrigation (Chauhan et al. 1987). A lot of research programs are already conducted to completely elucidate the molecular mechanism of drought tolerance and influence of climatic variation on intensity of drought stress, but the outcome of all these studies is presently inconclusive (Saxena et al. 2015). The physiological effects of drought stress in pigeonpea include decreased light interception, yield reduction and imbalance in dry matter partitioning (Lawn and Troedson 1990). The extreme moisture content is also observed to negatively affect the symbiotic nitrogen fixation (Kumar et al. 2014). The severity and duration of drought stress decides the extent of yield loss, such as if drought condition is terminal it will lead to flower drop which will thereby decrease pod setting (Muchow 1985; Chapman and Muchow 1985). Several mechanisms are observed to work in coordination for drought tolerance including osmotic adjustment, dehydration tolerance, decrease leaf area, reduced transpiration rate, etc. So, present information regarding drought tolerance in pigeonpea is not

sufficient for direct use in breeding programs. The conventional breeding for drought tolerance needs to focus on selection for accumulation of multiple traits in a single variety which includes pods/plant, seeds/pod, seed size and seed yield/plant (Choudary et al. 2011) along with established root system. Along with it, another preferable method is to shift towards developing varieties with drought escape mechanism where maturity duration matches with the soil moisture content. Genetic mechanism regulating drought tolerance in pigeonpea was defined, and it has been reported that a set of 10 genes regulates the expression of almost 51 drought-responsive proteins including universal stress proteins A(uspA)-like protein, Cation/H (+) antiporter proteins, uncharacterized proteins and U-box proteins (Sinha et al. 2016). Similar studies conducted by Varshney et al. (2012) lead to identification of almost 111 proteins having their role in combating drought stress (Pazhamala et al. 2015).

4.5.3.2 Waterlogging

Waterlogging is defined as a situation where soil is saturated with water. It is reported that pigeonpea seedlings are vulnerable to waterlogging stress (Sultana et al. 2013). It is observed that plants develop specific traits to combat waterlogging stress such as development of parenchyma and lenticels which expedite oxygen supply to plants (Hingane et al. 2015). A wide range of genetic variation exists in the pigeonpea to adapt for waterlogging condition (Sultana et al. 2013; Singh et al. 2016). Pigeonpea germplasm was screened for waterlogging tolerance, and several genotypes with waterlogging adaptation for longer period of time were identified (Sultana et al. 2013; Singh et al. 2016). The promising genotypes were AH-06-8, AH-07-74, ICPL 332 ICP 8859, AL 1843 and PAU 881. Along with it, certain genotypes had traits for both salinity and waterlogging tolerance (Singh et al. 2016). Investigation on the inheritance pattern for both salinity and waterlogging revealed that the traits are governed by single dominant gene, which depicts that similar molecular route can be followed to generate cultivars well adapted to both waterlogging and salinity stress (Subbarao et al. 1991; Perera et al. 2001).

4.5.3.3 Salinity

Salinity is the major abiotic stress affecting production of important crops including pigeonpea. Salinity refers to high salt ($NaCl/Na_2SO_4$) in the soil, and it is known to affect many physiological and biochemical pathways. Salinity affects the reproductive stage, and it advanced 50% flowering by approximately 2 weeks. Ill effects of salinity stress were observed more in early as compared to late maturing genotypes (Dua and Sharma 1996). Besides decreasing the flowering time, salinity also reduces the seed weight and pod number (Promila and Kumar 1982). A set of 120 genotypes of diverse adaptability was screened to combat salinity stress under laboratory conditions, and out of these, 5 accessions were found to be salt tolerant (Singh et al.

2016). Among wild species, *C. platycarpus*, *C. scarabaeoides* and *C. sericeus* were good sources of salinity tolerance. The major biomolecules which contributed for salt tolerance in these wild relatives include high concentrations of potassium ions, proline, free amino acids and soluble sugars (Subbarao et al. 1991; Waheed et al. 2006). Dominant genes are reported to regulate salinity tolerance in wild relatives such as *C. albicans* which can be transferred to the cultivated background (Choudary et al. 2011).

4.5.3.4 Temperature

Pigeonpea cultivars with long duration growth period (>200 days) are sensitive to low temperature especially at reproductive stage mainly affecting bud development and pollen dehiscence. It is observed that about 20 °C temperature is required for second flush of flower growth which ultimately alters the plant maturity and decreases the yield output. About 32 pigeonpea genotypes were identified which can tolerate temperature as low as 0 °C, and they exhibit normal reproductive cycle even at low temperature (Sandhu et al. 2007). This information is quite helpful to initiate breeding activities for low-temperature stress.

4.5.4 Breeding for High Protein Content

The issue of protein energy malnutrition is faced by population residing in developing countries, and this problem is accelerated due to increasing population and limited availability of quality protein, which warrant the development of biofortified cultivars. Pigeonpea generally contain 22% protein (Saxena et al. 2002) which can be exploited further for increase in protein content, but lack of genetic variation in terms of protein content is a major bottleneck (Manimekalai et al. 1979; Singh et al. 1984). Further, limited research is conducted on molecular mechanism for high protein content which is essentially required for targeted breeding program. So, two objectives for high protein pigeonpea breeding includes accessing diverse sources for variable protein content and depicting role of specific genes in enhancing protein content. It is reported that maternal genes strongly influence the protein content in F_1 generation (Dahiya et al. 1977; Durga 1989), and amount of overall protein expressed is decided by complementary or additive effect of at least 3–4 genes (Dahiya et al. 1977; Saxena 2008). It was observed that trait for low protein content was dominant over high protein content (Durga 1989). So, all the aspects of genetic information can be collectively implemented for generation of high protein cultivars. The wild relatives, *C. scarabaeoides*, *C. sericeous* and *C. albicans*, were reported to possess high protein content (28.5–30.5%) and used as donors in breeding high protein lines. The high protein content of wild relatives was found to have associated pleiotropic effect with small seeds (1.9–2.8 g/100 seeds), seed shape (flat, irregular), seed colour (grey, black), plant type (creeper, trailing) and perennial

nature with long maturity duration. Therefore, major objective of breeding program was to access those segregants which accumulate combination of high protein and desirable agronomic traits. Screening for desired traits leads to identification of some inbreds such as HPL 40-5 and HPL 40-17, with high protein content (28–32%), acceptable seed size (9–10 g/100 seeds) and brown-coloured round seeds, in F_9 generation (Saxena and Sawargaonkar 2015). This outcome was major milestone in pigeonpea breeding which established that yield, agronomic and nutritional traits can be simultaneously improved. Later on, it was observed that these high protein lines had accumulated high-quality protein as compared to control cultivars and this increase in protein quality was related to greater accumulation of essential amino acids especially sulphur-containing amino acids.

4.5.5 Speed Breeding

Normal pure line breeding takes more than 10 years to develop a new variety. Along with time it is also labour and resource intensive. So, there is a need to reduce the time span for variety development. Earlier many rapid generation advancement techniques have been developed and used in different crops to overcome the constraints of time and resources by postponing the selection till the attainment of homozygosity in the breeding populations. These include single seed descent method, modified pedigree method, multiple seed descent method, etc. In soybean, single pod descent method has been developed. Some workers have proposed the use of off-season nursery, but it is not possible in pigeonpea due to long-duration traditional pigeonpea germplasm, but it is now a viable option in case of early maturing pigeonpea germplasm (Saxena et al. 2019). Speed breeding is an ideal approach for accelerating varietal development (Li et al. 2018). This will help in reducing the time for development of varieties significantly by advancing breeding material through achieving rapid plant growth, flowering by optimizing environment. It has been well documented in many cereals, oilseeds and legumes. Gaur et al. (2007) reported three seed-to-seed generations can be taken in 1 year. Mobini et al. (2015) demonstrated that up to seven generations of faba bean and eight of lentil can be taken through application of growth hormones by inducing early flowering and harvesting immature seeds. But very scanty information is available in pigeonpea (Saxena 1996); however Saxena et al. (2017c) reported four seed-to-seed generations under controlled conditions where they suggested that immature seeds after 35 days of flowering should be harvested for proper germination. This research opened up the platform to use this speed breeding as potential technology to accelerate breeding of short-duration varietal development especially for pigeonpea-wheat rotation cropping system in northern part of India. Saxena et al. (2019) discussed an effective generation advancement program in pigeonpea where they showed that in 3 years up to F_7 generation can be reached using glass house facility, and in fourth year, uniform lines can be evaluated for yield performance. The proposed scheme is integrated with single seed descent method, accelerated generation

advancement in glass house and marker-based screening to shorten breeding cycle for varietal development in pigeonpea. More efforts will be required to overcome the limitations associated with this technology such as genetic erosion during generation advancement and difficulties in flower induction in late maturing genotypes in controlled conditions. Here technologies like genomic selection will help to bring this approach as cost effective.

4.6 Genomics and Molecular Breeding in Pigeonpea

In pigeonpea, first and second generations of molecular markers like restriction fragment length polymorphism (RFLP), amplified polymorphic DNA (RAPD) and fragment length polymorphism (AFLP) were used for genetic studies such as diversity analysis (Nadimpalli et al. 1993; Ratnaparkhe et al. 1995; Souframanien et al. 2003; Panguluri et al. 2006) and mapping of some important traits (Dhanasekar et al. 2010). Aruna et al. (2008) employed both hybridization (RFLP) and PCR-based marker systems (AFLP/SSR) to estimate genetic diversity in a collection of cultivated (*C. cajan*) and wild (*C. scarabaeoides*, *C. sericeus*, *C. reticulatus*) pigeonpea. Similarly, Sivaramakrishnan et al. (1997) used RFLP markers for identification of CMS and GMS lines.

The first set of large-scale DNA markers in pigeonpea was developed by Bohra et al. (2011). They developed 3072 SSR markers from BAC-end sequences (BESs). Earlier, the SSRs were developed in pigeonpea using conventional methods that were cumbersome and costly. The developed SSR markers were then used for construction of first SSR-based genetic linkage map for both wide (Bohra et al. 2011) and cultivated crosses (Gnanesh et al. 2011) and QTL discovery (Bohra et al. 2012). More recently, Bohra et al. (2017b) developed a set of 421 hyper variable SSR markers from the pigeonpea genome. The utility of these markers has been demonstrated in diversity analysis, trait mapping and hybrid purity testing. Sharma et al. (2018) assessed 96 pigeonpea genotypes including 15 male sterile, 13 maintainer and 68 germplasm lines using 44 SSR markers distributed over 11 linkage groups. Out of 44 SSRs, 33 were found to be polymorphic showing 75% polymorphism. PUSA 991 and ULA 11 were the most distant genotypes with highest dissimilarity coefficient (32%) which can further be utilized for the selection of desirable segregants.

In recent years, adoption of next-generation sequencing (NGS) technique has given a great impetus to the efforts on development of genomic tools in pigeonpea. Massively parallel sequencing has also allowed decoding of whole-genome sequence and whole-genome re-sequencing (WGRS) in pigeonpea. As a reference genome, a 605.78-Mb draft genome assembly of the pigeonpea genotype Asha (ICPL 87119) representing 72.7% of the entire pigeonpea genome was built through using a combination of Sanger and Illumina sequencing systems (Varshney et al. 2011). Asha, a medium-duration variety with high yield and resistance to Fusarium wilt and sterility mosaic disease, is widely cultivated in central and south zones of

India. Concerning the assembly statistics, the N50s of scaffolds and contigs are 516.06 kb and 21.95 kb, respectively. The genome assembly contains a total of 48,680 genes, of which 111 genes are predicted to be drought responsive. The GC and TE contents are predicted to be 32.8% and 51.67%, respectively. Availability of the reference genome paves the way for WGRS in pigeonpea. WGRS data on 104 pigeonpea lines was used to build Axiom *Cajanus* SNP array containing more than 56,000 SNPs uniformly distributed on all 11 chromosomes of the pigeonpea genome. The SNP array was then employed to investigate genetic architecture and diversity of 63 released cultivars and 40 other lines including germplasm and landraces (Saxena et al. 2018a, b). More recently, Varshney et al. (2017c) have re-sequenced whole genomes of 292 pigeonpea accessions including breeding lines, landraces and wild accessions. By finding 69 structural variations (SVs) as targets of selection, the study reports a greater role of SVs such as copy number variants (CNVs) and presence and absence variants (PAVs) during domestication and breeding of pigeonpea crop. They also conducted GWAS on the WGRS dataset and identified a total of 241 marker-trait associations (MTAs) for 8 agronomic traits including days to 50% flowering and days to 75% maturity and 100-seed weight. The presence of majority of these MTAs on CcLG09 (for instance, 64 of 86 MTAs for days to 50% flowering found on CcLG09) implies towards a significant impact during crop domestication and breeding on this particular pseudo-molecule.

By using NGS techniques, a variety of transcriptome assemblies have been built in pigeonpea, which not only offer a global view on the gene expression but also serve as a comprehensive resource for the development of functional DNA markers like expressed sequenced tag (EST)-SSRs, intron spanning regions (ITRs) and single nucleotide polymorphisms (SNPs). Through a comparative analysis, the total number of transcript assembly contigs (TACs) in the 4 transcriptome assemblies were 21, 434 (Kudapa et al. 2012), 48, 726 (Dubey et al. 2011), 43, 324 (Dutta et al. 2011) and 4557 (Raju et al. 2010) with 1510, 287, 1222 and 701 being the corresponding N50 (bp). A *C. cajan* gene expression atlas (CcGEA) has been developed (Pazhamala et al. 2017) that serves as a comprehensive catalogue of the candidate genes involved in key developmental stages of pigeonpea. Capturing the spatio-temporal expression of 28,793 genes, the CcGEA is built with 30 samples collected from 5 major stages of plant development (germinal, seedling, vegetative, reproductive) from the pigeonpea genotype Asha. Further network analysis led to the construction of a module comprising 28 flowering-related candidate genes, and subsequently important "hub" genes were identified such as *C. cajan 07765*, a pollen-specific SF3 gene and *C. cajan 35,396* coding for a H+-symporting sucrose transporter protein 2. Development of genomic resources like gene expression atlas complements the whole-genome sequence information to a great extent in order to bridge the phenotype-genotype gap.

Availability of polymorphic DNA markers and genetic linkage maps with moderate to high marker density has facilitated identification of MTAs for a range of agriculturally important traits in pigeonpea (Varshney et al. 2013). Bi-parental QTL analysis has been widely employed for trait mapping in pigeonpea (Bohra et al. 2019). The first QTL analysis in pigeonpea was performed in F_2 mapping

populations (ICP 8863 × ICPL 20097 and TTB 7 × ICP 7035) segregating for *Fusarium* wilt resistance (Gnanesh et al. 2011). They reported six QTL (qSMD1-qSMD6) on five LGs (1, 2, 3, 7, 9) with the phenotypic variations varying between 8.3% and 24%. Bohra et al. (2012) analysed three mapping populations (ICPA 2039 × ICPR 2447, ICPA 2043 × ICPR 2671 and ICPA 2043 × ICPR 3467) segregating for fertility restoration trait and found four QTLs (QTL-RF-1 to QTL-RF-4) on two LGs (6 and 11) explaining PV up to 24.17%. In another study, F_2 population was used by Sharma et al. (2018) to map fertility restorer gene in A_2 hybrid system utilizing 228 SSR markers. The Rf gene was mapped on linkage group 6 between CcM 1615 and CcM 0710 SSR markers with a distance of 3.1 and 5.1 cM, respectively (Fig. 4.3).

In recent years, high-density DNA marker data available from WGRS and genotyping by sequencing (GBS) have been used to elucidate the genetic underpinnings of important traits like resistance to FW and SMD. Singh et al. (2017) employed Indel-seq, an NGAs-based QTL analysis that combines WGRS with bulked segregant analysis (BSA), to identify a set of 16 Indels affecting 26 putative candidate genes for the 2 traits. Analysis of the multilocation and multi-year disease scoring data in combination with GBS data generated on three mapping populations (ICPL 20096 × ICPL 332, ICPL 20097 × ICP 8863 and ICP 8863 × ICPL 87119) led to the discovery of ten QTLs explaining up to 34.3% PV for SMD (Saxena et al. 2017a). The QTLs were detected on CcLGs 2, 3, 7, 10 and 11, with CcLG11 containing most promising candidate genes for targeted improvement of the SMD resistance. A similar GBS-based approach applied on 3 mapping populations (ICPB 2049 × ICPL 99050, ICPL 20096 × ICPL 332, ICPL 85063 × ICPL 87119) revealed 14 QTLs for FW resistance (Saxena et al. 2017b). GBS analysis of an F_2 population (ICPA 2039 × ICPL 87119) facilitated identification of one major QTL on CcLG08 for A4-CMS restoration (Saxena et al. 2018a, b). A strong agreement was established with earlier study by Bohra et al. (2012) regarding genomic location of the QTL responsible for fertility restoration. Similarly, mapping of the growth habit locus (*Dt1*) was facilitated on CcLG03 with GBS analysis of the population ICP 5529 × ICP 11605 (Saxena et al. 2017d). *Dt1* locus was demonstrated to control up to 61% variation of the trait. As evident from these mapping studies, the NGS-based

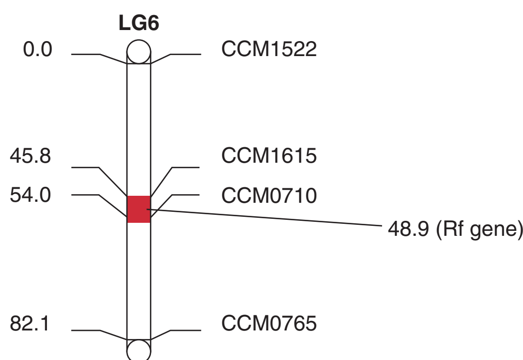

Fig. 4.3 Linkage map showing SSR markers CcM 1615 and CcM 0710 linked to fertility restorer gene (Adopted from Sharma et al. 2019)

approaches are found to be more efficient than the conventional QTL techniques in terms of time, cost and throughput. More recently, the genotyping of the RIL population (ICPL 99010 × ICP 5529) with Axiom 50 K SNP array enabled construction of a high-density genetic linkage map with 6, 818 SNP loci spanning 974 cM of pigeonpea genome. Further analysis of the population provided insights into the high-selfing flower and seed quality traits of pigeonpea. The study reported five QTLs for cleistogamous flower, whereas three and one QTLs were detected for seed shape and seed size, respectively, with the PV varying between 9.1% and 50.6% (Yadav et al. 2019).

Association mapping is a powerful technique for genetic dissection of trait architectures. In pigeonpea, limited studies have been reported on association mapping. The first study on association mapping in pigeonpea was reported by Mir et al. (2012) for determinacy trait. By scanning genomes of 94 pigeonpea genotypes with 6144 DArT and 786 SNP markers, the study provided a set of significant MTAs involving 19 SNP and 6 DArT markers influencing up to 8.58% and 14.53% PV, respectively. Another association mapping study in pigeonpea was performed on FW resistance (Patil et al. 2017a). They genotyped a set of 89 germplasm lines with 65 SSR markers, and an analysis of genotyping data in combination with 3-year wilt incidence data revealed 6 SSRs (HASSRs 8, 18, 30, 121, 128, 174) with significant association with FW resistance. Interestingly, association of the markers HASSR 8 and HASSR 121 was validated through analysis of an F_2 population of Bahar × KPL 43 (Patil et al. 2017b).

Genomic resources have also been developed to support hybrid pigeonpea breeding. CMS, a maternally inherited trait, has greatly facilitated hybrid breeding. Rearrangements in mitochondrial genome have been reported to be instrumental in CMS induction. Sequence information of organellar genomes (mitochondria and chloroplast) of pigeonpea has been published in recent years. Mitochondrial genomes of CMS line (ICPA 2039), fertile maintainer line (ICPB 2039), hybrid (IPH 2433) and a wild *C. cajanifolius* accession (ICPW 29) were sequenced, and a master circle 545.7 kb was assembled (Tuteja et al. 2013). The assembly harbours 51 genes of which 34 are predicted to be protein-coding genes. Further comparison of mitochondrial genome sequence information between CMS line and cognate maintainer lines suggested possible participation of 13 chimeric ORFs with male sterility. Association of a 10-bp deletion in the *nad7a* gene was demonstrated with A4-CMS based on expression profiling and structural variation analysis of 34 protein-coding mitochondrial genes (Sinha et al. 2015). Apart from this, various authors have developed sets of DNA markers that can be used for genetic purity testing of hybrids and their parental lines. Examples of SSR markers for hybrid purity testing include CcM0021, CcM0030 and CCB9 for GTH 1 (Patel et al. 2012); CCB4 and CCttc006 for ICPH 2438 (Saxena et al. 2010); CCB9, HASSR3, HASSR9, HASSR23, HASSR35, HASSR37 and HASSR43 for IPH 09-5 (Bohra et al. 2015); and CcGM18291, CcGM17648, CcGM12217 and CcGM16417 for IPH 15-03 (Bohra et al. 2017b).

4.7 Conclusion

Pigeonpea is an important food legume crop of semi-arid tropics. Breeding efforts over the last decade have yielded exciting results in the form of high-yielding and disease-resistant cultivars. However, there is a need to improve productivity gains of pigeonpea. Operational efficiency of the breeding programs needs to be improved. A variety of genomic tools have now been added to breeder's toolbox. Whole-genome sequencing and re-sequencing of multiple genomes are noteworthy achievements in this regard. Adoption of new tools and techniques like genomic resources and speed breeding protocols could help improving breeding efficiency. Speed breeding protocols need optimization for crops like pigeonpea. In parallel, development of heterotic pools is required to boost gains from hybrid breeding. Genome-wide prediction may be of immense significance in this regard. Improved genetic gain of pigeonpea breeding programs is the key to sustain the nutritional security and livelihood of the resource-poor farmers in developing world.

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Chapter 5
Genomics-Assisted Breeding Green Gram (*Vigna radiata* (L.) Wilczek) for Accelerating Genetic Gain

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5.1 Introduction

Pulses are the main sources of protein, and about 72% of the individuals, especially those who depend on vegetarian diet, are dependent upon pulses alone as a source of protein. Pulses continue to be major sources of protein in Indian diets and play a major role in sustaining agricultural growth. Apart from rich protein source, pulses possess several other qualities. They (1) improve soil fertility and physical structure, (2) fit in mixed/intercropping systems, (3) help in crop rotations, and (4) serve as nutritious fodder for milch and draught animals. Increasing pulse production is therefore important for food and fodder security, soil health (Sinclair and Vadez 2012; Foyer et al. 2016; Stagnari et al. 2017), and hence sustainable development. However, the productivity of pulses compared to their cereal counterparts is rather low. Low productivity of pulses is attributed to shortage and lack of timely availability of quality seeds, cultivation on marginal and sub-marginal lands, deficient/depletion in nutrients with low inputs, and poor post-harvest technology and storage infrastructure (Sharma et al. 2012; Joshi et al. 2017; Avinash and Patil 2018).

Green gram popularly known as mungbean is the third most important pulse crop after chickpea and pigeon pea in India. It is known by various vernacular names which include green gram, mungbean, golden gram, haricot mung, mungo amberique, haricot dore, feijao mungo verde, and mchoroko (Mogotsi 2006; Swaminathan et al. 2012). It has a diploid ($2n = 2x = 22$) chromosome number with a small genome size of 579 mb. It is predominantly a self-pollinating and fast-growing grain legume belonging to the family Fabaceae. Green gram being endowed with

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low input requirement and an ability to survive in a wide range of adverse soil and climatic conditions, it could be cultivated across different climatic and agro-climatic zones of India either as a sole or a relay intercrop. Being a short-duration legume, it is an ideal legume for intercropping, catch cropping, and relay cropping. It can be grown on a variety of soils ranging from sandy loam to black cotton soils having good drainage capacity. However, saline and alkaline soils are not suitable for cultivation. Green gram is regularly cultivated in all the seasons (Pratap et al. 2014, 2016; Allito et al. 2015).

Green gram is consumed in the form of whole pulse and split pulse which is prepared either separately as making roti or along with rice as moong dal khichdi, which forms an essential supplement of cereal-based diet. As processed food, it is utilized as flour, soups, and porridge, making it highly versatile for human diet. Green gram recipes are consumed throughout Asia, including the traditional Indian porridge dhal, in the form of sprouts and are consumed raw as fresh salad or vegetable in India, China, Bangladesh, the Philippines, Thailand, Southeast Asia, and Western countries; it is also popular as noodle salad known as Yum Woon Sen in Thailand and as sprout as side dish known as Sukjunamul in Korean and extract for soap industry in both India and China. Green gram is beneficial to animals as forage and diet of sheep; the haulms are used as livestock feed. In addition, mungbean forage is beneficial in the diet of sheep, without any adverse effects, and the haulms are used as livestock feed. The diversified utility of green gram has increased the global consumption by 22–66% and subsequent increase in annual production by a large percentage (Agboola and Fayemi 1972; Garg et al. 2004; Shanmugasundaram et al. 2009).

The raw and mature seeds of green gram are rich in nutrients including carbohydrates, proteins, fibers, minerals, vitamins, and antioxidants like flavonoids (quercetin-3-O-glucoside) and phenolics (Guo et al. 2012; Pooja et al. 2019). Green gram is a substantive source of dietary protein (24–28%) and carbohydrates (59–65%) on a dry weight basis and provides about 3400 kJ energy/kg grain. It is low fat and a source of high dietary fiber which realizes almost 40–70% of recommended daily fiber requirement. Summary of nutritional composition of green gram is presented in Table 5.1. This fiber content is known to reduce the food intake and helpful in obesity control. Despite its high dietary fiber content, green gram starch is easily digestible and also induces less flatulence as compared to other legumes such as chickpea (*Cicer arietinum*), pigeon pea (*Cajanus cajan*), and lentils (*Lens culinaris*), and thus this makes it more suitable to both children and aged persons as well (Sandhu and Lim 2008). In addition green gram has lower phytic acid (72% of total phosphorus content) than pigeon pea, soybean (*Glycine max*), and other cereals. Owing to its palatable taste and nutritional quality, it is being used as an iron-rich whole food source for baby foods (Chitra et al. 1995; Imtiaz et al. 2011). Comprehensive nutritive composition that includes macronutrients, vitamins, and minerals present in the form of cooked, raw, and sprouted raw forms of green gram is presented in Table 5.2.

Table 5.1 Summary of macronutrient composition of green gram

Sl. no.	Nutrients	Average[a]	Min.	Max.	References
1.	Moisture	9.80	4.10	15.20	Dahiya et al. (2015), Tsou and HSU (1978), Sampath et al. (2008)
2.	Crude protein (g/100 g) dm	23.80	14.60	32.60	Tsou and HSU (1978), Khatoon and Prakash (2004), Mubarak (2005), Barkoti and Bains (2007), Mallillin et al. (2008), Tang et al. (2014), Ganesan and Xu (2018), Vinod Kumar and Pandey (2018)
3.	Crude lipid (g/100 g) dm	1.22	0.71	1.85	Khatoon and Prakash (2004), Mubarak (2005), Tang et al. (2014), Ganesan and Xu (2018), Vinod Kumar and Pandey (2018)
4.	Crude fiber (g/100 g) dm	4.57	3.80	6.15	Mubarak (2005), Tang et al. (2014), Ganesan and Xu (2018), Vinod Kumar and Pandey (2018)
5.	Ash (g/100 g) dm	3.51	0.17	5.87	Sathe (1996), Mubarak (2005), Dahiya et al. (2015), Ganesan and Xu (2018)
6.	Carbohydrates (g/100 g) dm	61.00	53.30	67.10	Tang et al. (2014), Ganesan and Xu (2018), Vinod Kumar and Pandey (2018)
7.	Energy (kcal/100 g) dm	344.00	338.00	347.00	Dahiya et al. (2015)

[a]Mean value of all collected data

5.2 Origin, Domestication, and Distribution

According to Yajurveda, one of the Vedas of ancient Indian literature as early as 7000 BC, green gram is a native to India and Central Asia. The archeological evidences, its occurrence, and geographical distribution of wild and weedy types propose that the domestication and cultivation of green gram was initiated in the northwest and far south of India as early as 4000–6000 years ago. Based on diversity data of the cultivated green gram and the archeological evidences, green gram has been originated in India and Central Asia and is grown in these areas since prehistoric period (De Candolle 1886; Vavilov 1926; Singh et al. 1974; Vishnu-Mittre 1974; Fuller and Harvey 2006; Fuller 2007). Modern cultivated green gram has resulted from multiple rounds of domestication and selection and is currently distributed throughout southern and eastern Asia, Africa, and Austronesia (Vishnu-Mittre 1974; Lambrides and Godwin 2007). Its putative progenitor, *V. radiata* var. *sublobata*, is indigenous to the subtropical and tropical regions of northern and eastern Australia (Lawn and Cottrell 1988). It is widely distributed as a weed across many different areas including southern Africa, southern and eastern Asia, and Pacific islands of Indonesia and Australia (Table 5.3).

Table 5.2 Comprehensive nutritive composition including macronutrients, vitamins, and minerals in cooked, raw, and sprouted raw forms of green gram

Sl. no.	Different forms of green gram	Green gram cooked		Green gram raw		Green gram sprouted raw		References
		NV	RDA	NV	RDA	NV	RDA	
1.	Energy (kcal)	105.00	5%	347.0	17%	30.0	2%	Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
2.	Carbohydrates (g)	19.20	6%	62.6	21%	5.9	2%	Tang et al. 2014, Vinod Kumar and Pandey (2018)
3.	Fat (g)	0.40	1%	1.2	2%	0.2	0%	Ganesan and Xu (2018)
4.	Protein (g)	7.00	14%	23.9	48%	3.0	6%	Tsou and HSU (1978), Khatoon and Prakash (2004), Mubarak (2005), Barkoti and Bains (2007), Mallillin et al. (2008), Swaminathan et al. (2012), Tang et al. (2014), Dahiya et al. (2015), Ganesan and Xu (2018), Vinod Kumar and Pandey (2018)
Minerals								
5.	Calcium (g)	27.00	3%	132.0	13%	13.0	1%	Tang et al. (2014), Ganesan and Xu (2018)
6.	Phosphorus (mg)	99.00	10%	367.0	37%	54.0	5%	Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
7.	Iron (mg)	1.40	8%	6.7	37%	0.9	5%	Fatima and Kapoor (2006), Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
8.	Potassium (mg)	266.0	8%	1246.0	36%	149.0	4%	Watson (1977), Tsou and HSU (1978)
9.	Manganese (mg)	0.298	12%	1.035	45%	0.188	8%	Poehlman (1991), Ganesan and Xu (2018), Tsou and HSU (1978)
10.	Magnesium (mg)	48.00	26%	189.0	47%	21.0	0%	Kadwe et al. (1974), Prabhavat (1990), Ganesan and Xu (2018), Tsou and HSU (1978)

Vitamins								
11.	Vitamin A (I.U)	24.00	0%	114.0	2%	21.0	0%	Kylen and McCready (1975), Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
12.	Vitamin C (I.U)	1.00	2%	4.8	8%	13.2	22%	Tsou and HSU (1978), Dahiya et al. (2015), Ganesan and Xu (2018)
13.	Vitamin B 6 (I.U)	0.10	3%	0.4	19%	0.1	4%	Kylen and McCready (1975), Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
14.	Vitamin B 12 (I.U)	0.00	0%	0.0	0%	0.0	0%	Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
15.	Thiamine (mg)	0.20	11%	0.6	41%	0.1	6%	Abdullah and Baldwin (1984), Tsou and HSU (1978) Tang et al. (2014)
16.	Riboflavin (mg)	0.10	4%	0.2	14%	0.1	7%	Tang et al. (2014), Swaminathan et al. (2012), Dahiya et al. (2015), Ganesan and Xu (2018)
17.	Ash (g)	0.8	0.8	3.3	3.3	0.4	0.4	Tang et al. (2014), Ganesan and Xu (2018)

Nutritive value: NV percentage of RDA: RDA Mungbean nutrition profile (*Vigna radiata*), raw, mature seeds, values per 100 g. (Source: USDA National Nutrient data base)

US Department of Agriculture, Agricultural Research Service (2016) Nutrient Data Laboratory USDA National Nutrient Database for Standard Reference, Release 28. http://www.ars.usda.gov/nea/bhnrc/mafcl

Table 5.3 Distribution of germplasm of *Vigna* species and its genomic resources

Species name	Common name	Chromosome number	Origin areas	Cultivation	Genome sequence availability	Reference
Vigna aconitifolia	Moth bean	$2n = 2x = 22$	South Asia	India and the Far East	Not available	Adsule (1996)
Vigna angularis	Adzuki bean	$2n = 2x = 22$	East Asia	China, Japan, Korean peninsula	Available	Kaga et al. (2008), Kang et al. (2015)
Vigna mungo	Black gram	$2n = 2x = 22$	South Asia	South and Southeast Asia	Available	Gupta et al. (2013), Kang et al. (2015)
Vigna radiata	Mungbean	$2n = 2x = 22$	South Asia	South, East, and Southeast Asia	Not available	Nair et al. (2012)
Vigna reflexo-pilosa	Creole bean	$2n = 2x = 44$	Southeast Asia	Vietnam, Philippines (as pulse); India, Mauritius, and Tanzania (as forage)	Not available	Tomooka et al. (2002)
Vigna trilobata	Jungle bean	$2n = 2x = 22$	South Asia	Africa, Australia, Madagascar, Mauritius, and South America	Not available	Kaur and Kishore (2012)
Vigna trinervia	Tooapee (Thai)	$2n = 2x = 22$	South and Southeast Asia	Madagascar, South India, Sri Lanka, Myanmar, Malaysia, Sumatra, Java, Timor, and New Guinea	Not available	Tateishi (1985)
Vigna umbellata	Rice bean	$2n = 2x = 22$	Southeast Asia	Fiji, Australia, tropical Africa, Indian Ocean Islands, USA, Honduras, Brazil, and Mexico	Not available	Khadka and Acharya (2009)

5.3 Botany

Green gram included under the genus *Vigna* and species *radiata* is considered as Asian *Vigna* in the subgenus *Ceratotropis*. Research on cross compatibility based on seedling characteristics revealed that Asian *Vigna* consists of three isolated gene

pools (Lawn 1995). However, all the cultivated Asian *Vigna* species behave like same biological species and are able to provide viable hybrid seeds when crossed between several species of *Vigna*. All the species which belong to the Asian *Vigna* possess very specialized and complex floral organs which significantly differed among the species. However, a greater homology was revealed among the species belonging to Asian *Vigna* species with respect to vegetative morphology and growth habitat. *Vigna radiata* seems to be the most satisfactory seed parent when crossed with *Vigna angularis*, *Vigna umbellata*, *Vigna mungo*, and *Vigna trilobata* so as to obtain completely viable hybrid seeds. Further, *Vigna radiata* reciprocally crosses with the wild *Vigna radiata* var. *sublobata*, to produce viable hybrids.

Green gram is a small herbaceous annual varying between 1 and 3 feet in height. *Vigna radiata* L. belongs to the Fabaceae or Leguminosae family. The plant develops tap root system, central stem is erect, and side branches are semi-erect. Stem is covered with short hairs and the stems are ridged. The leaves are trifoliate and stipulate leaflets ovate or lanceolate. Green gram infloroscence is an axillary raceme with few flowers, bracts and bracteoles are present. Flowers are produced in cluster of 10-20 in axillary racemes, on long pedicels. Flowers are pale yellow in colour, 6-7 mm long; bisexual and zygomorphic, calyx campanulate with linear teeth; corolla papilionaceous; stamens 10 and diadelphous; ovary monocarpellary and syncarpous, many ovules, style filiform with oblique stigma. Green Gram pods are linear, globular, small and green cotyledons yellow and known as dal. 6–7 mm long; bisexual and zygomorphic, calyx campanulate with linear teeth; corolla papilionaceous; stamens 10 and diadelphous; ovary monocarpellary and syncarpous, many ovules, style filiform. Flowers are produced in a cluster of 10–20 in axillary racemes, on long pedicels. Stigma oblique, pods linear, globular, small and green cotyledons yellow and known as dal. Green gram has 40 different plant types varying in leaf size and color of flower, pod, and seed. Seed color is green, black, brown, or yellow. Seed surface is either dull or shining. Flower color is light yellowish – olive or olive yellow. Pod color is iron grey, olive gray, or snuff brown. Pods are 6–10 cm long, round, and slender with short pubescence. Seeds are small and nearly globular, usually green (Singh et al. 2016). Genetics, pattern of inheritance, and mode of gene action of various important traits of green gram are presented in Table 5.4.

5.4 Production and Productivity

In the world, green gram is predominantly produced in South, East, and Southeast Asia and in East Africa. The global green gram area is 7.3 Mha global production of 5.3 Mt with India and Myanmar each supplying about 30% of green gram, China 16%, and Indonesia 5%. The average global grain yield of green gram is quite low at 0.73 t ha^{-1} (World Vegetable Center 2018). In India, it is grown in an area of 4.07 Mha with a production of 1.90 million tons and productivity of 0.48 t ha^{-1}. The major green gram-producing states are Rajasthan, Maharashtra, Karnataka, Madhya

Table 5.4 Genetics, pattern of inheritance, and mode of gene action of various traits of green gram

Trait and its different states	Pattern of inheritance and mode of gene action	References
Growth habitat and plant type	Semi-spreading is dominant over erect habit controlled by single. Dominant > recessive gene	Sen and Ghosh (1959), Pathak and Singh (1963), Khattak et al. (1999), Singh et al. (2016)
Pubescence	Single dominant gene	Sen and Ghosh (1959), Murty and Patel (1973), Singh et al. (1985)
Nodulation	Additive and non-additive gene action	Singh et al. (1985)
Pigmentation	Single dominant/recessive gene, anthocyanin in hypocotyls governed by two supplementary genes	Pathak and Singh (1963), Misra et al. (1970), Mukherjee and Pradhan (2002)
Leaf traits	Single dominant gene; large leaflet is dominant over small leaflet; lobbed is dominant over entire type	Singh and Mehta (1953), Singh and Singh (1995), Talukdar and Talukdar (2003)
Stem fasciation	Single recessive gene	Dwiwedi and Singh (1990)
Inflorescence type	Simple types controlled by two dominant genes and compound types are double recessive homozygous; number of clusters controlled by single gene	Sen and Ghosh (1959), Singh and Singh (1970)
Flower color	Single dominant gene	Bose (1939)
Yield components	Additive and non-additive gene action	Singh and Singh (1972), Yohe and Poehlman (1975), Dasgupta et al. (1998), Khattak et al. (2002)
Pod color	Single dominant gene	Bose (1939), Sen and Ghosh (1959), Murty and Patel (1973)
Pod shattering	Single dominant gene	Verma and Krishi (1969)
Seed coat color	One or few genes; mottling governed by single gene	Khattak et al. (1999), Chen and Liu (2001), Lambrides et al. (2004)
Seed coat surface	Two complementary genes	Bose (1939), Sen and Ghosh (1959), Murty and Patel (1973)
Cotyledon color	Single recessive gene controls green color	Thakare et al. (1988)
Hard seededness	One or few dominant genes involved	Lambrides (1996), Humphry et al. (2005)
Pre-harvest sprouting	Additive and non-additive gene action, high G × E interaction	Durga and Kumar (1997)
Crop duration	Additive non-additive and epistatic gene action	Khattak et al. (2001)
Seed weight	Small is dominant over larger size	Sen and Murty (1960), Fatokun et al. (1992), Humphry et al. (2005)
Protein content	Additive and non-additive gene action	Chandra and Tickoo (1998)

Adopted and Modified from Singh et al. (2016)

Pradesh, Odisha, Tamil Nadu, Bihar, Andhra Pradesh, Gujarat, and Telangana in order of their highest contribution in Indian green gram.

5.5 Production Constraints

Mungbean yellow mosaic disease (MYMD) among the diseases and bruchids (*Callosobruchus* sp.) among the insect pests are the major green gram production constraints. Different begomovirus species infecting mungbean have been identified (Qazi et al. 2007). Bruchids (*Callosobruchus* sp.) are a major storage pest of green gram. The bruchids lay their eggs on pods in the field. Larvae hatch during storage and develop in a single bean to adulthood and then lay their eggs on the beans. In a few months storage time, bruchids can destroy all stored mungbean grains (Lambrides et al. 2000).

5.6 Genetic Resources

The availability of diverse genetic resources is the prerequisite for initiating, and genetic improvement in any crop and green gram is no exception to this. Several research institutes and universities are conserving green gram genetic resources for present and future needs. Green gram genetic resources are maintained at different centers throughout the globe, including the following: the University of the Philippines; AVRDC – The World Vegetable Center, Taiwan; the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences; National Bureau of Plant Genetic Resources of the Indian Council of Agricultural Research; and the Plant Genetic Resources Conservation Unit of the University of Georgia, USA (Ebert 2013; Kim et al. 2015). In addition, the University of the Philippines and the Rural Development Administration (RDA), Korea, hold duplicates of parts of the mungbean germplasm collection of AVRDC – The World Vegetable Center. AVRDC currently holds the world's largest collection of *Vigna* germplasm, comprising 12,153 accessions (Tables 5.5 and 5.6), which represent an important resource for inter-species hybridization. To enable efficient use of genetic resources and to increase access for breeders, mungbean core collections have been established in countries including China, India, the USA, and Korea. Very recently, AVRDC – The World Vegetable Center developed a core collection comprising 1481 accessions and a mini-core comprising 296 accessions (Table 5.7) (Schafleitner et al. 2015). The core collection was developed based on phenotypic characterization, while the mini-core was developed by molecular characterization using 20 SSR markers. In addition to these mungbean resources, researchers have begun utilizing mungbean-related species in crop improvement programs. For example, *V. mungo has* been used as a source of MYMVD for transfer into mungbean.

Table 5.5 Principal *Vigna* species in collection at AVRDC – The World Vegetable Center, Taiwan

Vigna species	Number of accessions
Vigna radiata	6742
Vigna mungo	853
Vigna umbellata	370
Vigna unguiculata	1587
Vigna angularis	2376

Kim et al. (2015)

Table 5.6 Summary of genetic resources maintained at different sources throughout the globe

Name of the international center	Country
University of the Philippines (holds duplicates of mungbean germplasm collection of AVRDC)	Philippines
AVRDC – The World Vegetable Center	Taiwan
The Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences	China
National Bureau of Plant Genetic Resources of the ICAR	India
Plant Genetic Resources Conservation Unit, University of Georgia	USA
Rural Development Administration RDA (Holds duplicates of mungbean germplasm collection of AVRDC)	Korea

Ebert (2013), Kim et al. (2015)

Table 5.7 Existing status of green gram germplasm resources both wild and related species at national and global level

Institute	Species	Global holding	National holdings		Total	References
			Indigenous	Exotic		
NBPGR Cultivated species	*Vigna radiata*	24,918	3567	537	4104	Singh et al. (2016)
	Vigna mungo	3767	3127	6	3133	Singh et al. (2016)
NBPGR Wild species	*Vigna species*		490		490	Singh et al. (2016)
AVRDC	*Vigna radiata*	6742	–	–	6742	Kim et al. (2015), Schafleitner et al. (2015)
	Vigna mungo	853			853	Kim et al. (2015)
	Vigna umbellata	370			370	Kim et al. (2015)
	Vigna unguiculata	1587			1587	Kim et al. (2015)
	Vigna angularis	2376			2376	Kim et al. (2015)
	Core collection	1481			1481	Odong et al. (2013), Kim et al. (2015), Schafleitner et al. (2015)
	Mini-core collection	296			296	Odong et al. (2013), Kim et al. (2015), Schafleitner et al. (2015)

5.7 Genetic Enhancement

5.7.1 Conventional Breeding

Conventional phenotype-based breeding efforts in India have resulted in the development of several varieties suitable for production in different agro-climatic zones of India (Table 5.8). Ankesh Kumar et al. (2017) involved F4 and F5 generations of RILs of two crosses, viz., Chinamung × BL-849 and Chinamung × LM-1668 which had contrasting response for powdery mildew resistance with 146 and 155 lines, respectively (Figs. 5.1 and 5.2). Out of 146 F4 RILs screened in cross Chinamung × BL-849, one of them was found to be highly resistant (R0), viz., C1-34-23. While in cross Chinamung × LM-1668 off the 155 F_4 RILs screened, 39 RILs were found to be moderately resistant (R2). Similar resistance response was observed in the lines C1-34-23 of the cross Chinamung × BL-849 and C2-14-11, C2-16-13 RILs of the cross Chinamung × LM-1668 which could further utilized in research for screening for yield-related traits to develop highly resistant breeding lines with high yielding ability in green gram (Figs. 5.3, 5.4, and 5.5).

Moderate resistance against MYMD has been found in the mungbean gene pool but was not sufficient for generating resistant varieties. Mutation breeding using moderately resistant accessions and hybrids derived from them resulted in several lines with high levels of resistance against MYMD (Table 5.9) (Ashraf et al. 2001). The line NM94, the result of a cross between a genotype derived from mutation breeding and a high yielding cultivar, is now registered as a MYMD-resistant line in various countries. However, in regions where MYMVD-urd bean strain is predominant, susceptibility of NM94 has been reported (Nair et al. 2017). Breeding programs in Pakistan and India resulted in several stable MYMVD-resistant lines. ML1628 was developed by the Punjab Agricultural University, Ludhiana, India, and resists to multiple species/strains of the virus-causing MYMVD (Nair et al. 2017). Genetic resistance against bruchid infestation has been found in mungbean and has been used to breed resistant varieties in China and Korea and at the WorldVeg (Hong et al. 2015; Yao et al. 2015; Schafleitner et al. 2016). Bruchid resistance of different sources were genetically mapped, and markers for selection of resistant lines in breeding programs are available (Schafleitner et al. 2016; Kaewwongwal et al. 2017).

The wild species are reservoirs of useful genes, which is not present in primary gene pool due to a genetic bottleneck that has occurred during domestication and modern breeding (Hawkes 1977; Doyle 1988; Tanksley and McCouch 1997; Kumar et al. 2011). The use of wild species in breeding has tremendous potential benefits (Hoisington et al. 1999). The valuable gene pool from wild species has been used by plant breeders for crop improvement (Tomooka et al. 2005; Kumar et al. 2011). Summary of potential sources of germplasm for various characters in *Vigna* species is presented in Table 5.10 (Singh et al. 2016). Green gram variety TC1966 is completely resistant to two species of bruchid beetles, *Callosobruchus chinensis* (adzuki bean weevil) and *C. maculatus* (cowpea weevil), which cause major damage to

Table 5.8 Green gram varieties released in India

Sl. no.	Variety	Year of release	Institution developed	Salient features of released varieties
1.	PKV Green gold (AKM 9911)	2007	Dr. PDKV, Akola	It is moderately resistant to pearl millet. Podding on top and easy to harvest
2.	TJM 3	2007	BARC and JNKVV	Resistant to powdery mildew, YMV, and *Cercospora* leaf spot
3.	PAU 911	2007	PAU, Ludhiana	Fairly resistant to MYMV, *Botrytis* leaf spot, and *Cercospora* leaf spot
4.	MGG 347	2009	ARS, Madhira	Tolerant to thrips, stem fly, YMV, *Cercospora* leaf spot
5.	MGG 207	2009	ARS, Madhira	Tolerant to stress and YMV
6.	Basanti	2010	CCSHAU, Hisar, Haryana	Suitable for rainfed and irrigated. Possess high protein content
7.	Pairy Mung	2010	IGKV, Raipur	Tolerant to YMV and resistant to powdery mildew up to podding and grain filling stage
8.	SML 832	2010	PAU, Ludhiana	Tolerant to thrips and MYMV
9.	DGGV 2	2012	UAS, Dharwad	Moderately resistant to shattering of pods and suitable for mechanical harvesting
10.	Shalimar Mung 2	2013	Srinagar Centre, SKUAST K	Resistant to Cercospora leaf spot and moderate resistant to aphid
11.	SGC 16	2014	RARS, Shillongani, AAU, Assam	Resistant to CLS and YMV
12.	BGS 9 (Somnath)	2014	UAS, Raichur	Moderately resistant to PM, bold seed, and long pod
13.	MH 318	2016	CCSHAU, Hisar	Resistant to MYMV, well under rice wheat rotation
14.	Pant Mung 8 (PM 9-6)	2016	GBPUAT, Pantnagar	Resistant to MYMV, CLS, and PM
15.	RMG 975 (Keshwanand Mung 1)	2016	RARI, Durgapura	Moderately tolerant to MYMV and tolerant to root-knot nematode
16.	GBM 1	2016	NAU, Gujarat	Moderately tolerant to MYMV
17.	VBN 8	2017	NPRC, Vamban	Semi-erect and determinate plant type with broad to narrow lanceolate terminal leaflet
18.	KM 2328	2018	CSAUAT, Kanpur	Stably resistant against MYMV, resistant to CLS, web blight, MB
19.	Pusa 1431	2018	IARI, New Delhi	Resistant to MYMV, resistant to CLS
20.	SGC 16	2018	AAU, Jorhat, Assam	Resistant to CLS and YMV, moderately resistant to WB
21.	GAM 5	2018	AAU, Anand	Highly resistant against YMV

Source: Annual Report, AICRP on MULLaRP, ICAR, IIPR, Kanpur. 2017–2018

Fig. 5.1 Recombinant inbred lines (RILs) of the cross Chinamung × BL-849 of F_4 generation plan laid out in augmented design along with checks, i.e., Pusa Baisakhi, Chinamung, BL-849 and KKM-3 of green gram

Fig. 5.2 Recombinant inbred lines (RILs) of the of cross Chinamung × LM-1668 of F_4 generation were sown with checks, i.e., Pusa Baisakhi, Chinamung, LM-1668 and KKM-3 of green gram

Fig. 5.3 Screening recombinant inbred lines (RILs) of various crosses for severity of powdery mildew disease in green gram

Fig. 5.4 Severity of powdery mildew disease in advanced breeding lines of green gram

mungbean during storage (Talekar 1988; Visarathanonth and Promsatit 1989; Somta et al. 2007). This variety has been used to develop a bruchid-resistant mungbean cultivar (Tomooka et al. 1992).

Fig. 5.5 Pod-yielding potentials of advanced breeding lines identified for its moderate resistance to powdery mildew and insect attack in green gram

Despite the systematic and continuous breeding efforts through conventional methods, substantial genetic gain in green gram could not be achieved. This is because most of the economically important traits including biotic and abiotic constraints are complexly inherited with large genotype × environment (G × E) interaction (Kumar and Ali 2006). Hence, a paradigm shift is needed in the breeding strategies to strengthen our traditional crop improvement programs. Utilization of genomic tools in conventional breeding programs such as DNA markers, genetic engineering, and genome editing is the way forward.

5.7.2 Genome Sequence of Green Gram Obtained Through EST-SSR Markers

Green gram was previously considered to be an orphan crop due to the limited number of available genomic resources compared to other legume crops. The first draft genome of mungbean (*Vigna radiata* (L.) R. Wilczek) was made available in 2014, and the estimated genome size is 421 Mb (Kang et al. 2014). The complete genome sequence of mungbeae enabled the researchers to develop large number of genomic breeder-friendly markers such as SSR and SNPs to facilitate accelerated phenotypic screening and hence augment the speed and precision of breeding for cultivars with enhanced economic values. In 1999, the first SSR marker for mungbean were reported by Yu et al. (1999) based on a search of the GenBank database, revealing six SSR sequences with five different types of motifs, including di-, tri-, and tetra-

Table 5.9 Sources of resistance to mungbean yellow mosaic virus (MYMV) and powdery mildew in green gram in India

Sl. no.	Variety	Year of release	Originating center	Salient features
1.	TM 96-2	2007	BARC and ANGRAU, Lam	Resistant to powdery mildew
2.	WBU 109 (Sulata)	2008	Berhampore (WB)	Resistant to MYMV
3.	IPU 02-43	2008	IIPR, Kanpur	Resistant to MYMV and powdery mildew
4.	NUL 7	2009	Nirmal Seeds	Resistant to MYMV and powdery mildew
5.	VBN (Gg) 3	2009	NPRC, Vamban, Tamil Nadu	Moderately resistant to YMV
6.	KM 2195	2010	CSAUAT, Kanpur	Resistant to YMV
7.	TM 2000-2	2010	BARC and IGKV, Raipur	Resistant to powdery mildew
8.	LU 391	2010	PAU, Ludhiana	Resistant to MYMV
9.	KUG 479	2010	ARS, Gurdaspur	Resistant to MYMV
10.	VBG 04-008	2011	TNAU, Vamban	Resistant to MYMV and powdery mildew
11.	TU 40	2011	Trombay Mumbai	Resistant to powdery mildew
12.	CO. (Gg) 8	2013	TNAU, Coimbatore	Resistant to YMV
13.	LBG 787	2016	ARS, Lam	Resistant to powdery mildew
14.	Utkarsh KM 11-584	2016	MS State Seed Corporation	Moderately tolerant to MYMV
15.	Yadadri (WGG 42)	2016	PJTSAU, Hyderabad	Resistant to MYMV
16.	Sri Rama (MGG 351)	2016	PJTSAU, Hyderabad	Moderately tolerant to MYMV
17.	ML 2056	2016	PAU, Ludhiana	Resistant to MYMV

Source: Annual Report, AICRP on MULLaRP, ICAR, IIPR, Kanpur. 2017–2018

nucleotide repeats (AT)n/(TA)n, (ATT)n/(AAT)n, (GGC)n/(GCC)n, (AGGG)n/(AGGG)n, and (CTTT)n/(AAAG)n, in a total length of 67.1 kb. Furthermore, the advent of next-generation RNA sequencing has enabled researchers to develop a rich pool of EST-SSR markers (Chen et al. 2015b) which may benefit genetic mapping, assessment of genetic diversity, and marker-assisted selection. Liu et al. (2016) employed Illumina paired-end sequencing to analyze transcriptomes of three different green gram genotypes. A total of 38.3–39.8 million paired-end reads with 73 bp lengths were generated. The pooled reads from the 3 libraries were assembled into 56,471 transcripts. Following a cluster analysis, 43,293 unigenes were identified with an average length of 739 bp and N50 length of 1176 bp. Of the unigenes, 34,903 (80.6%) had significant similarity to known proteins in the NCBI nonredundant protein database (Nr), while 21,450 (58.4%) had BLAST hits in the Swiss-Prot database (E-value < 10–5). Further, 1245 differential expression genes were detected among 3 green gram genotypes. In addition, they have also identified 3788

Table 5.10 Summary of potential sources of germplasm for various characters in *Vigna* species

Vigna species	Potential source for the character	References
V. riukinensis *V. reflexo-pilosa* *V. radiata* var. *sublobata* *V. umbellata* *V. tenuicaulis* *V. nepalensis*	Resistance to bruchid	Fujii and Miyazaki (1987), Tomooka et al. (1992, 2000), Kaga and Ishimoto (1998), Miyagi et al. (2004), Kashiwaba et al. (2003), Somta et al. (2008), Pratap et al. (2014), Singh et al. (2016)
V. stipulacea *V. reflexo-pilosa* var. *glabra*	Resistance to powdery mildew	Tomooka et al. (2000, 2002)
V. tenuicaulis	Low trypsin inhibitor activity	Konarev et al. (2002)
V. grandiflora	Chymotrypsin absent	Konarev et al. (2002)
V. radiata var. *sublobata*	High methionine content	AVRDC (1987), Babu et al. (1988)
V. radiata var. *sublobata*	High photosynthetic efficiency	Ignacimuthu and Babu (1987)
V. aconitifolia	Drought tolerance	Jain and Mehra (1978)
V. aconitifolia *V. riukinensis*	Heat tolerance	Tomooka et al. (2000, 2010), Egawa et al. (1999)
V. unguiculata ssp. *dekindtiana*	Insect resistance	Ehlers and Hall (1997)
V. radiata var. *sublobata*	YMV resistance	Singh and Ahuja (1977)
V. reflexo-pilosa var. *glabrescens*	Cucumber mosaic virus resistance	Tomooka et al. (2000)
V. reflexo-pilosa	Bean fly resistance	Tomooka et al. (2000)
V. radiata var. *sublobata*	High tolerance to saline and alkaline soils	Lawn and Cottrell (1988)
V. unguiculata ssp. *dekindtiana*	Resistance to pod bug	Koona et al. (2002)
V. vexillata	Resistance to cowpea insects pests	Birch et al. (1986)
V. radiata var. *sublobata*	No. of seeds/plant and pods/plant	Reddy and Singh (1990)
V. umbellata, *V. glabrescens*	Photo-/thermo-insensitivity	Pratap et al. (2012)

Adopted and modified from Singh et al. (2016)

expressed sequence tag-simple sequence repeat (EST-SSR) motifs that could be used as potential molecular markers. Among 320 tested loci, 310 (96.5%) yielded amplification products, and 151 (47.0%) exhibited polymorphisms among 6 green gram accessions. These transcriptome data and green gram EST-SSRs will serve as

a valuable resource for novel gene discovery and the marker-assisted selective breeding in green gram. Similarly, Chen et al. (2015a) identified 13,134 EST-SSRs in green gram which can be used as potential molecular markers, with mononucleotide A/T repeats being the most abundant motif class and G/C repeats being rare.

5.7.3 Genomics-Assisted Breeding

As a prelude to implement marker-assisted selection in breeding green gram, several attempts have been made to map the key quantitative trait loci controlling economically important traits using different types of DNA markers through both conventional linkage and association mapping approaches. The availability of the entire genome sequence and affordable high-performance genotyping tools, such as genotyping by sequencing, facilitates mapping of breeder desired traits (Kang et al. 2014; Singh and Singh 2017). Several genetic linkage maps have been constructed in green gram cultivars using RFLP, RAPD, and SSR markers (Fatokun et al. 1993; Menancio-Hautea et al. 1993; Humphry et al. 2002, 2005). The genetic linkage maps developed in green gram till date are mainly based on populations derived from F_2 or RILs from inter-subspecific crosses and BC F_1 or RILs from inter-subspecies crosses (Chaitieng et al. 2002). Of the three inter-subspecies green gram crosses ('VC3890A' × 'TC1966', 'Berken' × 'ACC41', and 'TC1966' × 'Pagasa 7') involved in the development of linkage maps, 'VC3890A', 'Berken' and 'Pagasa 7' are cultivated green gram types from *V. radiata* ssp. *radiate*, whereas 'TC1966' and 'ACC41' are accessions of the wild progenitor *V. radiata* ssp. *sublobata*. The size of the mapping populations involved in developing the linkage maps varied from 58 to 202 in case of green gram.

These maps have been used to map genes for azuki bean weevil resistance (Young et al. 1992) and seed color (Lambrides et al. 2004) and to identify QTLs for seed weight, hard seed, powdery mildew resistance (Young et al. 1992; Chaitieng et al. 2002; Kasettranan et al. 2010), and *Cercospora* leaf spot resistance. Despite many genetic linkage maps that were developed, the number of linkage groups reported has not coincided with the basic number of chromosomes in green gram (n = 11). In addition, the populations that were used to construct previous maps were small, and there were large distances between adjacent markers in some linkage groups. A genetic linkage map of green gram was constructed using 237 SSR markers from green gram and closely related species and 193 EST-SSR markers from soybean. This green gram map is the first map in which the number of linkage groups coincided with the haploid chromosome number. In total, 105 QTLs and genes for 38 domestication-related traits were identified, and QTLs with large effects (PVE ~20%) were distributed on 7 out of 11 linkage groups (Isemura et al. 2012).

The traits for which QTL has been mapped include resistance to salt tolerance (Chankaew et al. 2014), resistance to drought tolerance (Liu et al. 2017), resistance

to high seed starch content (Masari et al. 2017), resistance to high iron and zinc content (Singh et al. 2017a), resistance to powdery mildew (Poolsawat et al. 2017), resistance to MYMD (Singh et al. 2017b), and resistance to bruchid infestation (Schafleitner et al. 2016; Kaewwongwal et al. 2017). Schafleitner et al. (2016) obtained bruchid resistance data from RIL populations. More than 6000 single nucleotide polymorphic markers were generated through genotyping by sequencing (GBS) to map bruchid resistance genes. One highly significant quantitative trait locus (QTL) associated with bruchid resistance was mapped to chromosome 5, suggesting that TC1966 and V2802 (donor parents) contain the same resistance locus. Co-segregation of all markers associated with resistance indicated the presence of only one major resistance QTL on chromosome 5, while QTL analysis based on physical map positions of the markers suggested the presence of multiple QTLs on different chromosomes. The diagnostic capacity of the identified molecular markers located in the QTL to correctly predict resistance was up to 100%.

Singh et al. (2018) used recombinant inbred lines (RILs) derived from the cross between a susceptible cultivar Sonali and resistant wild relative of green gram (*Vigna radiata* var. *sublobota*) to map molecular markers linked with mungbean yellow mosaic Indian virus (MYMIV) resistance and yield attributing traits in green gram. Out of 224 molecular markers employed for the identification of polymorphism between parents, only 46 markers showed polymorphism between Sonali and *V. radiata* var. *sublobota*. Twenty-two polymorphic markers were used to construct a linkage map comprising 11 linkage groups. QTL analysis identified molecular markers linked with MYMIV resistance and agronomic traits, viz., no. of pods per plant, no. of seeds per pod, and 100 seed weight. Molecular markers identified to be linked with MYMIV were confirmed in 93 diverse green gram accessions screened for yellow mosaic disease. Such molecular markers linked to the MYMIV and yield attributing traits shall be useful in marker-assisted breeding for development of high-yielding green gram varieties resistant to MYMIV. Similarly, QTL mapping of 142 $F_{2:3}$ mungbean lines derived from a cross between BARImung 1 (low seed weight) and BARImung 6 (medium seed weight) was carried out. Single marker analysis suggested at least four loci controlling seed weight. Composite interval mapping consistently identified four QTLs – *qSWT1*, *qSWT6*, *qSWT8*, and *qSWT9* – on linkage groups 1, 6, 8, and 9 in both locations. These QTLs accounted for 5.80 to 19.96% and 8.31 to 33.72% of the seed weight variation depending on the location. *qSWT1*, *qSWT8*, and *qSWT9* were common to QTL for seed weight detected previously in green gram, while *qSWT6* was reported to be a new locus. *qSWT1*, *qSWT6*, *qSWT8*, and *qSWT9* are reported to be conserved in other *Vigna* crops (Mahbubul Alam et al. 2014).

For most mapped loci, validation of their value for breeding is still lacking. Another important direction in research of adaptation strategies by mungbean to stressful environments is wide crosses that aim to introgress traits from related wild species. One example is the introgression of MYMD immunity from *V. mungo* (Lekha et al. 2018). Crossing barriers are affecting this approach, but several *Vigna* species are cross-fertile (Kaur et al. 2017).

5.7.4 Genome-Wide Association Mapping

A pilot genome-wide association study of seed coat color in green gram was conducted by (Nobel et al. 2018) to characterize the genetic diversity, population structure, and linkage disequilibrium and signify its utility. A diversity panel of 466 cultivated accessions and 16 wild accessions were genotyped that enabled the identification of 22,230 polymorphic genome-wide SNPs, of which 16,462 were physically mapped across the 11 mungbean chromosomes. An average of 1497 SNPs was identified per chromosome (from 903 SNPs on chromosome 3 to 2306 on chromosome 7) with an average marker density of 57.81 SNPs/Mb. It was noticed that the level of polymorphism was considerably lower in the cultivated accessions in comparison to the wild accessions. LD decayed in ~100 kb in cultivated lines, a distance higher than the linkage decay of ~60 kb estimated in wild mungbean. It was concluded that five genomic regions associated with seed coat color in mungbean were identified, two of which were close to seed coat color genes in other species as well. This mungbean diversity panel constitutes a valuable resource for genetic dissection of important agronomical traits to accelerate mungbean breeding. This is the first high-resolution quantification of LD decay in mungbeans, defining the extent of LD within and between cultivated and wild mungbeans. The genetic diversity analysis, population structure, and LD analysis provide the foundation that can be used to broaden the genetic base of mungbean breeding material. The genome-wide association study provides an example of how the data can be used to identify genomic regions responsible for phenotypic traits. SNP markers provide a level of resolution to breeding programs far beyond traditionally used methods which relied solely on passport data such as geographical origin and pedigrees (Brown 1989) or genetic markers and quantitative phenotypic data to conduct cluster analysis of core collections (Bretting and Widrlechner 1995).

Currently, a number of such studies are, perhaps, in progress in many laboratories worldwide. The near-future completion of genome sequencing projects of crop species, powered with more cost-effective sequencing technologies, will certainly create a basis for application of whole genome association studies (Kim et al. 2015), accounting for rare and common copy number variants (CNV) (Estivill and Armengol 2007) and epigenomic details of the trait of interest in plants, which is widely being applied in human genetics with great success. This will provide with more powerful association mapping tool(s) for crop breeding and genomics programs in tagging true functional associations conditioning genetic diversities and, consequently, its effective utilization. Table 5.11 represents the genomic distribution of 22,230 single nucleotide polymorphisms (SNPs) physically mapped on 11 cultivated and wild mungbean chromosomes/unanchored scaffolds.

The integration of genomic tools and conventional breeding triggers new breeding strategies, like gene pyramiding, marker-assisted recurrent selection, marker-assisted pedigree selection, and genome selection (GS), which greatly accelerate the breeding. In recent years, genomics-assisted breeding (GAB) has become a powerful strategy for plant breeding. GAB enables the integration of genomic tools with

Table 5.11 Genomic distribution of 22,230 single nucleotide polymorphisms (SNPs) physically mapped on 11 cultivated and wild green gram chromosomes/unanchored scaffolds

Sl. no.	Chromosome	Size of chromosome (Mb)	Total no. of SNPs	% of total SNPs	No. of cultivated SNPs	No. of wild SNPs
1.	1	36.49	1643	7.39	835	267
2.	2	25.34	1332	5.99	538	340
3.	3	12.93	903	4.06	457	171
4.	4	20.78	1044	4.70	512	219
5.	5	37.09	1896	8.53	769	483
6.	6	37.41	1666	7.49	851	278
7.	7	55.45	2306	10.37	979	498
8.	8	45.72	2204	9.91	1072	405
9.	9	20.97	1208	5.43	638	181
10.	10	20.99	1099	4.94	454	282
11.	11	19.67	1161	5.22	570	239
12.	Total	332.84	16,462	74.05	7675	3363
13.	Average	30.26	1497	NA	698	306
14.	Unanchored	NA	5768	25.95	2423	1180

high-throughput phenotyping to assist breeding practices through molecular markers to facilitate the prediction of phenotype from genotype. GAB allows breeders to start out with a large population of only genotypically characterized offspring and then only use a selected subset for more expensive phenotypic evaluation (Cooper et al. 2014). In addition, genotypic evaluation can be done off season, e.g., in winter nurseries, where yield trials are usually not conducted, which also helps to speed up breeding. GAB is especially useful for the improvement of complex traits due to its advantages of high accuracy, direct improvement, short breeding cycle, and high selection efficiency. The ultimate goal of GAB is to find the best combinations of alleles (or haplotypes), optimal gene networks, and specific genomic regions to facilitate crop improvement (Xu et al. 2012). As such, GAB is promising to accelerate the generation of new plant varieties and promote the development of modern agriculture (Leng et al. 2017). Hence the use of these advanced techniques in plant breeding will greatly boost the crop improvement in mungbean (Table 5.12).

5.7.5 Transgenic Technology

The use of transgenic crops is especially required for those traits that are not easy to improve genetically through conventional approaches because of the lack of satisfactory sources of desirable gene (s) in crossable gene pools. However, the ongoing debate on biosafety and ethical issues involving use of transgenic crops for commercial cultivation slowed down the efforts to develop improved cultivars through transgenic technology.

Table 5.12 Examples of well-known online resources for genomic technologies

Name	URL
Next Gen Seek	http://nextgenseek.com
Bits of DNA	http://liorpachter.wordpress.com/seq
RNA-Seq Blog	http://www.rna-seqblog.com
Journal of Next Generation Sequencing & Applications	http://www.omicsonline.org/next-generationsequencing-applications.php
Blog @ Illumina	http://blog.illumina.com
Next-Gen Sequencing	http://nextgenseq.blogspot.com
Omics! Omics!	http://omicsomics.blogspot.com
CoreGenomics	http://core-genomics.blogspot.com
In Between Lines of Code	http://flxlexblog.wordpress.com
Kevin's GATTACA World	http://kevin-gattaca.blogspot.com
Next Generation Technologist	http://www.yuzuki.org

5.8 Future Prospects

This review will facilitate the development of strategies for breeding programs for enhancing crop productivity under resilient climatic conditions which in turn mitigate both biotic and abiotic stress factors enabling proliferation of broad-spectrum stress-tolerant crop species. Hence, this is an attempt to provide insight to the existing genomic breeding methodologies that would enhance the genetic gain in green gram. While it seems like many of these technologies are too far-fetched and futuristic to be true, however, the pace of technological achievement currently witnessed is unprecedented.

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Chapter 6
Breeding for High-Yielding and Disease-Resistant Urdbean Cultivars

Debjyoti Sen Gupta, Jitendra Kumar, Ashok Kumar Parihar, and Sanjeev Gupta

6.1 Introduction

Urdbean or blackgram (*V. mungo* L. Hepper) is a popular warm season pulse crop of India. Urdbean is cultivated throughout the country except the temperate regions. Based on the existing genetic diversity and distribution, Indian center is considered as the center of origin of this crop species (Arora 1985). The progenitor wild species of urdbean is *V. mungo* var. *silvestris* (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). This wild progenitor is spreading type with smaller seeds having prominent raised hilum. The present-day cultivated urdbean varieties were developed through accumulation of recessive mutant genes (Sen and Murty 1960; Smartt 1985). During domestication process, many adaptive traits like the dehiscent nature of pods and seed hardiness were selected out. Present-day urdbean varieties are high-yielding and multiple disease-resistant and have shorter maturity duration compared to what was grown 50 back (Rao and Jana 1974).

India produces and consumes most of the urdbean produced in the country as well as imports a large volume annually from neighboring countries like Myanmar (about 80% of total urdbean import). India produces about 3.26 million tons of urdbean annually from about 4.83 million hectares of area with an average productivity of 696 kg/ha (fourth advance estimates, DES, DAC & FW, GOI, 2018–19). Out of this 2.56 million tons were produced during "Kharif" season, and 0.70 million tons were produced during "Rabi" season (fourth advance estimates, DES, DAC & FW, GOI, 2018–19). Urdbean production contributes to about 14% of India's total pulse production (24 million tons). Urdbean is grown as winter crop in southern subtropical regions due to mild winter situation and as spring/summer/rainy season crop in

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northern India. Date of sowing of urdbean varies based on the local meteorological conditions, for example, Mane (2017) reported that early July sowing gives better yield than the early August sown crop in Parbhani, Maharashtra, India. In western part of the Uttar Pradesh state in India, urdbean is commonly sown in July or August, depending on the onset of pre-monsoon rain (Panotra et al. 2016). It requires moderate temperature (25–35 °C) with moderate to high humidity (70–90%) during vegetative growth and bright sunshine during reproductive stage with a well-distributed pattern of rainfall. Excessive continuous (6–7 days) rainfall during reproductive phase damages the crop yield to the extent of 100% sometimes (personal communication). Urdbean is more tolerant to waterlogging condition than mungbean; sometimes submergence of 2–3 days under water may not affect the crop severely. If drainage facility is not proper, urdbean can be grown in ridges which provide protection to the crop during excessive rainfall while the crop still in vegetative phase of growth. Irrigation facilities are required while growing urdbean in spring/summer seasons. During spring/summer, abrupt increase in maximum temperature reduces the vegetative growth and speeds up the reproductive phase, leading to low yields due to poor grain filling. Productivity is an issue in urdbean, and in countries like India, still the national average productivity (696 kg/ha) is low; however potential yield levels of newly bred urdbean varieties are quite high (1000–1200 kg/ha).

6.2 Major Producing Regions

6.2.1 The World

The area of traditional cultivation of urdbean is confined to the South Asia and adjacent regions (India, Pakistan, Afghanistan, Bangladesh, Thailand, Korea, Myanmar, etc.) Urdbean has been identified as a high-yielding pulse in many Asian countries (Smartt 1990). In Pakistan it is cultivated under a wide range of agroecological zones particularly under rainfed conditions.

6.2.2 India

The major producing states are Madhya Pradesh, Rajasthan, Uttar Pradesh, Maharashtra, Tamil Nadu, Andhra Pradesh, Telangana, Karnataka, Odisha, and West Bengal. Madhya Pradesh is the largest producing state contributing to about 40% of total country's output followed by Rajasthan and Uttar Pradesh with 16% and 10%, respectively. Highest productivity reported was from Andhra Pradesh and Telangana state (819 kg/ha) (Project Coordinator's Report 2018–19).

6.3 Centers of Origin

Cultivated urdbean or blackgram (*V. mungo* var. *mungo* (L.) Hepper) is believed to have been derived from its wild progenitor, *V. mungo* var. *silvestris* Lukoki, Maréchal, and Otoul (Chandel et al. 1984). It was reported further based on archeological evidence that urdbean originated in India (Zukovskij 1962) and domestication of urdbean might have occurred about few thousand years ago (Fuller and Harvey 2006).

Urdbean is reported to be originated in India with a secondary center of diversity in Southeast Asia. It is cultivated throughout Southeast Asia including Northern Malaysia, the Philippines, Thailand, and Myanmar. It is also being cultivated in neighboring countries of India, i.e., Afghanistan, Bangladesh, Bhutan, Pakistan, and Nepal. It is also grown but not extensively in Iran, Kenya, Malawi, and the United States. Genetic distance analysis revealed that cultivated urdbean was more closely related to wild urdbean from South Asia than that from Southeast Asia (Kaewwongwal et al. 2015).

6.4 Crop Systematics

The genus *Vigna* is a taxon in the 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), *V. mungo*(mungbean), and *V. unguiculata* (cowpea), and urdbean (*Viga mungo* L. Hepper) varieties can be categorized into three groups, i.e., *V. mungo* var. *mungo*, early maturing and large seeded; *V. mungo* var. *viridis* Bose, late maturing and greenish dull or shining-type seed; and *V. mungo* var. *silvestris* Lukoki, Maréchal, and Otoul, wild type. However, all possible types of recombinants are present today in cultivated types due to extensive breeding efforts done.

Botanical classifications of urdbean are as follows: kingdom, Plantae; division, angiosperms; subdivision, eudicots; class, rosids; order, Fabales; and, family, Fabaceae.

6.5 Species Relationship

The 21 yellow-flowered species of *Vigna* having origins and diversity in Asia are known as Asiatic *Vigna* species. Seven Asian *Vigna* species, i.e., mungbean (*V. radiata*), urdbean (*V. mungo*), adzuki bean (*V. angularis*), moth bean (*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*. In addition to morphological characters, seed proteins have been used to find species relationship among the *Vigna* species. Mungbean [*Vignaradiata* (L.) Wilczek] and urdbean [*V. mungo* (L.) Hepper] isozyme profiling showed that both are distinct in electromorphs of o-diphenol oxidase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and shikimate dehydrogenase, of several isoesterases and isoperoxidases. Isozyme study also confirmed the differentiation into distinct species at the progenitor level before domestication for both these crop species (Jaaska and Jaaska 1989). Molecular markers were used consecutively to establish or re-establish species relationship in *Vigna* by different workers. Intra- and interspecific variations were studied in the genus *Vigna* by RFLP (restriction fragment length polymorphism); however due to limited number of accessions used, the results could not be generalized (Fatokun et al. 1992). This study particularly showed the distinctness of cowpea section *Catiang*, Bambara groundnut (*V. subterranea*), subgenus *Plectotropis*, and Asiatic *Vigna* in subgenus *Ceratotropis* (Fatokun et al. 1992). Tun and Yamaguchi (2007) used sequence variation in noncoding regions of chloroplast genome to analyze species relationship of 15 species from 9 countries of the subgenus *Ceratotropis*. Most of the species in Myanmar are grouped into either adzuki bean group or mungbean group. Further, this study supported long back differentiation of *V. radiata* and *V. mungo* based on high degree of substitution and indel and microsatellite variation in the trnT-F sequences (Tun and Yamaguchi 2007). Kaewwongwal et al. (2015) while testing 534 *V. mungo* accessions found that gene diversity was highest in South Asia, followed by Southeast Asia, West Asia, and the Himalayan region. Further, SSR (simple sequence repeats)-based analysis showed that level of gene diversity of urdbean is comparable to that of mungbean and rice bean but lower than that of adzuki bean.

6.6 Plant Morphology and Floral Biology

It is an erect, suberect or trailing, densely hairy annual herb. The tap root produces a branched root system with smooth, rounded nodules. Urdbean is an annual food legume. It shows both erect and spreading growth habit. The pods are narrow, cylindrical, and up to 6 cm long. Urdbean is a highly self-pollinated crop with cleistogamy of up to 42% (Narasimhan 1929; Bose 1932; Puneglov 1968). Anthesis occurs in the early morning hours. Floral mutants are found with partial male sterility (Kumar et al. 2012). Mutants with male sterility have potential for use in a program to obtain hybrid seeds without emasculation.

6.7 Trait Inheritance

Systematic urdbean breeding was initiated in India with the start of All India Coordinated Pulses Improvement Project in 1966. Since then lots of trait improvement have been made in this crop species. One of the popular and early released (1970s) urdbean varieties was T9, a variety developed through selection from locally adapted land race from Bareilly, India. Breeding progress over the years was significant; however improvement of oligogenic traits was more visible than polygenic ones.

We will be discussing here modes of inheritance of different morphological traits like growth habit, leaf shape, pod pubescence, podding behavior, and flower structure. Urdbean plants are generally spreading, but breeding efforts developed present-day urdbean cultivars that are mostly semi-erect or erect. Erect plant type is not completely dominant over spreading type (Sen and Jana 1964). Similarly, in case of normal with induced mutant comparison, Rao et al. (1975) reported that induced dwarf mutant is controlled by a recessive gene. Leaves of urdbean are large, trifoliate, ovate, or lanceolate. Verma (1971) reported that ovate leaf shape is dominant over lanceolate leaf and is controlled by a single dominant gene. Singh and Singh (1971) observed that the hastate shape is dominant over ovate and is probably controlled by duplicate dominant genes. Rao et al. (1989) dealt with a mutant with multifoliate leaves in urdbean (6–7 leaflets/leaf) and observed that the trait is controlled by a single recessive gene. Muralidharan et al. (1990) found a fused leaf (cotton leaf) variant in urdbean and observed that the trait is recessive to ovate leaf shape and is controlled by a recessive gene. Pods of urdbean are most often pubescent or hairy. Pathak (1961) and Sirohi and Singh (1998) found that hairy pods are dominant to non-hairy pods and controlled by a single gene. Sen and Jana (1964) assigned the symbol g to a recessive gene controlling non-hairy pod surface. Pods in urdbean are generally found along the plant canopy or shy bearing type. However, due to breeding efforts above, canopy pod bearing types are recently developed in urdbean (personal communication, unpublished data). Two different pod orientations, i.e., main stem bearing and sympodial bearing types, are infrequently found in urdbean germplasm. Rao (1999) observed that the main stem bearing was under the control of single dominant gene which is incomplete in expression (1:2:1). Black-, brown-, and straw-colored pods are found in urdbean. Black pod color is the most common in urdbean germplasm as well as in breeding material. Straw pod color and brown pod color is recessive to black pod color and conditioned by a single gene (Sen and Jana 1964; Verma 1971). The crumpled petal character or small nonflowering bud or malformed flower or keel mutant was recessive to the normal allele and monogenic in inheritance (Appa Rao and Reddy 1976; Jana 1962). Kumar et al. (2012) studied the inheritance of protruded stigma in naturally occurring mutants and concluded that male sterility is controlled by a single recessive gene with pleotropic effects. Sen and Jana (1964) showed that brown seed coat color is recessive to green seed coat color and conditioned by a single gene. Arshad

et al. (2005) reported that brown seed coat color is dominant over green seed color. Shiny seed surface was dominant over dull seed surface (Sen and Jana 1964).

6.8 Genetics of Disease-Pest Resistance

The most devastating disease of urdbean is yellow mosaic disease. The first incidence of mosaic disease was reported on cowpea from India by Thomos (1937) and *Vigna* species in general by Mclean (1941). The disease has now become widespread and reported to cause severe yield losses annually to these crops (Varma and Malathi 2003). Altogether yield losses due to YMD in urdbean, mungbean, and soybean were estimated to be US $ 300 million per year (Varma and Malathi 2003). Growth reduction and yield loss are much severe when disease appears at early growth stages and may result in 100% yield loss. Reduction in growth components and grain yield also depends on crop variety, agronomic practices, alternative host, and vector population. The viral disease is transmitted through the white fly, *Bemisia tabaci*, and yield of the plants is affected drastically. Balaji et al. (2004) identified two DNA fragments, DNA A (KA30) and DNA 13 (KA21, 22, 27, 28, and 34). KA22 DNA B caused more intense yellow mosaic symptoms in urdbean, while K27 DNA B caused more intense yellow mosaic symptoms in mungbean. Therefore, DNA B is an important determinant of host range in urdbean. There are conflicting reports about the genetics of resistance to MYMV, claiming both resistance and susceptibility to be dominant. Monogenic dominant nature of resistance has been reported by several workers (Dahiya et al. 1977; Kaushal and Singh 1988a, b; Gupta et al. 2005, 2013). In some studies, the resistance was found to be mono or digenic recessive (Singh 1981; Dwivedi and Singh 1985; Verma and Singh 1986; Singh et al. 1987; Pal et al. 1991; Reddy and Singh 1995). However, only few workers mentioned which viral species of yellow mosaic infected the experimental material. It is important to note several yellow mosaic virus strains are present in India and they differ in pathogenicity. Another important disease in urdbean is powdery mildew disease (PMD). Powdery mildew caused by the fungal pathogen, *Erysiphe polygoniDC*, is a highly devastating disease of winter season urdbean crop. It is most important disease in urdbean grown under "rice-fallow" niches in southern India. Late sown urdbean crop also got infected with this disease sporadically in Northern states in India. Most conducive weather for this disease is when the average maximum temperature varied from 27 to 30 °C and relative humidity from 67 to 90% during the morning (Thakur and Agrawal 1995). Kaushal and Singh (1989) studied its genetics and reported that the resistance was controlled by a single recessive gene. This is in contrast to mungbean where powdery mildew resistance is controlled by two dominant genes (Reddy et al. 1994). Recently, Santosh (2016) in his thesis research reported that resistance to PMD is controlled by single gene with dominant gene action. Also, it was confirmed that PMD resistance in urdbean is not governed by maternal effects or cytoplasmic genes (Santosh 2016). Cercospora leaf spot (CLS) caused by fungi, *Cercospora canescens* and *C. cruenta*, is an important

disease during rainy season crop in urdbean. Kaushal and Singh (1991) indicated resistance to CLS is controlled by dominant gene. However, involvement of different species in causing *Cercospora* leaf spots complicates the genetic characterization of host resistance. The genetics of this trait in urdbean is not properly worked out. However, in another *Vigna* crop, cowpea (*V. unguiculata* ssp. *unguiculata*) resistance to *C. canescens* was reported to be controlled by single major recessive gene (Duangsong et al. 2016). Inheritance of disease resistance in urdbean to the leaf spots caused by *Colletotrichum truncatum* (Schw.) Andrus & Moore is controlled by single dominant gene (Kaushal and Singh 1988a, b). Another emerging disease is urdbean leaf crinkle virus (ULCV) disease. Nene and Kolte (1972) reported that this disease is caused by a mechanically transmissible and seed-borne virus. The symptoms are characterized by the following: enlargement of trifoliate leaves and crinkling of leaf lamina, delay in flowering, and no pod formation. This disease is emerging as a potent threat to urdbean cultivation next to YMV. Dubey et al. (1983) described the properties of ULCV and demonstrated that the virus was transmitted by *Aphis craccivora* and *Acyrthosiphon pisum*. Since the resistant germplasm are not yet identified, the genetics of resistance against this viral disease is not yet worked out. Bruchids (*Callosobruchus maculatus* and *C. chinensis*) are important stored grain pests causing substantial losses during storage. Dongre et al. (1996) indicated the presence of two dominant duplicate genes that are controlling resistance against *C. maculatus* in urdbean.

6.9 Breeding Objectives

Urdbean is grown either as a sole or intercrop during "Kharif" season, in "Rabi" season in rice fallow as sole crop, or in spring/summer season as sole crop where irrigation facilities are adequate. Initially breeding for resistance to diseases of economic importance, e.g., MYMV, was the main objective along with yield. This was done because the disease not only causes severe yield loss but also is difficult to be controlled by any other management measure. With the introduction of urdbean crop in rice fallow of coastal regions, breeding for resistance to PMD and CLS became the next priority in urdbean breeding. Among the invading insects, breeding for thrips was also taken up subsequently. However, attention also needs to be given to breeding for resistance to bacterial leaf spot, *Macrophomina* blight, and ULCV and tolerance to hairy caterpillar and blister beetle. Breeding for different seed colors as well as luster is also getting impetus. Under changing climate scenario, breeding for heat tolerance and photo-insensitivity is also becoming an important breeding objective. With the initiation of "National Initiative on Climate Resilient Agriculture" project in India, breeding materials for these traits are being generated. Improving the yield potential of the newly bred urdbean breeding materials is the prime objective in any urdbean breeding program.

6.10 Breeding Methods

Initial years of urdbean breeding in India and elsewhere witnessed use of conventional plant breeding methods to release urdbean varieties. These include mass selection, pedigree selection, backcrossing, and mutation breeding. Varieties released during initial years were direct selections from local land races. In urdbean varieties released in India from 1949 to 2000, more than 50% were developed from selections. Later, more emphasis was given on use of hybridization-based techniques to combine multiple traits in single variety to exploit newer niches like spring/summer cultivation or rice-fallow situations. Hence, the last two decades witnessed more 50% releases were hybridization based. Multiparent-based hybridization is presently initiated in many urdbean breeding programs to breed for urdbean varieties with broader genetic base. In this section we will be discussing different methods that are used or being used in urdbean breeding programs.

6.10.1 Mutation

In India, mutation breeding is facilitated by Bhabha Atomic Research Center (BARC), Mumbai. Varieties developed through mutation breeding include CO 4, Sarla, Vamban 2, TU94–2, Ujala (OBG17), and Prasad (B3–8-8). CO 4 was developed through mutation breeding and was having large seed size (6 gm/100 seeds) and early maturing (70–80 days) and was resistant to powdery mildew disease. Sarla was a mutant of T9 variety and was tolerant to mungbean yellow mosaic virus (MYMV) disease. Vamban 2 was also a mutant of T9 variety and was having hairy pod and early maturing (70–75 days) and was tolerant to drought stress. Ujala (OBG17) was a mutant of Prasad (B3–8-8) and was resistant to MYMV and CLS. Prasad was a mutant of T9 variety and was tolerant to MYMV and suitable for "Rabi" cultivation.

6.10.2 Intraspecific and Interspecific Hybridization

Intra- and interspecific hybridization was initiated to combine multiple traits in a single variety. Interspecific hybridization has become regular in most urdbean breeding programs with the use of improved hybridization techniques which have increased the possibilities of successful crosses. However, most of these interspecific crosses are with *V. radiata* or *V. mungo* var. *silvestris*. Other species are yet to be fully explored in distant hybridization of urdbean.

Disease Resistance Yellow mosaic disease, powdery mildew disease, *Cercospora* leaf spot disease, and leaf crinkle disease are major diseases of urdbean. In most cases field-level screening is done with the use of infector rows or susceptible

genotypes. Any sort of field resistance requires further laboratory-based or laboratory-controlled environment for reconfirmation. These mentioned diseases sometimes have localized isolates or even species-level variability which further complicates the host plant resistance breeding. Disease resistance breeding also requires interdisciplinary research with more emphasis on plant pathology and plant breeding techniques. In the following section, we will be discussing available host plant resistance in urdbean against these viral or fungal diseases and their role in varietal improvement.

Yellow mosaic disease (YMD) is caused by MYMV (mungbean yellow mosaic virus), MYMIV (mungbean yellow mosaic India virus), and HgYMV (horsegram yellow mosaic virus). In northern India, urdbean crop is mostly infected by MYMIV, and in the southern peninsula it is infected by MYMV. Due to increased seed mobility nowadays, presence of both the species in any part of the country cannot be ruled out. Donor for this disease has been identified and used in breeding programs to develop YMD-resistant urdbean varieties. All India Coordinated Pulses Improvement Project has mandated to release only urdbean varieties which are high yielding as well as resistant to YMD. Inheritance of resistance to YMD has been worked out by many workers (as discussed earlier); however single dominant gene is mainly involved in imparting YMD resistance. RAPD, SCAR, and ISSR markers for MYMV resistance have been have been developed in *Vigna* species (Selvi et al. 2006; Souframanien and Gopalakrishna 2006; Somta et al. 2009). This gene has been mapped at a distance of 12.8 cM from the SSR marker loci (Gupta et al. 2013); however finer mapping is required for its use in marker-assisted selection (MAS). Since the 1980s, a number of urdbean varieties with YMD resistance (recently released MYMV-resistant varieties are summarized in Table 6.1, and photographs of two recently released urdbean varieties from IIPR, Kanpur, are shown in Fig. 6.1) have been released. Cultivars Pant U 19, Pant U 30, and Pant U 35 released in 1980s derived their resistance from UPU 2, a selection from D-6-7 cultivar. Among MYMV-resistant cultivars released so far, most of them were bred using T9 as an agronomic base. Under this scenario breeding efforts in urdbean were diverted to diversify the background. For the last 10 years, a number of YMD-resistant varieties have been released in India with diverse background (Table 6.1). Breeding against YMD needs further refinement including use of molecular markers as well as agro-inoculation clones for working at the species level for this virus. Figure 6.2 depicts degree of genetic resistance where under natural disease incidence one urdbean accession is fully infected, whereas neighboring breeding lines were completely disease-free.

Powdery mildew is a major problem in coastal humid regions. In case of mungbean quantitative inheritance of resistance loci was reported (Chaitieng et al. 2002). The inheritance of resistance is reported to be controlled by a single recessive gene in urdbean (Kaushal and Singh 1989). 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

Table 6.1 MYMV-resistant high-yielding urdbean varieties released in India in the last 11 years (2008–2019)

State	Season	Varieties
Andhra Pradesh	Kharif	IPU 2–43, LBG 752, VBG 04–008, LU 39, LBG 787
	Rabi	LBG-709, TU 40, LBG 752, LBG 685, TBG 104
Assam	Kharif	WBU 109, SBC 40
Bihar and Jharkhand	Kharif	Birsa Urd 1, Pant U 31, WBU 109
	Spring/ summer	WBU-109, Pant U 31
Gujarat	Kharif	GU 1, NUL 7
Haryana	Kharif	UH1, Vallabh Urd 1, Mukund Urd 2
Himachal Pradesh and J&K	Kharif	Him Mash 1, Mukund Urd 2
Karnataka	Kharif	LU 391, VBG 04–008, IPU 2–43, DU 1, DBGV5, LBG 787
Madhya Pradesh and Chhattisgarh	Kharif	NUL 7, Indira Urd 1
	Spring/ summer	
Maharashtra	Kharif	AKU 15, NUL 7, PDKV Blackgold (AKU 1–10)
NEH states	Kharif	Pant U 31, Him Mash 1, Tripura Maskolai
Odisha	Kharif	IPU 2–43, OBG 31, VBG 04–008, LBG 787
	Summer/ spring	OBG 17
Punjab	Kharif	Mash 114, Pratap Urd 1, Mash 1008, Mash 479, Mash 391, Vallabh Urd 1, Mukund Urd 2
	Spring/ summer	KUG 479
Rajasthan	Kharif	Pant U 31, Pratap Urd 1, Vallabh Urd 1, Mukund Urd 2
	Spring/ summer	KUG 479
Uttar Pradesh and Uttaranchal	Kharif	Pant U 31, Vallabh Urd 1
	Spring/ summer	WBU 109
Tamil Nadu	Kharif	ADT 5, IPU 2–43, VBG 04–008, Co 6, Vamban 6, Vamban 7, MDU 1, LU 391, LBG 787
	Rabi-rice fallow	VBN-5, TU 40, LBG 787
West Bengal	Kharif	
	Summer/ spring	WBU 109, Pant U 31

Modified from PC Report, AICRP of MULLaRP, 208–19, IIPR, Kanpur

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.

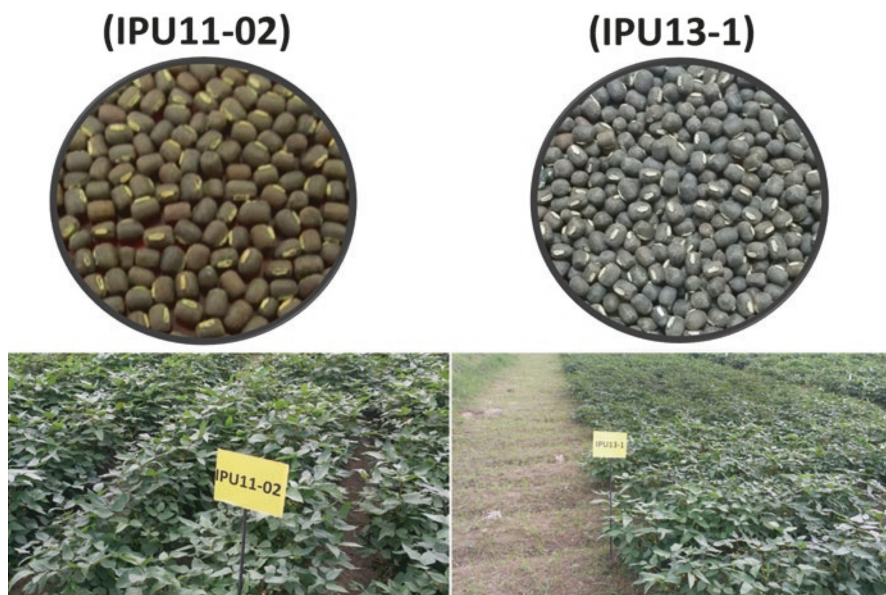

Fig. 6.1 Two recently released urdbean varieties from IIPR, Kanpur, India. (**a**) Variety IPU 11–02 (**b**) Variety IPU 13–1

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. cruenta* 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.

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 and Kolte (1972) reported 62–100% yield reductions depending upon the stage of growth at which the plant becomes infected. Prasad and coworkers (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.

Insect Resistance Thrips (*Megalurothrips distalis*) and stem flies (*Ophiomyia centrosematis* and *O. phaseoli*) are major pests of urdbean in all regions and seasons. The lepidopteran pod borer is particularly severe in central and southern states. Thrips feed on flowers, petioles, and stigmas, causing inflorescence deformity and premature flower shedding, while stem flies incite seedling mortality. Under heavy insect pressure, yield losses of susceptible cultivars can be as high as 40%. Highly

Fig. 6.2 Breeding against yellow mosaic disease (YMD) – under natural disease incidence one urdbean accession is fully infected, whereas neighboring breeding lines were completely disease free

resistant lines such as PDU 5, KB 63, UG 567, and UH 804 against thrips; UG 218, PDU 1, PDU 5, AKU 7, Co 305, UP 95–1, and LBG 707 against stem flies; and UG 737, PLU 557, and TAU I against pod borers have been identified. Despite single season observations in most of the reports, a long list certainly indicates wide variability in genotypic response to infestations by major insect pests in different regions. This necessitates systematic studies to identify stable resistant or tolerant genotypes to incorporate it in high-yielding cultivars.

Bruchids (*Callosobruchus chinensis* and *C. maculatus*) are very serious storage pests when no precautionary measures are taken during storage. No published report for resistant sources to bruchids is available in indigenous accessions. Instead, very high level of resistance to bruchids was found in few accessions of Pakistan (Rasul et al. 1989; Ashraf et al. 1991) and AVRDC material (AVRDC 1986, 1991). In this regard it is suggested that confirmed and widely reported sources of resistance to bruchids should be taken up for incorporation into high-yielding urdbean varieties. However, these various sources should be tested for degree of resistance before using them in breeding programs.

6.10.3 Use of "Omic" Technologies in Urdbean Breeding

6.10.3.1 Tissue Culture and Genetic Transformation

Conventional breeding programs are invaluable to provide regular supply of desirable varieties; however, to exploit molecular tools to introgress gene(s) which are not available in cross-compatible gene pool, efficient in vitro regeneration and transformation protocol are a prerequisite. Generally, pulses are comparatively recalcitrant to regeneration and transformation. In urdbean, progress has been achieved to the extent of callus formation and morphogenesis from different explants when placed on MS basal medium supplemented with hormones individually and in combination (Geetha and Rao 1997; Ignacimuthu et al. 1997; Franklin et al. 2000). In vitro screening techniques have been developed for resistance to *Cercospora canescens* (Kaushal et al. 1997) and tolerance to NaCl (Geetha et al. 1995) and polyethylene glycol (PEG) (Geetha et al. 1996; Geetha and Rao 1997). *Agrobacterium*-mediated transformation of urdbean was successfully demonstrated by Karthikeyan et al. 1996. cDNA clones encoding UDP-galactose-flavonoid 3–0-galactosyl transferase (UF 3 Gat) and genes encoding enzyme 1 (VmPE 1) were successfully sequenced, and their expression in cotyledons and other organs was investigated (Mato et al. 1998; Okamoto and Minamikawa 1999). Recently, in vitro regeneration of urdbean in variety "Sarala" was achieved through organogenesis using cotyledonary explants excised from 4-day-old seedlings on MS medium supplemented with 2.0 mg/l BAP. Seventy-five percent of regenerated plantlets could be fully grown (Adlinge et al. 2014). These progresses will be useful in urdbean breeding in near future.

6.10.3.2 Development of Molecular Markers

With the use of molecular markers like RAPD, SSRs, ISSRs, AFLP, and RFLP, genetic identity of germplasm or breeding materials is more precisely established. Molecular markers have been developed in urdbean initially by testing transferability of molecular markers developed in closely related species or markers derived from conserved sequence regions of distantly related species. Souframanien and Gopalakrishna (2009) transferred 49 microsatellite markers from cowpea (*V.unguiculata*), mungbean (*V. radiata*), adzuki bean (*V. angularis*), and common bean (*Phaseolus vulgaris*) to urdbean. While testing a panel of urdbean genotypes it was found that 42 out of 49 were polymorphic. In another study, Gupta and Gopalakrishna (2009) used 36 microsatellite markers from adzuki bean to genotype 20 urdbean genotypes and found that 33 (92%) markers were polymorphic. Gupta and Gopalakrishna (2010) used 65 cowpea-derived SSR markers to genotype different *Vigna* species, and 55 (85%) SSRs amplified urdbean DNA. This kind of high rate of marker transferability encouraged others like Gupta et al. (2013) where 361 SSR markers developed in different food legume species were tested for their

transferability among 24 diverse genotypes of urdbean. However, only 39 (16%) were polymorphic and 245 (68%) were monomorphic among the tested genotypes (Gupta et al. 2013). During 2015, Souframanien and Reddy developed 933 SSR markers from the urdbean transcriptome data and validated 55 primers, out of those 32 (58.2%) were polymorphic. These markers could be useful in urdbean breeding.

6.10.3.3 Use of Molecular Markers

Sivaprakash et al. (2004) used 7 AFLP primer combinations to detect genetic diversity among 26 urdbean landraces collected from Orissa state in India; the landraces clustered together into five groups based on their genetic affinities. Souframanien and Gopalakrishna (2004) compared RAPD and ISSR markers in assessing the genetic diversity in urdbean and found that ISSR markers were more informative. Genetic diversity was determined among gamma ray-induced mutants in urdbean using RAPD (25.8%) and ISSR (33.3%) markers (Souframanien et al. 2010). SSR markers were used many times to evaluate genetic diversity among urdbean accessions (Souframanien and Gopalakrishna 2009; Gupta et al. 2013). Kaewwongwal et al. (2015) evaluated 520 cultivated and 14 wild accessions of urdbean with 22 SSR markers. Wild urdbean species had higher gene diversity compared to cultivated urdbean species.

Use of molecular markers in gene tagging or mapping in urdbean is limited. MYMV resistance gene had been tagged or mapped by various workers in urdbean (Souframanien and Gopalakrishna 2006; Gupta et al. 2013).

6.11 Outlook

Vigna gene pool due to its rich diversity may aid in urdbean improvement. However, not much progress had been made in successfully hybridizing urdbean with other *Vigna* species, specifically which are in distant gene pool. Urdbean (*V. mungo*) is easily crossable with mungbean (*V. radiata*). Using different pre-breeding techniques like embryo rescue and hormonal manipulation, successful interspecific hybrids can be developed in urdbean. Many workers reported success in developing interspecific hybrids between different *Vigna* species like *V. radiata* x *V. glabrescens*, *V. radiata* x *V. mungo*, *V. radiata*x *V. umbellata*, and *V. umbellata* x *V. angularis* (Chen et al. 1983, 1989). These crossable species should be used to transfer many important traits like more seeds per pod that can be transferred from mungbean to urdbean. *V. trilobata* which has a greater number of pods per plant can be used in hybridization with urdbean for increasing the yield potential. Another important trait is bruchid (*Callosobruchus chinensis*, *C. sinensis*) resistance for which no resistance is reported to the best of our knowledge in cultivated urdbean varieties or germplasm accessions. Wild *Vigna* species could be explored for the

search of resistance allele against bruchids. There are ample evidences that suggest for source sink manipulation required for yield improvement. As far as applied plant breeding aspect is concerned, there is a need to integrate more physiological traits for yield improvement. Photosynthetic rate, net assimilation rate (NAR) at flowering, and biological nitrogen fixation are some of the traits which require immediate attention. Development of photo-thermo insensitive varieties is another priority area in urdbean breeding. Varieties with all these traits will be resilient toward climate changes. It will further help to expand this crop species in different ecological niches including rice fallows.

6.12 Major Crop Improvement Research Stations

National centers are (1) ICAR, Indian Institute of Pulses Research, Kanpur; and (2) State Agricultural University through AICRP in MULLaRP, IIPR, Kanpur, as coordinating headquarter.

International centers are (1) World Vegetable Center (AVRDC), Taiwan; (2) Kasetsart University, Bangkok, Thailand, and (3) National Agriculture and Food Research Organization, Japan.

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Chapter 7
Lentil Breeding in Genomic Era: Present Status and Future Prospects

Jitendra Kumar, Debjyoti Sen Gupta, and Pravin Tiwari

7.1 Introduction

Lentil, as an important pulse crop, is cultivated worldwide under rainfed conditions in cool season. It is a diploid ($2n = 2X = 14$) self-pollinated crop having a genome size of 4063 Mbp (Arumuganathan and Earle 1991). It is nutritionally rich having dietary proteins from 22% to 35% in their seeds besides minerals, fiber, and carbohydrates in plenty amount. High quality of the carbohydrates (i.e., low glycemic index) in their seeds makes its a healthier pulse for the people who are suffering from chronic diseases such as diabetes, obesity and cardiovascular. In India, it is currently cultivated on 1.49 mha and has a production of 1.61mt with an average productivity of 1006 kg/ha (AICRP on MULLaRP 1918–19). The systematic and continuous conventional breeding approaches led to increase its productivity nearly two times since 1960–1961 (539 kg/ha) (AICRP on MULLaRP 2018–19). Though a significant increase in the productivity of lentil has been observed in the past years, compared to average productivity of the world (>1500 kg/ha), it is still low. This low productivity is caused by several reasons including its poor competitive ability to weeds, higher flower drop rate, pod shedding, and several biotic and abiotic factors in India (Sharpe et al. 2013). High genotype × environment (G × E) interactions on the expression of important quantitative traits are also one of the reasons of the slow gain in lentil (Kumar and Ali 2006). The current climatic changes have also become a threat to the lower production and productivity of lentil. Therefore, strengthening the conventional breeding programs with new tools and techniques is urgently required for making improvement in the productivity of lentil.

Genomics has emerged as a potential way to increase the genetic gain in crop plants (Li et al. 2018; Nepolean et al. 2018; Shamshad and Sharma 2018; Cobb et al. 2019; Voss-Fels et al. 2019). Therefore, in the past years, considerable

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attention has been paid on development of genomic resources for accelerating the genetic gain in pulse crops (Bohra et al. 2014), and next-generation sequencing provided genome sequences of major pulse crops in the past few years (Varshney et al. 2012, 2013; Kang et al. 2014; Kreplak et al. 2019). These genomic resources help to accelerate the genetic gains in legumes and translated genomics in agriculture (Varshney et al. 2015, 2018). During the past few years, considerable efforts have also been made to develop the genomic resources including availability of genome sequences, transcriptome sequences, SNP markers, SSR markers, mapping populations, and genes/QTLs for important traits in lentil (Bett et al. 2014; Kumar et al. 2014, 2015, 2018a, b, c; Singh et al. 2017a, b, 2019a, b). These genomic tools and technologies have opened up new ways for exploiting them in lentil breeding. In the present chapter, a present and future prospect of lentil breeding has been discussed in the light of current genomics era.

7.2 Genomic Resources

During the past few years, efforts have been made to develop genomic resources in lentil, which are discussed below.

7.2.1 Molecular Markers

Exploitation of genomics via marker-assisted selection required linkage maps based on molecular markers and then their close association with trait of interest. In lentil, various types of molecular markers have been developed and used that have been classified broadly in three classes including (i) hybridization based, (ii) PCR based, and (iii) sequencing based.

7.2.1.1 Hybridization-Based Molecular Markers

It includes RFLP markers that are first-generation molecular markers. These markers have not been used widely in lentil, and few studies reported their use in the development of linkage maps (Havey and Muehlbauer 1989; Eujayl et al. 1998a).

7.2.1.2 PCR-Based Molecular Markers

These are second-generation molecular markers that include RAPD (random amplified polymorphic DNA), SCAR, and SSR (simple sequence repeat) markers. The random amplified polymorphic markers have been used widely in lentil for genetic mapping, establishment of genetic relationships, and analysis of genetic diversity

(Abo-Elwafa et al. 1995; Andrahennadi et al. 1995; Sharma et al. 1995; Eujayl et al. 1997; Chowdhury et al. 2001; Hoque et al. 2002; Hoque and Hasan 2012; Erdoğan 2015; Mbasani-Mansi et al. 2019). In lentil, RAPD markers showed association with *Ascochyta* blight resistance and have been converted into SCAR markers (Chowdhury et al. 2001). Development and use of PCR-based simple sequence repeat markers are comprehensive in lentil like other crops due to several advantages such as co-dominant, high level of polymorphism, and easy handling over the other PCR-based makers. These SSR markers have been developed either from random genomic regions (genomic SSR; Hamwieh et al. 2009 Andeden et al. 2015; Bakır and Kahraman 2019) or from functional regions (EST-SSR or genic SSR; Kaur et al. 2011; Gupta et al. 2016). In lentil both genomic and functional markers have been used widely for genetic diversity analysis and mapping of genes/QTLs for traits of agronomic interest (Tullu et al. 2008; Gupta et al. 2012a; Kushwaha et al. 2013; Roy et al. 2015; Singh et al. 2016a, b, c; Ates et al. 2018b; Kumar et al. 2018a, b; Tsanakas et al. 2018; Polanco et al. 2019; Singh et al. 2019b).

In the recent years, second-generation sequencing technology carrying out transcriptome sequencing of lentil permitted large-scale unigene assembly and SSR marker discovery (Kaur et al. 2011). A set of 2393 EST-SSR markers developed in lentil using this technology and a subset of 192 EST-SSR markers have been validated across a panel of 12 cultivars with the 47.5% polymorphism (Kaur et al. 2011). In recent times, transcriptome cDNA library sequencing using Illumina GA/GAIIx system has provided a potential alternative. As a result of transcriptome sequencing, massive data was obtained in the form of about 847,824 high-quality sequence reads and the transcriptome assemblies with 84,074 unigenes (Singh et al. 2017c).

7.2.1.3 Sequencing-Based Markers

Single nucleotide polymorphism (SNP) markers are sequencing-based third-generation molecular markers. These markers essentially required sequencing of two or more genotypes for detecting polymorphism after their development in a particular species. Different strategies have been used for detection and validation of SNP markers in crop plants (Mammadov et al. 2012). Initially, gene-based SNPs have been discovered by using resequencing of unigene-derived amplicons or using the available EST database following their validation by PCR (Batley et al. 2003; Wright et al. 2005). They used first-generation sequencing technology based on Sanger's sequencing method. In lentil, kompetitive allele specific PCR (KASP) methodology has been used to detect the SNPs using available EST database ((Fedoruk et al. 2013; Sharpe et al. 2013). However, advancement in next-generation sequencing technologies led to the development of different second- and third-generation sequencing technologies, which provided opportunities of discovering SNPs more rapidly and cost-effectively (Kaur et al. 2011; Sharpe et al. 2013). Use of next-generation sequencing approaches led to the discovery of ~44,879 SNP markers using Illumina Genome Analyzer (Sharpe et al. 2013), and discovery of

high-density SNP markers in lentil has facilitated the establishment of ultra HTP genotyping technologies such as Illumina GoldenGate (GG), which can accommodate more than 1000 SNPs in GG platforms (Sharpe et al. 2013; Kaur et al. 2014). However, during the past few years, transcriptome analysis based on current next-generation technologies has speed up the discovery of SNPs in lentil, and a vast amount of SNPs have been detected from coding regions of lentil genome (Sharpe et al. 2013; Kaur et al. 2014; Singh et al. 2017c, 2019b). In lentil, SNP makers have been used in the development of linkage maps, genetic diversity analysis, and association with traits of agronomic importance (Lombardi et al. 2014; Sudheesh et al. 2016; Khazaei et al. 2017, 2018; Pavan et al. 2019). More recently, genotyping by sequencing detected 6693 SNPs. These SNPs differentiated Mediterranean gene pool of lentil according to geographical patterns and phenotypic traits that indicated routes of introducing lentil cultivation in Mediterranean countries after domestication and further lentil population structure have been shaped due to selections (Pavan et al. 2019).

7.2.2 Lentil Genome Sequence

Considerable progress has been made in decoding the genomes of different legume crops, and draft genome sequences have been released in the past few years (Bauchet et al. 2019). For accessing the genetic and genomic resources of legumes, a number of web database have been developed publicly (Bauchet et al. 2019). In lentil, an initial draft of 23x coverage produced scaffolds covering over half the genome (2.7 Gb of the expected 4.3 Gb), and recent additional 125x coverage has been developed (Bett et al. 2014). The lentil genome assembly v1.0 of this draft genome has been released in January 2016. It has seven pseudo-molecules anchored through the use of six high-density genetic linkage maps, and this draft genome has been assembled from genomic and RNA sequencing data that have been carried out at several institutions across the world using different technologies. All information related to this draft genome sequence is available on the KnowPulse web portal (http://knowpulse.usask.ca) for facilitating in-depth genetic and genomics studies in lentil (Bett 2016). Another effort has also been made to develop a draft genome of lentil in Australian cultivar PBA Blitz. This draft genome has a total of 337.7 Gbp (c. 85x coverage) of high-quality sequences, and its assembly is comprised of 352,065 scaffolds and 444,011 singletons with N50 value of 94.4 kb, resulting a total of 2.3 Gbp. This draft genome also represented by seven pseudo-molecules having a similarity of 99% with earlier reference genome sequence of lentil (Kaur et al. 2016).

7.2.3 Mapping Populations and Linkage Maps

An association of a molecular marker with gene(s)/QTL(s) is required polymorphic molecular markers, a mapping population (biparental or multi-parental mapping populations or diverse panel of genotypes), phenotypic data of interested trait, linkage maps if its biparental or multiprental mapping population, and computer softwares. In lentil, biparental mapping populations have been developed for various traits and used to construct linkage maps based on molecular markers. This information has been used further for marker-trait association analysis (Kumar et al. 2019). Moreover, a rapid generation advancement technology allows four to five generations per year in lentil (Mobini et al. 2014). This can boost the development of genetic resources for genomics-enabled improvement. For genetic mapping of agronomically important traits, a number of linkage maps have been developed in the recent past years (see Kumar et al. 2019). Zamir and Ladizinsky (1984) developed the first genetic map in lentil. However, Havey and Muehlbauer (1989) developed the first DNA marker-based linkage map. After that, a number of genetic linkage maps have been developed in lentil using different markers including RAPD, AFLP, RFLP, SSR, and ITAP gene-based markers and morphological markers (Eujayl et al. 1998a; Rubeena et al. 2003; Durán et al. 2004; Hamwieh et al. 2005; Rubeena et al. 2006; Phan et al. 2007; Gupta et al. 2012b; Andeden et al. 2013). These linkage maps have been reviewed in details by earlier workers (Kumar et al. 2015, 2019). An intra-specific linkage map of 216 SSR loci covering 1183.7 cM distance with an average marker density of 5.48 cM was constructed using three mapping populations (Verma et al. 2015). However, advancement of genome sequencing technologies resulted in development of SNP markers, which have been used to develop high-density linkage maps in lentil (Table 7.1). First, SNP markers have been mapped along with other molecular markers (Sharpe et al. 2013; Kaur et al. 2014). However, subsequently cost-effective next-generation sequencing led to the development of linkage maps that are based solely on SNP markers (Gujarai-Verma et al. 2014; Temel et al. 2014; Sudheesh et al. 2016; Aldemir et al. 2017; Bhadauria et al. 2017). Recently a high-density consensus map has been developed in lentil that is comprised of 9793 SNP markers covering a total of 977.47 cM. In this linkage map, an average distance between two markers is 0.10 cM, and it has seven linkage groups representing seven chromosomes of the lentil genome (Ates et al. 2018a). In another linkage map, 5385 DNA markers have been placed in a biparental population used in above consensus map, which covered a total map length 973.1 cM, with an average distance between markers of 0.18 cM (Ates et al. 2018b). Transcriptome-based next-generation sequencing helped to develop the SNP markers from functional genes, which have been used to construct a high-density interspecific (*Lens culinaris* × *L. odemensis*) genetic map in lentil (Polanco et al. 2019). In this map, 6153 markers have been placed on ten linkage groups. These markers are grouped into 4682 unique bins and covered 5782.19 cM length (Polanco et al. 2019).

Table 7.1 List of molecular linkage maps based on SNP markers in lentil

Crossing species	Type and size (in parenthesis) of population	Mapped length (cM) and no. of loci (in parenthesis)	References
Precoz × WA 8649041	RIL (101)	540 (519)	Temel et al. (2014)
Indianhead × Northfield; Indianhead × Digger; Northfield × Digger	RILs (117, 112, 114)	2429.6 (689)	Sudheesh et al. (2016)
L01-827A (*L. ervoides*) × IG 72815 (*L. ervoides*)	RIL (94)	740.9 (543)	Bhadauria et al. (2017)
ILL 8006" × "CDC Milestone"	RIL (118)	497.1 (4177)	Aldemir et al. (2017)
CDC Redberry × ILL7502; ILL8006 × CDC Milestone; PI320937 × Eston	RIL (120; 118; 96)	977.47 (9793)	Ates et al. (2018a)
CDC Redberry × ILL7502	RIL (120)	973.1 cM (5385)	Ates et al. (2018b)
L. culinaris cv. Alpo × *L. odemensis* accession ILWL235	RIL (78)	5782.19 (6306)	Polanco et al. (2019)

Marker trait association is commonly studied either in a population garneted by crossing between two or more parents or in a natural diverse population. In lentil, association of molecular markers has been established for a number of traits following above both approaches (Table 7.2). The linkage maps developed in biparental populations help to know the distance of a linked marker with trait of interest, while phenotypic contribution of a particular genotype based on its frequency in a natural diverse population indicates association of a marker with trait of interest. Although considerable efforts have been made in lentil to establish marker trait association, their use is not taken effectively in lentil breeding program due to long distance of associated marker from trait of interest, low phenotypic variation explained, and poor reproducibility of markers across the background. For instance, a loose association of SSR59-2B marker with *Fusarium* wilt (Fw) at a distance of 19.7 cM restricted its use in breeding program (Eujayl et al. 1998b; Hamwieh et al. 2005). For several other traits, flanking markers located >10 cM distance could not be used in marker-assisted breeding program, although they explained high phenotypic variance. In these cases, fine mapping can make them useful for crop improvement in lentil. The 6306 SNP markers were developed through transcriptome analysis used for genotyping of a F_7 RIL population derived from a interspecific cross (*L. culinaris* cv. Alpo × *L. odemensis* accession ILWL235). The first interspecific map developed from these SNP markers used to precisely identify candidate genes for seed coat spotting pattern, flower color, and stem pigmentation and QTLs for flowering time, seed size and *Ascochyta* blight resistance in lentil (Polanco et al. 2019). In this study, lentil genome sequence has been used to identify candidate genes, and two candidate genes have also been identified for stem pigmentation in lentil. For this trait, genetic analysis in the interspecific crosses also reported that two

Table 7.2 Molecular markers linked to genes/QTLs controlling important genetic distance of flanking markers and phenotypic variance explained (PVE) by linked QTL in lentil

Traits	Molecular markers	PVE (%) by linked markers*	References
Ascophyta blight resistance	RAPD	90	Ford et al. (1999)
	RAPD, AFLP, ISSR	~50	Rubeena et al. (2006)
	AFLP, RAPD	41	Tullu et al. (2006)
	ITAP, SSR, ISSR	61	Gupta et al. (2012a)
	SNP, SSR	52–69	Sudheesh et al. (2016)
Anthracnose	SNP	8.9–24.8	Bhadauria et al. (2017)
Earliness	RAPD, AFLP, SSR	37–46	Tullu et al. (2008)
Plant height	RAPD, AFLP, SSR	31–40	Tullu et al. (2008)
	RAPD, AFLP, SSR, morphological	38.2	Fratini et al. (2007)
Branches at the first node	RAPD, ISSR, AFLP, SSR, morphological	91.7	Fratini et al. (2007)
Total no. of branches	RAPD, ISSR, AFLP, SSR, morphological	54	Fratini et al. (2007)
Height at the first node	RAPD, ISSR, AFLP, SSR, morphological	33.3	Fratini et al. (2007)
Flowering time	RAPD, ISSR, AFLP, SSR, morphological	90.4	Fratini et al. (2007)
Pod dehiscence	RAPD, ISSR, AFLP, SSR, morphological	81.3	Fratini et al. (2007)
Seed weight	Morphological	18.2	Fratini et al. (2007)
Seed diameter	RAPD, ISSR, AFLP, SSR, morphological	37	Fratini et al. (2007)
Winter hardiness	RAPD, ISSR, AFLP	20.45	Kahraman et al. (2010)
Cotyledon color class (Yc)	SNP, SSR, seed color loci	23	Fedoruk et al. (2013)
Seed thickness	SNP, SSR, seed color loci	84	Fedoruk et al. (2013)
Seed diameter	SNP, SSR, seed color loci	~ 60	Fedoruk et al. (2013)
Seed plumpness	SNP, SSR, seed color loci	~ 50	Fedoruk et al. (2013)

(continued)

Table 7.2 (continued)

Traits	Molecular markers	PVE (%) by linked markers*	References
Days to 50% flowering	SNP, SSR, seed color loci	~ 34	Fedoruk et al. (2013)
	SSR, SRAP, RAPD	24.2	Saha et al. (2013)
Hundred seed weight	SSR, SRAP, RAPD	17.5	Saha et al. (2013)
Plant height	SSR, SRAP, RAPD	15.3	Saha et al. (2013)
Seed diameter	SSR, SRAP, RAPD	32.6	Saha et al. (2013)
Stemphylium blight resistance	SSR, SRAP, RAPD	46	Saha et al. (2010)
	SNP	9.9–18.3	Bhadauria et al. (2017)
Boron tolerance	EST-SNP	71	Kaur et al. (2014)
Iron concentration	SNP	5.9–14.0	Aldemir et al. (2017)
Mn concentration	DArT	16.1–24.1	Ates et al. (2018b)
Milling quality traits	SSR, SNP		Subedi et al. (2018)
Seed coat spotting, flower color, stem pigmentation, time to flowering, seed size, *Ascochyta* severity	SNPs	23.1–84.2	Polanco,et al. (2019)

complementary genes are involved for stem pigmentation in lentil (Kumar and Gupta 2019). In a study, QTL mapping analysis identified six QTLs for Mn concentration that explained 15.3–24.1% of the phenotypic variation. Among these QTLs, four QTLs were associated with the markers at a distance of less than 1.1 cM. These QTLs can be useful for development of micronutrient-enriched lentil genotypes (Ates et al. 2018b). Association mapping or comparative gene mapping helps to identify the markers linked tightly to or present within the gene of interest. These markers can be used directly in breeding program without validation. Therefore, in lentil, both these approaches have been used to establish marker trait association, and genes/QTLs for several traits have been identified during the past years (Fedoruk et al. 2013; Kumar et al. 2018a, b). Kumar et al. (2018a) identified functional markers (EST-SSR) associated with flowering time in lentil, and the sequence of express sequence tags (ESTs) belonging to these markers showed their similarity with the gene sequences that have important roles in controlling flowering time in other crops. In another study, association mapping analysis identified QTLs for agronomic traits including the primary branches/per plant, days to maturity, pods/plant, secondary branches/plant, 100-seed weight, yield/plant, and reproductive duration

(Kumar et al. 2018b). In lentil, the genome-wide analysis identified *LcMLO1* and *LcMLO3*, and comparative analysis revealed similarity of these genes with MLO genes involved in controlling powdery mildew response in other species (Polanco et al. 2018).

7.2.4 Transcriptome Analysis for Identification of Candidate Genes

Next-generation sequencing (NGS) platforms have opened up the new opportunity of obtaining the genome sequences and transcriptomes. NGS-based transcriptome analysis identified candidate genes expressed under biotic and abiotic stress conditions in lentil (Singh et al. 2017c, 2019b). For heat tolerance, candidate genes related to physiological process and phenotype of pollen, cell wall, and secondary metabolism have been identified in lentil through transcriptome analysis of heat-sensitive and heat-tolerant genotypes (Singh et al. 2019b). In this study, the genes encoding plasmodesmata callose-binding protein 3 (PDCB), phosphatidylinositol/phosphatidylcholine transfer protein SFH13, CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase 1 chloroplastic, probable glycerol-3-phosphate acyltransferase 2 (GPAT2), O-acyltransferase, and phosphatidylcholine diacylglycerol choline phosphotransferase showed their upregulation under heat stress conditions. A gene encoding pyruvate phosphate dikinase identified under heat stress produced phosphoenolpyruvate (PEP) metabolite, which is an essential compound of shikimate pathway. This pathway produces secondary metabolites involved in heat tolerance (Singh et al. 2019b). In another study, a total of 11,435 upregulated and 6934 downregulated transcripts have been identified through transcriptome analysis of drought-sensitive and drought-tolerant genotypes in lentil. For functional classification of DEGs, KEGG pathway annotation analysis extracted a total of 413 GO annotation terms where 176 were within molecular process, 128 in cellular, and 109 in biological process groups (Singh et al. 2017c).

7.2.5 Comparative Genome Analysis

Comparative genome analysis has also been studied in lentil, and its genome demonstrated close relationship with other legume crops (Weeden et al. 1992; Phan et al. 2007; Choudhary et al. 2009). This syntenic relationship helped in the development of molecular markers, establishment of phylogenetic relationships, mapping of genes, and identification of candidate genes. STMS markers developed in chickpea, *Trifolium*, *Medicago* (36%), *Pisum,* common bean, pigeonpea, and soybean have shown their transferability in lentil (Pandian et al. 2000; Reddy et al. 2009; Datta et al. 2011). The close genomic relationship between *M. truncatula* and

lentil genome helped to enrich an existing intra-specific lentil genetic map with EST-SSR markers (Gupta et al. 2012a). EST-based ITAP markers (500 ITAP markers) developed from *M. truncatula*, *Lupinus albus*, and *G. max* could be applied to lentil (Phan et al. 2007). The comparative genomic analysis identified orthologous gene loci of *ELF3* in lentil. This gene has been identified for controlling differences in photoperiod response between wild and domesticated pea (Weller et al. 2012). In lentil, genes for boron tolerance have been identified on the basis of a comparison of the flanking markers SNP 20002998 and SNP 20000246 with the *Arabidopsis thaliana* and *M. truncatula* genome sequences (Kaur et al. 2014). Association mapping analysis identified EST-SSR markers for flowering time in lentil, and a comparative analysis of associated EST sequence with closely related genome sequences led to identification of candidate genes for flowering time in lentil (Kumar et al. 2018a).

7.3 Transgenic Development

Genes of the cross incompatible species/genera can only be used in the genetic improvement through transgenic approach. Therefore, efforts have been made to establish genetic transformation protocol in lentil, and it has been done through *A. tumefaciens*-mediated gene transfer (Lurquin et al. 1998) and biolistic transformation including electroporation (Chowrira et al. 1996) and particle bombardment (Gulati et al. 2002; Mahmoudian et al. 2002). The first fertile transgenic plant of lentil has been generated on MS medium by Gulati et al. (2002). This plant has been micrografted and transplanted in soil. Using *A. tumefaciens*-mediated transformation, herbicide-resistant transgenics have been developed in lentil (Khatib et al. 2007). In another study, transgenic lentil plants via *Agrobacterium*-mediated transformation have been developed by transferring the marker genes such as the *nptII* and *gus*A genes that are stably transmitted in the subsequent generations (Akcay et al. 2009). In these studies, efforts have been to establish transformation techniques. However, the *DREB1A* gene has been transferred for the first time for making genetic improvement for drought and salinity tolerance through transgenic approach in lentil (Khatib et al. 2011). This study confirmed stable inheritance of the transferred gene and *bar* marker gene and also confirmed the expression of genes for salt tolerance in transgenic plants.

7.4 Future Prospects of Lentil Breeding in Current Genomics Era

During the past few years, development of genomic resources including markers, candidate genes, linkage maps, and markers associated with economically important traits have been speed up for lentil improvement. Molecular markers have been used to assess genetic diversity in lentil (Udupa et al. 1999; Abe et al. 2003; Hamwieh et al. 2009; Dikshit et al. 2015; Idrissi et al. 2015; Khazaei et al. 2016; Kumar et al. 2016). However, results obtained from these studies could not be practically used in lentil breeding programs. In a study, molecular markers have been used to identify hybridity in lentil and detected only 21% plants as true hybrids. Use of molecular markers for identification of true hybrids in F_1 can enhance the efficiency of plant breeders (Solanki et al. 2010). Therefore, molecular markers should be routinely used in breeding program for saving the time and money that are required to grow a population from self-fed or admixed plants.

Marker-assisted introgression of QTLs/genes controlling economically important traits is key use of genomics in breeding program. In lentil, molecular genetic mapping led to association of molecular markers with genes/QTL controlling a trait of interest and markers having tight association (<1.0 cM) and explained high phenotypic variation can be useful for lentil breeding program. For example, QTLs explaining more than 47% of total phenotypic variation among three QTLs that accounted 47% (*QTL*-1 and *QTL*-2) and 10% (*QTL*-3) of disease variation can be useful in marker-assisted breeding. Therefore, in the future, such QTLs can be used in breeding program. The efforts are also required to identify the environmental responsive QTLs because quantitative traits are influenced by both genetic and environmental effects. For this, there is a need to develop trait-specific RILs or near isogenic lines (NILs) that are more suitable for accurately dissecting major QTLs, minor QTLs, environmental responsive QTLs, and interactive QTLs. For *Ascochyta* blight, three QTLs each were detected for resistance at seedling and pod/maturity stages (Gupta et al. 2012a). These QTLs together accounted 34 and 61% of the total estimated phenotypic variation and demonstrated that resistance at different growth stages is potentially controlled by different genomic regions. The flanking markers identified may be useful for MAS and pyramiding the different resistance genes into elite backgrounds for developing cultivars having resistance throughout the cropping season. One QTL each for the seed weight (*qSW*) and seed size (*qSS)* explaining 48.4% and 27.5% of phenotypic variance, respectively was identified in lentil. These QTLs were located on average 5.48 cM from the marker indicating close marker-trait association and hence can be useful in marker-assisted breeding for improving the seed size and weight (Verma et al. 2015). Next-generation sequencing has accelerated the development of functional SSR and SNP markers, and have been used in marker-trait association analysis. NGS-based transcriptome sequencing identified candidate genes for heat and drought tolerance. Validation of these genes is required so that they could be utilized in breeding program for developing climate-smart lentil cultivars. Next-generation sequencing is not used widely for

different purposes in lentil compared to other legume crops but its use in future can speed up genomic based lentil breeding program.

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Chapter 8
Chickpea Breeding for Abiotic Stress: Breeding Tools and 'Omics' Approaches for Enhancing Genetic Gain

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8.1 Introduction

Among the various grain legumes, chickpea ranks the third most important grain legume grown globally and is harvested 11.5 million tons from 14.56 million hectares annually (Merga et al. 2019). It plays important role by acting as a cheap source of protein and essential micronutrients, thus providing nutritional security to the human population across the world (Graham and Vance 2003; Jukanti et al. 2012). Besides, as a member of legume family, it plays great role in improving soil nitrogen content through root-residing rhizobacteria that fix atmospheric nitrogen (Zahran 1999). Given the global climate change, crop yield including chickpea production is seriously challenged that causes great concern for global food security (Wheeler and von Braun 2013). Among the various abiotic stresses drought, temperature extremities and salinity stresses are the major constraints to chickpea yield (Gaur et al. 2012; Vadez et al. 2012; Jha et al. 2014a, 2017). Drought alone causes 50% yield loss in chickpea, and 15–20% yield loss is caused by extreme temperature events (Varshney et al. 2014). In future, events of drought episodes and heat stress events could further aggravate this problem. In the past, extensive efforts of conventional breeding approach have delivered several chickpea genotypes conferring various abiotic stress tolerance (Malhotra and Singh 1991; Gaur et al. 2012). However, this progress is limited, and not matched by the increasingly growing human demand required presently. To meet this rising demand, designing of chickpea genotypes with broad genetic base and higher resilience for various biotic and

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abiotic stresses is urgently needed (Tester and Langridge 2010). Among the several approaches, exploring the chickpea genetic variability existing across the genepool and landraces possessing adaptive traits conferring various abiotic stresses could be a sustainable approach. Similarly, advances of chickpea genomic resources especially molecular markers remain instrumental in pinpointing the chromosomal location residing traits of various abiotic stresses and assisting in genetic dissection of these traits precisely (Vadez et al. 2012; Varshney et al. 2014; Jha 2018; Paul et al. 2018b). Likewise, SNP marker generated from draft chickpea genome sequence and re-sequencing could allow conducting GWAS for underpinning the various haplotypes carrying the abiotic stress tolerance loci across the whole genome level (Varshney et al. 2013a, 2019; Thudi et al. 2014). In parallel, efforts of functional genomics have provided great opportunity to unveil the possible candidate gene(s) along with their possible role in response to various abiotic stresses in chickpea (Varshney et al. 2009; Garg et al. 2016; Kudapa et al. 2018; Kumar et al. 2019). High-throughput phenotyping of various traits contributing to abiotic stress could enable us in understanding of plant response to various abiotic stresses (Furbank and Tester 2011). Moreover, advanced breeding techniques, viz. genomic selection, speed breeding and genome editing tool, could speed up the desired genetic gain in chickpea under various abiotic stresses (Pennisi 2013; Watson et al. 2018; Hickey et al. 2019; Samineni et al. 2019).

8.2 Drought Stress

Chickpea is mostly grown under rain-fed condition; consequently it suffers from terminal drought stress especially in the arid and semi-arid regions across the globe (Leport et al. 1999; Siddique et al. 1999; Gaur et al. 2012). All the essential biological processes including photosynthesis, respiration, transpiration and other essential biochemical processes are seriously challenged by drought stress that ultimately affect crop yield significantly in all plant including chickpea (Kaiser et al. 1981; Farquhar and Sharkey 1982; Chaves 1991; Yordanov et al. 2000). Imposition of drought stress during the reproductive phase remains most important affecting all the essential reproductive processes ranging from pollination to fertilization rendering chickpea yield loss seriously (Leport et al. 1999; Jha et al. 2014a). In parallel impact of drought stress also affects biological nitrogen fixation processes through symbiotically active rhizobacteria resulting in significant chickpea yield loss (Serraj et al. 1999).

8.2.1 Genetic Sources and Advanced Breeding Strategies Combating Drought Stress

Significant genetic variability for drought adaptive traits across the various genepool has been recorded in chickpea (Canci and Toker 2009; Krishnamurty et al. 2010). By assessing various drought selection indices based on grain yield under contrasting water regime, several important drought-tolerant chickpea genotypes were identified (Kanouni et al. 2002; Pouresmael et al. 2013; Jha et al. 2017; Rani et al. 2020). Likewise, based on drought escape mechanism, important chickpea genotypes, viz. ICC 96029 and ICC 96030, have been explored and are being promisingly used in chickpea breeding programme for transferring earliness traits to elite genotypes for drought stress tolerance (Kumar and Abbo 2001) (see Table 8.1). Likewise, a wide range of genetic variability for root and root-related traits was

Table 8.1 List of chickpea genotypes contributing in various abiotic stress tolerance

Abiotic stress	Sources of tolerance	References
Drought	ICC 96029, ICCV 2, ICC 4958, ICC 4958, ICC 8261, ACC 316 and ACC 317, FLIP03-145C, ILC3182 and ILC588, Gokce, Neelam, Bakhar-2011	Kumar and Rao (1996), Kumar and Abbo (2001), Krishnamurthy et al. (2003), Kashiwagi et al. (2005, 2006a)
		Macar and Ekmekci (2009), Hamwieh and Imtiaz (2015), Pang et al. (2017a), Farooq et al. (2018)
Cold	ILC 3470, FLIP 82-64C	Malhotra and Singh (1991)
	ILC 8262, ILC 8617,FLIP 87-82C	Singh et al. (1997)
	C. pinnatifidum, C. judaicum	Clarke et al. (2004)
	C. echinospermum	Sharma and Nayyar (2014)
	Sonali and Rupali	Farooq et al. (2017)
	ICC16349	
	Punjab 2008	
	İnci	Arslan et al. (2018)
Heat	ILC 482, Annegiri, ICCV 10, ICCV 88512, ICCV 88513, ICC 4958, ICC 14778, ICC 1205, ICC 456	Srinivasan et al. (1996), Dua (2001), Krishnamurthy et al. (2010)
	ICC 1205,ICC 15614, Katila, Vaibhav, Avrodhi, RVG 203, JAKI 9218, JG 130,ICCV0 7118, ICC1356	Devasirvatham (2012), Gaur et al. (2012), Devasirvatham et al. (2013), Kaushal et al. (2013), Jha and Shil (2015), Jha et al. (2015, 2018a, c, 2019b)
Salinity	L 550, H 355, JG 62, ICC 1431, ICC 15610, ICC 5003, ICC 4593, ICC 12155	Lauter and Munns (1986), Tejera et al. (2006), Ltaief et al. (2007), Vadez et al. (2007, 2012)
	ICC 10755, ICC 13124, ICC 13357, ICC 15406, ICC 15697, ICC 1431, Genesis 836,CC 7323, ICC 95	Serraj et al. (2004a, 2004b), Kotula et al. (2015), Khan et al. (2016), Kotula et al. (2019)
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recorded under water stress in chickpea (Kashiwagi et al. 2005). Several important parameters ranging from high root biomass, root length, root weight, root volume to root density were explored as important drought adaptive traits in ICC 4958 and ICC 8261 genotypes (Kashiwagi et al. 2005, 2008). In this context, Serraj et al. (2004a) recorded considerable genetic variation for various root traits in large chickpea core collection giving great opportunity to design drought-tolerant chickpea genotype. Relying on various physiological traits, viz. water-use efficiency and $\Delta^{13}C$ discrimination, significant amount of genetic variation has been explored, and how these traits could be potentially harnessed for designing drought-tolerant chickpea has been argued (Kashiwagi et al. 2006b; Krishnamurthy et al. 2007; Upadhyaya et al. 2012). Currently, among the various innovative breeding schemes employed for improving genetic base and creating better recombinants for better yield gain and stress resilience, multi-parent advanced generation intercross (MAGIC) breeding scheme is receiving great attention in various crop breeding programmes including chickpea (Huang et al. 2015). This technique has been initiated to develop drought tolerance in chickpea by incorporating ICC 4958, JG 130, ICCV 10, JAKI 9218, JG 130, JG 16, ICCV 97105 and ICCV 00108 well-adapted parents in crossing programme (Samineni et al. 2017; Devasirvatham and Tan 2018). Likewise, considerable genetic variation for drought stress has been explored in *C. anatolicum*, *C. microphyllum* and *C. songaricum* chickpea wild species (Toker et al. 2007). Thus, these species could be promisingly incorporated in breeding programme through pre-breeding for harnessing higher drought adaptation capacity in chickpea.

8.3 Effects of Heat Stress

Results of global climate change and heat stress are posing serious challenge to crop yield including chickpea globally (Beta and Gerats, 2013; Jha et al. 2014b, 2017). Heat stress affects all the growth stages ranging from germination, anthesis, pollination and reproductive processes to grain yield (Jha et al. 2014a, b). Among all the stages, reproductive stage remains the most vulnerable to heat stress (above 35 °C) in chickpea leading to inhibition in pollen germination, affecting pollen fertility and inhibiting fertilization, thus resulting in poor pod and seed setting (Devasirvatham et al. 2012, 2013, 2015; Kaushal et al. 2013). This ultimately causes significant yield loss in chickpea.

8.3.1 Genetic Sources and Progress of Heat Tolerance in Chickpea

Considerable genetic variation contributing in heat stress tolerance has been recorded in chickpea. Important chickpea genotypes, viz. ACC316 and ACC317 (Canci and Toker 2009) and ICC14346 (Upadhyaya et al. 2012), contributing to heat stress tolerance have been reported based on early phenology that helps in escaping heat stress. ICC1205, ICC15614 and ICCV92944 displayed higher pollen viability and fertility at reproductive stage which could be promisingly used for transferring these traits to high-yielding yet heat-sensitive chickpea genotypes (Devasirvatham et al. 2012, 2013; Kaushal et al. 2013). Similarly, substantial amount of genetic variability for various yield and yield-related traits was captured under heat stress in chickpea (Jha et al. 2015, 2017; Jha and Shil 2015; Paul et al. 2018a). Relying on various yield-related parameters such as high pods/plant, high pod filling and grain yield, ICC 4958, RVG 203, RVG 202, JAKI 9218 and JG 130 (see Table 8.1) genotypes exhibited superior performance under heat stress condition (Krishnamurthy et al. 2010; Jha et al. 2018a). Likewise, the genotype JAKI 9218 displayed better heat stress tolerance based on genotype × genotype × environment biplot analysis (Jha et al. 2019a). Hence these above-mentioned genotypes could be potentially incorporated in breeding programme for enhancing heat stress resilience in chickpea. Concurrently, incorporation of CWRs for heat stress adaptive traits could further broaden the genetic base of chickpea for developing heat stress-resilient chickpea varieties.

8.4 Salinity Stress

Soil salinity stress is one of the increasing problems across the globe due to increasing evidences of land degradation; indiscriminate practices of irrigating farm lands lead to high deposition of toxic ions (Na^+ and Cl^-) in cultivable lands (Ismail and Horie 2017; Jha et al. 2019a, b, c, d). Like other crop chickpea is also sensitive to salinity stress. It impairs all key biological processes including photosystem II and nutritional imbalance and causes cellular toxicity due to excessive loads of toxic ions leading to cell death (Flower et al. 2010; Khan et al. 2015, 2016; Kotula et al. 2015; Pushpavalli et al. 2016). Reproductive processes are seriously inhibited because of deficiency in supply of photosynthate to pod and seed growth and seed size under salinity stress in chickpea (Flower et al. 2010; Kotula et al. 2015; Khan et al. 2017). Thus, it affects all the growth stages ultimately resulting in yield loss in chickpea.

8.4.1 Genetic Sources for Salinity Tolerance in Chickpea

Significant progress has been achieved for exploring genetic variability for salinity stress tolerance in chickpea (Vadez et al. 2007; Krishnamurthy et al. 2011; Turner et al. 2013). Several important genotypes such as C 10, C 14, C 16 and C 17 served as key source of salinity tolerance at germination stage. Based on higher shoot biomass accumulation and other vegetative growth advantages, ICC 10755, ICC 13124, ICC 13357 genotypes (Serraj et al. 2004b) and ICC 30, ICC 8980 and ICC 903 (Maliro et al. 2008) showed promising result under salinity stress, while JG 62, ICC 1431 and ICC 15610 genotypes (Vadez et al. (2007, 2012) and Genesis 836, ICC 7323 and ICC 95 (Atieno et al. 2017) exhibited superior yield under salinity stress. Considerable genetic variation for salinity has been harnessed from cultivated chickpea genotypes; however, crop wild relatives (CWRs) of chickpea have been limitedly explored for developing salinity stress tolerance in chickpea.

8.5 Impact of Cold Stress in Chickpea

Chickpea suffers from cold stress when it faces chilling (3–8 °C) or even freezing temperatures that result in arresting of germination process, affecting seedling vigour negatively during early growth establishment (Siddique and Sedgley 1986). Evidences of oxidative stress-driven damage, cellular membrane damage and low chlorophyll content are also noted under low-temperature stress (Kumar et al. 2011). Importantly, reproductive phase also suffers seriously from cold stress as it causes abortion of flower bud; inhibition of pollen tube growth lowers stigma receptivity; reduction in pod inhibits seed filling processes (Srinivasan et al. 1999; Nayyar et al. 2005). Under northern South Asia and South Australia condition, chickpea is grown in winter and faces low-temperature stress during reproductive stage (Berger et al. 2005, 2012), while in Mediterranean region, chickpea faces low-temperature stress during vegetative phase (Berger et al. 2005).

8.5.1 Genetic Resources for Cold Tolerance

Harnessing of chickpea germplasm for cold tolerance remains an important approach for designing cold-tolerant chickpea. Chickpea genotypes imparting cold tolerance such as ICC 96030 and ICC 96029 relying on escape mechanism (Kumar and Rao 2001); ICCV 88502 and ICCV 88503 based on pod setting under cold stress (Srinivasan et al. 1998); and Sonali and Rupali based on availability of viable pollen under cold stress have been reported (Clarke et al. 2004) (see Table 8.1). Additionally, CWRs of chickpea, viz. *C. reticulatum*, *C. echinospermum*, *C. judaicum* and *C. pinnatifidum*, serve as promising source for conferring cold tolerance in

chickpea (Singh and Ocampo 1997; Abbo et al. 2002; Clarke and Siddique 2004; Berger et al. 2012). Thus, the cold-tolerant controlling genomic region harbouring in the above-mentioned species could be harnessed through pre-breeding activities.

8.6 Physiological Trait Breeding and High-Throughput Phenotyping for Abiotic Stress Tolerance

Plant breeders primarily practice empirical selection mostly focusing on 'yield trait' for improving crop yield in crop breeding programme. However, a plethora of physiological traits that could be harnessed for enhancing genetic diversity, plant adaptation and ultimately genetic gain under various stresses have been discussed (Cossani and Reynolds 2012; Reynolds and Langridge 2016). A substitute series with plethora of physiological traits ranging from phenological traits, shoot and shoot-related traits, carbon discrimination to various root and root-related adaptive traits that could be harnessed for increasing future genetic gain in crop plant including chickpea under various abiotic stresses (see Table 8.2). Under drought stress and heat stress, various shoot and shoot-related traits, viz. stomatal conductance, canopy temperature, biomass and pods/plant, remain a major area of research to improve the chickpea genetic yield (Kashiwagi et al. 2006a; Zaman-Allah et al. 2011; Purushothaman et al. 2016). Likewise, root traits ranging from root length, root biomass and other related traits have been harnessed for better water-use efficiency under drought in chickpea especially in south and central Indian condition where chickpea faces terminal drought stress (Kashiwagi et al. 2005, 2015; Chen et al. 2017). Likewise, biochemically chickpea produces several biochemicals and antioxidants for mitigating the challenges of reactive oxygen species-derived toxicity under drought, heat, cold and salinity stresses (Macar and Ekmekci 2009; Mafakheri et al. 2010; Farooq et al. 2017, 2018). However, at genetic level these physiological traits are limitedly studied. Thus, in future a thorough study of these physiological traits at genetic level could open up a great opportunity to design climate-resilient chickpea genotype.

8.6.1 Conventional Breeding Efforts for Developing Abiotic Stress-Tolerant Chickpea

In the past to elucidate the genetic mechanisms involved in controlling drought (Farshadfar et al. 2008; Mannur et al. 2009), heat (Jha et al. 2018a), cold (Malhotra and Singh 1991) and salinity stress (Ashraf and Waheed 1998), both classical and conventional breeding approaches have been extensively devoted. However, success of developing abiotic stress-tolerant chickpea via conventional breeding approaches remains slow and limited due to complex inheritance of these abiotic stresses.

Table 8.2 Relevance of various physiological traits contributing drought, heat, cold and salinity stress adaptation in chickpea

Physiological traits	Related with	Reference
Early phenology (early flowering, early podding)	Drought escape/conservative water-use strategy	Canci and Toker (2009), Hamwieh and Imtiaz (2015)
	Also associated to heat and cold escape	Pang et al. (2017a)
Rate of partitioning	High grain yield	Purushothaman et al. (2016)
Crop growth rate	High water harvest	Purushothaman et al. (2016)
Shoot biomass	High shoot biomass at maturity contribute in higher grain yield under drought, heat and salinity	Maliro et al. (2008), Kashiwagi et al. (2015)
Pod abortion and Seed filling	High seed/grain yield could help in drought and heat stress tolerance	Pang et al. (2017b)
Biomass partitioning	Greater biomass partitioning to grain helps in drought and heat stress tolerance	Krishnamurthy et al. (2013a, b, 1999), Purushothaman et al. (2016), Serraj and Sinclair (2002)
Pod number	High pod number causes increase in grain yield and contributes in heat, drought and salinity stress tolerance	Vadez et al. (2012), Purushothaman et al. (2016)
Pod production	Number of pods/plant is more effected at early stage than late stage under drought stress	Leport et al. (2006)
Specific leaf area	SLA has positive effect on grain yield at reproductive stage	Purushothaman et al. (2016)
Cell membrane stability	Related to drought, heat and cold tolerance	Srinivasan et al. (1998, 1999)
Canopy temperature depression	Cooler canopy contributes in drought avoidance and has positive association with seed yield under drought stress, and it also contributes in heat stress tolerance	Kashiwagi et al. (2008), Purushothaman et al. (2015), Zaman-Allah et al. (2011)
Canopy conductance	Associated to both heat and drought stress tolerance	Sivasakthi et al. (2017)
Carbon isotope discrimination	Transpiration efficiency	Kashiwagi et al. (2006b), Krishnamurthy et al. (2013b)
Recycling of CO_2 inside the pod	Maintain seed filling	Ma et al. (2001)
Antioxidants enzymes, proline, anthocyanin content, trehalose, sucrose and nonreducing sugars	Increase in antioxidant enzymes, proline, trehalose and anthocyanin content during vegetative stage causes drought and cold stress tolerance	Macar and Ekmekci (2009), Mafakheri et al. (2010), Kaur et al. (2017), Farooq et al. (2017, 2018)

(continued)

Table 8.2 (continued)

Physiological traits	Related with	Reference
Relative water content	Increase in relative water content causes drought stress tolerance	Macar and Ekmekci (2009)
Chlorophyll content, carotenoid content	Higher chlorophyll content, carotenoid content helps in heat stress tolerance	Mafakheri et al. (2010)
(Na^+ and K^+) ion uptake	(Na^+ and K^+) ion uptake cause drought tolerance	Talebi et al. (2013)
Chlorophyll a fluorescence F_O, F_M, PSII, ETR, F_V/F_M	Enable in preventing PSII photochemistry from damage and helps in both drought and heat stress tolerance	Macar and Ekmekci (2009)
Plant transpiration rate	Low plant transpiration rate helps in conserving soil water	Sivasakthi et al. (2017, 2018)
Transpiration efficiency	It decides ultimate yield	Kashiwagi et al. (2006b)
Early vigour	Associated to both heat and drought stress tolerance	Sivasakthi et al. (2017, 2018)
Pollen traits (pollen viability, fertility, pollen tube germination)	High pollen viability and fertility under heat stress are associated to heat stress tolerance	Devasirvatham et al. (2013, 2015)
Abscisic acid (ABA)	Under drought increase in ABA causes closure of stomata, thus reducing assimilate production that leads to inhibition of seed set	Pang et al. (2017b)
Root architectural trait prolific root system, root branch, root density root depth, root area, root volume, etc.	Prolific root system is associated to grain yield	Kashiwagi et al. (2005)
	Deep rooting helps in using conserved soil moisture from subsoil and helps in avoiding terminal drought stress	Kashiwagi et al. (2006a, b), Varshney et al. (2013b), Kashiwagi et al. (2005), Chen et al. (2017)

Subsequently advances in genomics and precise phenotyping assisted in deciphering the complex mechanisms involved in these abiotic stresses in chickpea are discussed below.

8.6.2 Genomic Resources and QTL Mapping for Drought Stress Tolerance

Unprecedented advances of chickpea genomics have greatly benefitted in genetic dissection of various traits of breeding importance including drought stress (Varshney et al. 2013b, 2014). Based on biparental mapping scheme, several QTLs (ranging from days to flowering, various physiological traits to yield traits) related to drought stress tolerance have been elucidated (Rehman et al. 2011; Hamwieh et al. 2013; Varshney et al. 2014, 2013b; Srivastava et al. 2016) (see Table 8.3).

Table 8.3 List of QTLs contributing in various abiotic stress tolerance in chickpea

Abiotic stress	Mapping approach used	Identified QTLs	Type of markers	Statistical method used	References
Drought	Biparental	15 QTLs	SSR		Rehman et al. (2011)
Drought	Biparental	93 QTLs	SSR	Composite interval mapping-epistatic mapping (ICIM-EPI)	Hamwieh et al. (2013)
Drought	Biparental and backcross	'QTL-hotspot'	SSR, AFLP		Varshney et al. (2013b)
Drought	Biparental	'QTL-hotspot'	SSR	Composite interval mapping	Varshney et al. (2014)
Drought	GWAS	312 significant MTAs	DArT, SNP	Mixed linear model	Thudi et al. (2014)
Drought	Biparental	164 main-effect QTLs	SNP, CAPS,	Composite interval mapping	Jaganathan et al. (2015)
			dCAPS, SSR		
Drought	Biparental	QTL-hotspot_a(15 genes)	SNP	ICIM-ADD mapping method	Kale et al. (2015)
		QTL-hotspot_b(11 genes)			
Drought	Biparental	3 candidate genes	SNP		Singh et al. (2016)
Drought	Biparental	12 QTLs	SNP		Srivastava et al. (2016)
Drought	Biparental	21 QTLs	SNP	Composite interval mapping	Sivasakthi et al. (2018)
				ICIM-ADD	
Drought	GWAS	Several MTAs	SNP		Varshney et al. (2019)
Heat stress	Biparental	4 QTLs	SNP	Composite interval mapping	Paul et al. (2018b)
Heat stress	Biparental	2 QTLs	SSR	Composite interval mapping	Jha et al. (2019c)
Heat stress	GWAS		SSR	Mixed linear model, generalized	Jha et al. (2018b)
				linear model	
Heat stress	GWAS	Several MTAs	SNP		Varshney et al. (2019)
Cold stress	Biparental	3 QTLs	SNP	Composite interval mapping	Mugabe et al. (2019)
Salinity	Biparental	Several QTLs contributing	SSR	Composite interval mapping	Vadez et al. (2012)

(continued)

Table 8.3 (continued)

Abiotic stress	Mapping approach used	Identified QTLs	Type of markers	Statistical method used	References
		in salinity stress tolerance			
Salinity	Biparental	–	SSR, SNP	Composite interval mapping	Puspavalli et al. (2015)

Among the drought stress tolerance QTLs, a 'hot spot QTL' on CaLG04 controlling several important drought-related traits is worth mentioning (Varshney et al. 2014). Subsequently release of chickpea genome sequences and availability of high-throughput SNP markers allowed precise and accurate mapping of drought-controlling genomic regions and elucidation of underlying candidate genes responsive to drought stress in chickpea (Jaganathan et al. 2015; Kale et al. 2015; Srivastava et al. 2016). In the context, by employing genotyping by sequencing approach, the 'hot spot QTL' was further bipartitioned into *QTL-hotspot_a* (contained 15 genes) and *QTL-hotspot_b* (contained 11 genes) QTLs (Kale et al. 2015). Likewise, availability of high number of markers allowed genome-wide association approach to explore several novel alleles related to drought stress tolerance through marker-trait association analysis (Thudi et al. 2014; Varshney et al. 2019). Thus, further fine mapping of these genomic regions could lay the spring board for future cloning drought tolerance QTLs in chickpea.

8.6.3 QTLs Contributing to Heat and Cold Stress Tolerance

To elucidate the genetics of heat stress tolerance Jha et al. (2019b), investigated the gene action study controlling various yield and phenological traits under heat stress condition revealing prevalence of both additive and nonadditive gene action. However, as heat stress tolerance is controlled by many QTLs/genes and is highly responsive to G × E interaction, its genetics remains elusive. Thus, for better understanding of heat stress tolerance in chickpea, Jha et al. (2019c) reported one QTL-controlling primary branch number and one QTL for chlorophyll content under heat stress based on SSR marker analysis in DCP92-3 × ICCV92944 mapping population. Likewise, employing SNP marker, several QTLs controlling total filled pods/plot, total seeds/plot, biomass and total % of pod setting were elucidated on *CaLG05* and *CaLG06* chromosomes by conducting QTL analysis in ICC15614 × ICC4567 mapping population (Paul et al. 2018b). Considering cold tolerance, limited genomic resources have been reported in chickpea (Saeed and Darvishzadeh 2017). In the recent past, Mugabe et al. (2019) elucidated three QTLs for freezing tolerance on LG1B, LG3 and LG8 and CT Ca-3.1 (on LG3) and CT Ca-8.1 (on LG8) developed from ICC 4958 × PI 489777 population (see Table 8.3).

8.6.4 Genomic Resources for Elucidating Salinity Stress in Chickpea

Substantial progress on elucidation of genetics control of salinity tolerance has been reported in chickpea. However, due to its complex genetic inheritance, availability of genomic resources has enabled in underpinning salinity stress-controlling genomic regions in chickpea and thus improved our understanding of the genetic basis of salinity tolerance. First QTLs derived from JG 62 (tolerant) × ICCV 2 (sensitive) recombinant inbred lines derived mapping population-controlling seed yield under salinity stress on LG3 and LG6 were reported (Vadez et al. 2012). Subsequently, QTL analysis through SSR and SNP marker allowed unfolding of two key genomic region governing salinity stress tolerance on CaLG05 and on CaLG07 (Pushpavalli et al. 2015). The authors also unearthed several salinity stress-responsive putative candidate genes from this investigation.

8.6.5 Advances in Functional Genomics for Underpinning Various Abiotic Stress-Responsive Candidate Genes

Since the last decade, various functional genomic platforms have been established in chickpea including c-DNA libraries, gene expression microarray (Mantri et al. 2007; Varshney et al. 2009) and RNA-seq technologies (Agarwal et al. 2016; Garg et al. 2015, 2016; Badhan et al. 2018; Mahdavi Mashaki et al. 2018; Kudapa et al. 2018; Kumar et al. 2019) for expression analyses that allowed underpinning the candidate genes and their possible functions for various traits including abiotic stresses. Relying on microarray results, Mantri et al. (2007) and Varshney et al. (2009) laid the foundation for preliminary idea on expression of various genes participating in drought stress and cold stress response in chickpea. Subsequently arrival of NGS-based RNA-seq provided great opportunity for thorough understanding of various candidate genes (*Ca_04561*, *Ca_04564*, *Ca_04569*, etc.), TFs (bHLH, leucine-rich repeat, EREBP, MYB, WRKY and NAC), DEGs (1624, 1562 and 2592 genes) and various novel molecular players involved in drought stress response (Hiremath et al. 2011; Garg et al. 2015, 2016; Srivastava et al. 2016; Badhan et al. 2018; Mahdavi Mashaki et al. 2018; Kumar et al. 2019). Moreover, recently several drought-responsive candidate genes were elucidated from *Cicer arietinum* gene expression atlas (CaGEA) of ICC4958 genotype (Kudapa et al. 2018). Likewise, Varshney et al. (2009) and Molina et al. (2011) developed salinity stress-responsive ESTs resources in chickpea, while several DEGs, candidate genes and novel pathways related to salinity stress have been deciphered by various research groups (Kohli et al. 2014; Garg et al. 2016; Kashyap et al. 2018) that provided novel insight into salinity tolerance mechanism in chickpea. Considering heat stress several candidate genes, viz. *Ca_25811*, *Ca_23016* and *Ca_09743* (Table 8.4), contributing in heat stress tolerance have been unearthed from various reproductive

Table 8.4 List of differentially expressed genes/ESTs involved in various abiotic stress tolerance in chickpea

Abiotic stress	Differentially expressed genes/ ESTs	Technique/platform used	References
Drought	1562 genes, 2592 genes	Illumina HiSeq 3000	Badhan et al. (2018)
Drought	1624 differentially expressed genes	Illumina platform	Kumar et al. (2019)
Drought	20,162 ESTs	-	Varshney et al. (2009)
Drought	3062 unigenes	Suppression subtraction hybridization	Deokar et al. (2011)
Drought	44,639 differentially expressed tentative unique sequences	Roche/454 and Illumina/ Solexa	Hiremath et al. (2011)
Drought	7532unitags and 880 unitags	SuperSAGE	Molina et al. (2008)
Drought	4053 and 1330	Illumina HiSeq 2000 platform	Garg et al. (2016)
Drought	261(shoot) and 169(root)	Illumina TrueSeq RNA	Mahdavi Mashaki et al. (2018)
Salinity	3798 and 4460 ESTs	cDNA library	Varshney et al. (2009)
Salinity	363 and 106 transcripts	deepSuperSAGE	Molina et al. (2011)
Salinity	1376 and 3660 DEGs	Illumina HiSeq 2000	Garg et al. (2016)
Salinity	5 miRNAs	Illumina Genome Analyzer IIx	Kohli et al. (2014)
Salinity	–	RNA-Seq libraries	Kashyap et al. (2018)
Heat stress	Five HSP 90	–	Agarwal et al. (2016)

tissues in chickpea (Agarwal et al. 2016). Thus, the growing repertoire of functional genomics could further increase our understanding of various complex gene networks and novel pathways related to various abiotic stresses in chickpea.

8.6.6 Scope of Sequencing and Re-sequencing Efforts for Investigating Abiotic Stress Tolerance-Related Haplotype Assembly

Release of draft chickpea genome sequence (Jain et al. 2013; Varshney et al. 2013) and re-sequencing efforts of global chickpea germplasm (Thudi et al. 2016; Varshney et al. 2019) have offered great opportunity to explore the drought stress tolerance-related haplotype assembly across the whole genome level. Results of re-sequencing efforts of global chickpea germplasm have enabled in identification of several genomic regions including *TIC*, *REF6*, aspartic protease, *cc-NBS-LRR* and

RGA3 potential candidate genes controlling drought stress and heat stress-related traits in chickpea (Varshney et al. 2019). Thus, the SNPs derived from re-sequencing efforts could further unveil the candidate genes controlling various abiotic stresses in chickpea.

8.6.7 Progress and Hope of Novel Breeding Technologies for Designing Abiotic Stress-Tolerant Chickpea

To enhance the desired genetic gain in various crops including chickpea, novel breeding schemes such as marker-assisted back crossing (MABC), marker-assisted recurrent selection (MARS), MAGIC, genomic selection, speed breeding and genome editing tools are worth mentioning (Varshney et al. 2013a; Roorkiwal et al. 2018; Hickey et al. 2019 for details see Jha et al. 2019d).

Advances of chickpea genomic resources allowed implication of molecular markers for marker-assisted breeding for various traits ranging from biotic and abiotic stresses. In the context, incorporation of *QTL-hotspot* genomic region controlling drought stress tolerance from ICC 4958 into an elite chickpea cultivar JG 11 is notable instance of marker assisted selection (MAS) in chickpea (Varshney et al. 2013b). Likewise, MARS scheme has been employed for increasing the genetic base for improving drought tolerance in chickpea at ICRISAT, Patancheru, India. However, these MAS-based schemes remain restricted to transferring major effect QTLs, thus failing to transfer minor effect QTLs (Hayes et al. 2009). Subsequently, outcome of draft chickpea genome sequence and re-sequencing efforts has allowed further enrichment of molecular marker repertoire in chickpea, thus facilitating high-density genotyping for acquiring novel insights into various complex traits like drought, yield, etc. in chickpea (Roorkiwal et al. 2018). Genomic selection allows estimation of 'genomic estimated breeding value' of test sets based on trait information of 'training population' having prior information of both genotyping and phenotyping by using various prediction models (Meuwissen et al. 2001; Newell and Jannink 2014). This novel breeding scheme has been employed for enhancing genetic gain in chickpea evaluated at various locations under drought stress (Roorkiwal et al. 2016, 2018; Li et al. 2018). Likewise, genome editing tool could be used to edit the desired genomic region controlling abiotic stresses. However, this innovative approach is yet to be used for developing abiotic stress-tolerant chickpea.

8.6.8 High-Throughput Phenotyping (HTP) for Increasing Genetic Gain Under Abiotic Stresses

Significant progress has been achieved for genotyping various crop plants however; measurement of plant response to various abiotic stresses both at spatially and timely remains one of the major hurdles for improving crop yield (Furbank and Tester 2011). As plant abiotic stresses are controlled by various 'small effect QTLs' and are highly affected by G × E and genotype-phenotype-envirotype interactions that makes their genetic study complex (Xu 2016). Thus, HTP/phenomics approach has been introduced for accurate characterization/phenotyping of various complex traits including abiotic stresses at higher resolution through studying at multilevel, multidimension and multiscale for increasing genetic gain in crop plant (Houle et al. 2010; Furbank and Tester 2011; Zhao et al. 2019). Among the various techniques used in high-throughput phenotyping, RGB imaging, hyperspectral imaging, thermal imaging, unmanned aerial system, etc. remain noteworthy (James and Sirault 2012; Bodner et al. 2017; Yang et al. 2017). Notable instances of HTP for screening for phytophthora root rot, ascochyta blight disease, canopy conductance and plant vigour under water stress and salinity stress have been reported in chickpea (Atieno et al. 2017; Sivasakthi et al. 2017; Amalraj et al. 2019; Zhang et al. 2019). However, HTP has been limitedly used for measuring other relevant traits involved in drought and heat stress response in chickpea. Thus, establishment of HTP facilities for drought and heat stress response could enable us in increasing genetic gain in chickpea under various abiotic stresses.

8.7 Conclusion and Perspective

Ensuring global food security feeding the increasing human population under the unpredictable global climate change is becoming a great challenge to the plant breeding community (Tester and Langridge 2010). Increasing evidences of severe drought and extreme temperature-related events are challenging crop yield including chickpea globally (Jha et al. 2017, 2019d).

Incorporation of various adaptive traits from CWRs and landraces needs great priority for increasing the genetic base of chickpea for climate resilience (Mickelbart et al. 2015). Since the last decade, advances in chickpea genomic resources have played a great role in genetic dissection of various abiotic stresses. Thus, these resources allowed to practice MAS for various abiotic stresses especially drought tolerance (Varshney et al. 2013b). Concurrently, availability of draft and reference genome sequences of cultivated and wild chickpea and re-sequencing efforts of global collection of chickpea germplasm and landraces could offer great opportunity to explore the various copy number variations and structural variations associated to various abiotic stress-tolerant allele(s)/QTLs (Varshney et al. 2019). Rapidly increasing number of SNP markers across the whole genome has allowed

employing genomic selection to enhance genetic gain in chickpea under various abiotic stresses. Additionally, novel breeding schemes such as MAGIC, nested association mapping and MARS could increase the chances of obtaining novel recombinants for improving chickpea yield under various abiotic stresses (Gaur et al. 2012). Likewise, CRISPR/Cas9-based genome-editing tool promises manipulation of traits gene/allele at desired position precisely. Ultimately, rigorous screening of improved breeding lines with desired tolerant gene(s)/ QTLs through HTP phenotyping facilities should be done under both natural and controlled condition across the various locations for several years for stable expression of tolerant gene(s)/QTLs. Thus, these innovative breeding technologies could allow better designing of climate-resilient chickpea genotypes for accelerating the desired genetic gain required for feeding the increasing global population under the changing global climate.

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Chapter 9
Recent Advances in Mungbean Breeding: A Perspective

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9.1 Introduction

Green gram [*Vigna radiata* (L.) Wilczek], also known as mungbean, green bean, mashbean, golden gram and green soy, an important annual legume belongs to family Fabaceae, subfamily Papilionoideae, genus *Vigna*. It has diploid chromosome number $2n = 2x = 22$ (Karpechenko 1925). It is the native of Indo-Burma region of Hindustan centre (Vavilov 1926). It is well suited to dry areas, mainly under irrigated conditions. Hundred grams of raw green gram contains 10 g moisture, 24 g protein, 60 g carbohydrates, 348 kilo calories of energy, 1 g fat, 3 g minerals, 1 g fibre, 83 mg vitamin A, 0.82 mg thiamine, 0.15 mg riboflavin and 2.4 mg nicotinic acid, and it is having important metal ions, viz. 75 mg calcium, 405 mg phosphorus and 4 mg iron. It possesses some important amino acids, viz. tryptophan 260 mg, lysine 1664 mg, methionine 252 mg, phenylalanine 1421 mg, threonine 758 mg, valine 1246 mg, leucine 1687 mg and isoleucine 1058 mg (Dahiya et al. 2014). The total area under green gram in the world during 2018 was 60.12 lakh hectares; production was about 30.12 lakh tonnes and productivity of about 1024 kg per hectares (Anonymous 2018). The total area under green gram in India during 2018 was 43.27 lakh hectares, production of about 21.65 lakh tonnes and productivity of 467 kg per hectares. From 2015–2016 onwards, increased trend in area and production has been observed. Out of total area under green gram, Rajasthan shares highest area of about 42% area and highest production of about 39%. The 10 states, namely, Rajasthan, Madhya Pradesh, Maharashtra, Bihar, Karnataka, Tamil Nadu, Gujarat, Andhra Pradesh, Odisha and Telangana, have collectively contributed about more than 80% to the total India's green gram production (Anonymous 2018). In India, green gram is grown in poor fertile soils

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where other crops cannot be grown (Kumar et al. 2010a). The production is mainly affected by some of the production constraints including pod shedding, pod shattering, indeterminate growth, unproductive plant types and low harvesting index. Also the green gram production has been affected by several biotic stresses, i.e. pest and diseases. So far 20 diseases of green gram have been documented; among them viral diseases are the most detrimental. Yellow mosaic is the utmost destructive yield reducing viral disease of green gram. MYMV could cause up to 85% yield damage when infection occurs from fourth week of seeding. The crop is also affected by fungal diseases, viz. powdery mildew, bacterial disease viz., anthracnose, etc. (Dash et al. 2018). In green gram, so far 84 pests were identified, among which storage pests particularly bruchid species, namely, *Callosobruchus maculatus*, *Callosobruchus chinensis* and *Callosobruchus analis*, cause significant damage during storage. Per cent losses caused by bruchids is about 30–40%, within a short period of 3 months, and it continued up to 100%, when no protection is taken (Sekar and Nalini 2017a, b). So determining the source of varietal resistance could be helpful in avoiding the storage losses of pulses, and that could be eco-friendly as compared to chemical control measures. Further yield being a complex trait is influenced by many component characters. So the absence of sufficient genetic variability in economically important measureable traits is considered to be one of the major barriers in yield improvement of green gram. Numerous breeding strategies have been employed for enrichment of genetic variability in green gram, but hybridization and induction of mutations are considered to be relatively promising to select appropriate variants from the segregating population. Mutagenesis in association with recombination breeding offers a viable option to improve adapted variety by crossing with donors of seed yield components thereby releasing variability hidden in the conserved gene block. Of late with many genomic tools and resources for legumes becoming increasingly available, they can be used to accelerate genetic improvement of green gram. Crop breeders face the challenge of breeding input-responsive, widely adoptable and climate-resilient varieties of crop plants and developing such varieties at a faster pace based on the target environment and consumer preferences. Therefore, integrating conventional breeding approaches with molecular breeding tools is helpful to develop varieties with pyramided features. The success of deployment of the marker technology to deal with the circumventing constraints in green gram productivity depends on the availability of suitable marker systems in pulses.

9.2 Production Constraints in Mungbean

9.2.1 Biotic Stress

The major constraints for achieving higher yield are inherently low genetic yield potentiality, indeterminate excessive vegetative growth, asynchronous maturity, low harvest index, defective plant type, low partitioning efficiency, small seed size and

susceptibility to biotic and abiotic stresses. Since green gram is being grown under marginal conditions of moisture stress and less fertile lands, natural selection played a major role in determining the plant type and other characteristics of this crop than human selection even long after the crop domestication (Khattak et al. 2001a, b). Diseases are the major problem for green gram cultivation which has a devastating effect on both quantity and quality of the product. Green gram suffers from many diseases caused by fungi, bacteria, viruses, nematodes and also abiotic stresses. Among these, foliar diseases such as powdery mildew, anthracnose, *Cercospora* leaf spot, mungbean yellow mosaic virus (MYMV) and crinkle virus are more prevalent.

Powdery mildew is caused by a fungal pathogen *Erysiphe polygoni*. It is one of the widespread diseases of green gram. More severe symptoms of this diseases are usually observed on 35–40-day-old crop (during flowering and pod stage) causing up to 21% yield loss (Rakhonde et al. 2011). White powdery patches appear on leaves and other green parts which later become dull coloured. When the infection is severe, both the surfaces of the leaves are completely covered by whitish powdery growth. Severely affected parts get shrivelled and distorted. In severe infections, foliage becomes yellow causing premature defoliation (Fig. 9.1).

Anthracnose is a major problem of green gram cultivated in hot and humid areas. The disease has been reported from all major green gram growing regions of India in mild to severe forms. Also, in tropical and sub-tropical areas, it causes considerable damage by reducing seed quality and yield. In northern Karnataka losses in yield and disease incidence of anthracnose have been estimated to be in the range of 24–67% (Deeksha and Tripathi 2002) and 18.20–86.57%, respectively (Laxman 2006). *Colletotrichum lindemuthianum* is the causal organism which is septate, hyaline and branched fungi. The disease appears on all aerial parts of the plant at any stage of its growth. The disease appears on all aerial parts and at any stage of plant growth. The Circular, black, sunken spots with dark centre and bright red orange margins appear on leaves and pods (Fig. 9.2). In severe infections, the affected parts wither off. Seedlings get blighted due to infection soon after seed germination.

Cercospora leaf spot of green gram is widespread in India. *Cercospora canescens* attack a wide range of pulses including green gram which causes 23–27% yield

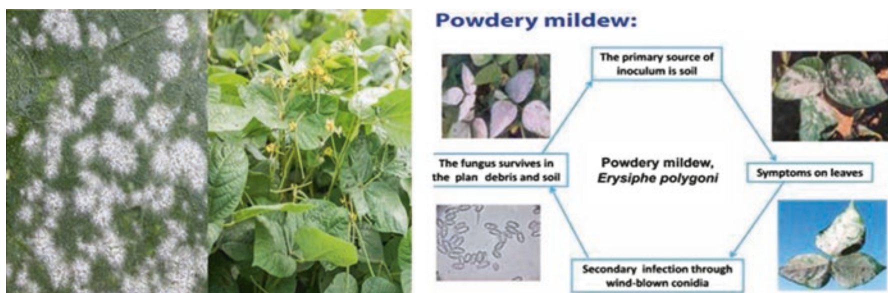

Fig. 9.1 Symptoms of powdery mildew of green gram caused by *Erysiphe polygoni* and disease cycle

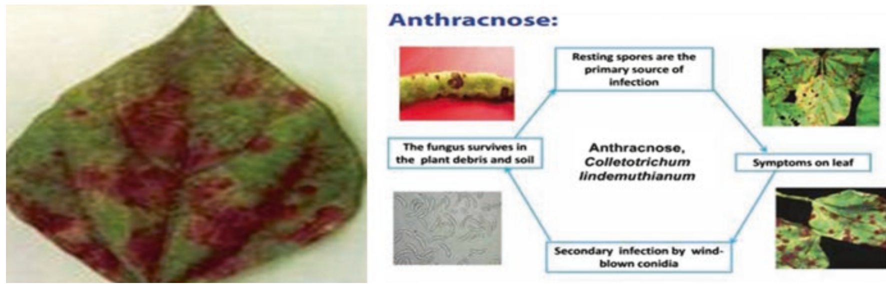

Fig. 9.2 Symptoms of anthracnose on green gram caused by *Colletotrichum lindemuthianum* and disease cycle

Fig. 9.3 Symptoms of *Cercospora* leaf spot on green gram caused by *Cercospora canescens* and disease cycle

loses. Spots produced are small and numerous in numbers with pale brown centre and reddish brown margin. Similar spots also occur on branches and pods. Under favourable environmental conditions, severe leaf spotting and defoliation occurs at the time of flowering and pod formation (Fig. 9.3).

Mungbean yellow mosaic virus (MYMV) is the most devastating disease of mungbean in India and other countries in South Asia. It causes yellow specks and spots on the leaves. The leaves emerging from the apex show bright yellow patches interspersed by green areas. Later on these spots enlarge and in severe cases entire foliage becomes yellow (Fig. 9.4).

Leaf crinkle virus is a seed-borne disease of green gram which causes 2–93% yield loss as the virus affects the development of whole plant including inflorescence. But the extent of damage depends on the cultivar used and stages of infection of the host. The disease manifest itself at the second or third trifoliate stage with the development of light green colour of leaves followed by crinkling after 1–2 weeks. The virus is transmitted through sap, seed and vectors such as aphids (*Aphis craccivora*), white flies (*Bemisia tabaci*) and beetles. This disease is managed by hot water treatment of seeds and vector control.

Fig. 9.4 Mungbean yellow mosaic virus (MYMV), a white fly (*Bemisia tabaci*) transmitted geminivirus belonging to the begomovirus

The yield loss from the viral diseases in pulses accounts up to 80%, while the MYMV alone causes losses up to 80–100% in mungbean. Management of this disease is only possible by the way of reducing the vector, viz. white fly population, using insecticides which are ineffective under severe infestations making a complete destruction of the virus difficult. Therefore, development and use of virus resistance cultivars turn out to be most effective against mungbean yellow mosaic virus (MYMV).

Despite green gram being a short duration crop, nearly 85 insect pests attack mungbean from field to storage. A sizeable proportion of mungbean production is damaged by an array of destructive pests, a notable group of which are the storage pests. Among them, bruchids belonging to the genus *Callosobruchus* (Coleoptera: Bruchidae) are most destructive storage pest in green gram which causes severe damage in storage condition. The genus *Callosobruchus* attacks grain legumes during both pre- and postharvest stages all over the world; but in India, *C. maculatus*, *C. chinensis* and *C. analis* are predominant pest species of the genera. The bruchid *C. maculatus* (F.) is a species of beetle known commonly as the cowpea weevil or pulse beetle. The economic losses of bruchids in various pulses ranged from 30 to 40% within a period of 6 months, and when left unattended losses could be up to 100% (Dongre et al. 1996a, b; Akinkurolere et al. 2006).

Although chemical control remains the most effective means of controlling bruchids in large-scale storage, but the ruthless impacts of insecticidal residues on the human health and environment are most serious impediments for the use of chemical pesticides as a source of pest control (Sarwar 2012). For the safe storage of pulses, this is essential to determine the source of resistance so that resistant factors can be utilized in breeding programme. The best alternative and promising eco-friendly approach to reduce the pesticide dependency is to develop host-plant resistance by transferring the resistance gene from the resistance source to the susceptible through hybridization.

9.2.2 Abiotic Stress

Mungbean is often grown in marginal lands with limited inputs making it prone to a number of abiotic stresses causing tremendous yield losses. For the adaptation of a crop to new environments, tolerance to abiotic stresses is more important than the biotic stresses. Abiotic stresses affect plant metabolism, disrupt cellular homeostasis and uncouple major physiological and biochemical processes (Arora et al. 2002; Srivalli et al. 2003).Terminal heat and drought stresses may lead to considerable flower drop and thus to reduced pod set. Excess moisture and water logging conditions may lead to lodging of crop besides making it vulnerable to a number of diseases and insect pests, resulting in reduction of yield and quality of the grains. Although the abiotic stresses have been the major constraints in increasing the productivity of this crop, only limited work has been carried out on breeding for tolerance to these stresses.

9.2.2.1 Drought Stress

Water stress affects various physiological processes associated with growth, development and economic yield of a crop (Hsiao and Acevedo 1974a, b; Begg and Turner 1976). Water deficit disturbs normal turgor pressure, and the loss of cell turgidity may stop cell enlargement that causes reduced plant growth. Water deficit may change the pattern of growth. Due to water deficit, often the root-shoot ratio increases, leaf area index decreases and the thickness of cell walls and amount of cutinization and lignifications increase. Mungbean is reported to be more susceptible to water deficits than many other grain legumes, mostly because this crop is generally cultivated under rainfed conditions. Therefore, productivity is severely affected by water stress conditions, particularly in the spring and summer grown crops.

In many reports, mungbean is documented as relatively drought tolerant, though it may be a case of escape due to its short duration. The crop is more sensitive to drought during the flowering periods, and it is one of the major limiting factors which contribute to instability and low productivity of mungbean. Severe drought reduces vegetative growth, flower initiation and pod set (Morton et al. 1982). Osmoregulation is an easily measurable process, and it has been found to be significantly associated with drought tolerance (Morgan 1984). The full osmoregulation is reported to be positively and significantly correlated with grain yield. In those plants which face water stress, the osmotic potential tends to go down. Genotypic variation in the root system has been reported in mungbean. A deeper and extensive root system helps mungbean to combat the moisture stress conditions. If genetically determined, the characters could be useful in breeding new mungbean cultivars to grow in declining soil moisture situations.

9.2.2.2 Water Logging Stress

Mungbean cannot withstand water logging; particularly during the early stages of growth (Tickoo et al. 2006). Extensive losses have also been observed when the plants are young. Flooding or water logging reduces oxygen concentrations around the roots of the submerged plants and restricts nodule activity and nitrogen fixation. Thus, mungbean is not suited to the wet tropics when the annual precipitation is >1000 mm (Fernandez and Shanmugasundaram 1988). The heavy rain coupled with strong winds damages the mature crop causing severe yield losses. Plants of mungbean die due to water logging, and if they survive, they get severely affected by fungal diseases and insect pests (Tickoo et al. 2006). Haqqani and Pandey (1994) in their studies on response of mungbean genotypes to water stress and irrigation at various growth stages observed that a decrease in leaf area index and increase in specific leaf weight, leaf water potential, osmotic potential and root length density (RLD) were drought avoidance mechanisms, which help in the survival of the crops during the reproductive stage for seed development when the moisture has been depleted in rice soils.

9.2.2.3 Temperature Stress

Mungbean is a short duration crop and can be grown over a range of environments. Keeping this in view, there is a need to develop photoperiod- and temperature-insensitive varieties which can be grown in dry as well as wet seasons. This is important because mungbean in general is sensitive to varying photoperiod and temperature regimes. There are genotypic differences also for response to changing photoperiods. While most of genotypes usually flower in photoperiods of 12–13 h, flowering is progressively delayed as the photoperiod is extended. As the photoperiod is lengthened from 12 to 16 h, flowering in some short-season early strains may be delayed to only a few days; but in photoperiod sensitive strains, it may be delayed to as many as 30–40 days and some strains may even fail to flower (Poehlman 1991). High temperature stress is reported to have a direct negative impact on flower retention and consequently on pod formation (Kumari and Varma 1983). Flower shedding is very common in mungbean crop, and the extent of flower shedding has been reported up to 79% (Kumari and Varma 1983). Screening and selection of mungbean genotypes, which can retain maximum number of flowers and produce productive pods during high temperature (>40 °C), are essential to increase its production in the country. However, only limited basic information is available about mungbean flower shedding, and no work has been carried out for breeding of mungbean for maximum flowers' retention capability under high temperature (>40 °C). Increased flower shedding under high temperature, precipitation and desiccating winds during flowering period in legumes have been reported by different researchers (Sinha 1977; Tickoo et al. 1996; Rainey and Griffiths 2005). Significant flower shedding above 40 °C in mungbean was reported by Tickoo et al. (1996). Khattak

et al. (2009) reported absence of resistance to flowers shedding under high temperature in mungbean. In this crop, flowers are borne in clusters of 10–20 in axillary or terminal racemes and come in different flushes. Generally, a higher mean temperature hastens flowering, while a lower mean temperature is expected to delay it at all photoperiods. However, this relationship may not hold true for all strains of mungbean as observed by Aggarwal and Poehlman (1977).

9.2.2.4 Salinity Stress

Among abiotic stresses, salinity stress is an important stress, and it causes significant reduction in mungbean production (Abd-Alla et al. 1998; Saha et al. 2010). Salt stress is reported to cause decline in seed germination, root and shoot lengths, fresh mass and seedling vigour (Misra et al. 1996; Promila and Kumar 2000). Further, salt injury also leads to pronounced symptoms like enhanced chlorosis, necrosis and decreased content of chlorophyll A, B and carotenoids (Wahid et al. 2004). Saha et al. (2010) reported that NaCl stress caused a drastic effect on the roots as compared to shoots, accompanying reductions in length, number of root hairs and branches, while the roots became stout, brittle and brown in colour. Salt stress caused a gradual reduction in chlorophyll, carotenoid pigment contents and chlorophyll fluorescence intensity, while the superoxide dismutase and catechol peroxidase activities increased under stress in both roots and leaves. NaCl stress, possibly combined with other types of stress that accompanied the experimental conditions, resulted in organ-specific changes in polyamine biosynthesis and content in mungbean plants (Friedman et al. 2006). Every aspect of the morphological, physiological and biochemical pathway is strongly related to soil salinity which affects yield.

Mungbean has the distinct advantage of being a short duration pulse crop, which can grow in a wide range of soils and environments. Owing to these qualities, it has tremendous scope for horizontal expansion and can be a bonus to farmers in those agricultural lands which remain fallow for 2–3 months after the harvest of the main crops. However, being sensitive to thermo- and photoperiods, drought, salinity and water logging, its widespread adoption by the farmers is not gaining ground. Therefore, there is an immediate need for the evaluation of mungbean germplasm as well as its wild relatives for identification of donors having genes for resistance to the above stresses. Incorporation of genes from closely related species for resistance or tolerance to water, temperature as well as soil-related stresses and nutrient use efficiency should be the top priority for mungbean breeders. There is a need to define the range of preferential adaptation of the cultivated, wild and related species to various abiotic stresses. In such a case, field screening is a powerful tool for evaluation of germplasm and breeding materials for their direct use in the improvement of mungbean to abiotic stresses.

9.2.2.5 Pre-harvest Sprouting

The pre-harvest sprouting is sometimes referred to as field weathering, and it occurs due to adverse physical and chemical changes in seed following its exposure to rainfall and high humidity. In view of the substantial losses caused by pre-harvest sprouting, it is imperative to develop pre-harvest sprouting -tolerant varieties and there by minimize yield losses due to viviparous germination. The average productivity of this crop is low and uncertain due to neglected management and poor adoption of the production technology due to the risk of pre-harvest sprouting. Sometimes losses due to pre-harvest sprouting will be as high as 60–70%. High yielding varieties developed/identified in recent years, despite their high yield potential, could not increase/stabilize the yields of this crop due to lack of resistance to pre-harvest sprouting. Therefore it is essential to develop resistant or tolerant varieties to pre-harvest sprouting by understanding the mechanism/genetics of resistance. Information on the genetics of pre-harvest sprouting and the traits responsible for pre-harvest sprouting can be made through studies on genetics of resistance to pre-harvest sprouting and the traits imparting resistance to pre-harvest sprouting through a line x tester programme.

A prolonged rainy period at maturation often results in poor seed quality due to fungal infestation, sprouting of seeds within pods and discolouration of seeds. Premature sprouting is a serious problem in mungbean in the tropics (Fernandez and Shanmugasundaram 1988). Small pod beak and angle, thick pod wall, low rate of moisture absorption by pod wall, hard seededness and higher cuticular wax content on pod wall were found to impart resistance to pre-harvest sprouting (Naidu et al. 1996). A moderate level (15–20 days) of hard seededness may be useful in contributing to tolerance to weather damage. Transient hard-seededness is common in mungbean; the level of hard seededness in mungbean has been observed to be the highest at harvest, and it declines with storage. It has been observed that the hard-seeded character in the wild progenitor of mungbean, V. *radiata* var. sublobata, is governed by a dominant gene Hd_1Hd_1 (Singh et al. 1983). Most of the mungbean genotypes are prone to shattering. The indeterminate flowering habit of this crop leads to a spread of flowering and pod maturity on a single plant over the entire reproductive phase. Consequently, pods which develop at the earliest flower may shatter prior to 100% pod maturity. To avoid shattering, often the pods are hand-picked. Therefore, it may be desirable to identify donors and incorporate gene(s) for non-shattering. ‘Pant Moong-1’ is tolerant to shattering. Its harvesting can be delayed by 7–10 days, so as to allow the maturity of pods from second flush of flowers (Singh and Sharma 1984). Verma and Krishi (1969) showed that shattering is completely dominant to the non-shattering and probably conditioned by a single gene. The long duration cultivars with more reproductive flushes would give more stable yields because flowering would be continued over the longer period (Chowdhury and Haque 1977). However, such cultivars would require additional pickings and would be prone to lodging, shattering and sprouting.

9.3 Crop Improvement Strategy

Low production level of green gram invites sincere and serious attention of the scientists engaged in agricultural development programmes. Among the various technological options available to increase the production and productivity of pulses in general and green gram in particular, breeding high yielding varieties deserves greatest attention. To meet the increasing demand, an increase in production of pulses through the development of stable high yielding varieties, which can be achieved by using elite parental genotypes. Pulses, in general, are location specific and varieties developed elsewhere are not suitable everywhere. Hence, there is a need to test genotypes for their suitability in specific region. A study on genetic divergence is important to identify suitable variety, as well as the magnitude of variability and heritability and association of characters and characters contributing to genetic diversity.

The entire success of plant breeding programme of any crop largely depends on the wide range of variability present in that crop. It is the range of genetic variability in respect of important economic characters present in the population upon which is based on the effectiveness of selection.

In a self-pollinating crop like green gram, the diversification of genotypes is expected to be limited compared to cross-pollinated crops. The divergence studies of parents involved and their F_1s may provide a tool for selection for ensuring occurrence of more number of desirable attributes in progenies and also for planning future crossing programme.

Lack of adequate genetic variability in economically important quantitative traits are considered to be one of the major impediments in yield improvement of mungbean. Several approaches have been taken up for enhancement of genetic variability in green gram, and induction of mutation is considered to be quite promising. Gamma rays which are the physical mutagens, are non-particulate ionizing radiations with high energy and possess penetrable capacity in biological tissues which have been successfully employed in mutation breeding. Majority of mutant varieties (64%) were developed by the gamma rays (Ahloowalia et al. 2004).

These aspects are important and useful in crop improvement as they help plant breeders in identification of best lines for qualitative and quantitative characters for selection and recombination breeding programme.

9.3.1 Germplasm Resources

Large ex situ germplasm collections generally harbour a wide range of crop diversity. AVRDC – the World Vegetable Center is holding in trust the world's second largest mungbean (*Vigna radiata*) germplasm collection with more than 6700 accessions. To enhance the access of breeders to the diversity of the crop, mungbean core and mini core collections have been established. The core collection of 1481 entries has been built, and a mini core set of 289 accessions is maintained at AVRDC.

Ex situ germplasm collections are essential to conserve plant genetic resources for food and agriculture. Mungbean genetic diversity is safeguarded in various germplasm collections; the five largest collections are held at the University of the Philippines; AVRDC – the World Vegetable Center, Taiwan; the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences; the All India Coordinated Research Project of the Indian Council of Agricultural Research; and the Plant Genetic Resources Conservation Unit of the University of Georgia, USA. Both the University of the Philippines and the Rural Development Administration (RDA), Korea, hold parts of a duplicate of the mungbean germplasm collection of AVRDC – the World Vegetable Center.

9.3.2 Breeding Goals

Development of *Cercospora*, powdery mildew and yellow mosaic virus disease-resistant genotypes with high yield potential: Development of pest resistance genotypes with special emphasis on bruchid resistance: Development of short duration, photo and thermo insensitive varieties suitable for growing across all the three seasons and paddy fallows: Development of varieties suitable for mechanical harvesting: Development of climate resilient genotypes: Resurgence of apion beetle due to climate change.

The breeding procedures applicable to mungbean are the typical breeding procedures applicable to any self-pollinated crop. Accordingly, these include pure line selection, hybridization followed by handling of the segregating generations as per pedigree, bulk, backcross or single seed decent method, depending upon the requirement. To take the advantage of growing two generations/year in India (rainy season and spring – summer crop), it would be desirable to resort to SSD method of advancing the generation. Maturity wise, one should aim at 90–115 days so that the maturing crop does not get caught in rains in September, instead it should mature in first fortnight of October. For *rabi* and summer crops, the maturity duration should be 60–70 days, while for spring crop, it could be around 80–90 days. Mutation breeding could also be utilized to improve mungbean, using 40–50 kR of gamma rays or 0.2–0.3% EMS or any other mutagen.

9.4 Breeding Procedures

In self-pollinated crops, the conventional methods of handling segregating populations like pedigree or bulk methods do not provide any opportunity for reshuffling of genes. Hence, any unfavourable associations observed in early segregating generation like in F_2 are likely to persist through the filial generations. The biparental mating in early segregating generations like F_2 helps in breaking these unfavourable associations and recovering the desirable recombinants. Kampli et al. (2002) in their

study on effect of intermating in early segregating generations on character association have reported that comparison of correlation coefficient among characters studied within biparental mating and those within F_3 populations revealed that correlation coefficient in biparental mating are generally of higher magnitude than in F_3 population. Further they have concluded that impact of intermating on association pattern has clearly brought out its importance in altering the association pattern involving some important components of yield to the breeder's advantages enabling him to increase the efficiency of selection for improving productivity.

Mutagenesis in association with recombination breeding offers a viable option to improve adapted variety by crossing with donors of seed yield components thereby releasing variability hidden in the conserved gene blocks. Kajjidoni et al. (2008) have compared variability generated through different mating schemes and combination of mating and irradiation to improve productivity of black gram. Irradiated populations of single crosses exhibited higher phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) values for clusters per plant and pods per plant traits. Variability generated by irradiation appeared to add to the recombination variability for two traits such as clusters per plant and pod length. Irradiated single cross (F_2M_2) progenies had higher frequency of superior progenies for pods per plant, 100 seed weight and seed yield per plant compared to other hybridized populations involving two or more than two parents revealing the importance of irradiation in creation of desirable variability. Further they have also reported selected superior progenies isolated in F_2 and F_2M_2 (112) and in F_3M_3 and F_3 generations (135) were advanced to the F_5 generation, and on their evaluation found that 29 advance breeding lines were superior. Out of 29 lines, 18 originated from irradiated single crosses and 5 lines from single crosses without irradiation and 6 lines from hybridized progenies involving more than two parents revealing the importance of irradiation in creation of desirable variability. The stability analysis involving 29 advanced breeding lines revealed the stable performance of DBS-14, DBS-16, DBS-24 and DBS-26 genotypes over environments with better mean performance for seed yield. Genotype DBS-15 had highest seed protein content (27.20%), which was followed by DBS-12 (26%) compared to high yielding check TAU-1 (19.68%).

9.4.1 Breeding for Resistance to Biotic Stresses

9.4.1.1 Breeding for Resistance to Diseases

Among biotic factors, diseases are the most destructive. Green gram is attacked by about 26 diseases worldwide, and yield losses up to 44% have been estimated due to diseases. Viral, bacterial and fungal diseases are of economic importance in South Asia, Southeast Asia and sub-Saharan Africa (Taylor et al. 1996; Singh et al. 2000; Raguchander et al. 2005; Mbeyagala et al. 2017). Mungbean yellow mosaic disease (MYMD) is an important viral disease of mungbean (Singh et al. 2000). MYMD is

caused by several begomoviruses, which are transmitted by whitefly *Bemisia tabaci* (*Gennadius*) (Hemiptera: Aleyrodidae) (Nair et al. 2017). The major fungal diseases are *Cercospora* leaf spot (CLS) [*Cercospora canescens* Ellis & G. Martin], powdery mildew (*Podosphaera fusca* (Fr.) U. Braun & Shishkoff, *Erysiphe polygoni* (Vaňha) Weltzien) and anthracnose (*Colletotrichum acutatum* (J.H. Simmonds), *C. truncatum* (Schwein.) Andrus & Moore, *C. gloeosporioides* (Penz.) Penz. & Sacc). Dry root rot [*Macrophomina phaseolina* (Tassi) Goid] is an emerging disease of mungbean. The less important ones are web blight (*Rhizoctonia solani* Kuhn), *Fusarium* wilt (*Fusarium solani* (Mart.) Sacc) and *Alternaria* leaf spot (*Alternaria alternata* (Fr.) Keissl) (Ryley and Tatnell 2011). Halo blight (*Pseudomonas syringae* pv. *phaseolicola*), bacterial leaf spot (*Xanthomonas campestris* pv. *phaseoli*) and tan spot (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*) are the important bacterial diseases. The economic losses due to MYMD account for up to 85% yield reduction in India (Karthikeyan et al. 2014). Dry root rot caused 10–44% yield losses in mungbean production in India and Pakistan (Kaushik and Chand 1987; Bashir and Malik 1988). Reports of yield losses of 33–44% due to Rhizoctonia root rot (Singh et al. 2013a) and 30–70% due to anthracnose (Shukla et al. 2014) from India were estimated. Yield losses due to CLS were 97% in Pakistan and different states of India (Iqbal et al. 1995; Chand et al. 2012) and 40% due to powdery mildew (Khajudparn et al. 2007). Among the minor fungal diseases, 20% yield loss was reported due to *Fusarium* wilt (Anderson 1985) and 10% due to *Alternaria* leaf spot (Mahalingam et al. 2013). A survey of mungbean fields throughout China between 2009 and 2014 reported average yield reductions of 30–50% and total crop failure in severely infected fields due to halo blight. Halo blight is an emerging disease in China and Australia. In Iran 70% incidence (Osdaghi 2014) and in India 30% incidence (Kumar and Doshi 2018) of bacterial leaf spot (*X. phaseoli*) have been reported. Studies were carried out to investigate the efficacy of bactericides, fungicides, bio-fungicides and botanicals in seed treatment and foliar spray and impact of cultural practices to reduce mungbean diseases. Deployment of varieties with genetic resistance is the most effective and durable method for integrated disease management.

Breeding for Resistance to Viral Diseases

Green gram also harbours different viruses, namely, alfalfa mosaic virus, bean common mosaic virus, cucumber mosaic virus, leaf crinkle virus, leaf curl virus, mosaic mottle virus and mungbean yellow mosaic virus. Among all the viruses, mungbean yellow mosaic virus (MYMV) is the most destructive one. It is one of the most vicious diseases of green gram and has been a devastating biotic stress in India for more than five decades. It is caused by a group of Geminiviruses belonging to the genus *Begomovirus* of the family Geminiviridae. They are transmitted through whitefly in a persistent manner. The economic losses due to this virus account up to 85% in green gram which is spreading faster towards newer areas.

Presently in India, nearly all the varieties are susceptible to mungbean yellow mosaic viruses, and rate of infection may vary from 10% to 100% (Nene 1972). It depends upon the susceptibility of the variety, time of infection, population of virus transmitter (*Bemisia tabaci*) and other favourable conditions. Varma et al. (1992) has shown that an annual loss of US$ 300 million was caused by 'mungbean yellow mosaic virus' by reducing the yield of mungbean, black gram and soybean. Yellow mosaic is reported to be the most destructive viral disease not only in India but also in Pakistan, Bangladesh, Sri Lanka and contiguous areas of Southeast Asia.

Mungbean yellow mosaic virus (MYMV), a whitefly (*Bemisia tabaci*)-transmitted geminivirus belonging to the begomovirus having monopartite (one ~2.9 kb DNA) or bipartite genome (two ~2.6 kb DNAs referred to as 'DNA-A' and 'DNA-B'), causes disease in a number of leguminous crops in India and Southeast Asia especially green gram, black gram and soybean. Basakdash et al. (2004) used six YMV-tolerant black gram lines ('VM1'-'VM6') developed from a highly susceptible genotype 'T-9' and F_2 population to identify DNA markers linked to YMV tolerance. A RGA primer pair RGA-1-F-CG/RGA1-R amplified a 445 bp fragment only in homozygous tolerant and the heterozygous lines differentiating the YMV tolerant and susceptible parents and was found to be linked to YMV tolerance. The 445 bp marker was sequenced and named 'VMYR1'. The predicted amino acid sequence showed highly significant homology with the NB-ARC domain present in several gene products involved in plant disease resistance, nematode cell death and human apoptotic signalling. On further evaluation of more RGA primer pairs, Maiti et al. (2011) identified two markers amplified using RGA primer pairs (RGASF1/RGASR1 and RGA22F2/RGA24R2) referred to as YR4 and CYR1 linked to MYMV resistance. Both these resistance linked markers are part of the open reading frames (ORFs) and possess conserved motifs of the NB-ARC domain having sequence homology with other virus resistance genes. Both the markers were validated using green gram and black gram genotypes by multiplex PCR and showed YR4 to be partially linked and CYR1 to be completely linked. Souframanien and Gopalakrishna (2006) identified a tightly linked ISSR marker (ISSR811 1357) using a RIL mapping population (F_8) that was 6.8 cM away from the MYMV resistance gene loci. Sequence characterized amplified region (SCAR) primers designed (YMV1-F and YMV1-R) from this ISSR marker distinguished the MYMV-resistant and MYMV-susceptible plants in RIL population, agreeing well with the phenotypic data. The ISSR811 1357 marker was also validated using diverse black gram genotypes differing in their MYMV reaction. In green gram, F_2 population derived from a cross between 'ML267' DNA markers and their nucleotide sequence linked with MYMV resistance gene in black gram and green gram which can be used in marker-assisted breeding for MYMV resistance.

Research into resistance to MYMD has been underway since 1980 with mutant genotypes developed from local germplasm by mutation breeding (gamma irradiation) at the National Institute for Agriculture and Biology, Pakistan, which later led to the development of the popular NM series varieties including NM 92 and NM 94. Researchers reported that in mungbean, the genetic resistance against MYMD is governed by a single recessive gene (Reddy 2009), a dominant gene (Sandhu et al. 1985),

two recessive genes and complementary recessive genes (Pal and Jana 1991). The mungbean variety NM 92 showed a resistant reaction against MYMD due to a single recessive gene (Khattak et al. 2000). Dhole and Reddy (2012) reported that two recessive genes governed the segregation ratio in the F_2 population in six crosses between resistant and susceptible genotypes. However, F_2 and F_3 populations developed through an interspecific [TNAU RED × VRM (Gg) 1] and intraspecific [KMG 189 × VBN (Gg)] crosses showed role of a single recessive gene in MYMD resistance (Sudha et al. 2013). Saleem et al. (1998) in their study with F_2 populations derived from crosses between two local lines (NM 92- and NM 93-resistant to MYMD) and four exotic lines (VC-1973A-, VC-2254A-, VC-2771A- and VC-3726A-susceptible to MYMD) found that susceptibility and resistance were controlled by a single genetic factor and that susceptibility was dominant over resistance. Similar results were recorded by Jain et al. (2013) in F_2 and F_3 populations of crosses between five susceptible (LGG 478, KM6 202, PUSA 9871, K 851 and KM6 204) and four resistant (KM6201, Sonamung, Samrat, and KM6 220) lines, and it was reported that the inheritance was governed by single dominant gene. However, two recessive genes were found to be responsible for MYMD resistance in the populations developed from crosses between two resistant (Satya and ML 818) and two susceptible (Kopergoan and SML 32) cultivars (Singh et al. 2013b). However, in the study of Mahalingam et al. (2018), two dominant genes governed MYMD resistance in the crosses between resistant (SML 1815, MH 421) and susceptible [VBN (Gg) 3, VBN (Gg) 2, LGG 460, RMG 10-28 and TM 96-2] genotypes. The major genes controlling MYMD resistance in the two crosses (KPSI × BM 6 and BM1 × BM 6) using six (P_1, P_2, F_1, F_2, BC_1, and BC_2) generations were estimated within 1.63–1.75 loci (Alam et al. 2014). It is important to identify the strain/species of the virus causing the disease to make comparison between the different studies done. In repeated samplings over consecutive years in India, Nair et al. (2017) reported genetic similarity of MYMV strains from mungbean to a strain from Urdbean [*Vigna mungo* (L.) Hepper] (MYMV-Urdbean) dominant in North India, strains most similar to MYMV-*Vigna* predominant in South India and *Mungbean yellow mosaic India virus* (MYMIV) strains predominant in Eastern India. The resistance sources of mungbean genotypes to MYMD can be used as potential donors and to develop mapping populations for the development of potential markers for MYMD. For the development of resistant lines, researchers have deployed plant-breeding methods with traditional methods of disease screening. In this regard, marker-assisted selection (MAS) is the most promising technique for disease-resistant cultivar development. The study of genotypic diversity and the discovery of linked markers for *R* gene and quantitative trait loci (QTL) maps construction through molecular markers has improved the adeptness in the breeding programmes conferring resistance for MYMD (Sudha et al. 2013). Basakdash et al. (2004) developed a yellow mosaic virus resistance linked marker named 'VMYR1' in mungbean. Among the parents, one pair, resistance gene analog (RGA) 1F-CG/RGA 1R (445 bp DNA) of gene was found to be polymorphic out of 24 pairs of RGA primers screened. In F_2 and F_3 families, the polymorphisms were found to be linked with YMV-reaction. Binyamin et al. (2015) used sequence characterized amplified region-based markers linked with the MYMD-resistance gene for

the screening of mungbean genotypes against the disease. In the resistant and tolerant genotypes, marker amplified desired bands were reported, while no amplification was observed in susceptible genotypes. Maiti et al. (2011) identified two MYMD-resistance marker loci, *CYR1* and *YR4* completely linked with MYMD-resistant germplasm and co-segregating with MYMD-resistant F_2 and F_3 progenies. Holeyachi and Savithramma (2013) identified random amplified polymorphic DNA (RAPD) markers linked with MYMD recombinant breeding lines. They reported that out of 20 random decamers, only 10 primers showed polymorphism between parents China mung (S) and BL 849 (R) and, among them, only one primer (UBC 499) amplified a single 700 bp band in the resistant parent (BL 849) that was absent in susceptible genotype (China mung). Kalaria et al. (2014) studied the polymorphism by using 200 RAPD and 17 inter-simple sequence repeat (ISSR) markers. Among RAPD markers, OPJ-18, OPG-5 and OPM-20 and in ISSR DE-16 were found to be potential ones, as they produced 28, 35, 28 and 61 amplicons, respectively. The resistant genotypes NAUMR1, NAUMR2, NAUMR3 and Meha were clearly separated from the susceptible cultivar, GM4. In another study, five QTLs based on simple sequence repeats (SSR) markers were investigated against MYMD; of them, three were from India (*qYMIV1, qYMIV2* and *qYMIV3*) and two were from Pakistan (*qYMIV4* and *qYMIV5*) (Kitsanachandee et al. 2013). The QTL, *qYMIV1* explained 9.33% variation in disease response. Similarly, *qYMIV2* explained 10.61%, *qYMIV3* explained 12.55%, *qYMIV4* explained 21.55% and *qYMIV5* explained 6.24% variations in the disease response. Two major QTLs controlling genes on linkage group 2 (*qMYMIV2*) and 7 (*qMYMIV7*) resistant to MYMD were reported. These QTLs were conferring resistance in both F_2 and BC_1F_1 populations with a coefficient of determination (R^2) of 31.42–37.60 and 29.07–47.36%, respectively (Alam et al. 2014). Markers linked to QTLs in this study will be useful in marker-assisted breeding for the development of MYMD-resistant mungbean varieties. During the growing season, plant breeders can conduct repeated genotyping in the absence of disease incidence by applying linked marker-assisted genotyping. This technique will save labour and time during the introgression of MYMD resistance through molecular breeding, as phenotyping against begomoviruses is complex, labour and time-consuming. New donors of MYMD resistance have also been identified from interspecific sources (Chen et al. 2013; Nair et al. 2017).

Breeding for Resistance to Fungal Diseases

Researchers screened mungbean genotypes against fungal diseases from different countries in controlled and field conditions in order to identify sources of resistance. It may be noted that screening of mungbean genotypes against powdery mildew and *Cercospora* leaf spot diseases has been much explored. However, little work has been done on the identification of sources of resistance against anthracnose and dry root rot and needs to be addressed as future priorities. Screening of mungbean genotypes against fungal diseases were carried out under natural conditions, except for dry root rot, Khan and Muhammad (2007) it screened in laboratory conditions.

Efficient breeding for fungal stresses requires readily available resistant germplasm and markers linked with QTL regions or major genes that can be employed in marker-assisted selection (MAS). In mungbean, *Cercospora* leaf spot and powdery mildew molecular markers have been identified for application in breeding programmes. However, QTLs or molecular markers for dry root rot and anthracnose have not been investigated. Both qualitative and quantitative modes of inheritance have been reported for resistance to powdery mildew Kasettranan et al. (2009). Single dominant gene control of resistance to powdery mildew was reported (AVRDC 1979; Khajudparn et al. 2007; Reddy 2009), while Reddy et al. (1994) reported that two major dominant genes control the resistance. Chaitieng et al. (2002) and Humphry et al. (2003) found that one QTL conferred the resistance to powdery mildew, while Young et al. (1993) reported three QTLs linked with powdery mildew resistance. Young et al. (1993) made the conclusion from studying the mapping population developed from mungbean line VC3890 as a resistance parent. The population developed from a cross between KPS 2 (moderately resistant) and VC 6468-11-1A (resistant) mungbean genotypes was investigated by Sorajjapinun et al. (2005), and they reported additive gene action control of resistance. Kasettranan et al. (2010) identified SSR marker-based QTLs such as *qPMR-1* and *qPMR-2* associated with resistance to powdery mildew. One major QTL on the linkage group 9 and two minor QTLs on linkage group 4 were identified in mungbean line V4718 (Chankaew et al. 2013). The mapping population against powdery mildew developed from mungbean line RUM5 resulted in two major QTLs on LG6 and LG9 and one minor QTL on LG4 (Chankaew et al. 2013). Fine mapping with populations developed from crosses between highly susceptible and highly resistant parents would be reliable for the identification of reliable markers.

Reports on quantitative genetic control of resistance to CLS (Chankaew et al. 2011) and a single recessive gene control (Mishra et al. 1988) have been reported. One major QTL (*qCLS*) for CLS located on linkage group 3, which explained 66–81% phenotypic variation was reported (Chankaew et al. 2011) using F_2 (CLS susceptible cultivar Kamphaeng Saen1, KPS1 × CLS-resistance mungbean line, V4718) and BC_1F_1 [(KPS1 × V4718) × KPS1] populations.

Powdery Mildew

Powdery mildew disease also creates forced maturity of the infected plants which results in heavy yield losses. It is a serious problem in all the areas having rice-based cropping systems of the country. Powdery mildew caused by the obligate biotrophic fungus *Erysiphe polygoni* is an airborne disease of worldwide distribution, being particularly important in climates with warm dry days and cool nights. One of the main foliar diseases that affect the production of green gram and black gram is powdery mildew, caused by fungus *Erysiphe polygoni* D.C. Severe infection by powdery mildew occurs in cool, dry months when it can reduce the yield of green gram by 20–40% (Reddy et al. 1994). Molecular marker studies of powdery mildew in green gram have indicated both qualitative and quantitative inheritance. Young et al. (1993) used RFLPs to map genes in green gram that confer partial resistance

to the powdery mildew fungus. F_3 lines derived from a cross involving cross between a moderately powdery mildew resistant ('VC3980A') and a susceptible ('TC1966') green gram parent were assayed in the field for powdery mildew response, and the results were compared to the RFLP genotype data, thereby identifying powdery mildew response associated loci. A total of three genomic regions were found to have an effect on powdery mildew response, together explaining 58% of the total variation. One marker showing a strong association with powdery mildew response was sgK472, located on LG 3 of green gram. The study of Humphry et al. (2003) identified a major locus conferring powdery mildew resistance in line 'ATF 3640'. 147 F_7 and F_8 RILs derived from a cross between 'Berken' (highly susceptible) and 'ATF 3640' (highly resistant) were screened for powdery mildew under glasshouse condition. RFLP linkage map constructed with 52 loci generated by 51 probes were used to identify a single major locus flanked by markers LpCS82 and VrCS73 on linkage group K. This locus peaked approximately 1.3 cM from marker VrCS65, explained 86% of the total variation in the resistance response to the pathogen. However, location of this QTL did not coincide with any QTLs reported by Young et al. (1993). Kasettranan et al. (2010) identified two QTLs controlling the disease resistance in a RIL population of 190 F_7 lines. The population was developed from the cross between a susceptible cultivar, 'Kamphaeng Saen 1', and a resistant line, 'VC6468-11-1A'. Reaction to the disease was evaluated for resistance in field and greenhouse conditions. Analysis of variance revealed that 15 SSR loci on three linkage groups were associated with the resistance. Composite interval mapping consistently identified two QTLs on two LGs, *qPMR-1* and *qPMR-2,* conferring the resistance. *qPMR-1* and *qPMR-2* accounted for 20.10% and 57.81% of the total variation for plant response to the disease, respectively. Comparison based on common markers used in previous studies suggested that *qPMR-2* is possibly the same as the major QTL reported earlier using another resistant source. The SSR markers closely linked to *qPMR-1* (CEDG282 and CEDG191) and *qPMR-2* (MB-SSR238 and CEDG166) are useful in MAS for green gram powdery mildew resistance.

Anthracnose: Colletotrichum lindemuthianum (Sexual Stage – Glomerella lindemuthianum)

The disease appears on all aerial part parts and at any stage of plant growth. Circular, black, sunken spots with dark centre and bright red orange margins on leaves and pods. In severe infections, the affected parts wither off. Seedlings get blighted due to infection soon after seed germination. Anthracnose in green gram causes qualitative as well as quantitative losses (Sharma et al. 1971). Anthracnose is the main cause of yield loss induced by pathogens in this crop if left uncontrolled. Losses in yield due to anthracnose have been estimated to be in the range of 24–67% (Deeksha and Tripathi 2002). Even disease may reach up to 18.2–86.57% severity which is reported from northern Karnataka (Laxman 2006). Anthracnose of green gram caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore occurs severely in Indo-gangetic plains every

year. Development of markers for dry root rot and anthracnose is needed for fast track development of disease-resistant lines. Insect-resistant sources of few insects such as bruchids and whiteflies are available, which are being used in breeding programmes to develop insect-resistant mungbean. However, there is every possibility of the introgression of undesired traits from these resistant sources to the cultivars. In order to have stable disease- and insect-resistant mungbean for a specific disease or pest, a synergy between the conventional breeding techniques and molecular technologies is very important (Schafleitner et al. 2016). Identification of molecular markers will help in the evaluation of the diseases and pest resistance and reduce our dependency on the phenotypic data, which might be laborious in big trials.

Breeding for Resistance to Bacterial Diseases

Bacterial pathogens are seed-borne and can persist in crop residue. Varietal resistance is recognized as the corner stone of integrated disease management. Little work has been done on the screening of mungbean genotypes against bacterial diseases and identifying genetic markers associated with bacterial diseases in mungbean. From India, Patel and Jindal (1972) evaluated 2160 genotypes of mungbean for resistance to bacterial leaf spot (*X. phaseoli*) and reported that Jalgaon 781, P 646, P 475 and PLM 501 mungbean genotypes were resistant. From Pakistan, 8 out of 100 mungbean genotypes were reported as resistant against bacterial leaf spot disease under field conditions (Iqbal et al. 1991, 2003). Munawar et al. (2011) screened 51 genotypes against bacterial leaf spot disease in Pakistan and found NCM11-8, NCM 15-11, AZRI-1 and 14,063 mungbean genotypes as resistant in natural incidence of the disease. In their field evaluation, few genotypes such as NCM 258-10, NCM-21, NCM 11-6, AZRI-06 and NCM 11-3 showed moderate resistance reaction.

The inheritance of bacterial leaf blight is governed by a single dominant gene (Thakur et al. 1977). Patel and Jindal (1972) reported that in mungbean genotypes Jalgaon 781, P 646, P 475 and PLM 501, the inheritance of resistance to bacterial leaf blight (BLB) was monogenic dominant. While QTLs were identified for bacterial leaf blight disease in other crops like chickpea (Dinesh et al. 2016), no records are available on QTLs of mungbean against bacterial disease. Screening for halo blight and tan spot has been carried out by the Australian breeding programme in both controlled (glasshouse) and field conditions to identify useful donors as well as resistant progenies. Identification of genetic markers/QTLs associated with halo blight, tan spot and bacterial leaf spot disease resistance in mungbean will accelerate the development of resistant commercial cultivars. These markers can be established through genome-wide association studies using large, diverse mungbean mapping populations' representative of worldwide germplasm (Schafleitner et al. 2015).

Leaf Spot: Cercospora canescens

This is an important disease of green gram, and it usually occurs in a severe form, causing heavy losses in yield. Spots produced are small, numerous in numbers with pale brown centre and reddish brown margin. Similar spots also occur on branches and pods. Under favourable environmental conditions, severe leaf spotting and defoliation occur at the time of flowering and pod formation.

The crop experiences several stresses predominated by a leaf spot caused by *Cercospora canescens* which is a recognized destructive disease of the crop worldwide for inflicting qualitative and quantitative losses ranging from 23% to 96% under natural epiphytotic conditions. Until now very few varieties of green gram have been found resistant or moderately resistant to *C. canescens,* though several improved varieties have been developed through selection, hybridization and mutation.

One of the most important diseases affecting green gram production in Asia is the *Cercospora* leaf spot (CLS), a foliar disease caused by the biotrophic fungus *Cercospora canescens* Illis & Martin. The fungus initially causes spotting on green gram leaves; the spots increase in number and size during flowering, but the increment is 8 Advances in Green gram and Black gram Genomics most rapid at the pod-filling stage. In susceptible varieties, infection expands rapidly resulting in premature defoliation and reduction in size of pods and seeds and thus cause yield losses of up to 50% if devoid of protection (AVRDC 1984). The progress in selecting CLS-resistant genotypes in breeding programmes is still limited. This is mainly due to the fact that CLS occurs only in the rainy season, which is the primary growing season for green gram. Moreover, field evaluation for resistance can be done in only one season per year; albeit green gram is considered among the shortest season crop in the world and can be grown 3–4 times a year. Molecular markers linked to the gene controlling resistance can aid selection and advance the generation year-round (Collard and Mackill 2009). Chankaew et al. (2011) identified QTL for *Cercospora* resistance using F_2 ('KPS1' × 'V4718') and BC_1F_1 [('KPS1' × 'V4718') × 'KPS1'] populations developed from crosses between the CLS-resistant green gram 'V4718' and CLS- susceptible cultivar 'Kamphaeng Saen 1' (KPS1). CLS resistance in F_2 and BC_1F_1 populations was evaluated under field conditions during the wet seasons. Sixty-nine polymorphic SSR markers were analysed in the F_2 and BC_1F_1 populations. Segregation analysis indicated that resistance to CLS is controlled by a single dominant gene. Single regression analysis in the F_2 and BC_1F_1 identified seven SSR markers, namely, CEDC031, CEDG044, CEDG084, CEDG117, CEDG305, VR108 and VR393, associated with CLS resistance ($P < 0.01$). All of them were located on LG 3, except CEDG044 which was located on LG 11. The R^2 of the markers ranged from 6.11% (CEDG044) to PCR markers and their nucleotide sequence linked with powdery mildew resistance gene in green gram (Souframanien and Dhanasekar 2014). While composite interval mapping consistently identified one major QTL (qCLS) for CLS resistance on LG 3 in both F_2 and BC_1F_1 populations, *qCLS* was located between markers CEDG117 and VR393 on LG 3 at 26.91 and 24.91 cM. It accounted for 65.5–80.53% of the disease

score variation depending on seasons and populations. An allele from 'V4718' increased the resistance. The SSR markers flanking *qCLS* will facilitate transfer of the CLS resistance allele from 'V4718' into elite green gram cultivars.

9.4.1.2 Breeding for Resistance to Insects

An estimated 8.5% of the pulse production in India is lost during postharvest handling and storage. Postharvest damage to mungbean seed from bean weevils or bruchids (*Callosobruchus sp*.) is common, causing losses of up to 100%.

Insect pests attack mungbean at all crop stages from sowing to storage and take a heavy toll on crop yield. Some insect pests directly damage the crop, while others act as vectors of diseases. The economically important insect pests in mungbean include stem fly, thrips, aphids, whitefly, pod borer complex, pod bugs, and bruchids (Swaminathan et al. 2012). Stem fly (bean fly), *Ophiomyia phaseoli* (Tryon), is one of the major pests of mungbean. Other species of stem fly that infest mungbean include *Melanagromyza sojae* (Zehntner) and *Ophiomyia centrosematis* (de Meijere) (Talekar 1990). This pest infests the crop within a week after germination, and under epidemic conditions, it can cause total crop loss (Chiang and Talekar 1980). Whitefly *B. tabaci* is a serious pest in mungbean and damages the crop either directly by feeding on phloem sap and excreting honeydew on the plant that forms black sooty mould or indirectly by transmitting MYMD. Whitefly's latent period is less than 4 h and a single viruliferous adult can transmit the MYMV within 24 h of acquisition and inoculation. The male and female whiteflies can retain the infectivity of the virus for 10 and 3 days, respectively. Further, *B. tabaci* complex consists of 34 cryptic species (Boykin and De Barro 2014). Whitefly causes yield losses between 17% and 71% in mungbean (Marimuthu et al. 1981; Chhabra and Kooner 1998; Mansoor-Ul-Hassan et al. 1998). Thrips infest mungbean both in the seedling and in flowering stages. The seedling thrips are *Thrips palmi* Karny and *Thrips tabaci* Lindeman and the flowering thrips are *Caliothrips indicus* Bagnall and *Megaluro thrips* spp. During the seedling stage, thrips infest the seedling's growing point when it emerges from the ground, and under severe infestation, the seedlings fail to grow. Flowering thrips cause heavy damage and attack during flowering and pod formation. They feed on the pedicels and stigma of flowers. Under severe infestation, flowers drop and no pod formation takes place. Spotted pod borer, *Maruca vitrata* (Fab.), is a major insect pest of mungbean in the tropics and subtropics. With an extensive host range and distribution, it is widely distributed in Asia, Africa, the Americas and Australia (Zahid et al. 2008). The pest causes a yield loss of 2–84% in mungbean amounting to US$30 million (Zahid et al. 2008). The larvae damage all the stages of the crop including flowers, stems, peduncles and pods; however, heavy damage occurs at the flowering stage where the larvae form webs combining flowers and leaves (Sharma et al. 2000). Cowpea aphid, *Aphis craccivora* Koch., sucks plant sap that causes loss of plant vigour and may lead to yellowing, stunting or distortion of plant parts. Further, aphids secrete honeydew (unused sap) that leads to the development of sooty mould on plant parts. Cowpea aphid also acts as a

vector of bean common mosaic virus. Bruchids are the most important stored pests of legume seeds worldwide. They infest seeds both in field and in the storage; however, major damage is caused in storage. Bruchid damage can cause up to 100% losses within 3–6 months, if not controlled (Tomooka et al. 1992; Somta et al. 2007). Twenty species of bruchids have been reported infesting different pulse crops (Southgate 1979). Of these, the Azuki bean weevil (*Callosobruchus chinensis* L.) and cowpea weevil (*Callosobruchus maculatus* Fab.) are the most serious pests of mungbean. The cryptic behaviour of bruchids where the grubs feed inside the legume seeds makes it easy to spread them through international trade.

A number of specific insect resistance mechanisms have been identified in legumes although they have not yet been widely integrated into mainstream breeding programmes (Edwards and Singh 2006). Insect pest resistance in crops generally comprises four important mechanisms (i) antixenosis; (ii) antibiosis; (iii) tolerance; and (iv) escape. Tolerance and escape are resistance mechanisms relevant for field infestations but not for storage insect pests of grain crops. However, the processes of resistance involve morphological, physiological and/or biochemical mechanisms which range from simply minimizing the effect of insect attack to adversely affecting the insects' cellular processes, growth and development (Singh 2002). According to Edwards and Singh (2006), legumes as a group employ an array of direct and indirect defences including morphological barriers, secondary metabolites and anti-nutritional compounds. Antibiosis expressed with the adverse effects upon larvae of storage pests feeding on seed of a resistant host plant may also involve morphological, physiological and biochemical features of the host plant or their combination (Panda and Khush 1995). Antibiosis may lead the insect pest to death.

Green plants utilize principal metabolic pathways and biochemical cofactors for converting carbon dioxide and water to sugars and nitrogen to amino acids and to synthesize nucleotides, lipids and simple organic acids. These primary plant metabolites serve as the starting materials for the biosynthesis of secondary metabolites (polymers like lignins and tannins, alkaloids, quinines, etc.) that play an important role in the seed defence against insects such as repellents, feeding inhibitors and anti-nutritional factors (Panda and Khush 1995). In wild species of *Phaseolus*, for instance, arcelin, phytohema glutinin and α-amylase inhibitor genes or anti-nutritional proteins collectively called lectins were identified, cloned and sequenced and deployed in breeding programmes (Morton et al. 2000; Tomooka et al. 2000; Acosta-Gallegos et al. 2008; Edwards and Singh 2006). Lectins are often resistant to proteolytic activity and function by binding to chitin or to carbohydrate targets in the insect's mid gut, thereby blocking nutrient assimilation (Edwards and Singh 2006). The α-amylases inhibitor gene from *Phaseolus* may be the single most studied mechanism providing resistance to legume seed beetles. The α-amylases are major digestive enzymes in some bruchids like the adzuki bean beetle that feed on seeds of legumes. To cope with such insect pests, some species of *Phaseolus* and *Pisum* have developed seed protection systems involving secondary metabolites like α-amylases inhibitors (Morton et al. 2000; Yamada et al. 2003; Edwards and Singh 2006).

Antixenosis refers to non-preference of the insect pest due to unsuitability of the host for oviposition, growth and/or survival due to some morphological or biochemical factors in the host. Morphologically, varieties with smooth, soft and thin seed coats may be more preferable for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Ahmed et al. 1989; Shaheen et al. 2006), but there is still some controversy and doubt about considering these features as universal indicators of resistance (Lale and Kolo 1998; Somta et al. 2007; Srinivasan and Durairaj 2007). For instance, Desroches et al. (1995) found that the seed coat in a faba bean (*Vicia faba*) acts like a physical barrier against penetration by *C. chinensis* and C. *maculatus*. They found that only 45–58% of the neonate larvae perforated through the seed coat to the cotyledons. A similar type of resistance against *C. maculatus* was also reported on cowpea (Edde and Amatobi 2003). On the other hand, Lale and Kolo (1998) observed that resistance to *C. maculatus* in three cultivars of cowpea was conferred mainly by a combination of reduced oviposition and egg hatching which may be a reflection of chemical rather than physical characteristics of the seed coat. Host plants may also pose nutritional, physiological and ecological hurdles on the insects (Panda and Khush 1995).

Breeding for Bruchid Resistance

Callosobruchus maculatus (F.) are the important pests of mungbean and cause damage in the field and in storage. Bruchid infestation reduces the nutritional and market value of the grain and renders seeds unfit for human consumption, agricultural and commercial uses. These pests are controlled mainly by fumigation with highly toxic chemicals such as carbon disulphide, phosphene and methyl bromide or by dusting with several other insecticides, which leave residues on the grain, thus, threatening food safety. Some plant-based extracts have been found useful in controlling bruchids, but are not fully successful due to their short-term activity, rapid degradability and potentially negative effect on seed germination. Although some wild sources of bruchid resistance in mungbean have been reported, which have been used to develop bruchid-resistant lines, undesirable genetic linkages threaten the proper exploitation of genetic diversity from wild germplasm into commercial cultivars. Further, biotype variation in bruchids has rendered some mungbean lines susceptible that otherwise would have been resistant to the pest. Host plant resistance is a cost-effective and a safe alternative to control bruchids in mungbean and is associated with morphological, biochemical and molecular traits. These traits affect insect growth and development, thereby reducing the yield losses by the pests. Understanding the defence mechanisms against insect pests could be utilized in exploiting these traits in crop breeding. However, the processes of resistance involve morphological, physiological and/or biochemical mechanisms which range from simply minimizing the effect of insect attack to adversely affecting the insects' cellular processes, growth and development.

AVRDC researchers have identified 2 black gram accessions (VM 2011, VM2164) that are 26 highly resistant to bruchids (*C. chinensis* L.) as well as 2 mungbean accessions (V2802, V2709) 161 that recently have been confirmed to possess complete resistance to both *C. chinensis* L. and *C. maculatus*. However VM 2164 had significantly higher (26 vs. 24 TIU) trypsin inhibitor activity than susceptible genotypes. 97.3 transfer of bruchid resistance from black gram into popular mungbean 4 lines can be accomplished efficiently through interspecific or intraspecific crosses aided by use of 5 markers linked to bruchid resistance genes. Bruchid-resistant mungbean lines are expected to be made available for testing within 3 years.

The genes responsible for bruchid resistance in two wild green gram strains, 'TC1966' and 'ACC41,' have been mapped. The *Br* gene conferring resistance to *C. chinensis* in TC1966 that was initially mapped on LG 8 flanked by RFLP markers sgA882 and mgM151 at a distance of 3.6 cm and 6.5 cm, respectively, was subsequently mapped to LG 9 at a distance of 0.2 cm with the marker Bng143 and 0.9 cm with the marker Bng110. Two-point linkage analysis of RFLP marker data from F_2 DNA showed significant association of six RFLPs (pA352, pR26, pA882, pM151a, pA315, pA257). Bruchid resistance was located to a single locus on LG 8 between marker pA882 and pA315 at a distance of 3.6 cm and 27 cm away from the former and latter, respectively. QTL mapping of bruchid resistance identified the only region on LG 8 to be significantly associated with a LOD value of 15.3, attributing 87.5% of the total phenotypic variation (Young et al. 1992). Menancio-Hautea et al. (1993) constructed a RFLP linkage map of green gram and located bruchid resistance gene to a 13 cm interval flanked by RFLP markers. Bruchid resistance from 'TC1966', incorporated into cultivated green gram 'Osaka-ryokuto', conferred simultaneous inhibitory activity against the bean bug, *Riptortus clavatus* Thunberg, and was characterized by the presence of a group of novel cyclopeptide alkaloids, called vignatic acids. Kaga and Ishimoto (1998) constructed linkage map for *Br* and the vignatic acid gene (*Va*) using RAPD and RFLP probes developed from linked RAPD markers. *Va*co segregated with bruchid resistance and mapped to single locus at the same position as the cluster of markers and 0.2 cm away from *Br.* Their finding suggests that a dominant gene or a cluster of genes controls the production of vignatic acids analogs. However, the study also showed vignatic acids producing lines with susceptible reaction suggesting that vignatic acids are not the principal factors involved in conferring resistance (Kaga and Ishimoto 1998). Kaga and Ishimoto (1998) showed eight RAPD markers were significantly associated and three RAPD (BEXA08, BEXA99 and BEXC49) to be tightly linked to resistance gene. Chen et al. (2007) developed 200 RILs (F_{12}) involving bruchid resistance accession 'TC1966' and MYMV-resistant variety 'NM92'. Ten RAPD markers (UBC66, UBC 168, UBC 223, UBC 313, UBC 353, OPM04, OPU11, OPV02, OPW02 and OPW13) were found associated with the bruchid resistance through BSA. Four (OPW02, UBC223, OPU11 and OPV02) of these that were closely

linked (Table 8.4) were cloned and transformed into SCAR and cleaved amplified polymorphism (CAP) markers. Seven co-dominant CAPs developed from the identified RAPD markers showed tighter linkage with the *Br* gene than the original RAPD. Molecular markers linked with bruchid resistance in *Vigna radiata* var. *sublobata* and their validation have been reported by Sutanu et al. (2011). They have employed STS marker, STSbr1, in screening of 50 green gram accessions and found high efficiency in screening of bruchid-resistant genotypes also. They have further reported that STS br1 will be useful for marker-assisted selection and germplasm screening for development of bruchid-resistant green gram.

9.5 Recent Advances in Mungbean Breeding

9.5.1 Use of Biotechnological Tools to Complement Conventional Breeding

Despite the systematic and continuous breeding efforts through conventional methods, substantial genetic gain in green gram production and productivity has not been achieved. The major yield constraint in green gram is high genotype and environment (G XE) interactions on the expression of important quantitative traits leading to slow gain in genetic improvement and yield stability, besides severe losses caused by susceptibility of pulses to biotic and abiotic stresses. The very much debated biosafety and ethical issues involving use of transgenic crops for commercial cultivation suggest that molecular marker-aided conventional methods of breeding may be the main short-term option for increasing productivity. One way is to utilize genomics tools in conventional breeding programmes involving molecular marker technology in selection of desirable genotypes or growing of transgenic crops. The use of transgenic crops is especially required for those traits that are not easy to improve genetically through conventional approaches because of the lack of satisfactory sources of desirable gene(s) in crossable gene pools. However, available high yielding diverse lines need to be used as base material for incorporating some useful attributes/genes from otherwise unadapted cultivars, lines or wild relatives. The present view of researchers is that the efficiency and effectiveness of conventional breeding can be significantly improved by using molecular markers. Nowadays, deployment of molecular markers is handy to a conventional plant breeder as it is routinely used worldwide in all major cereal crops as a component of breeding because of the availability of a large amount of basic genetic and genomic resources (Gupta et al. 2010). Use of molecular marker technology can give real output in terms of high yielding genotypes in pulses because high phenotypic instability for important traits makes them difficult for improvement through conventional breeding methods.

9.5.2 Genomic Resources

9.5.2.1 Development and Use of Markers

During the last three decades, DNA-based marker systems such as restriction fragment length polymorphisms (RFLPs), RAPD, SSRs or microsatellites, AFLPs, single-nucleotide polymorphism (SNP) and DArT have become available. Among these marker systems, RAPD, RFLP and AFLP are commonly employed for marker-trait association and diversity analysis in pulses, but their use for MAS is not preferred by conventional plant breeders because of the poor reproducibility, difficulty in handling, requirement of highly skilled person and use of radioactive elements for generating these markers (Gupta et al. 2010). Only PCR (polymerase chain reaction)-based SSR and SNP markers have been preferred by breeders because these markers can easily be employed in genotyping of large segregating populations in a cost-effective manner and with minimum infrastructure facilities. Also, high reproducibility and user-friendliness make them preferential to traditional plant breeders for MAS (Gupta et al. 2010). In many other crops, these have been extensively utilized (Kumar et al. 2010a, b), but their use is still limited in pulses (Varshney et al. 2009; Saxena et al. 2010). Therefore, in recent years, attention has been paid to develop more markers in pulses considered as orphan legumes (Hamwieh et al. 2009; Varshney et al. 2009). To reduce the cost of development of these markers, close phylogenetic similarity has encouraged researchers to transfer SSR markers from one pulse crop to the other (Datta et al. 2010; Reddy et al. 2010). Different types of markers such as RFLPs, RAPDs, AFLPs, SSRs and ISSRs have been used in discerning genetic diversity and developing linkage maps in these crops. In green gram, eight genetic linkage maps have been developed so far, but no map contained enough markers to resolve all the 11 linkage groups. Markers have been used for tagging and mapping of genes and QTLs for resistance against mungbean yellow mosaic virus, powdery mildew and *Cercospora* leaf spot diseases, bruchids and seed traits. Comparative genome mapping between green gram and several other legumes including azuki bean, common bean, cowpea, soybean and lablab revealed various levels of macrosynteny. Comparison between blackgram and azuki bean maps revealed high degree of genome colinearity. Efforts have been made in developing BAC libraries in green gram to facilitate map-based cloning of genes and QTLs. High-throughput sequencing technologies have led to the partial nuclear genome sequencing (100 Mb) and complete sequencing of chloroplast and mitochondrial genomes of green gram. Annotation of transcriptome sequences for functional genes has been carried out in green gram. The ESTs and genomic data based from closely related legumes will be helpful in developing high-throughput markers such as SSRs and SNPs. These resources have the potential to accelerate gene discovery and mapping and assist molecular breeding in these crops. In green gram, RFLP markers have been used to map (Young et al. 1993) or identify a new source of resistance (Chaitieng et al. 2002) to powdery mildew disease. Humphry et al. (2003) identified RFLP markers linked to major powdery mildew resistance

locus, while Fatokun et al. (1992) studied orthologous seed weight genes using RFLP. Besides requiring large quantity of DNA for analysis, the time and labour-intensive RFLP requires radioactive-labelled probes that limit their wide application in spite of their high polymorphism. RFLP markers of both DNA and random genomic clones of green gram were reported by Young et al. (1992). These RFLPs together with those from common bean, cowpea and soybean have been extensively used in green gram and or black gram genome mapping. Souframanien et al. (2003) studied intra- and interspecific variations in the DNA internal transcribed spacer (ITS) region using RFLP and found no variation within cultivated *V. mungo* species, while interspecific variation was detected among wild *Vigna* species. RAPD markers have been used for the identification of green gram cultivars and for assessing the genetic diversity (Santalla et al. 1998; Lakhanpaul et al. 2000). Assessment of genetic diversity using RAPD analysis shows close similarity among green gram cultivars (Lakhanpaul et al. 2000). The study revealed narrow genetic base of Indian cultivars probably due to repeated use of limited ancestors in their pedigrees. This observation has further been confirmed using RAPD (Afzal et al. 2004; Betal et al. 2004). Significant polymorphism among gamma ray-induced mutants have been observed using RAPD (25.8%) and ISSR (33.3%) markers in black gram (Souframanien et al. 2002). Yu et al. (1999) reported the abundance and variation of microsatellite DNA sequences in *Phaseolus* and *Vigna*. The cross amplification of soybean SSRs in *Vigna* species was studied by Peakall et al. (1998), and they found that there was 3–13% cross-amplification. Only recently microsatellite or SSR markers have been developed from green gram (Kumar et al. 2002a, b; Miyagi et al. 2004; Gwag et al. 2006). However, SSRs from azuki bean, common bean and cowpea can be used in both green gram and black gram. As high as 72.7% and 78.2% of the azuki bean SSRs amplify green gram and black gram genomic DNA, respectively, while 60.6% of common bean SSRs amplify green gram genomic DNA (Souframanien and Gopalakrishna 2009). Gupta and Gopalakrishna (2009) demonstrated that the azuki bean microsatellite markers are highly polymorphic and informative and could be successfully used for genome analysis in black gram. Gupta and Gopalakrishna (2010) reported the transferability of functional unigene-derived SSR markers in cowpea (*Vigna unguiculata*) to other *Vigna* species including green gram and black gram. ISSR markers have been successfully utilized for analysis of repeat motifs in green gram (Singh et al. 2000), genetic relationships in the genus *Vigna* (Ajibade et al. 2000) and varietal identification in black gram (Ranade and Gopalakrishna 2001). Singh (2003) revealed narrow genetic base of Indian cultivars using ISSR. ISSR markers were comparatively more efficient than RAPD in assessing genetic diversity among black gram cultivars (Souframanien and Gopalakrishna 2004). AFLP marker study also reiterated the low genetic diversity in green gram. High polymorphism was obtained with +3 than with +2 primers. Saini et al. (2004) reported that long primers (18–22 bases) in comparison to the 10-mer primers could efficiently dissect the genetic diversity and relationships in green gram germplasm.

With many genomic tools and resources for legumes becoming increasingly available, a more detailed and in-depth genome mapping of green gram is crucial for their genetic improvement. The current genetic linkage maps of green gram

display an inadequate level of marker density. To improve the utility of such maps, it will be required to further saturate the map with additional markers. High degree of colinearity and conservation in genome organization among legume species can be exploited for cross-species utilization of identified marker/genes/DNA sequence from other legume species.

Mungbean yellow mosaic virus (MYMV), a whitefly (*Bemisia tabaci*)-transmitted geminivirus belonging to the begomovirus having monopartite (one ~2.9 kb DNA) or bipartite genome (two ~2.6 kb DNAs referred to as "DNA-A" and "DNA-B"), causes disease in a number of leguminous crops in India and South East Asia especially green gram, black gram and soybean. Basakdash et al. (2004) used six YMV tolerant black gram lines ('VM1'-'VM6') developed from a highly susceptible genotype 'T-9' and F_2 population to identify DNA markers linked to YMV tolerance. A RGA primer pair RGA-1-F-CG/RGA1-R amplified a 445 bp fragment only in homozygous tolerant lines, differentiating the YMV tolerant and susceptible parents and was found to be linked to YMV tolerance. The 445 bp marker was sequenced and named 'VMYR1'. The predicted amino acid sequence showed highly significant homology with the NB-ARC domain present in several gene products involved in plant disease resistance, nematode cell death and human apoptotic signalling. On further evaluation of more RGA primer pairs, Maiti et al. (2011) identified two markers amplified using RGA primer pairs (RGASF1/RGASR1 and RGA22F2/RGA24R2) referred to as YR4 and CYR1linked to MYMV resistance. Both these resistance linked markers are part of the open reading frames (ORFs) and possess conserved motifs of the NB-ARC domain having sequence homology with other virus resistance genes. Both the markers were validated using green gram and black gram genotypes by multiplex PCR and showed YR4 to be partially linked and CYR1 to be completely linked. Souframanien and Gopalakrishna (2006) identified a tightly linked ISSR marker (ISSR811 1357) using a RIL mapping population (F_8) that was 6.8 cm away from the MYMV resistance gene loci. Sequence characterized amplified region (SCAR) primers designed (YMV1-F and YMV1-R) from this ISSR marker distinguished the MYMV-resistant and MYMV-susceptible plants in RIL population, agreeing well with the phenotypic data. The ISSR811 1357 marker was also validated using diverse black gram genotypes differing in their MYMV reaction. In green gram, F_2 population derived from a cross between 'ML267'and 'CO-4' was screened with RAPD primers and identified one marker OPS7 900 to be associated with YMV resistance (Selvi et al. 2006).

9.5.2.2 Bacterial Artificial Chromosome (BAC)

BAC libraries have been widely used in different aspects of genome research. However, there are only few reports of BAC library in *Vigna* species. Miyagi et al. (2004) constructed two green gram BAC libraries that together gave a 3.5 × coverage of the 587 Mb genome. The libraries were constructed from both *radiata* ssp. (green gram) using genotypes 'ACC41' and 'ATT3640' and its wild progenitor *sublobata* ssp. (golden gram) by cloning the DNA in pBelo BacII vector with an

average insert size of 107 and 113 kb size. Two PCR-based markers were developed closely linked to a major locus conditioning bruchid resistance, by screening these libraries using RFLP probes, including Mgm 213 that is very closely linked (1.3 cm). This information should aid in the introgression of this resistance locus into agriculturally elite cultivars. These libraries could also facilitate development of other PCR-based markers linked to other desirable traits. In the near future, the BACs of pulse crops should have potential applications in pulse comparative genomics and functional genomics as well owing to the macro- and microsynteny widespread within legumes (Souframanien and Dhanasekar 2014).

Similarly molecular Markers and QTL linked with MYMV have been reported widely in mungbean, but inheritance study MYMV resistance gene has revealed that different sources may have different MYMV resistance gene; thus markers identified using one source of MYMV resistance gene may not work for another source. Resistance gene analog (RGA) markers YR4 and CYR1 were associated with resistance to MYMIV in black gram (*Vigna mungo* (L.) Hepper) reported by Maiti et al. (2011). CYR1 is proposed as part of the candidate disease resistance (R) gene (Maiti et al. 2011). Recently, the R gene CYR1 was fully isolated from black gram (Maiti et al. 2012). Dhole and Reddy (2013) reported that SCAR marker MYMVR-583 is associated with MYMV resistance in mungbean. This marker is 6.8 cm from the resistance gene. Gupta et al. (2013) reported that CEDG180 is linked to MYMIV resistance in black gram at a distance of 12.9 cm. Chen et al. (2013) identified four major QTLs on three different linkage groups for MYMIV resistance using AFLP and SSR markers.

9.5.2.3 EST-SSRs

The EST databases available for many crop species provide a valuable resource for the identification and development of SSR markers. The sequences available in these databases can be mined for SSR repeats, thereby reducing the time and cost in developing microsatellite-enriched libraries. EST-SSRs, being part of the genes, are more useful as genetic markers because they represent variation in the expressed portion of the genome. EST-SSRs have been developed in a large number of plant species including chickpea (Choudhary et al. 2009), soybean (Hisano et al. 2007), common bean (Hanai et al. 2007) and *Medicago* spp. (Eujayl et al. 2004). Like genomic SSR markers, EST-SSR markers could be used for a variety of applications such as molecular mapping, gene tagging and genetic diversity analysis (Varshney et al. 2005). In addition, EST-SSR markers show a high rate of transferability to related species or genera owing to the higher conservation of expressed sequences across species (Varshney et al. 2005). Hence, SSR markers developed in one species can be used in related species for which sufficient sequence information is not available for marker development. However, owing to large redundancy in the public EST databases, multiple sets of markers can be developed for the same locus. This problem can be circumvented by clustering the ESTs into a non-redundant set of gene-oriented clusters called unigenes. Primer pairs successfully developed from

cowpea unigene SSRs were demonstrated to show cross species amplification and polymorphism in green gram, black gram and other *Vigna* species (Gupta and Gopalakrishna 2010). The unigene SSR markers developed in this study showed a high rate of transferability (88%) to other *Vigna* species, indicating the conservation of microsatellite sequences in the genus *Vigna* during evolution. These SSR markers would be helpful in the development of a saturated genetic linkage map and tagging genes in green gram (Souframanien and Dhanasekar 2014).

9.5.2.4 Intron Length Polymorphism (ILP)

Like other molecular markers, ILP markers can be used for a variety of applications like molecular mapping, gene tagging, genetic diversity analysis and comparative studies. In addition, ILP markers show a high rate of transferability to related species owing to a higher conservation of EST sequences across species. ILP markers were developed from cowpea EST. One hundred ten PCR primers targeting one or more introns were developed from randomly chosen cowpea EST sequences and showed cross-species amplification and polymorphism in green gram, black gram and other related *Vigna* species (Gupta et al. 2012).

9.5.2.5 RNAi Technology

Gene silencing technologies using RNA interference (RNAi) or virus-induced gene silencing have been developed to study the expression or inhibition of the candidate genes (Wesley et al. 2001). RNAi technology offers a new and innovative potential tool for plant breeding for resistance/tolerance to biotic and abiotic stresses through the introduction of small non-coding RNA sequences that are able to regulate gene expression in a sequence-specific manner (Fig. 9.3; Dubrovina and Kiselev 2019). The suppression of expression of a specific gene provides an opportunity to remove or accumulate a specific trait in plants that would lead to biochemical or phenotypic changes, which in turn provide resistance/tolerance to plants against biotic and abiotic stresses. Furthermore, RNAi-mediated gene silencing techniques can be used by plant breeders to suppress genes in full or partially using specific promoters and construct design (Senthil-Kumar and Mysore 2010). In RNAi technology, the candidate gene activity is disrupted and/or silenced in a sequence-specific manner by introducing constructs that generate double-stranded RNAs. Though this technology is generally used as a pest and disease control strategy on the pest aspect, the plant-mediated or host-induced RNAi (HI-RNAi) can be used to develop the engineered crop plant material with hair-pin RNAi vector to produce dsRNA that would target the insect and pathogen genes. When the insect feeds on the plant parts, the entry of dsRNA into the insect gut will induce the RNAi activity and silence the target gene in the insect pest. Further, RNAi can be used to alter the gene expression in plants involved in resistance against diseases (Senthil-Kumar and Mysore 2010) and abiotic stresses (Abhary and Rezk 2015). Haq et al. (2010) studied the silencing

of complementary-sense virus genes involved in MYMV replication in soybean by targeting a complementary-sense gene (ACI) encoding replication initiation protein (Rep) against mungbean yellow mosaic India virus. Similarly, Kumar et al. (2017) generated cowpea plants with resistance to MYMV using RNAi technology, which contained three different intron hairpin RNAi constructs. RNAi technology has been used against a number of insect pests such as *H. armigera* by targeting the *CYP6AE14* gene 9 (Mao et al. 2007). When transcriptional factor genes of *H. armigera* were targeted by HI-RNAi, a significant reduction in mRNA and protein levels was observed that resulted in deformed larvae and larval mortality (Xiong et al. 2013). Additionally, this technology has been implicated in increasing the production of unique secondary metabolites, increasing the shelf life of the fruits, improving crop yield and improving insect and disease resistance (Abhary and Rezk 2015). Sunkar and Zhu (2004) reported that in *Arabidopsis* plants, miRNAs are involved in tolerance against abiotic stress including cold, drought and salinity. They further showed that exposure to higher salinity levels, dehydration, cold and abscisic acid upregulated the expression of miR393. While RNAi technology can be used to improve biotic and abiotic stress resistance/tolerance in mungbean, large-scale field studies are needed to study any potential risks of this technology.

Although some progress in genome sequencing has been made in green gram, it is still far behind the other major legume crops such as soybean, cowpea and common bean or, even their relative but less important, azuki bean. Next generation sequencing platforms have already made their strides in green gram sequencing, and complete sequences of chloroplast, mitochondria, partial genome sequence and transcriptomic resources are now available in public domain. This coupled with complete genome sequencing in green gram can generate large-scale SNPs, SSRs and Initro on length polymorphic markers, which can help to saturate the linkage maps. They are expected to enhance molecular breeding such as marker-assisted backcrossing and marker-assisted recurrent selection. This will also be helpful in development of climate resilient cultivars in the present context of climate change, resistant to serious insects and diseases and with tolerance to adverse environmental conditions. This will lead to enhanced crop productivity in these crops and ensure progress towards attaining nutritional security (Souframanien and Dhanasekar 2014).

9.5.2.6 Application of Plant Phenomics

Farmers and plant breeders have been selecting the best genotypes based upon their phenotype for a very long time. However, the traditional phenotyping methods deal with either one or few specific plant characteristics at a given time and do not allow a thorough functional analysis of constituent traits linking genotype with the phenotype. Furthermore, the invasive, labour-dependent and time-intensive nature of phenotyping for many traits make measuring these traits in segregating generations difficult, thereby delaying selection to later generation and decreasing the breeding efficiency. In plant breeding, field experiments at multiple locations are

indispensable to evaluate the adaptability of new candidate genotypes and to examine the pattern of genotype X environment interaction. Plant phenotyping needs to generate high-quality quantitative data on the dynamic response of a genotype to the environment to adapt to the needs of modern breeding. Increased accuracy, precision and throughput at all levels, while reducing costs and minimizing labour through automation, remote sensing, data integration and experimental design, is the trend in modern plant phenotyping. Many next generation and high-throughput plant phenotyping platforms (HTPPs) were developed to measure trait values accurately and assess variation among individuals after realizing the need for rapid and precise phenotyping of multiple traits. Consequently, HTPPs enabled better approaches to address the relationship between traits, plant development, growth and reproduction under various conditions. The strides in plant phenotyping have been so swift that high-throughput phenotyping using non-invasive technologies is now a rapidly advancing field. This is based on various imaging techniques to record plant structure, estimate biomass and analyse phenology, plant health, tissue water relations, transpiration, photosynthetic activity and others. The phenotyping systems can operate in a field setting or in a controlled environment, where plants are automatically weighed and watered. Low-cost, automated and semiautomated methods for data acquisition and analysis are now being developed that cost-effectively provide physiological and morphological data. These HTPPs collect accurate observations using modern tools with high precision and automation and allow simultaneous analysis of the massive generated data. This leads to a better understanding of the whole phenome of the plant under a wide range of environmental and growth conditions (Pratap et al. 2019).

Precise plant phenotyping using next generation high-throughput plant phenotyping platforms (HTPPs) has significantly improved our understanding of plant growth and development, the response of genotypes towards changing environments, designing newer plant types and ultimately leading to the development of better plants addressing the constraints of phenotyping bottleneck. The vast amount of genomic resources developed in a plant species can now be linked with its phenotypes using the modern HTP approaches available through automated phenotyping platforms. Several public- and private-funded phenomics projects are currently underway, and evidently, a large amount of money is invested in such projects which need to be amply justified by the development of large-scale high-quality phenotypic data. Success of such phenomics projects will depend upon several factors including the species; population and traits used for phenotyping; the degree of genetic diversity; the phenotypic assays performed and methods of collection; storage and interpretation of data; and the extent to which the generated data is available in the public domain and preserved for future use. Likewise, the involvement of a multidisciplinary team comprising biologists, engineers and statisticians will increase our competitiveness in generating the best possible quality data and utilizing it in multifarious ways. Analysis of a large amount of data requires user-friendly supporting platforms. The establishment of International Phenotyping Network in setting up standards, indexing and searchable features would pave the way for efficient management of the stored data. Simulation studies can play an important role

in understanding the dynamic response of plants to changing environments as these have the potential for optimizing sensor acquisition and evaluation of the robustness of algorithms prior to field measurements. The idea of virtual phenotyping has already been deployed in clinical therapy, in which the genetic information is transformed into the most likely associated phenotype and also finds great promise in crop plants. In plants also, the option of integrated simulation environments – such as Robotics Operating System (ROS 2013) – have been applied for simulating the data acquisition for different sensors in a field-like situation. Plant phenotyping also encounters certain limitations or disadvantages such as high data generation and processing costs, complex handling algorithms limiting practical application and lack of efficient analysis pipelines. Unlike genotyping, many traits in plant phenotyping are interrelated and therefore are dependent on each other. For example, canopy temperature is related to plant water status and water use efficiency, stomatal conductance, transpiration rate, leaf area index and others. However, this trait itself is dependent on the developmental phase of the plant or crop, time of the day and the season, and therefore its measurement also affects the interpretation of the related traits. The collection, storage and retrieval of vast amounts of phenotyping data is still a challenge irrespective of the method applied for phenotyping. Integration of data from different users and different phenotyping platforms poses an additional challenge to utilize such data generated which therefore needs attention. Since phenomics uses several types of sensors simultaneously, systematic data acquisition is crucial from the beginning of experiments to develop efficient input data for interpretation of crop properties. Keeping in view the importance of seed in early plant establishment, plant stand and biomass, analysis of seed-related traits and root system architecture are likely to gain more impetus, especially in high-resolution phenotyping. Although phenotyping has mostly been applied to cereals and other major field crops, food legumes, forage and turf species offer a vast potential to determine their response to dynamic environments, and therefore non-invasive phenotyping methodologies hold great promise in these species. Next generation phenotyping is an emerging discipline and if properly integrated with genomics will be crucial in quantifying plant growth and development in real time as well as metabolic pathways governing these processes. Virtual phenotyping is now emerging as an important tool to reduce the complexity of sensor-based high-throughput phenotyping. Extrapolation of the experimental setup before establishing field trials will further improve our efficiency in designing climate-resilient genotypes. Therefore plant phenomics can be successfully utilized to exploit the gains of genomics (Pratap et al. 2019).

Agricultural scientists face the dual challenge of breeding input-responsive, widely adoptable and climate-resilient varieties of crop plants and developing such varieties at a faster pace. Integrating the gains of genomics with modern-day phenomics will lead to increased breeding efficiency which in turn offers great promise to develop such varieties rapidly. Plant phenotyping techniques have impressively evolved during the last two decades. The low-cost, automated and semiautomated methods for data acquisition, storage and analysis are now available which allow precise quantitative analysis of plant structure and function and genetic dissection

of complex traits. Appropriate plant types can now be quickly developed that respond favourably to low input and resource-limited environments and address the challenges of subsistence agriculture (Pratap et al. 2019).

9.5.2.7 Genomic Survey Sequences (GSS) in Green Gram

Sequenced crop plants are the good resources in identification of important genes for quality and insect pest and disease resistance, resistance to abiotic stresses like temperature, drought, salinity, etc. which are possible to transfer to cultivable background by combining traditional and molecular breeding methods. Genome Survey Sequences (GSS) are nucleotide sequences similar to EST's that the only difference is that most of them are genomic in origin, rather than mRNA, while expressed sequence tags sequences represent the expressed region of the genome. These GSS and EST sequences are used for the identification of "functional molecular markers" (FMM) which are associated with trait of interest and may be transferable in closely related genera. Genomic Survey Sequences of green gram [Vigna radiata (L.) R. Wilczek] are available online in public domain from NCBI (http://www.ncbi.nlm.nih.gov) for public use. Due to generation and availability of huge genomic information online of the crop plants, the computational studies, i.e. performed on computer or via computer simulation, are important areas of interest for genomics researchers for comparative genomics study. Domains can be thought of as distinct functional and/or structural units of protein. The identification of a conserved domain footprint may be the only clue towards cellular or molecular function of a protein, as it indicates local or partial similarity to other proteins, some of which may have been characterized experimentally. Conserved domains (CD) contain conserved sequence patterns or motifs, which allow for their detection in polypeptide sequences (www.ncbi.nlm.nih.gov/Structure/cdd). It has been suggested that domain combinations are evolutionarily conserved and evolution creates novel functions predominantly by combining existing domains (Dudhe et al. 2012). The conserved domain database (CDD) is a compilation of multiple sequence alignments representing protein domain conserved in molecular evolution (Marchler-Bauer et al. 2002). Computational identification of conserved domains from genomic survey sequences in green gram has been emphasized by Eruvuri et al. 2016. They report that with recent advances in the field of genome sequencing, analysis and availability of large genomic data in the public domain, attempts are made to survey the presence of the conserved domains, superfamilies and multidomains having putative functions identified from green gram [Vigna radiata (L.) R. Wilczek]. Genomic Survey Sequences (GSS) using computational tools are beneficial in the area of comparative genomics for the identification of important genes and also development of functional molecular markers in identified genes for green gram and its related crops improvement. The Genome Survey Sequences (GSS) of green gram [Vigna radiata (L.) R. Wilczek] are available online in public domain from NCBI (http://www.ncbi.nlm. nih.gov) which can be downloaded in FASTA format and used for further analysis.

9.6 Future Breeding Emphasis

In spite of its importance and wide cultivation and continuous crop improvement activities, the overall productivity of green gram is very low. This is due to several biotic, abiotic and physiological constraints. Thus, modification and design of advanced breeding strategies are essential. Some of the most required breeding activities can be:

- Development of short duration, photo- and thermo-insensitive varieties suitable for growing across all the three seasons and paddy fallows
- Breeding bio-fortified green gram genotypes with higher seed protein and mineral and methionine levels
- Breeding for target traits, viz. insect resistance with special emphasis on bruchid resistance
- Disease resistance especially MVMV and other fungal and bacterial foliar diseases, parasitic weeds (*Striga* and *Alectra*) and drought tolerance
- Development of varieties suitable for mechanical harvesting

9.7 Conclusions

Despite the incessant efforts to evolve genotypes for a specific target, be it a biotic or abiotic stress tolerance, ideal plant type, the success achieved is less due to the cumulative effect of several stresses owing to sudden outbreak of pests and diseases due to climatic changes across various growth stages of plants, right from seed germination, field establishment, flowering, pod initiation for yield potential, targeting a specific stage and or all the biotic and abiotic stresses is very challenging. In this endeavour, there is a possibility of introgression of undersirable trait due to linkage drag. Therefore a synergy between the conventional breeding methods and molecular breeding approaches is very essential. Identification of molecular markers will help in evaluation for the target trait and reduce the complete dependency on phenotypic data, which might have a confounding effect. Further employing the molecular markers can help in transferring the target traits from the related legumes such as azuki bean, rice bean and black gram to mungbean. Introgression of multiple genes into the same cultivar becomes very important. Further to avoid strain or biotype development, gene pyramiding must be preferred by breeders to develop mungbean with resistance to diseases and insect pests. Breeding mungbean lines for stressful environments is very important. While in particular stress dominates a population of environments, many of the agroecologies are featured by multiple stresses. This often makes a particular agroecology unique for which systemized solutions are essential. For making the best combination of abiotic stress and the traits to incorporate, it is essential to have insight on the fundamental mechanism for stress tolerance from intrinsic physiological and biochemical perspectives. We aim to develop root systems that help plants to withstand moisture deficits by drawing water from

the deeper soils. Screening for various abiotic stresses needs to be more precise and stringent to identify robust donor(s) for these traits. The identified donors need to put in use by the breeders at a faster pace. Plant type(s) having a deep root system, early maturity span, erect stature with sympodial pod-bearing, multiple pods per cluster and longer pods with many nodes and shorter internodes will help in withstanding heat and drought-related stresses. Of late, converging various modern technologies like infrared thermography, automated robotics, camera images and computational algorithms, which all make components of high-throughput phenotyping facilities (phenomics and phenospex), can facilitate high throughput phenotyping for stress tolerance (Pratap et al. 2019).

Molecular approaches are becoming handy in revealing resistance/tolerance mechanisms, which will help in modifying mungbean plants to suit the biotic and abiotic stresses. Genome-wide association studies (Breria et al. 2019) would help in better understanding of the genetic basis of the phenotypes. Association mapping for biotic- and abiotic-resistant/biotic- and abiotic-tolerant traits is highly important to identify the desired haplotypes in performing association mapping on a panel of adapted elite breeding lines. This will provide the ample justification to utilize these lines directly in breeding programmes. The selection of favourable haplotypes through MAS will be helpful in reducing the phenotyping material in the advanced breeding generations and increase the breeding efficiency. There must be systematic efforts towards exploring physiological and biochemical regulations of biotic and abiotic stresses and studying the whole profile of genes, proteins and metabolites imparting resistance/tolerance so that the same can be manipulated to develop improved cultivars of mungbean.

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Chapter 10
Genetic Advancement in Dry Pea (*Pisum sativum* L.): Retrospect and Prospect

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10.1 Introduction

Field pea or dry pea (*Pisum sativum* L.) is one of the important, highly productive cool season food legume crops grown around the world to consume as food, feed and fodder (Dahl et al. 2012; Warkentin et al. 2015; Holdsworth et al. 2017; Rubiales et al. 2019). It has yellow, green and orange cotyledon varieties which are consumed by human being in various forms such as *soup*, *chat*, *chhola*, *dal*, *stew*, *snacks*, vegetables and flour, whereas the whole seed is used as animal feed (Dahl et al. 2012; Parihar et al. 2016; Singh et al. 2018). Since it is an excellent source of protein, starch and fibre, therefore, it is being widely used as an ingredient in many food industries around the world (Dixit et al. 2014; Gupta and Parihar 2015; Parihar et al. 2016). It is a good source of proteins (21.2–32.9%) and carbohydrates (56–74%) along with vitamins, essential amino acids and micronutrients. It is considered as one of the cheapest sources of easily digestible protein for human and livestock consumption owing to the absence of major anti-nutritional factors. The seed coat and cotyledon are the dietary fibre-rich part of seed, i.e. water-insoluble and water-soluble fibre, respectively (Reichert and MacKenzie 1982; Guillon and Champ 2002; Tosh and Yada 2010; Parihar et al. 2016). In case of micronutrients, the potassium is the most prevailing element followed by phosphorus, magnesium and calcium. The dry pea is also a good source of other micronutrients such as Fe (97 ppm), Se (42 ppm), Zn (41 ppm) and Mo (12 ppm) (Reichert and MacKenzie 1982). In addition, dry pea also has sizeable amount of folate (101 μg per 100 g) with many vitamins (Dang et al. 2000; Hedges and Lister 2006). It has many health benefits

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such as helps in prevention and management of type 2 diabetes (Marinangeli et al. 2009; Marinangeli and Jones 2011), reduces and stabilizes bold cholesterol (Daveby et al. 1998; Ekvall et al. 2006), improves cardiovascular health (Slavin 2008; Singh et al. 2013) and also has cancer combating and antioxidant properties (Kalt 2001; Kleijn et al. 2001; Boker et al. 2002; Steer 2006). Besides, it helps in weight management and betterment of gastrointestinal function (Fernando et al. 2010; Tosh and Yada 2010; Lunde et al. 2011). Given nutritional quality makes dry peas as important international food commodities, which cater the dietary requirement of resource poor undernourished individuals of developing countries (FAOSTAT 2011). The production of dry pea has been unstable during recent past decades due to many prevalent biotic and abiotic stresses. Of them, biotic stresses are powdery mildew, rust, ascochyta blight, *Fusarium* root rot, common root rot and Fusarium wilt, while abiotic stresses are high temperature, drought and cold. Since majority of the pulses including dry pea are cultivated under low-input agriculture around the world. Dry peas produced under these conditions by resource-poor farmers are more vulnerable to attack by biotic and abiotic stresses. The high-input farmers have more resources to stride against these stresses through the use of recently developed technologies (fertilizer, irrigation, pesticides and management strategies). However the application of such inputs and management can increase cost of cultivation which ultimately reduces profit of farmers and also has negative impact on environment, and even many pests are not effectively controlled with chemical treatment. Hence, incorporating host plant resistance mechanism in the crop through conventional, molecular and genomic-assisted breeding strategies is the most economically efficient way of tackling these stresses. Therefore, in this chapter, we have covered the present scenario of dry pea cultivation, present status of trait-specific genetic improvement happened in dry pea over the years and their future perspectives towards sustainable dry pea production for nutritional security of resource poor farmers.

10.2 Dry Pea Area, Production, and Productivity Scenario at Worldwide

Dry pea is being cultivated around the world about in 94 countries (Smýkal et al. 2012), and the total production and area of dry peas at worldwide is at present approximated to be 16.20 mt and 8.14 mha, respectively, increasing trend observed during 2007–2017 (Fig. 10.1). In addition, the production and area of green peas is 20.69 mt and 2.66 mha, respectively. The top 10 major share-holding countries in production are Canada (21.85%), Russian Federation (10.31%), China (7.53%), India (4.32%), the USA (4.26%), France (3.77%), Ukraine (2.90%), Australia (1.91%), Ethiopia (1.85%) and Germany (1.17%) (Fig. 10.2). Region-wise production situation at global level witnessed that the Americas (38.6%) are accountable for highest share in total production followed by Europe (34.4%) and Asia (19.0%)

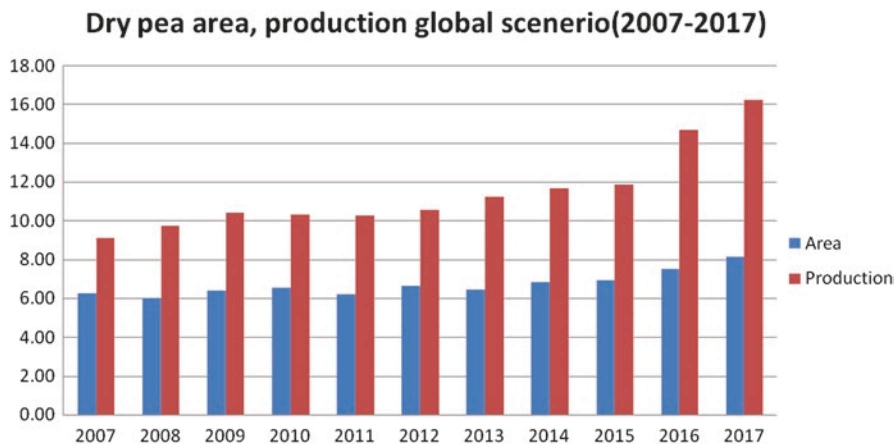

Fig. 10.1 Dry pea area and production trend during 2007–2017 at worldwide

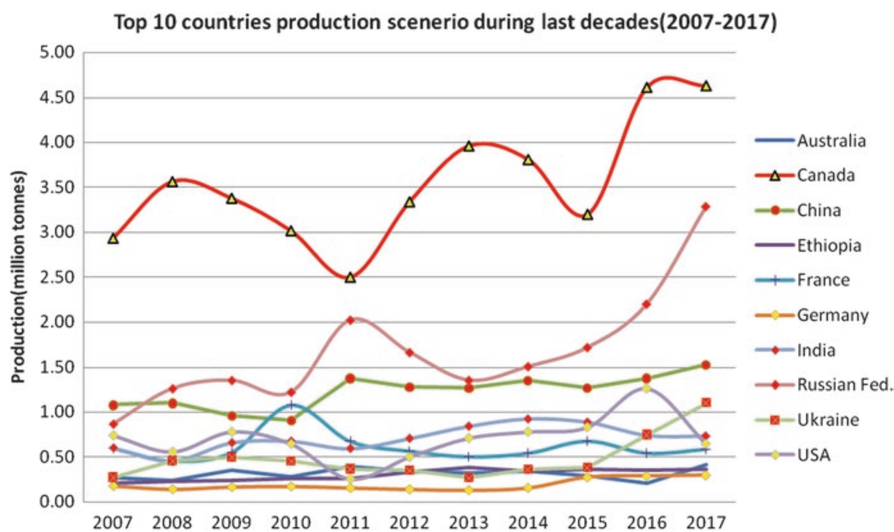

Fig. 10.2 Top 10 countries dry pea production trend during 2007–2017

(FAOSTAT 2019). During the past five decades, the yield gain is just 15.3 kg/ha/year in dry pea at global level, much lower than other crops, which demonstrating that least concentration is invested on dry pea improvement programme. In addition, the yield gain in Canada is 2.0% which is greater than the yield gain in most of the crops at global level witnessed large investment has been made in pea research programme over the years (Rubiales et al. 2019). However, the global dry pea productivity has been increased more than 36% during 2007–2017 and currently in tune of 1.9 tonnes/ha. The countries having highest productivity are the Netherlands (4877 kg/ha), Denmark (4463 kg/ha), Belgium (3824 kg/ha), Ireland (3571 kg/ha),

Germany (3487 kg/ha) and France (3222 kg/ha). On the contrary, in other dry pea-growing countries like India, China, Australia and Myanmar, productivity is low as compared to above-mentioned countries varied between 1000 and 2000 kg/ha. Some of the countries like the USA, Finland, Brazil, Ireland, Belgium, Pakistan and the Netherlands portrayed negative tendency in production during 2007–2017, while the opposite trend has been recorded for the Russian Federation, Ukraine, Germany, Canada, Denmark, India, Australia, China and Myanmar where production showed increase. The highest increase in production and productivity has been recorded in Russian Federation, Ukraine, Germany, Australia, China, Ethiopia, Canada, Belgium and Denmark, while the decrease recorded in Finland, the USA, Italy, Ireland and Pakistan. Interestingly, the Netherlands is the only country where production decreased in spite of substantial increase in productivity.

10.3 Systematic, Origin, and Domestication

The pea is a self-pollinated diploid ($2n = 14$, $x = 7$) annual crop and its a member of third largest flowering plant family Leguminosae, largest subfamily Papilionoideae and the tribe Fabeae (Doyle et al. 1997; Lavin et al. 2005; Lewis et al. 2005). The tribe Fabeae comprised of five genera such as *Lathyrus*, *Lens*, *Vicia*, *Pisum* and *Vaviloviaformosa* (Smýkal et al. 2011; Mikič et al. 2013; Rubiales et al. 2019). The genus *Pisum* L. mainly have three species such as cultivated pea (*P. sativum* subsp. *sativum*) with its five subspecies (*elatius*, *sativum*, *humile*, *arvense* and *hortense*), Ethiopian pea (*P. abyssinicum*) and *P. fulvum* (Maxted and Ambrose 2001; Warkentin et al. 2015; Trněný et al. 2018). These species are cross-compatible and produce hybrids; however, the fertility level may be subsidized owing to karyological and nuclear – cytoplasmic incompatibility (Ben-Ze'ev and Zohary 1973; Bogdanova et al. 2015). In addition, based on crossing ability, the genus *Pisum sativum* contains the following subspecies which are considered as varieties, namely, *P. sativum* L. var. *hortense* (garden pea), *Pisum sativum* L. var. *arvense* (field pea), *Pisum sativum* L. var. *macrocarpum* (whole pod edible pea) and *Pisum sativum* L. var. *syriacum* (wild form) (Nasiri et al. 2009; Mohan et al. 2013).

The Near East and Mediterranean region is considered as the primary centre of origin/diversity for pea where two wild species, i.e. *P. fulvum* and *P. sativum* subsp. *elatius* are cultivated today also. The distribution of *P. fulvum* is restricted to the Middle East (Ladizinsky and Abbo 2015), while wild pea (*P. sativum* subsp. *elatius*) is noticed all over the Mediterranean basin and the maximum diversity available in the Near East, which is accounted as the centre of pea diversity (Smýkal et al. 2017). The secondary centres of diversity are the upland Asiatic region of the Hindu Kush with the long-vined Afghan types, and the upland regions of Ethiopia and Yemen, with *P. abyssinicum (*Rubiales et al. 2019). Further, the cultivation of pea expands from the Fertile Crescent to today's Russia, North and West Europe, Greece and Rome. Simultaneously, pea cultivation has extended eastward to Persia, India and China (Makasheva 1979; Chimwamurombe and Khulbe 2011). Most recently,

P. humile has been incorporated as extra taxa and at present exist only in secondary habitats (Abbo et al. 2013).

The archaeological facts witnessed that the pea is the world oldest grain legume and domesticated about 10,000 years ago in the Near East and Central Asia (Baldev 1988; Zohary and Hopf 2000). During early civilization in the Middle East and Mediterranean, it was consumed with cereals as important dietary components (Abbo et al. 2010; Rubiales et al. 2019). In Europe, it has been grown since the Stone and Bronze Ages and in India from 200 BC (De Candolle 2007). Over the years due to domestication, several changes happened in plant type such as from indeterminate, tall, slender, bushy or climbing types with small and coloured seeds to short, determinate mechanical harvested crop with large seeds without tannins (Smýkal et al. 2018). Cultivated pea is described by characters resulted from domestication, like non-dehiscing pods and lack of seed dormancy (Abbo et al. 2013; Smýkal et al. 2014; Trněný et al. 2018). Similarly, based on uses, peas have been classified in many groups such as the mature round seed with yellow, green, red cotyledon varieties typically used in the dehulled/split form in foods which is known as field pea or dry pea. The large seeds, blocky shape, green and yellow cotyledons are different from wrinkled type called as marrowfat field pea used for snacks and mushy pea. The mottled seed coat (maple) and high biomass (forage) types are consumed as feed and fodder for birds and animals (Warkentin et al. 2015; Rubiales et al. 2019).

10.4 Available Genetic Resources at Global Level

Genetic resources and their judicious utilization is the quintessential step towards development of high-yielding varieties with targeted traits. In pea approximately 98,000 pea accessions comprising commercial varieties, breeding lines, landraces, mutant stock and wild species are existing in different gene banks at global level, of them 59,000 are unique (Smýkal et al. 2013; Warkentin et al. 2015; Rubiales et al. 2019) The five largest active *Pisum* germplasm-holding institutions include National Institute for Agricultural Research (INRA) of France held at Dijon (8839 accessions); Australian Temperate Field Crop Collection, Horsham, having 7432 accessions; N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia, holds 6790 accessions; the US Department of Agriculture (USDA) (6827 accessions); and International Center for Agricultural Research in the Dry Areas (ICARDA) holds 6105 accessions (Table 10.1). There are other national collection centres of pea germplasm in different countries' national gene banks such as in Germany (5343 accessions), Italy (4558 accessions), China (3837 accessions), India (3609 accessions), the UK (3567 accessions), Poland (2896 accessions), Sweden (2849 accessions) and Bulgaria (2100 accessions). Furthermore, the national gene banks maintain more than 1000 germplasm accessions of *Pisum* available in at least nine other countries also. Among all the countries, Australia has the least duplicative and most diverse ex situ collection so far for *Pisum*. The busiest

Table 10.1 List of major dry pea germplasm collections (>3000 accessions) institutions at global level

S.N.	Name of institutions/ organization	Number of accessions	Share (%) of total accessions		
			Commercial varieties	Wild species	Others (breeding lines, landraces and mutant stock)
1	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russia	6790	98.0	–	2.0
2	INRA CRG Légumineuse à grosses graines, Dijon, France	8839	14.9	0.7	62.1
3	Australian Temperate Field Crop Collection, Horsham, Australia	7432	15.7	2.8	81.0
4	Plant Germplasm Introduction and Testing Research Station, Pullman, USA	6827	22.0	1.2	59.1
5	International Center for Agricultural Research in the Dry Areas, Aleppo, Syria	6105	19.4	3.7	74.4
6	Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany	5343	56.3	0.9	35.6
7	Istituto del Germoplasma, Bari, Italy	4558	–	–	100.0
8	Institute of Crop Sciences, CAAS, China	3837	13.9	–	86.3
9	ICAR-National Bureau of Plant Genetic Resources, New Delhi, India	3609	5.8	–	61.8
10	John Innes Centre, Norwich, UK	3567	30.0	10.3	34.9

Source: Warkentin et al. (2015)

websites for supplying germplasm are the JI Centre (JIC; http://www.jic.ac.uk/germplasm/) and the USDA (http://www.ars-grin.gov/npgs/). Both the portals have the highest proceeds of international requisition of readily available *Pisum* accessions. In addition, there are other exciting national collections of pea germplasm, for example, in Israel the gene bank having a collection of wild relative's *P. fulvum* and *P. sativum* subsp. *elatius* var. *pumilio* collected in the Middle East. The land races are contributed highest in total germplasm available at international level. Interestingly, the tiny share (about 2%) of conserved germplasm accessions represents wild pea (Smýkal et al. 2013; Warkentin et al. 2015). Of them, 706 accessions belongs 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). Wild *Pisum*

species and subspecies are reservoir of many useful traits, for instance, pea seed weevil resistance (Clement et al. 2002; Byrne et al. 2008; Clement et al. 2009), rust (Barilli et al. 2010), powdery mildew resistance (Fondevilla et al. 2007b) and many other yield components (Mikič et al. 2013). The commercially least favoured germplasm such as pigmented flower and pigmented seed coat have been confirmed as an outstanding sources of *Aphanomyces* root rot resistance (Hamon et al. 2011) and *Fusarium* root rots (Weeden and Porter 2007; Grunwald et al. 2003). There are several international collection databases, which having important information of pea, such as European Cooperative Programme for Plant Genetic Resources (ECPGR), Germplasm Resources Information Network (GRIN), System-wide Information Network for Genetic Resources (SINGER) and GRIN-Global. Most recently, numerous databases, namely, Cool Season Food Legume Database (https://coolseasonfoodlegume.org; Washington State University) and KnowPulse (https://knowpulse.usask.ca; University of Saskatchewan), have been developed to store and share information related to phenotypic and genotypic data sets. To speed up germplasm evaluation and their judicious utilization, eight core collections have been made in Australia, China, the Czech Republic, France, Poland, Spain, the UK and the USA (Warkentin et al. 2015; Rubiales et al. 2019).

10.5 Genetic Improvement of Important Agronomic Traits (Retrospect)

Genetic improvement in grain yield with stability is a major objective of plant breeders across the crops. Grain yield is an intricate attribute influenced by many traits directly or indirectly. In dry pea breeding program, the improvement in overall productivity has been mainly approached through breeding for tailoring plant type (especially lodging resistance and plant height), resistances to key biotic (powdery mildew, rust, ascochyta blight, etc.) and abiotic (heat, drought and cold) stresses.

10.5.1 Breeding for Lodging Resistance

Earlier plant type in pea was used to be tall type with bulky vegetative growth. Over the years dramatic development has been embraced by researcher in pea plant type by reducing plant height from 1–2 m to 0.3–0.6 m. In spite of considerable dwarfing of pea plant, the lodging earlier remains major problems due to high biomass (Davies 1977a, b; Donald and Hamblin 1983; Amelin et al. 1991). Therefore, the alternative strategy to get lodging resistance is the development of 'semi-leafless' pea cultivars (Fig. 10.3) using 'afila' leaf type, which proved superior to 'leafless' in photosynthetic capacity; equivalent to that of the wild type is considered possibly the best achievement made in pea breeding (Duparque 1996). The lodging changes

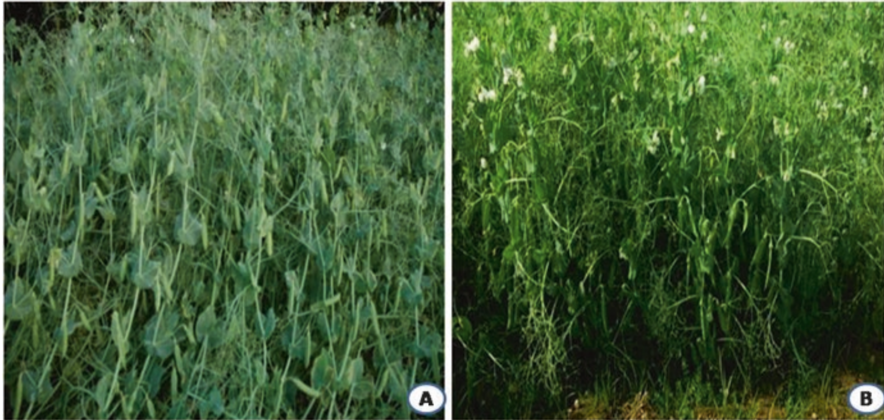

Fig. 10.3 Semi-leafless tall and dwarf dry pea varieties. (**a**) Variety, Aman. (**b**) Variety, IPFD 12-2

the canopy microclimate congenial for fungal disease development, condenses photosynthetic ability of the plants, declines harvest efficiency and amplifies harvest cost; consequently, it is considered as a serious constraint towards field pea production (Heath and Hebblethwaite 1985; Warkentin et al. 2001a; Xue and Warkentin 2001; Taran et al. 2003; Zhang et al. 2006). Given situations can cause up to 74% grain yield loss in some dry pea cultivars and also affect quality of seed (Armstrong et al. 1999; Warkentin et al. 2001b; Amelin and Parakhin 2003; Hashemi et al. 2003; Singh and Srivastava 2018). The semi-leafless plant type significantly increased lodging resistance or standing ability of pea cultivars which reduced grain yield losses and canopy disease severity (Wang et al. 2002; Banniza et al. 2005; Singh and Srivastava 2018). Thus, the semi-leafless type is preferred by most pea producers and has become the dominant leaf type in commercial cultivars. Such cultivars also increased the interest of farmer towards cultivating pea as a quality food and feed at worldwide. Most cultivars released during the recent decades have the semi-leafless leaf type (Mikić et al. 2006, 2011). Complete to partial shift has been made in many countries from 'leafy' cultivars to 'semi-leafless' cultivars. The first commercial deployment of the semi-leafless (*afila*) trait was done during the 1970s in Europe with the development of Solara cultivar. During recent period, 'semi-leafless' pea cultivars accounted 95%, 80% and 30% of the total dry pea production in Canada, European Union and Russia, respectively. It has to be noticed that *afila* improve the lodging resistance, but increased stem strength is also a very important trait (Banniza et al. 2005; Tayeh et al. 2015). In addition, it is also suitable for cultivation under diverse climatic conditions, particularly low and high temperature (McPhee and Muehlbauer 2007; McPhee et al. 2007; Mikić et al. 2011). Such cultivars contributed significantly in substantial increment of the total pea cultivation area in many countries, i.e. Canada, India, Australia and China (Mikić et al. 2007; Warkentin et al. 2015). A number of varieties were released with semi-leafless trait which helps

in increased production potential of dry pea in India (Dixit and Parihar 2014; Dixit et al. 2014; Gupta and Parihar 2015; Parihar and Dixit 2017; Parihar et al. 2019).

10.5.2 Breeding for Dwarf Type

Wild pea and most of the older cultivated varieties have tall plant type, which had high biomass and severe lodging problems leading to disease severity (Donald and Hamblin 1983). One developmental mutant (*le-1*) shortened internode length by reducing 3β-hydroxylation of GA_{20} to GA_1 (Ingram et al. 1984; Ross et al. 1989; Martin et al. 1997). The most of modern varieties have shortened internodes or dwarf plant type due to the incorporation of dwarf gene (*le-1*). A similar phenomenon has been exploited during *Green Revolution* in wheat and rice, which is associated with gibberellin (GA) pathway (Martin et al. 1997). Vasileva et al. (1980) reported that dwarf cultivars have greater lodging resistance than tall cultivars since they have short internode length. The dwarfing Mendel's *le-1* mutation, affecting gibberellin biosynthesis, seems to be the only dwarf gene/allele that has been used by pea breeders; another allele, *le-3*, is described as less severe than *le-1* (Ross and Reid 1991). Its effect on yield and lodging resistance is also assessed since *le-1* adds a slightly depressing effect on yield (Burstin et al. 2007) while having a highly beneficial effect on lodging. The dwarfing gene has been successfully incorporated in pea breeding especially in India which enhanced productivity through improved response to fertilizers, irrigation and dense plant population. The first dwarf and semi-leafless variety HFP 4 (Aparna) has been developed in 1988 from the cross of T 163 with an exotic line EC 109196. Later, HFP 4 in combination with EC 109185 and Flavanda led to the development of dwarf variety, HFP 8909 and Swati, respectively (Dixit and Gautam 2015). It also resulted in the development of dwarf leaflet less variety KPMR 144-1 (Sapna) from hybridization with Rachna. At the end of the twentieth century, a dwarf and landmark variety of dry pea HUDP-15 developed which is the product of the cross (PG 3 X S 143) X FC 1 and has resistance against powdery mildew and good tolerance to rust and ruled the seed chain for long time span (Dixit et al. 2014). Sincere efforts have been made, and a number of high-yielding dwarf type varieties, viz. IPFD 99-13, IPFD 1-10, IPFD 10-12, IPFD 12-2, IPFD 11-5 and IPFD 6-3, have been developed (Anonymous 2019).

10.5.3 Breeding for Biotic Stresses

The productivity of dry pea is limited by large number of biotic stresses. These included fungal, viral, bacterial pathogen causing diseases and various insect-pests and nematodes. Of them, fungal diseases with more than 28 fungi species are the most common and devastating (Reiling 1984). Some of these are powdery mildew, rust, root rots, wilt, stem/pod rot, ascochyta blight, etc. (Bohra et al. 2014). These

diseases occur in almost all pea-growing regions of the world and can cause significant crop losses when conditions are favourable for their development. Keeping this in view, the progress made in breeding for diseases resistance in field pea has been presented in this section of chapter.

10.5.3.1 Powdery Mildew

Powdery mildew is a serious constraint to dry pea production in pea-growing areas worldwide and largely incited by *Erysiphe pisi* (Gritton and Ebert 1975; Smith et al. 1996; Kraft and Pfleger 2001; Sun et al. 2016, 2019). Earlier, only *Erysiphe pisi* was the only known causal agent of dry pea powdery mildew, but during recent past two other fungi such as *Erysiphe baeumleri* and *Erysiphe trifolii* have also been designated as casual organism for powdery mildew disease with similar symptoms on pea plant (Ondřej et al. 2005; Attanayake et al. 2010; Fondevilla and Rubiales 2012; Sun et al. 2019). This is an airborne disease and turns into more serious threat in temperate and tropical climatic conditions with warm dry days and cool nights (Smith et al. 1996; Davidson et al. 2004; Fondevilla and Rubiales 2012; et al. 2016). It causes 25–80% losses in total grain yield and also reduces total biomass, number of pods per plant, number of seeds per pod, plant height, number of nodes and seed quality under congenial conditions for disease expansion (Munjal et al. 1963; Singh et al. 1978; Warkentin et al. 1996; Katoch et al. 2010; Fondevilla and Rubiales 2012; Ghafoor and McPhee 2012). As symptoms this disease basically developed a white powdery coating on surface of leaves, stems and pods (Fig. 10.4) (Singh et al. 1978; Bilgrami and Dube 1982; Agrios 1988; Kazmi et al. 2002). The delayed planting and late-maturing varieties are more vulnerable to powdery mildew (Gritton and Ebert 1975; Tariq et al. 1983; Davidson et al. 2004; Fondevilla and Rubiales 2012).

Owing to their economic importance, a large number of methods to control powdery mildew have been proposed, including cultural practices, the use of resistant

Fig. 10.4 Powdery mildew infected plants of dry pea

varieties and fungicide application. However, the control efficacy of chemical and agronomic practices is restricted by many factors. Therefore, use of resistant varieties has become the first choice due its efficiency, low cost, eco-friendly and qualitative resistance nature (Fondevilla and Rubiales 2012; Ghafoor and McPhee 2012). First time powdery mildew resistance was recognized by Harland (1948) in the pea landrace Huancabamba which genetically controlled by a single recessive gene. Since then, screening and genetic analysis of resistance to pea powdery mildew have been performed almost for more than 60 years (Fondevilla and Rubiales 2012; Sun et al. 2016). Many resistant pea accessions have been identified and characterized their gene(s) for resistance to *E. pisi*. Different levels of resistance to *E. pisi* have been reported, but only three genes for resistance have been reported so far, of them two recessive, namely, *er1* and *er2*, and one dominant *Er3* (Heringa et al. 1969; Fondevilla et al. 2007c; Parihar et al. 2013). Among them *er1* gene exists in maximum resistant pea accessions, while e*r2* gene is harboured only in few resistant accessions (Tiwari et al. 1997). The *Er3* is a recently identified dominant gene from a wild relative of pea (*P. fulvum*) that has recently been successfully introduced into cultivated pea (*P. sativum*) (Fondevilla et al. 2007a, b, c; Fondevilla and Rubiales 2012). Most pea breeding programmes depend on *er1*, and it is based on pre-penetration resistance (Fondevilla et al. 2006). Both monogenic and digenic recessive models for powdery mildew resistance have been reported by many researcher (Harland 1948; Heringa et al. 1969; Saxena et al. 1975; Kumar and Singh 1981; Liu et al. 2003; Sharma 2003). Several researchers reported linkage between the *er1* locus and various morphological and molecular markers and used them to place the *er1* gene on pea chromosome VI (Sarala 1993; Dirlewanger et al. 1994; Timmerman-Vaughan et al. 1994). Similarly, different types of marker, i.e. RAPD, SCAR and SSR, linked with powdery mildew resistance gene er1 have been reported as given in Table 10.2 (Tiwari et al. 1998; Rakshit et al. 2001; Janila and Sharma 2004; Ek et al. 2005; Pereira and Leitão 2010; Tonguc and Weeden 2010; Nisar and Ghafoor 2011). The recessive *er1* locus due to loss-of-functional alleles of plant-specific *MLO* (*Mildew Resistance Locus* O) governed powdery mildew resistance in pea (Humphry et al. 2011; Pavan et al. 2013).

Most recently, a new allele of *er1* which is named as *er1-6* has been reported by using cDNA sequence of *PsMLO1* gene. Subsequently, the resistance allele *er1-6* in landrace G0001778 has been confirmed by resistance inheritance analysis using mapping populations derived from G0001778 × Bawan 6. Finally, a SSR marker specific to *er1-6* has been developed which could be used in pea breeding for marker-assisted selection (Sun et al. 2016). Similarly, Sun et al. (2019) reported two novel *er1* alleles, *er1-8* and *er1-9*, in the germplasm accessions G0004839 and G0004400, respectively. These alleles were identified using inheritance analysis and genetic mapping with F2- and F2:3-derived populations, respectively. In addition, codominant functional markers specific to *er1-8* and *er1-9* have been developed and validated in populations and pea germplasms. These results would improve our understanding of *E. pisi* resistance in pea germplasms worldwide and provide powerful tools for marker-assisted selection in pea breeding.

Table 10.2 Details of markers/QTLs linked with different important traits in pea

Trait	Marker name/marker type	Gene/QTLs	References
Fusarium root rot *(Fusarium solani* f.sp. *pisi*)	AA416/SSR, AB60/SSR	*Fsp-Ps 2.1; Fsp-Ps3.2; Fsp-Ps3.1, Fsp-4.1 Fsp-Ps3.3; Fsp-Ps7.1*	Coyne et al. (2015, 2019), Feng et al. (2011)
Rust (*Uromyces fabae*)	AA446/SSR, AA505/SSR, AD146/SSR, AA416/SSR	*Qruf, Qruf1, Qruf2*	Singh et al. (2015), Rai et al. (2016)
	SC10–82360/RAPD, SCRI-711000/RAPD	*Ruf*	Vijayalakshmi et al. (2005), Rubiales et al. (2011)
	F7XEM4a/SRAP		Saha et al. (2010), Rubiales et al. (2011)
Rust (*U. pisi*)	OPY111316/RAPD, OPV171078/RAPD	*Up1*	Barilli et al. (2010), Rubiales et al. (2011)
	AD280/SSR, 3567800/ DArT, 3,563,695/ DArT, 3,569,323/ DArT	*UpDSII, UpDSIV, UpDSIV.2*	Barilli et al. (2018)
Fusarium wilt (*Fusarium oxysporum.* f.sp. *Pisi*), race 1	H19/RAPD, Y14/RAPD, Y15/ RAPD, p254/RFLP, p248/RFLP, p^{2}27/RFLP, p10μ/RFLP		Dirlewanger et al. (1994)
	Y15_*999/*SCAR, Y15_1050/ RAPD/ACG: CAT_222/AFLP, ACC: CTG_159/AFLP		Okubara et al. (2005), McClendon et al. (2002)
	AD134_213/SSR, AA5_225/ SSR, AA5 _235/SSR, AB111/ SSR, AD73/SSR, AA484/SSR AD85_178/SSR		Loridon et al. (2005)
	THO/CAPS, AnMtL6, Mt5_56, PR X1TRAP13, TC112650/SSR, TC112533/SSR		Jain et al. (2013, 2015)
	Fw_Trap_480/SCAR, *Fw_ Trap_340/SCAR*, *Fw_Trap_220/ SCAR*		Kwon et al. (2013)
	PSAS/SSR		Burstin et al. (2001)
Fusarium wilt, race 2	PSMPSAD171/ SSR		McPhee et al. (2004)
	AC22_185/SSR, AD171_197/ SSR, AB70_203/SSR, AD180_60/SSR	*Fnw 4.1, Fnw 3.1*	Mcphee et al. (2012)
Fusarium wilt, race 5	U693_400*Fwf/* SCAR		Okubara et al. (2002)

(continued)

Table 10.2 (continued)

Trait	Marker name/marker type	Gene/QTLs	References
Powdery mildew	p236/RFLP, PD10650(RAPD to SCAR)	er-1, er-2, er-3	Dirlewanger et al. (1994)
	Sc-OPO-181200/SCAR, Sc-OPE-161600/SCAR		Frew et al. (2002)
	OPU-17/RAPD, ScOPD-10_{650} / *SCAR,* ScOPL61600/SCAR, OPO-$18_{1200/}$RAPD, OPE-16_{1600}/ RAPD, OPL-$6_{1900/}$RAPD		Janila and Sharma (2004), Tiwari et al. (1998), Loridon et al. (2005)
	AB71/SSR, AD59/SSR, AD60/ SSR/SCAR, ScOPO18-1200/ SCAR, ScOPX04-880/SCAR, ScOPE16-1600/SCAR		Timmerman-Vaughan et al. (1994), Tiwari et al. (1998), Sun et al. (2019)
	PSMPSAD51/SSR, PSMPSA5/ SSR, PSMPSAA374e/SSR		Ek et al. (2005), Pereira and Leitão (2010), Sun et al. (2019)
	PSMPSAA369/SSR, c5DNAmet/ gene marker		Sun et al. (2015)
	AD60/SSR, ScOPX04880/ SCAR, ScOPD-10_{650} */SCAR*		Srivastava et al. (2012), Sun et al. (2019)
	SNP1121/SNP		Sun et al. (2016)
	OPW04_637/RAPD, OPC04_640/RAPD, OPF14_1103/RAPD, OPAH06_539/RAPD, SCW4637/SCAR, SCAB1874/ SCAR		Fondevilla et al. (2008a)
Common root rot	N14.950/RAPDs, U326.190/ RAPD, E7M4.251/AFLPs, E2M4.292/AFLP, E3M3.167/ AFLP	*Aph 1, Aph 2, Aph 3*	Pilet Nayel et al. (2002, 2005)
	P393/RFLP, PgmF$_{_390/}$Isozyme		Weeden et al. (2000)
	PSARGDECA_F/SSR	*Ae-Ps4.5, Ae-Ps7.6, Ae-Ps2.2, Ae-Ps5.1, Ae-Ps3.1, Ae-Ps1.2,* Ae-Ps4.1	Hamon et al. (2011, 2013), McGee et al. (2012), Lavaud et al. (2015, 2016), Desgroux et al. (2016, 2018), Kwon et al. (2012)
	Ps115429/SNP		Desgroux et al. (2018)
	AA505/SSR, AB101/SSR	Ae26, Ae27	Boutet et al. (2016)

(continued)

Table 10.2 (continued)

Trait	Marker name/marker type	Gene/QTLs	References
Ascochyta blight	p227/RFLP, p105/RFLP, p236/RFLP	QTL	Dirlewanger et al. (1994)
	c206/RFLP, M02-835/RAPD, sM2P5-234/SCAR M27/SCAR, J12-1400/RAPD, C12-680/RAPD, W17-150/RAPD, P346/RFLP, sY16-112/SCAR1 M2P2-193/AFLP sB17-509/SCAR, S15-1330/RAPD	*Asc1.1, Asc2.1, Asc3.1, Asc3.2, Asc4.2, Asc4.3, Asc5.1, Asc7.1, Asc7.2, Asc7.3*	Timmerman Vaughan et al. (2002, 2004)
	V03-1200/RAPD, PSm PSAA175/SRR, PSMPSAA 163.2/SSR, PSMPSAA399/SSR, G04-950/RAPD	*mpIII-1, mpIII-3, mpVa-1, mpVII-1, mpVI-1*	Prioul et al. (2004)
	Sc33287_25420/SNP, Sc34405_60551/SNP, Sc33468_44352/SNP, Sc12023_67096/SNP	*abIII-1, abI-IV-2,* abI-IV-2.1, abI-IV-2.2	Jha et al. (2017)
	IIccta2/AFLP, *IVccc1*/AFLP, *VIACCT1*/AFLP		Taran et al. (2003)
Drought	*A6/SSR, AA175/SSR, AC74/SSR, AD57/SSR, AB 141/SSR, AB64/SSR, Psblox2/SSR, PsAAP2-SNP4/SSR, DipeptIV-SP1/SSR*	*rwclF-1, rwclF-3, rwcsF-1, audpc_rwcs-2, rwcsF-2, rwclF-2, audpc_rwcs-1, audpc_rwcl*	Iglesias-Garcia et al. (2015)
Frost	AD59/SSR, AD141/SSR, AA200/SSR, AD159/SSR	*WFD3.1, WFD 5.1, WFD 6.1*	Lejeune-Henaut et al. (2008)
	AA67/SSR, *AGL20a/SSR,* AD141/SSR, *SucSyn/SSR,* AA475/SSR, I01.600/SSR, AB64/SSR, *AGL20a/SSR*	*WFDcle.a, WFDmon.a WFDcle.b, WFDmon.b, WFDcle.c, WFDmon.c, FD164.a, FD164.b, FD164.c*	Dumont et al. (2009)
	EST1109/SSR		Liu et al. (2017)

A second monogenic recessive resistance locus *er2* was identified earlier by several researchers (Heringa et al. 1969; Ali et al. 1994; Tiwari et al. 1997). It has not been used commercially since the resistance breakdown because the pathogen virulence influenced by day/night temperatures and age of plants (Tiwari et al. 1997; Fondevilla et al. 2006; Rana et al. 2013). The resistance governed through *er2* gene is mainly based on post-penetration cell death complemented by a reduction of percentage penetration success in mature leaves (Fondevilla et al. 2006). Different molecular markers like AFLP, RAPD and SCAR linked to *er2* gene are available

which can be used in breeding programme for marker-assisted selection after validation (Tiwari et al. 1999; Katoch et al. 2009).

The availability of saturated consensus map, associated molecular markers and diagnostic marker for different important traits are very precious resources for dry pea breeding programme. Recently, Sudheesh et al. (2014) developed saturated genetic linkage maps using SNP and SSR markers in two RIL populations. A consensus map constructed by combining data of these maps with previously published integrated map. The consensus structure has 2028 loci scattered across seven linkage groups (LGs), with a cumulative length of 2387 cM at an average density of one marker per 1.2 cM. Trait dissection of powdery mildew resistance identified a single genomic region (PsMLO1) of large size in the same genomic region on Ps VI, which is inferred to correspond to the *er1* gene. The identified candidate gene validated in resistant and susceptible genotypes as putative diagnostic marker for powdery mildew resistance which would be used in dry pea molecular breeding programmes.

The third gene dominantly inherited powdery mildew resistance (*Er3*) identified in *Pisum fulvum* and has been introduced successfully into the adapted *P. sativum* (Sharma and Yadav 2003; Fondevilla et al. 2007a, c). Its resistance mechanism mainly based on the high frequency of cell death that occurs both as a rapid response to infection and a delayed response that follows the colony establishment (Fondevilla et al. 2007a, c). Dominant molecular marker like RAPD that tightly linked to *Er3* has been identified and converted into SCARs (Fondevilla et al. 2008a) for their utilization in pea breeding. Still, breeders are dependents on a single gene *er1* for powdery mildew resistance which is not safe; therefore, pyramiding of more than one gene in a single background is instantly required. In addition, other species including *E. trifolii* also infects pea and break*er1* resistance, which deserves urgent attention to sustain dry pea production (Fondevilla et al. 2013).

10.5.3.2 Rust

Pea rust has been considered as a serious disease since the mid-1980s, and it is scattered around the world in all pea-growing countries (Barilli et al. 2010). This disease incited either by *Uromyces viciae-fabae (Pers.)* j.Schrot (Arthur 1934) or U. *pisi* (Pers.) Wint (EPPO 2009; Barilli et al. 2009a, b, c, 2010, 2018; Rubiales et al. 2011, 2019; Singh et al. 2015; Das et al. 2019). In the tropical and subtropical regions *U. viciae-fabae* is prevalent, where weather is warm-humid which remains suitable for the manifestation of both uredial and aecidial stage (Pal et al. 1980; Singh et al. 2004; Kushwaha et al. 2006). These conditions usually coincide with the flowering or podding stage of crop and favour rust outbreak (Kushwaha et al. 2007; EPPO 2009; Singh et al. 2015). Contrarily, in temperate regions, it infected pea at seedlings stage and later developed under field conditions (Emeran et al. 2005; Barilli et al. 2007, 2010). *U. viciae-fabae* is an obligate parasitic fungus that only infected legume species crops such as pea, faba bean, lentil and vetches (Cummins 1978), whereas *U. pisi* is heteroecious fungi ubiquitous in cool climatic condition, and it completes life cycle on *Euphorbia esula* and *Euphorbia*

cyparissias (Pfunder and Roy 2000; Rubiales et al. 2019). This disease under amiable environmental circumstances vigorously spread over aerial part, i.e. leaves, stipules, pods and stem and distressed physiological and biochemical processes of plants which subsequently lead to reduction in photosynthesis (Fig. 10.5). Consequently, most of leaves fall down, and pods remain undeveloped, which resulted into more than 30% yield losses (EPPO 2009; Barilli et al. 2010). The best strategy to stabilize the productivity of pea crop is to go for host plant resistance and grow rust-resistant varieties. Complete resistance for rust yet to be reported and partial resistance or incomplete resistance is the only best available option. However, sincere efforts have been made for screening pea germplasm towards rust, but none of the genotype was found completely free from infection, while genotypic differences for rust intensity were observed (Narsinghan et al. 1980; Singh and Srivastava 1985; Gupta 1990; Anil Kumar et al. 1994). Sources of incomplete resistance to *U. pisi* from 2759 pea accession have been identified under both field and controlled conditions (Barilli et al. 2009c). Resistance to pea rust is mainly due to a restriction of haustorium development, and none of the pea accessions is observed free from rust infection (Singh and Srivastava 1985; Chand et al. 2006; Barilli et al. 2009a, b, c).

The number of genotypes with incomplete or partial resistance against *U. viciae-fabae* has been reported (Vijayalakshmi et al. 2005; Chand et al. 2006; Kushwaha et al. 2006; Das et al. 2019). Rust resistance in pea is governed by single dominant gene (*Ruf*) (Katiyar and Ram 1987; Tyagi and Srivastava 1999; Vijayalakshmi et al. 2005). Further, this trait seems to be controlled by polygenic nature of gene action in addition to the reported oligogene *Ruf* (Singh and Ram 2001). Singh et al. (2012) found that single gene shows partial dominance along with minor and 2–3 additive genes. Pea breeders have used the reported partial resistance sources in their breeding programme and developed number of high-yielding varieties with partial rust resistance suitable for different agro-climatic conditions.

The occurrence of rust is significantly influenced by environmental conditions during disease contamination and further development. This is the major constraint

Fig. 10.5 Rust infected plant parts of dry pea

in proper screening and identification of rust resistance stable genotypes. Therefore, use of molecular marker and QTL would allow indirect selection of genotypes independent of weather conditions. Some rust-associated marker and QTL with rust have been reported as presented in Table 10.2, which seems to be controlled by one major gene and one minor QTL (Vijayalakshmi et al. 2005; Barilli et al. 2010; Rai et al. 2011). A single major gene *Ruf* responsible for this partial resistance has been identified which is flanked by two RAPD markers, SC10-82_{360} and SCRI-71_{1000}, with 10.8 and 24.5 cM distance, respectively, but both markers were not close enough to the gene of interest to allow marker-assisted selection for rust resistance (Vijayalakshmi et al. 2005; Rai et al. 2011). A linkage map was developed by Barilli et al. (2010) using a F_2 population of two *Pisum fulvum* lines. A QTL (*Up*1) associated with resistance to pea rust caused by *U. pisi* was identified on LG III. The two RAPD flanking markers OPY 11_{1316} and $OPV17_{1078}$ are located at the position 26.9 and 46.3 cM, respectively. Both the markers are not close to QTL; therefore their subsequent conversion in SCAR markers could permit a reliable marker-assisted selection for rust resistance. Rai et al. (2011) reported the quantitative nature of resistance of pea rust caused by *U. fabae*. ARIL population was used (population size – 136) which derived from the cross between HUVP 1 (susceptible) and FC 1 (resistant) pea genotypes. A linkage map was developed using simple sequence repeat (SSR), sequence-tagged site (STS) and random amplified polymorphic (RAPD) markers covering 634 cM of genetic distance on the seven linkage groups of pea with an average interval length of 11.3 cM. They reported one major (Qruf) and one minor (Qruf1) QTL associated with rust resistance located on LG-7 using composite interval mapping (CIM). Also reported two flanking SSR markers AA505 and AA446 (10.8 cM) for major QTL. The minor QTL was environment-specific and only detected in polyhouse. It was flanked by two SSR markers, AD146 and AA416 (7.3 cM). Therefore, the SSR markers flanked QTL Qruf would be useful in future for marker-assisted selection for pea rust (*U. fabae*) resistance.

The validation of associated marker and QTLs is quintessential step before accommodation of them in marker-assisted programme to reduce risk and cost of programme. Therefore, the four reported SSR markers (AA446 and AA505 flanking the major QTL, Qruf; AD146 and AA416 flanking the minor QTL, Qruf1) by Rai et al. (2011) were validated in 30 diverse pea genotypes. The results revealed that the QTL, Qruf flanking markers were able to identify all the resistant genotypes when used together, except for Pant P 31, while SSR markers AD146 and AA416 flanking the minor QTL, Qruf1 were able to identify all the pea resistant genotypes during validation, except for HUDP-11 by AD146 and Pant P 31 by AA416. Similarly, SSR markers AA446 and AA505 were able to cull all the susceptible pea genotypes, except IPFD 99–13, HFP 9415 and S-143. SSR markers AD146 and AA416 were together able to identify all the pea susceptible genotypes used for validation, except KPMR 526, KPMR 632 and IPFD 99–13. The validation clearly indicated that the above-mentioned SSR markers can be used in MAS of pea rust resistance (Singh et al. 2015).

Recently, Barilli et al. (2018) used RIL population of *P. fulvum* for *U. pisi* and genotyped by Diversity Arrays Technology. Finally, an integrated linkage map was

developed using total 12,058 markers (9569 high-quality DArT-Seq and 8514 SNPs) which were distributed into seven linkage groups. The CIM revealed three QTLs (UpDSII, UpDSIV and UpDSIV.2) distributed over two linkage groups that were associated with the rust disease. First two QTLs were constantly detected both in adult and seedling plants under controlled conditions. The third QTL (UpDSIV.2) was environmentally specific and also situated on the LGIV identified only in seedlings plant under controlled conditions.

10.5.3.3 Ascochyta Blight

Ascochyta blight (AB) (commonly acknowledged as 'black spot disease') is incited by a complex of fungal species that includes *Ascochyta pisi*, *Peyronellaea pinodes* (syn. *Mycosphaerella pinodes*), *Phoma medicaginis* var. *pinodella*, *P. koolunga* and *P. glomerata* (Kraft and Pfleger 2001; Davidson et al. 2009; Aveskamp et al. 2010; Li et al. 2011; Khan et al. 2013; Liu et al. 2013; Tran et al. 2014; Sivachandra Kumar and Banniza 2017). Of them *P. pinodes* is the most prevalent and devastating fungus caused 28–88% yield damage under wet climatic conditions (Bretag et al. 1995a; Tivoli et al. 1996; Xue et al. 1997; Garry et al. 1998; Rubiales et al. 2019). It is one of the most severe diseases of field peas, and it is distributed worldwide, including almost all of the major pea-growing areas (Bretag et al. 2006; Parihar et al. 2013). In general ascochyta blight complex reduces grain yield 10–60% depending on environmental conditions in different growing regions (Wallen 1965, 1974; Tivoli et al. 1996; Xue et al. 1996; Bretag et al. 2006; Liu et al. 2016). This disease complex develops range of symptoms on seedling and all aboveground pea plant parts, including necrotic leaf spots, stem lesions, shrinkage and dark-brown discoloration of seeds, blackening of the base of the stem, foot rot and pod spot. It also causes slightly hollow, circular, tan coloured lesions with dark brown margins that occur on the leaves, pods and stems (Chilvers et al. 2009; Davidson et al. 2009; Li et al. 2011; Tran et al. 2014). All the pathogens are seed-borne in nature that can also survive on infected plant debris which play a crucial role in disease transmission in uninfected areas of developing crop (Tivoli and Banniza 2007; Parihar et al. 2013; Liu et al. 2016;). Seed-to-seedling transmission under controlled conditions is up to 100% for *P. pinodes* (Xue 2000) and 40% for *A. pisi* (Maude 1966). Most importantly *Ascochyta* spp. can survive on pea seed coats for several years (Bretag et al. 1995b), and particularly for *A. pisi*, it was estimated that the fungus will be dissect from seed after 5–7 years of seed storage in cool and dry conditions (Wallen 1955).

The incidence of the disease under field conditions is highly influenced by agronomic traits including lodging and plant height (Taran et al. 2003; Banniza et al. 2005; Le May et al. 2009; Jha et al. 2013, 2016). Therefore, development of resistant cultivars is the best management strategy for ascochyta blight in peas since it is most practical, effective and economical approach (Zimmer and Sabourin 1986). However, sincere efforts have been made, but none of the material from cultivated pea could show complete resistance against ascochyta blight fungi. Therefore,

cultivars that are highly resistant to ascochyta blight have not yet been developed. Although, some potential genotypes out of more than 3500 cultivated pea accessions with low- to moderate-level resistance were identified (Kraft et al. 1998; Zhang et al. 2006). On the contrary, high level of resistance was reported in wild pea (*P. fulvum*) accession (Clulow et al. 1991; Wroth 1998; Fondevilla et al. 2005; Jha et al. 2012). Further, Fondevilla et al. (2005) also identified the high level of resistance in accession P651 (*P. fulvum*) compared to other wild peas (*P. sativum* ssp. *elatius* and *P. sativum* ssp. *syriacum*) accessions. Later on, Jha et al. (2012) recognized promising accessions AB resistance from *P. fulvum* and *P. sativum* ssp. *elatius* through appraisal of 44 wild pea accessions. Of them, the most promising accession was P651 belong to *P. fulvum* and utilized for resistance breeding (Sindhu et al. 2014; Jha et al. 2016). The nature of inheritance so far reported for AB resistance is polygenic (Xue and Warkentin 2001; Prioul et al. 2004; Fondevilla et al. 2007b; Prioul Gervais et al. 2007; Carrillo et al. 2014; Timmerman Vaughan et al. 2016; Jha et al. 2017), and this has hampered the AB resistant cultivar development programme (Rubiales and Fondevilla 2012). Furthermore, different QTL mapping studies have identified numerous genomic regions (see Table 10.2) involved in the control of resistance and confirming the polygenic nature of resistance (Timmerman Vaughan et al. 2002, 2004; Taran et al. 2003; Prioul et al. 2004; Fondevilla et al. 2008b). QTLs were also identified from a cross involving wild pea, *P. sativum* subsp. *syriacum* (Fondevilla et al. 2008a, b, 2011; Carrillo et al. 2014). The candidate genes co-locating with QTL for resistance to *M. pinodes* have also reported (Prioul Gervais et al. 2007). Further, Jha et al. (2015) reported a significant association of SNPs detected within candidate genes PsDof1 (PsDof1p308) and RGA-G3A (RGA-G3Ap103) for AB resistance. Similarly, Jha et al. (2016) reported nine QTLs associated with AB resistance in an interspecific pea population (PR-19) developed from a cross between Alfetta (*P. sativum*) and wild pea accession P651 (*P. fulvum*), of them two QTLs abIII-1 and abI-IV-2 were consistent across locations and/or years.

QTL mapping in several pea crosses designated genomic regions associated with AB resistance; nevertheless, these QTLs cover large regions and may not be effective for use in MAS programme. Similarly, the large number of markers associated with resistance genes has been identified, but none of them tightly linked to the targeted gene of interest (Michelmore 1995). Recombination could occur between a marker and QTL if markers are not tightly linked to genes (Collard et al. 2005). Therefore, high resolution or fine mapping of QTLs should be used to recognize more tightly linked that can be precisely used for MAS (Mohan et al. 1997). For fine mapping, an advanced mapping population, like near-isogenic lines (NILs), through consecutive backcrossing is need to be developed. An alternative and more efficient method proposed by Tuinstra et al. (1997) is development of heterogeneous inbred family (HIF) populations, which is more efficient method than the NILs.

Most recently, Jha et al. (2017) fine mapped the abIII-1 and abI-IV-2 QTLs using a high-density SNP-based genetic linkage map and examine identified markers in HIF populations. Selective genotyping was performed in 51 PR-19 recombinant inbred lines using genotyping-by-sequencing (GBS), and the resultant high-density

genetic linkage map was utilized to recognize eight new SNP markers within the abI-IV-2 QTL, whereas no additional SNPs were identified within the abIII-1 QTL. Two HIF populations HIF-224 (143 lines) and HIF-173 (126 lines) were developed from F6 RILs PR-19-224 and PR-19-173, respectively. The HIF populations ascertained under field conditions in which a wide range of variation for reaction to AB resistance observed. HIFs were genotyped using SNP markers within targeted QTLs. The genotypic and phenotypic data of the HIFs were used to identify two new QTLs, abI-IV-2.1 and abI-IV-2.2 for AB resistance within the abI-IV-2 QTL. These QTLs individually accounted for 5.5–14% of the total phenotypic variation. Resistance to lodging was also associated with these two QTLs. In addition, five and three additional SNP markers identified in QTLs abI-IV-2.1 and abI-IV-2.2, respectively, by fine mapping will be useful in marker-assisted selection for development of pea cultivars with improved AB resistance. This approach has been extensively adopted in several species such as *Arabidopsis*, soybean and maize for fine mapping of QTLs (Meng et al. 2008; Bai et al. 2010; Todesco et al. 2010; Coles et al. 2011; Dwiyanti et al. 2011; Watanabe et al. 2011; Bouteillé et al. 2012).

10.5.3.4 *Fusarium* Root Rot

Fusarium root rot, caused by *Fusarium solani* f.sp. *pisi*, is a cosmopolitan disease of pea occurred in almost all pea-growing areas around the world and considered as major limiting factor in production (Kraft et al. 1988, 1996; Backhouse et al. 2001; Kraft and Pfleger 2001; Grunwald et al. 2003; Hamid et al. 2012; Porter et al. 2015). *Fusarium* root rot of peas, caused by *F. solani* f.sp. *pisi*, was first reported as a serious pathogen in the USA (Bisby 1918; Jones 1923). The disease may damage peas produced in both dry and wet fields and has been reported that it reduced yield up to 60% under suitable circumstances (Kraft and Pfleger 2001; Kraft 2001; Chang et al. 2004; Porter 2010). This disease is distinct from Fusarium wilt, caused by *F. oxysporum* f.sp. *pisi*, but sometimes occurs in combination with other diseases of peas also (Zaumeyer and Thomas 1957). The compact and warm soil (18–24 °C soil temperature) conditions are most suitable for *Fusarium* root rot development in pea-growing regions around the world (Kraft and Roberts 1969; Kraft and Giles 1979; Kraft and Wilkins 1989; Kraft and Boge 2001). The symptoms above the ground include yellowing of aerial parts start from the base and progress towards upper side. The black to brown lesions developed on stems where the cotyledons are attached to the stem and eventually, and it causes root and stem rot followed by necrosis and death of leaves (Kraft 1994). In case of *Fusarium* root rot, wilting of plants is not commonly happened, but it shortened the growth of plants and induces force maturity (Hagedorn 1991; Oyarzun 1993; Hamid et al. 2012; Porter et al. 2015).

Breeding disease resistance varieties is considered to be the basic prerequisite for improving and stabilizing yield of grain legumes (Ranalli 2003). But, so far complete resistance to this disease has not been reported in pea, but a number of sources of partial tolerance have been found (Kraft et al. 1988; Gretenkort and Helsper 1993; Hwang et al. 1995; Grunwald et al. 2003; Porter 2010; Porter et al. 2015).

Interestingly, most of the accessions with pigmented flowers have tendency of greater partial resistance to *F. solani* f.sp. *pisi* than white-flowered cultivars (Kraft 1975; Grunwald et al. 2003). Detailed data about sources of resistance to *Fusarium* diseases in wild *Pisum* species and accessions are not available. However, a set of ten accessions of wild *P. sativum* subspecies along with varieties was examined for resistance to *F. Solani* under controlled conditions (Kraft and Roberts 1970; Lebeda and Švábová 1997; Grunwald et al. 2003; Coyne et al. 2008; Porter et al. 2014). Genetic resistance offers one of the best strategies to control this root-rotting fungus. Complete resistance to pathogen was not recorded, but very high level of resistance was observed (Lebeda and Švábová 1997). It has polygenic nature of inheritance; therefore, development of resistant varieties becomes more complicated (Lockwood 1962; Muehlbauer and Kraft 1973; Kraft 1992). However, the genetics of the quantitative partial resistance is little bit studied with just few QTL reports published so far for *Fsp* as given in Table 10.2 (Hance et al. 2004; Feng et al. 2011). First a RIL population derived from cross between JI 1794 and Slow (*P. sativum* subsp. *sativum*) has been used. The segregation patterns reults revealed that the tolerance to *F. solani* was multigenic in JI 1794 and also identifed one QTL for *Fusarium* root rot tolerance that near to *Le* gene.

Feng et al. (2011) developed RIL population (71) of dry pea, derived from crosses between a resistant cultivar 'Carman' and a susceptible cultivar 'Reward'. To discover markers linked with the resistance, a total of 213 SSR markers were used, and of them only 14 markers were polymorphic between the two parents. QTL analysis reported a QTL that explained 39.0% of the phenotypic variance in the RIL population and flanked by markers AA416 and AB60 on LG VII. The microsatellite markers that are closely linked to this QTL may be useful for marker-assisted selection to develop cultivars with superior *Fusarium* root rot resistance. Additionally, five QTL were also reported on pea LGs II, III, IV, VI and VII (Hance et al. 2004; Weeden and Porter 2007).

Recently, Coyne et al. (2015) used a RIL population and employed composite interval mapping (CIM) for QTL detection. A total of five QTL were identified, and of them one QTL is detected consistently over the years. The multiyear QTL *Fsp-Ps2.1* contributed a significant portion of the phenotypic variance (22.1–72.2%), while a second QTL, *FspPs6.1*, contributed 17.3% of the phenotypic variance. The other single growing season QTLs are of additional interest as they co-segregate with previously reported pea-*Fusarium* root rot resistance QTL. QTL *Fsp-Ps2.1*, *Fsp-Ps3.1*, *Fsp-4.1* and *Fsp-Ps7.1* are flanked by codominant SSRs and may be useful in marker-assisted breeding of pea for high levels of partial resistance to Fsp. Most recently the previously identified QTL *Fsp-Ps 2.1* has been confirmed in two RIL populations by Coyne et al. (2019). They identified three QTLs such as *Fsp-Ps 2.1, Fsp-Ps3.2* and *Fsp-Ps3.3* using CIM. The first QTL *Fsp-Ps 2.1* explains 44.4–53.4% of the variance with a narrow confidence interval of 1.2 cM. The second and third QTL *Fsp-Ps3.2* and *Fsp-Ps3.3* are closely linked and explain only 3.6–4.6% of the variance. All of the alleles are belong to the resistant parent PI 180693. The confirmation of *Fsp-Ps 2.1* now in two RIL populations and SNPs associated with this region makes it a good target for marker-assisted selection in

pea breeding programmes to develop high levels of partial resistance for *Fusarium* root rot caused by *Fusarium solani* f.sp. *pisi.*

10.5.3.5 Fusarium Wilt

Fusarium wilt is inflicted by soil-borne fungus *Fusarium oxysporum*. f.sp. *pisi* (van Hall) Snyd. & Hans., which is a serious production threat and dispersed around the world (Haglund 1984; Kraft 1994; McClendon et al. 2002; Sharma et al. 2010; Rubiales et al. 2015). It enters into the host vascular system through root tips or wound, subsequently causes chlorosis of the leaves and stems, wilting, and collapse of the root systems (Bishop and Cooper 1983; Beckman 1987; Correll 1991; Benhamou and Garand 2001; Haglund and Kraft 2001; Haglund 2001; Zvirin et al. 2010). Fusarium wilt is an economically significant disease causes losses in dry pea up to 100% under favourable conditions (Aslam et al. 2019). The early symptoms are yellowing of lower leaves and reduced plant growth which eventually leads towards wilting of complete plants. A soil temperature of 23–27 °C is most suitable for proper wilt development. This fungal species has a total of 11 different races which are described on the basis of virulence (Armstrong and Armstrong 1974; Gupta and Gupta 2019). Of these, races 1 and 2 are widely distributed, while races 5 and 6 are, to date, scattered only in some specific regions (Infantino et al. 2006; Bani et al. 2018). Fusarium wilt race 1 is one of the major races among the four pathogenicity groups on pea (Kraft and Pfleger 2001). This pathogen is soil-borne and can survive in the soil in the absence of pea crop for longer time (Skovgaard et al. 2002; Roncero et al. 2003; Bani et al. 2018; Gupta and Gupta 2019). The soil-borne fungal diseases are mainly controlled by the integration of different disease management procedures. Among these methods, the use of resistant cultivars is widely recognized as the safest, most economical and most effective crop protection method (Ciancio and Mukerji 2008; Rubiales et al. 2015; Gupta and Gupta 2019). Mcphee et al. (1999) reported 62 and 39 resistance accessions for race 2 and race 1, respectively, from core collection. One of the wild progenitors, PI 344012, possessed resistance to races 1 and 2. The genetic resistance to Fop races 1, 5, and 6 is conferred by single dominant genes, whereas resistance to race 2 is quantitative (Mcphee et al. 1999, 2012; Bani et al. 2012, 2018; Rispail and Rubiales 2014). The resistance controlled by a single dominant gene has been incorporated successfully into many varieties (Mcphee 2003). The transfer of quantitative resistance in susceptible cultivar is complicated where molecular marker can play a crucial role because the selection process is time -consuming and labour- intensive for such traits. Therefore, recent developments in genomics research have provided scope for searching, using, and selecting naturally occurring resistance against Fusarium wilt in cool season food legumes with the help of molecular tools (Kamboj et al. 1990; McClendon et al. 2002; Infantino et al. 2006; Kumar et al. 2011; Smýkal et al. 2012).

Therefore, numerous dominant molecular markers like RAPD, SCAR and AFLP for race 1 (*Fw*) locus have been identified as presented in Table 10.2 (McClendon et al. 2002; Okubara et al. 2005). The inheritance of resistance to race 5 is conferred

by single dominant gene, *Fwf* (Hagedorn 1989). For race 5 Coyne et al. (2000) used a RIL population and identified a locus which was associated with resistance for race 5 (*Fwf*). Similarly, a total of 14 markers including 5 morphological, 1 isozyme and 9 RAPD co-segregated with *Fusarium* race 5 resistance gene (*Fwf*) within a 123 cM interval. Of these, one tightly linked RAPD marker, i.e. U693a, located at distance of 5.6 cM and about 8.5 cM closer than previously identified marker (Okubara et al. 2002). The gene conferring resistance to *F. oxysporum* race 1 in pea, *Fw*, which is located on linkage group (LG) III and widely used in breeding programmes. Similarly, Loridon et al. (2005) placed Fw between two SSR markers AA5_225 and AD134_213 at 2.7 and 2.5 cM distances, respectively. Because both the markers situated at relatively larger distance from Fw, hence, both the SSR markers are not suitable for reliable marker-assisted selection (MAS) of Fw. Later on, three sequence-characterized amplified region (SCAR) markers were developed using the target region amplified polymorphic (TRAP) marker technology and mapped close to Fw in a population developed from PI 179449 and 'Green Arrow' using a bulk segregant analysis approach (Kwon et al. 2013). These three markers, *Fw_Trap_480*, *Fw_Trap_340* and *Fw_Trap_220*, are located only 1.2 cM away from Fw locus. However, use of these markers in MAS is dubious because of the dominant nature of these markers. Codominant markers such as CAPS makers are more suitable for MAS in plants since they can distinguish heterozygotes from homozygotes (Jiang 2013). Therefore, Jain et al. (2015) developed a breeder-friendly functional codominant cleaved amplified polymorphic sequence (CAPS) marker, THO, which can be used in pea breeding programmes for selection of resistance to *F. oxysporum* race 1. By using this marker, dry pea breeder can select lines with more than 94% accuracy from mapping populations and advanced pea breeding lines. This marker, in combination with other gene-based markers (AnMtL6, Mt5_56 and PRX1TRAP13) developed from conserved sequences of closely related legume species, lays the foundation for candidate gene identification through comparative mapping.

10.5.3.6 Common Root Rot

Common root rot of field pea incited by the soil-borne fungus *Aphanomyces euteiches* Drechs. is one of the serious constraints to pea production (Jones and Drechsler 1925; Mcphee 2003; Pilet Nayel et al. 2005; Desgroux et al. 2016; Wu et al. 2018). This pathogen has been mainly reported as a yield-limiting factor in major dry pea cultivation countries such as the USA, Europe and most recently in Canada (Papavizas and Ayers 1974; Wicker and Rouxel 2001; Wicker et al. 2003; Chatterton et al. 2015; Desgroux et al. 2016). This pathogen can cause severe root damage, wilting and substantial yield losses under wet soil conditions (Wu et al. 2018). Two main pathotypes of *A. euteiches* were reported, and both pathotypes cause honey brown necrosis symptoms on pea roots and epicotyls, resulting in dwarfism, foliage yellowing and then death of plants in highly infested fields (Wicker and Rouxel 2001). The conventional disease management strategies, such as crop rotations and

seed treatments, are unable in full prevention of this disease under favourable conditions, due to the durability of the pathogen oospores (Papavizas and Ayers 1974), which can infect field pea plants at any growth stage. Therefore, development of resistant cultivars has been considered as a major objective in dry pea breeding programme. Pea lines partially resistant to *A. euteiches* were identified from germplasm screening and breeding programmes (Gritton 1990; Kraft 1992; Davis et al. 1995; Malvick and Percich 1998a, b; Kraft 2000; Kraft and Coffman 2000a, b; Pilet Nayel et al. 2007; Conner et al. 2013). The reported resistant accessions were incorporated into breeding programmes during the last three decades to develop breeding lines (Roux-Duparque et al. 2004; Moussart et al. 2007), recombinant inbred lines (RILs) (Pilet Nayel et al. 2002, 2005; Hamon et al. 2011, 2013; McGee et al. 2012) and near-isogenic lines (NILs) (Lavaud et al. 2015). But breeding for tolerance to common root rot has been difficult because of the polygenic nature of the tolerance and also associated with some undesirable traits like long internodes, anthocyanin content and late-flowering (Marx et al. 1972; Pilet Nayel et al. 2002). Therefore application of different molecular marker has become important to speed up and reduces the cost of breeding programme. Different types of molecular marker are identified (Table 10.2), for example, Weeden et al. (2000) found a gene MN313 located on the linkage group IV near P393 which has a significant influence on the expression of tolerance to common root rot. The nature of inheritance of partial resistance to *A. euteiches* in pea has not been extensively studied. Therefore, RIL population (127) derived from the cross Puget (susceptible) × 90-2079 (partially resistant) was used and genotyped using automated AFLPs, RAPDs, SSRs, ISSRs, STSs, isozymes and morphological markers. Subsequently, developed genetic map and identified seven genomic regions, including a major quantitative trait locus (QTL), *Aph1*, along with two other year-specific QTLs, namely, *Aph2* and *Aph3* associated with partial resistance to *Aphanomyces* root rot and explained 47%, 32% and 11% of the variation, respectively. The remaining two QTLs were environment-specific and mapped near the *R* (wrinkled/round seeds) and *af* (normal/afila leaves) genes. However, the integration of these QTL for MAS in European breeding programmes has been questionable, since partial resistance of 90-2079 was not effective in French field conditions (Pilet Nayel et al. 2002). To evaluate the specificity and consistency of already identified QTLs in previous study (*Aph1*, *Aph2* and *Aph3*), the same mapping population was evaluated under greenhouse and field conditions with two isolates (the USA and French). By using previously reported genetic map, a total of ten QTL were identified for resistance in greenhouse conditions to the two isolates. Among these *Aph1*, *Aph2* and *Aph3* were previously detected for partial field resistance in the USA. *Aph1* and *Aph3* were detected with both isolates and *Aph2* with only the French isolate. The consistency of the detected resistance QTL, i.e. *Aph1*, *Aph2* and *Aph3*, suggested the usefulness of these in marker-assisted selection (Pilet Nayel et al. 2005). Hamon et al. (2011) used two RIL mapping populations (178 individual in each), derived from crosses between 552 or PI180693 (partially resistant) and Baccara (susceptible), to identify QTL for *Aphanomyces* root rot resistance. They identified a total of 135 additive-effect QTL corresponding to 23 genomic regions and 13 significant epistatic interactions

associated with partial resistance to *A. euteiches* in pea. Of the 23 additive-effect genomic regions identified, 5 were constantly detected and showed high stability towards *A. euteiches* strains and other external factors. These results confirmed the complexity of inheritance of partial resistance to *A. euteiches* in pea and suggested to use steady QTL in marker-assisted selection programme to increase current levels of resistance to *A. euteiches* in pea breeding, since development of durable plant genetic resistance to pathogens through QTL pyramiding and diversification requires in-depth knowledge of polygenic resistance within the available germplasm. The polygenic partial resistance to *Aphanomyces* root rot, caused by *Aphanomyces euteiches*, is already confirmed in individual mapping populations (Pilet Nayel et al. 2002, 2005; Hamon et al. 2011). However, there are no data available regarding the diversity of the resistance QTL across a broader collection of pea germplasm. Therefore, Hamon et al. (2013) performed a meta-analysis using previously reported 244 individual QTL in three mapping populations (Puget × 90–2079, Baccara × PI180693 and Baccara × 552) and in a fourth mapping population in this study (DSP × 90–2131), which detected 27 meta-QTL for resistance to *A. euteiches*. In addition, 11 stable meta-QTL have been identified which highlight 7 highly steady genomic regions. Furthermore, seven resistance meta-QTLs were identified; of them six were highly consistent, co-segregated with morphological/phenological alleles. Alleles accountable for the resistance were often associated with unwanted alleles for dry pea breeding (Marx et al. 1972; Pilet Nayel et al. 2002).

QTL validation is an important and often ignored step prior to subsequent research in QTL cloning or marker-assisted breeding for disease resistance in plants. Therefore, Lavaud et al. (2015) validate seven recently identified QTL in different genetic backgrounds and also assess the effects of various resistance alleles. In this study near-isogenic line (NIL) population was evaluated for resistance to two reference strains of the main *A. euteiches* pathotypes under controlled conditions. The NILs carrying resistance alleles at the major-effect QTL *Ae-Ps4.5* and *Ae-Ps7.6*, either individually or in combination with resistance alleles at other QTL, showed significantly condensed disease severity compared to NILs without resistance alleles. Resistance alleles at some minor-effect QTL, especially *Ae-Ps2.2* and *Ae-Ps5.1*, were also validated for their individual or combined effects on resistance. The effect of QTL x genetic background interactions were observed high for QTL *Ae-Ps7.6* in the winter cultivar. The pea NILs are a novel and valuable resource for further understanding the mechanisms underlying QTL and their integration in breeding programmes.

The proper understanding of the effects of resistance QTL on pathogen development is an important concern for the construction of QTL combination strategies to increase durability disease resistance in plants. Therefore, recently, Lavaud et al. (2016) investigated the effect of the main *A. euteiches* resistance QTL in NILs on different steps of the pathogen life cycle. Significant effects of several resistance alleles at the two major QTLs *Ae-Ps7.6* and *Ae-Ps4.5* were observed on symptom appearance and root colonization by *A. euteiches*. Some resistance alleles at three other minor QTLs (*Ae-Ps2.2*, *Ae-Ps3.1* and *Ae-Ps5.1*) significantly decreased root colonization. The combination of resistance alleles at two or three QTLs including

the major QTL *Ae-Ps7.6* (Ae-Ps5.1/Ae-Ps7.6 or Ae-Ps2.2/Ae-Ps3.1/Ae-Ps7.6) had an increased effect on delaying symptom appearance and/or slowing down root colonization by *A. euteiches* and on plant resistance levels, compared to the effects of individual or no resistance alleles. This study recommended that single resistance QTL can affect different steps of the disease growth cycle and that their actions could be pyramided to increase partial resistance in future pea varieties. Further studies are needed to investigate QTL effects on different steps of the pathogen life cycle, as well as the efficiency and robustness of pyramiding strategies with QTL which come out to act on the similar stage of the pathogen cycle.

Genome-wide association (GWA) mapping has recently emerged as an important move towards refining the genetic basis of polygenic resistance to plant diseases, which are being used in integrated strategies for durable crop protection. Linkage mapping studies reported quantitative trait locus (QTL) controlling resistance to *A. euteiches* in pea (Pilet Nayel et al. 2002, 2005; Hamon et al. 2011, 2013). Nevertheless, the confidence intervals (CIs) of these QTLs remained large and were often linked to undesirable alleles, which limited their application in breeding. Therefore, to refine and validate the previously reported QTLs and to identify new loci, Desgroux et al. (2016) used GWA with 13,204 SNPs from the recently developed GenoPea Infinium® BeadChip. The GWA analysis identified total 52 QTL of small confidence intervals associated with resistance to *A. euteiches*, using the recently developed multi-locus mixed model. The analysis validated six of the seven previously reported main *Aphanomyces* resistance QTLs and detected novel resistance loci. The previously reported linkages between resistance alleles and undesired late-flowering alleles for dry pea breeding were mostly confirmed, but the linkage between loci controlling resistance and coloured flowers was broken due to the high resolution of the analysis. A high proportion of the putative candidate genes implicit resistance loci encoded stress-related proteins, and others suggested that the QTLs are concerned in diverse functions. Similarly, Desgroux et al. (2018) used a comparative genome-wide association (GWA) of plant architecture and resistance to *A. euteiches* in a collection of 266 pea lines contrasted for both traits. The collection was genotyped using 14,157 SNP markers from recent pea genomic resources. A total of 11 genomic intervals were significantly associated with resistance to *A. euteiches* confirming several reliable formerly known major QTLs. One SNP marker, mapped to the major QTL *Ae-Ps7.6* was associated with both resistance and root system architecture (RSA) traits. This marker is associated with the resistance-enhancing allele along with an increased total root projected area. The identify pea lines, QTL, closely linked markers and candidate genes for RSA loci can be used to reduce *Aphanomyces* root rot severity in future pea varieties.

10.5.4 Breeding for Abiotic Stresses

The major abiotic stresses which are now becomes serious issue in sustainable production of dry pea under climate change scenario are extremities of temperature (low and high), moisture extremities (drought and flood) and salinity conditions (Rubiales et al. 2019). These stresses have full potential to negatively affect the seed yield and its quality at significant levels. The selection of resistance genotypes for abiotic stresses is cumbersome owing to the oscillation of environmental conditions over the locations and years. Besides, the growth stage of the crop at the time the stress comes can result in dramatic changes in response and injury level (Monti et al. 1993). Therefore, evaluations of crop in controlled environments have been commenced to estimate precisely plant response to a specific stress. Importantly, the testing of pea materials in extreme field situations where a specific stress is assured while other abiotic stress can be avoid or minimized can be productive and improve breeding efficiency (Sadras et al. 2012). In this section we will discuss status of the major abiotic stresses such as heat/high temperature, drought and frost.

10.5.4.1 Heat Stress

Grain legumes play a vital role in different cropping systems towards ensuring food security for alarmingly increasing human population (Foyer et al. 2016; Considine et al. 2017). However, according to the IPCC report in 2018, global average temperature over the last 5 years (2014–2018) has been increased by 1.04 °C compared to the preindustrial base line and will reach 1.5 °C as soon as by 2030 (IPCC 2018). Accordingly, legume growth and development will be subjected to recurrent and harsh heat stress (Zinn et al. 2010; Vadez et al. 2012).

The elevated temperature beyond the threshold level especially at critical growth stages causes a significant loss in productivity and quality of produces (Wahid et al. 2007; Bita and Gerats 2013; Farooq et al. 2017; Liu et al. 2019a). The optimal temperature for grain legume crops range 10–36 °C, above which severe losses in grain yield can take place (Siddique 1999). High leaf temperatures condense plant growth and limit crop yields. It is estimated up to 17% decrease in grain yield for each degree Celsius increase in average growing season temperature (Lobell and Asner 2003). On the basis of climatic requirements, dry pea comes in cool season category of grain legumes (Oram and Agcaoili 1994). Cool season grain legumes are more sensitive to high temperature than warm season grain legumes (Hall 2001).

Elevated ambient temperature above 25 °C during dry pea life cycle reduces seed yield by reducing plant growth, number of flowering nodes, number of pods per plant and abortion of flowers and young pods and by speeding up the crop life cycle towards maturity (Boswell 1926; Lambert and Linck 1958; Karr et al. 1959; Stanfield et al. 1966; Nonnecke et al. 1971; Jeuffroy et al. 1990; Guilioni et al. 1997, 2003; Sadras et al. 2012; Bueckert et al. 2015). The high temperature negatively affects photosynthesis and growth of pea with substantial genotypic difference

(McDonald and Paulsen 1997). In pea (*Pisum sativum* L.), photosynthetic activity is detained at 40 °C (Georgieva and Lichtenthaler 1999). Similarly, reduction in net photosynthesis rate beyond 35 °C temperature in pea leaves has been noticed, and at 45 °C net photosynthesis reduced up to 80% (Haldimann and Feller 2005).

The heat stress exaggerated under field conditions by other environmental and management factors (Bonada and Sadras 2015). The increased temperatures caused seed yield reduction in dry peas by reducing flowering to maturity period (Bueckert et al. 2015) indicating that earlier flowering with prolonged flowering duration would result in greater heat tolerance (Huang et al. 2017). The longer flowering period supports the idea that greater plasticity in crop phenology would contribute to greater yield under stress conditions proposed by Turner et al. (2001). The severe heat stress (33 °C day–30 °C night for 2 days) caused rapid abortion and abscission of reproductive organs in pea under controlled conditions (Guilioni et al. 1997). Using a 12 h photoperiod, high night temperatures (24 °C day–30 °C night) caused 25% yield loss in dry pea, as opposed to 8% loss for high day temperatures (32 °C day–15 °C night (Karr et al. 1959). In other experiments, elevated day temperatures ranging from 24 to 33 °C did not affect the number of seeds per pod nor the seed to ovule ratio in dry pea, whereas severe heat stress significantly reduced these parameters when day temperatures increased from 33 to 36 °C (Jiang et al. 2015). It was also suggested that seed development was most affected to the exposure of high temperatures for 5–10 days after opening of the flower at the first node (Jeuffroy et al. 1990). High temperatures during flowering caused reduction in pea grain yield by reducing fruitful node and number of pods per plant (Pumphrey et al. 1979; Duthion et al. 1987; Laconde et al. 1987). Exposure to high temperatures reduces in vitro pollen germination percentage and pollen tube length in field pea (Petkova et al. 2009; Lahlal et al. 2014; Jiang et al. 2015, 2017a). Therefore, exposure to a severe temperature of 36 °C in a growth chamber under cool fluorescent lights was recommended for future screening of pea genotypes for assessment of their heat tolerance using in vitro pollen germination (Jiang et al. 2015, 2017a).

Most importantly, every 1 °C increase in mean temperature during flowering stage could reduce yield to the tune of 0.6 tonnes/ha (Ridge and Pye 1985). Similarly, Pumphrey and Raming (1990) suggested yield loss in pea varying from 16 to 67 kg/ha vis-á-vis a temperature increase between 27 and 35 °C. Additionally, biological nitrogen fixation is severely affected above 40 °C in pea (Michiels et al. 1994). Pea production starts to suffer a reduction when the maximum daytime air temperature exceeds 25 °C (Guilioni et al. 2003). When air temperature is over 30 °C for just a few hours a day, the damage to plants is regarded as moderately severe and severe when maximum air temperature exceeds 35 °C for similar periods (Munier Jolain et al. 2010). Sousa Majer et al. (2004) found that high temperature reduced the protective capacity of the transgenic peas by reducing the production of a-amylase inhibitor 1 (α-AI-1). The plants exposure to high temperatures produces 27% less seeds than the controls. In the transgenic peas, the level of α-AI-1 as a percentage of total protein was reduced on average by 36.3% in the high-temperature treatment. If crop exposed to high temperature during flowering and seed filling stages under field condition, it reduces membrane stability index (28.8%), plant height (60.2%),

total biomass yield (61.7%), seed yield (68.9%) and harvest index (19.3%). Based on the minimum reduction in observed traits, genotypes, KPF 103, DMR 15 and IPFD 4-6, were found to be having comparatively higher amount of resistance towards high temperature stress. IPFD 99-7, IPFD 3-17, IPFD 2-6, IPFD 1-10, HUDP 16 and DPR 13 were adjudged to moderately resistant for high temperature stress as they were having more than 75.0% yield stability index (Vijaylaxmi 2013). Jiang et al. (2018) used two cultivars ('CDC Golden', 'CDC Sage') and exposed them to 24/18 °C (day/night) continually or to 35/18 °C for 4 or 7 days. The given heat stress altered stamen chemical composition, reduced pollen and ovule viability. Pollen appears susceptible to stress, and ovule fertilization and embryos are less susceptible to heat, but further research is warranted to link the exact degree of resilience to stress intensity. Recently, Jiang et al. (2019) reported that the heat stress reduced the number of pollen grains per anther, induced smaller pollen grains and increased ROS production in pollen grains, but it did not affect ROS accumulation in ovules and ovule number per ovary. Heat exposure when young floral buds were visible at the first reproductive node was more detrimental to flower retention, seed set, pod development and seed yield compared to heat exposure started later when flowers at the second reproductive node were fully open. Overall, the high temperature stress negatively affects pollen development and seed set. Heat stress reduced pollen viability, in vitro pollen germination and pollen tube length in field pea (Jiang et al. 2015, 2018, 2019; Jiang 2016).

Since flowering stage is the most sensitive for heat stress, therefore, to increase seed setting efficiency, pea genotypes are being selected for viable pollen production (Jiang et al. 2017a), viable ovules, successful pollination (Jiang et al. 2015, 2017a). The preliminary screening methods are only based on limited number of genotypes (2–24 genotypes), and easily observable traits are being adopted for the examined material (Jiang et al. 2017a). In addition, drought and heat normally comes together causes severe reduction of grain yield (Bueckert et al. 2015). Other traits that are being used to develop heat and drought resistance are pod wall ratio and proxy measurements for crop growth rate from vegetation indices such as NDVI (Sadras et al. 2013), PRI and WBI and leaf wax (Bueckert and Clarke 2013; Tafesse 2018). Most recently, it has been well established that the knowledge of structural-chemical composition of the leaf cuticle is of immense interest in stress physiology (Sánchez et al. 2001), because when pea leaves are exposed to high temperature, the cuticular compounds may respond to heat stress by changing composition or amount, as emphasized in Suseela and Tharayil (2018) and Heredia-Guerrero et al. (2018). Liu et al. (2019b) used attenuated total reflection (ATR)-Fourier-transform infrared (FTIR) spectroscopy, a non-invasive technique, to investigate and quantify changes in adaxial cuticles of fresh leaves of pea varieties exposed to heat stress. Results reported considerable diversity in spectral-chemical makeup of leaf cuticles within commercially available dry pea varieties, and they responded differently to high growth temperature, revealing their diverse potential to resist heat stress. The ATR-FTIR spectral technique can, therefore, be further used as a medium-throughput approach for rapid screening of superior cultivars for heat tolerance.

In addition, other measurements which are suitable for automation of phenotyping are canopy temperature (infrared thermometry and thermal images), lodging (red green blue images) and height, either for abiotic stress impact or for a factor linked to biotic stress (disease and disease ratings). The studies conducted in controlled conditions are in unnatural environments and expensive but still remains valuable as screening methods for trait validation. In contrast, more genotypes can be evaluated in the field condition with low cost and precise phenotyping for canopy measurements, but environmental affects need to be understood for proper interpreted.

Based on visual observation, the selection of physiological traits associated with plant response to high temperature, selection for grain yield and more recently marker-assisted selection (MAS) are the important selection methods mainly used to develop heat-tolerant materials through breeding (Howarth 2005). Of them selection and improvement through MAS is most précised and robust technique with better efficiency compared to other approaches. Therefore, a panel of 92 diverse pea cultivars was evaluated across 9 environments and genotyped using 1536 single-nucleotide polymorphisms (SNPs) arranged in a GoldenGate array. The population structure analysis developed three subpopulations, and association analyses identified a total of 60 SNPs significantly associated ($-\log 10\ p \geq 4.3$) with various reproductive development-related traits. Of them, 33 SNPs were associated with the onset of flowering, 8 SNPs with pod development and 19 SNPs with the number of reproductive nodes. Also found 12 SNPs linked with days to flowering and 2 SNPs associated with duration of flowering which were overlapped with the SNP markers associated with the number of reproductive nodes. Genomic regions associated with variation for reproductive development-related traits identified in this study provide grounds for future genetic improvement in pea (Jiang et al. 2017b). Heat tolerance is a quantitative trait, therefore, identification of associated QTLs and their judicious utilization is an important strategy for accelerating breeding programme for the development of heat tolerant genotypes. So far, any QTLs directly related to heat tolerance have not been reported in dry pea. However, considerable progress has been made regarding QTL mapping for heat tolerance in major crops including wheat (*Triticum aestivum* L.) (Mason et al. 2010; Pinto et al. 2010) and rice (*Oryza sativa* L.) (Jagadish et al. 2010; Ye et al. 2012). In case of legume crops, QTLs associated with heat tolerance have been detected in chickpea and cowpea (Lucas et al. 2013; Paul et al. 2018). In case of pea with the use of next-generation sequencing technology and high-density genetic maps (Leonforte et al. 2013; Duarte et al. 2014; Sindhu et al. 2014), identification of QTLs linked to heat tolerance traits has become possible. Recently, Huang et al. (2017) used a RIL populations (107) developed from the cross of dry pea cultivars CDC Centennial and CDC Sage and developed a genetic linkage map consisting of 1024 loci with a total coverage of 1702 cM using SNP markers. Ten QTLs were found constantly over more than one environment, five for flowering traits and five for yield component traits. A stable QTL at Linkage Group 6b for days to flowering was detected over four environments. The QTLs for flowering duration, TSW and reproductive node number were different

between normal and late seeding, which implies that different mechanisms were involved under the contrasting environments.

10.5.4.2 Drought Stress

Drought or water stress is an imperative environmental limitation that reduces quality and quantity of the yield (Boyer 1982; Ali et al. 1994). The reduction in grain yield due to moisture stress is reported 25% under field conditions (Sánchez et al. 1998). Water stress causes reduction in plant growth rate, stem elongation, leaf expansion and stomatal movements (Hsiao 1973). Furthermore, it causes changes in a number of physiological and biochemical processes governing plant growth and productivity (Daie 1988). Previously, it was reported by many researcher that peas are more sensitive to moisture stress during flowering and pod filling stage than the vegetative stage (Salter 1962, 1963; Maurer et al. 1968; Pumphrey and Schwanke 1974). Later on it was found that the timing of water stress is less important than the actual intensity of the deficit (Zain et al. 1983; Jamieson et al. 1984; Martin and Jamieson 1996). The timing of water stress does not influence the decline in pea grain yield but affects the total dry matter (DM) production. If moisture stress occurred before flowering, the total DM is reduced more than if it occurred after flowering. However, this total yield reduction is completely compensated by an increase in individual seed weight and consequently an increased harvest index (Martin and Jamieson 1996). The shoot-to-root ratio of drought-resistant cultivars remains significantly smaller than the sensitive plants in both control and drought treatments (Grzesiak et al. 1997). In case of plant type, the dwarf types have more drought resistant than tall type (Iwaya-Inoue et al. 2003).

Earlier, the semi-leafless type were considered more tolerant to water stress than conventional leafy- type varieties and it was supposed that the reduced leaf area of the semi-leafless varieties is the main factor (Semere and Froud Williams 2001). But, Gonzalez et al. (2001) examined the background of phenomena and noticed that total leaf area and transpiration rate per plant are not significantly different in both plant types. In addition, osmolarity at tissue level is similar among different leaf structure, whereas at the epidermal vacuole level, tendrils of the semi-leafless have a higher osmolarity than conventional plant type of pea. On the semi-leafless plants, the tendrils are about 40% of the total leaf; thus, its more efficient osmotic adjustment might be involved in improving water use efficiency under water stress (Gonzalez et al. 2001). Nevertheless, under water stress only, stipules of semi-leafless pea plants exhibited significantly better ability to boost osmolarity by accumulation of potassium, magnesium and chloride as compared to other leaf structures (Gonzalez et al. 2002). The Epicuticular wax load of cultivars increased significantly under drought conditions, and it is supported by increased residual transpiration rate (Sánchez et al. 2001). In drought condition, the roots of field pea go deeper in the soil than those under irrigated conditions (Benjamin and Nielsen 2006). However, osmotic stress induced by PEG 6000 resulted in shortening of primary root and increase of lateral root number (Kolbert et al. 2008).

Drought stress negatively affects the number and distribution of seeds developed on the basal phytomers of drought-stressed pea plants than on control plants (Guilioni et al. 2003). If moisture stress appears 1 week after development of first pods, then it leads to 79% reduction in number of seeds than the controls (De Sousa-Majer et al. 2004). According to Iturbe-Ormaetxe et al. (1998), severe water stress almost completely inhibited photosynthesis and damaged the photosynthetic system. Net photosynthesis was also decreased by water stress during the stress period. The relationship between total seed numbers and plant growth rate during critical period for seed set suggests that pea can adjust the number of reproductive sinks in a balance with assimilate availability in the plant (Guilioni et al. 2003). Although yield was reduced when drought stress exists during flowering and pod filling, the size and distribution of seeds are not affected constantly (Sorensen et al. 2003). The plant height and leaf area are not influenced significantly, but the drought stress decreased the fresh and dry weight of the pea and especially the relative leaf water content. The decrease in relative leaf water content is the main factor in reduced growth in drought-treated plants (Alexieva et al. 2001).

In moisture stress conditions, pea demonstrated major reduction in photosynthesis (78%), transpiration (83%) and glycolate oxidase activity (44%) and minor reduction in the chlorophyll a, carotenoids and soluble protein content (Moran et al. 1994) which might lead to reduction in various morphological traits and overall grain yield. The chlorophyll content to some extent increased, while the amounts of anthocyanins were not affected in water-stressed pea plants. The soluble phenols in leaves increased noticeably under drought stress (Alexieva et al. 2001). Water stress led to full disruption of the chiral macroaggregates of the light harvesting chlorophyll a/b pigment-protein complexes (LHCIIs) measured by circularly polarized chlorophyll luminescence (CPL) in detached pea leaves (Gussakovsky et al. 2002). Sucrose content of seeds is also increased by water stress (Sorensen et al. 2003). Contrarily, recently it has been reported that chlorophyll and protein contents in leaves decreased significantly with increased water stress, while the proline and malondialdehyde (MDA) contents elevated as a result of water shortage in pea leaves. Drought stress noticeably improved the activities of superoxide dismutase, catalase and peroxidase but slightly changed the activity of ascorbate peroxidase (Karatas et al. 2012).

The capability of plant to cope up with moisture stress condition determines its yield potential in a specific environment. There are mainly three strategies, i.e. escape, avoidance and tolerance of crops to sustain in moisture restricted conditions (Turner et al. 2001). Of these strategies, the initial two stand firm against stresses, while in third crop it has to survive with sizeable loss in productivity. Given approaches can be used in breeding programme to develop genotypes that would perform well under limited water conditions. The avoidance by escape approach is mainly based on earliness in terms of flowering and maturity, and therefore, it is the first preference of breeders. Because early flowering is often associated with early maturity, early flowering-early maturing crops cannot respond to higher moisture environments, and the yield performance of early-flowering genotypes can be low (Khan et al. 1996). Dry pea can perform well under water stress conditions if the

crop flowers early and pod filling occurs when plant water status is adequate (drought escape mechanism); accordingly, development of genotypes with vigorous early growth, flowering and pod set is necessary (Khan et al. 1996). Therefore, greater plasticity in phenology with early flowering would be always helpful (Turner et al. 2001). The pea breeding programme in many countries is presently selecting more stress-resistant cultivars with high yield potential by earliness and prolonged flowering duration. The drought avoidance tactic is delayed water loss using stomatal conductance, leaf area and any non-transpirational water loss from leaves. Leaf area is a significant factor due to extensive adaptation of the *afila* trait. The semi-leafless trait has many advantages in water-deficit situations owing to reduced leaf area (Rodriguez Maribona et al. 1990, 1992; Sánchez et al. 2001). Semi-leafless genotypes are enabled to maintain stomatal conductance for long time in water stress, maintain canopy temperature low and yield more compared to the normal leafed genotype under water stress condition (Alvino and Leone 1993). Genetic diversity exists for root architecture and water uptake ability (Armstrong et al. 1994; Thorup Kristensen 1998), but none of the programme has selected superior genotypes based on rooting. The increased ABA production was a preferred feature in the mid-1980s and successful in maize and wheat, which is related with stomatal control in stress and results in high yield, but only in some environments (Read et al. 1991). Thus, breeder must be cautious when selecting for improved ABA. Association between yield performance under water stress and osmoregulation ability of pea genotypes has been proved (Neumann and Aremu 1991; Rodriguez Maribona et al. 1992). The relationship between growth and osmotic adjustment and turgor maintenance was observed at seedlings stage under water stress induced by 46 mM polyethylene glycol (PEG) 6000 (Sanchez et al. 2004). The assessment of turgor maintenance at the early stages of development could be used to recognize drought-tolerant genotypes (Sanchez et al. 2004).

In dry pea, grain numbers are most decisive, and crop has maximum sensitivity to stress in the period between the start of flowering and the beginning of seed filling (Guilioni et al. 2003; Jeuffroy et al. 2010; Lecoeur and Guilioni 2010; Sadras et al. 2012). Overall, water stress in pea crop reduced plant height, internode length and leaflets size. The canopy colour changes to pale green since drought reduces nitrogen fixation or uptake. Tips of leaflets can die, flower buds and flowers may abort, and the life cycle is shortened resulting in fewer pods with fewer seeds (Rubiales et al. 2019). Therefore, development of new varieties with wider adaptation ability including drought tolerance is the prime endeavour of pea breeding programme (Abd-El Moneim et al. 1990). Drought tolerance is a multifaceted phenomenon in which different adaptations mechanism are involved (Sánchez et al. 2001); thus, it is quintessential to reveal mechanisms responsible towards drought tolerance and enhancement of crops performance in water stress situations. The use of molecular markers for the indirect selection of breeding lines reduces the time required for selection process compared to direct screening under greenhouse and field conditions (Dita et al. 2006). So far none of the study has been addressed the genetics of adaptation to drought in pea or reported QTLs for this trait. Therefore, recently Iglesias-Garcia et al. (2015) reported the quantitative genetics of drought adaptation

in pea and identify the genomic regions controlling the trait. They assessed drought symptoms and relative water content in soil (RWCS) and leaves (RWCL) in a RIL population. They identified 10 quantitative trait loci (QTLs) associated with the traits accounted individually between 9% and 33% of the phenotypic variation depending on the variable ascertained and altogether between 20% and 57%. A set of reproducible markers linked to these QTLs (*A6*, *AA175*, *AC74*, *AD57*, *AB141*, *AB64*, *Psblox2*, *PsAAP2-SNP4* and *DipeptIV-SNP1*) has been dissected. The SSR marker associated with the drought adaptation QTLs could be useful for MAS in drought adaptation breeding programmes.

10.5.4.3 Frost Stress

Frost stress is one of the major abiotic stresses causing a significant problem at vegetative and reproductive stage in pea (Shafiq et al. 2012; Liu et al. 2017). In temperate environment, frost during winter or early spring can severely damage or kill seedlings (Swensen and Murray 1983; Badaruddin and Meyer 2001; Meyer and Badaruddin 2001; Stoddard et al. 2006). Frost is the situation in which temperature goes abruptly below 0 °C during the vegetative and reproductive stage according to planting time, and this shock is usually experienced with low overnight temperatures that last between 2 and 8 h before the cold acclimation of crop (Rubiales et al. 2019). Frost damage cause permanent injury such as destruction of cell membrane system or loss of photosynthetically active tissue when plants are not acclimated (Chen et al. 2004; Menon et al. 2015; Liu et al. 2017). In cold acclimation, crop plants developed an adaptation mechanism to withstand cold which is induced by low, non-freezing temperatures (Levitt 1980; Xin and Browse 2000). Severe radiant frost is a hazard during reproductive stages, causing ice formation within plant cells or tissues (Ridge and Pye 1985). Frost at reproductive stage can damage or kill buds, flowers and pods and can reduce seed weight which leads to overall reduction in grain yield (Ridge and Pye 1985). Under wet conditions, physical frost damage on plants can promote infection by *Pseudomonas syringae pv. pisi*, the causal agent of bacterial blight (Knott and Belcher 1998). The frost tolerant of field pea at the vegetative stage decrease gradually with increasing age (Badaruddin and Meyer 2001; Meyer and Badaruddin 2001), and the sensitivity towards frost increases after flower initiation (Lejeune-Henaut et al. 1999).

Genetic variation has been reported for frost tolerance in dry pea for seedling (Bourion et al. 2003), vegetative stage (Lejeune-Henaut et al. 2008) and reproductive stage (Shafiq et al. 2012). Shafiq et al. (2012) identified five accessions ATC 104, ATC 377, ATC 968, ATC 3992 and ATC 4204 originated at different countries, which showed the highest frost tolerance at flowering stage with the production of least numbers of abnormal seeds. Dry pea is exhibited moderate freezing tolerance with LT50 (temperature that kills 50% of seedlings) of −4.5 °C in comparison to forage legumes (Meyer and Badaruddin 2001), while some winter hardy varieties of pea are found to be able to adapt to a temperature range between −8 and −12 °C (Homer and Sahin 2016; Auld et al. 1983). The evaluation was also done for winter

hardiness in a set of 58 accessions of pea germplasm under both field and laboratory conditions in Turkey and identified genotypes with differential survival at −8 °C among which the higher level of winter hardiness was selected for future cultivar development (Homer and Sahin 2016). Recently, a large-scale evaluation of 3672 pea germplasm for cold tolerance was executed in the field condition in China and found that genotypes from winter production regions showed a higher level of cold tolerance than those from spring production regions and identified a collection of genetic resources for cold tolerance of pea in China (Zhang et al. 2016). Selecting frost-resistant genotypes is possible in controlled conditions up to −5 °C (Shafiq et al. 2012) and in the field under naturally occurring radiation frost (Davies and Pham 2017).

The frost-tolerant accessions identified in these studies may be useful as parents for developing resistant cultivar for frost and mapping population for identification and tagging of candidate gene for frost tolerance, since understanding of the genetic nature of frost tolerance is a prerequisite for the development of frost-tolerant pea cultivars. In addition, breeding winter cultivars requires the combination of freezing tolerance as well as high seed productivity and quality. The flowering locus *Hr* is suspected to influence winter frost tolerance in pea by delayed floral initiation until the main winter freezing periods over (Avia et al. 2013; Dhillon et al. 2010). In pea, Lejeune-Henaut et al. (2008) reported six QTL region referred to as *WFD 1.1* to *WFD 6.1*, among which three (*WFD 3.1*, *WFD 5.1* and *WFD 6.1*) are steady among the different experimental conditions, confirming oligogenic determinism of frost tolerance in pea. A major QTL of pea frost tolerance on LGIII is located in the vicinity of the *Hr* locus. *Hr* is a gene controlling plant response to photoperiod (Weller et al. 2012). This gene is an essential component of frost avoidance, since it delays the vegetative tore productive stage transition until longer days, when the risk of frost occurrence is lower. The co-locations between WFD QTL and QTL for physiological traits have been also discovered on LGV and VI (Dumont et al. 2009). Klein et al. (2014) also confirmed the quantitative inheritance of frost tolerance and detected a total of 161 QTLs which explained 9–71% of the phenotypic variation across the six environments for all traits studied. Two clusters of QTL mapped on the linkage groups III and one cluster on LGVI revealed the genetic links between phenology, morphology, yield-related traits and frost tolerance in winter pea. QTL clusters on LGIII highlighted major developmental gene loci (*Hr*and *Le*), and the QTL cluster on LGVI explained up to 71% of the winter frost damage variation. This suggests that a specific architecture and flowering ideotype defines frost tolerance in winter pea. However, two reliable frosts tolerant QTL on LGV were detected, and these are independent of phenology and morphology traits, showing that different protective mechanisms are involved in frost tolerance. These results suggest that frost tolerance can be bred independently to seed productivity and quality. Most recently, Liu et al. (2017) performed marker-trait association analysis for frost tolerance with 267 informative SSR markers and identified 16 accessions as the most winter-hardy based on their ability to survive. Population structure analysis revealed two subpopulations plus some admixtures in the 672 accessions. Association analysis detected seven markers that repeatedly had associations with frost tolerance in at

least two different environments with two different statistical models. In addition, one of the markers is the functional marker EST1109 on LG VI which is supposed to co-segregate with a gene involved in the metabolism of glycoproteins in response to chilling stress and may provide a novel mechanism of frost tolerance in pea. These winter-hardy germplasms and frost tolerance-associated markers will play a vital role in marker-assisted breeding for winter-hardy pea cultivar. The consistently reported QTLs/marker can be used as interesting targets for marker-assisted selection.

10.6 Future Perspectives

Notwithstanding considerable efforts have been made to improve its productivity; the average productivity of this crop at global level is now to the tune of 1.9 tonnes/ha which is quite low as compared to other leading countries likely the Netherlands, Denmark, Belgium, Ireland, Germany, France and Canada. In dry pea, breeders around the world have been focused largely on three traits, viz. tendril (*afila*), dwarf plant type and powdery mildew resistance. These three traits are being extensively used in the breeding programme, which has resulted in the development of a number of high-yielding varieties resistant to powdery mildew with diverse plant type. In spite of that, during the last five decades, the yield gain is just 15.3 kg/ha/year in dry pea at global level, which is much lower than other crops, indicating that least attention was paid towards pea research. On the contrary, the yield gain in Canada is 2.0% which is greater than the yield gain in most of the crops at global level witnessed large investment in pea breeding programme over the years (Rubiales et al. 2019). Therefore, there are few more areas of interest that need greater attention in future and are discussed below.

1. *Multiple disease resistance*: Dry pea is vulnerable towards different biotic stresses such as powdery mildew, rust, ascochyta blight, *Fusarium* root rot, Fusarium wilt and *Aphanomyces* root rot which have increased over the years. Therefore, it is urgently required to incorporate multiple diseases resistance (region-specific) in a single variety in future dry pea breeding programme to increase the productivity.
2. *Better standing ability:* The stem of pea plant is very weak and coupled with huge mass of foliage on the upper side and accumulation of massive pods which leads to lodging as the lean stem is unable to hold it in upright position. Hence, any heritable system that can make the base of pea plant anatomically strong and thick enough, which can keep plant standing erect till full maturity, will undoubtedly boost its yield potential.
3. *New uses*: The dry pea crop in some parts at global level has a limited direct consumption pattern, and the uses of grain are not diversified. Therefore, new uses of dry pea have to be found if the popularity of this crop has to increase.

4. *Multi-purpose:* Another aspect of pea breeding could be to initiate breeding programmes for multipurpose (food-feed-fodder). There is no doubt that in addition to its use as protein source for human being, the demand for cattle/poultry feeds and fodder will increase manifold in this country. A cheap pulse like dry pea could play a crucial role in such a situation.
5. *Earliness:* Nowadays, earliness is becoming another trait of economic importance in every crop; through that, the productivity per unit time and per unit area can be increased. The early varieties with dwarf semi-leafless type plant can be planted with higher crop density and good standing ability. Perhaps this would be the most ideal situation to maximize yields not only per unit time but also per unit area.
6. *Abiotic stress tolerance*: The major abiotic stresses which are now become serious issue in sustainable production of dry pea under climate change scenario are high temperature, drought and frost (Rubiales et al. 2019). Therefore, there is urgent need to develop abiotic stress tolerance varieties with high yield potential to get more production of field pea.
7. In future the extensive utilization of similar kind of parent should be avoided in hybridization programme and needs to include diverse parents in dry pea breeding programme to develop new plant types with high levels of resistance to biotic and abiotic stresses, and earliness, thus, dry pea would be adapted better to the changing climatic scenario. The value added dry pea varieties, i.e. low flatulence, high iron and zinc are the quintessential need of future to popularize this crop. Thus, the future needs of dry pea breeding will be to develop lodging resistance, early maturing, biofortification, heat, drought and frost tolerance and disease-free varieties with yellow and green cotyledon (for human consumption) as well as pigmented and mottled seed coat (for feed and fodder purpose). Furthermore, most importantly, major/minor genes or QTLs have been identified responsible for different traits including important biotic and abiotic stresses. It would be advisable to concentrate further on large-scale high-throughput screening of germplasm for identification of genes/QTLs and their tightly linked markers for various targeted traits with high precision using different advance mapping populations. Further, the introgression of these resistant sources in good genetic agronomic background should be done with the help of marker-assisted selection to accelerate field pea breeding programme efficiently and more precisely. It is believed that conventional breeding approaches will remain the mainstay in combating these stresses. However, new tools of genomic selection, genome editing, gene mapping, gene cloning and genetic transformation offer opportunities to create new gene combinations to overcome losses due to biotic and abiotic stresses.

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Chapter 11
Translational Genomics and Breeding in Soybean

Anita Rani and Vineet Kumar

11.1 Introduction

Soybean breeders have developed various breeding methodologies and have made tremendous progress in development of superior genotypes. Development of superior soybean varieties using traditional methods is very tedious as selection procedure of desirable genotypes using conventional approaches is complicated, inefficient and uncertain due to difficulties related to phenotyping procedures, which can be time-consuming, expensive, unreliable for traits with a low heritability and subject to genotype × environment (G × E) interactions (Francia et al. 2005). Advances in molecular genetics and genomics have revolutionized breeding procedures for development of superior genotypes. The cost- efficient development of next-generation sequencing (NGS) technologies has facilitated the discovery of single nucleotide polymorphism (SNP) markers in genes that affect traits of interest as a large number of SNPs have been located near or within genes or gene structural variants, revealing allelic variations (Collard and Mackill 2008). Several sequence-based datasets have been generated by re-sequencing efforts in soybean (Lam et al. 2010; Li et al. 2013; Chung et al. 2014; Qiu et al. 2014; Zhou et al. 2015; Valliyodan et al. 2016). While these datasets have been proven very useful for the understanding of genome architecture and dynamics as well as facilitating the discovery of genes, an obligation for, and challenge to, the scientific community is to translate genome information to develop products, i.e. superior lines for trait(s) of interest (Varshney et al. 2015). Development of SNP-based marker assays such as SoySNP6K Infinium BeadChip (Akond et al. 2013), the NJAU 355 K SoySNP array (Wang et al. 2016), SoySNP50K iSelect BeadChip (Song et al. 2013; Vuong et al. 2015; Zhang et al. 2015) and Axiom SoyaSNP array for ~180,000 SNPs (Lee et al. 2015), identification of gene-specific functional markers (de Moraes et al. 2006;

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Lenis et al. 2010; Pham et al. 2011; Reinprecht et al. 2011; Xia et al. 2012) and SSR markers linked to various useful traits (Yu et al. 1994; Kim et al. 2006; Hyten et al. 2007; Monteros et al. 2007; Rani et al. 2011, 2013, 2017, 2018; Yang and Gai 2011; Kumar et al. 2014) are important steps towards translational genomic and breeding of soybean variety with desired traits.

11.2 Flowering and Maturity Duration

Soybean is a typical short-day (SD) plant. It flowers when the day length becomes shorter than a critical duration. A shorter day length accelerates not only flowering initiation of photoperiod-sensitive soybean varieties but also reduces the maturity duration by speeding up all the reproductive phases. Switching photoperiod-sensitive soybean varieties from SD to long-day (LD) conditions leads to flowering reversion (Han et al. 1997; Washburn and Thomas 2000; Wu et al. 2006). Fourteen major genes/loci (Table 11.1) affecting soybean flowering and maturity period have been mapped till now: E1 (Cober and Voldeng 2001; Molnar et al. 2003), E1La and E1Lb (Xia et al. 2012), E2 (Mahinur et al. 1995; Cregan et al. 1999), E3 (Molnar et al. 2003), E4 (Abe et al. 2003; Molnar et al. 2003),E5 (Dissanakaya et al. 2016), E6 (Li et al. 2017), E7 (Cober and Voldeng 2001; Molnar et al. 2003), E8 (Cober et al. 2010), E9 (Kong et al. 2014), E10 (Samanfar et al. 2017), J (Sijia et al. 2017) and GmAGL1 (Zeng et al. 2018). The functional allele of all of these genes except E6, E9, J and GmAGL1 delays both flowering and maturity, while the non-functional counterpart accelerates. These *loci* have different roles under different photoperiods. LD strengthens while SD weakens these maturity genes (Wang et al. 2008). Functional role of six genes has been deciphered. E1 gene was identified as a transcription factor which functions as a flowering repressor with a putative nuclear localization signal and a B3-related domain (Xia et al. 2012). Two E1L genes, E1La and E1Lb (*Glyma04g24640.1/Gm18g22670*), showed an expression pattern similar to E1, suggesting that these genes might be regulated by a similar mechanism (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). E2 is an orthologue of *Arabidopsis thaliana* flowering gene GIGANTEA (Watanabe et al. 2011). E3 and E4 are phytochrome genes. E3 is GmPhyA3 (Watanabe et al. 2009) and E4 is GmPhyA2 (Liu et al. 2008). J is the orthologue of *Arabidopsis thaliana* EARLY FLOWERING 3 (Sijia et al. 2017). GmFT4 is the most likely candidate gene at a newly identified maturity locus, E10 (Samanfar et al. 2017). In addition, two homologues of soybean Flowering Locus T (FT) genes, GmFT2A and GmFT5A, were identified and coordinately regulate flowering (Kong et al. 2010). J depends genetically on flowering repressor E1, and J protein physically associates with the E1 promoter to downregulate its transcription, relieving repression of two important FLOWERING LOCUS T (FT) genes and promoting flowering under short days (Sijia et al. 2017). In soybean, at least ten FT homologues have been identified (Kong et al. 2010). GmFT2a and GmFT5a were found to be strictly

Table 11.1 Known early genes and their position

Locus	Functional gene	Mapped position	References
E1	*Glyma06g23040.1*	Present on Chr 6 near Satt365 SSR marker	Cober and Voldeng (2001), Molnar et al. (2003), Yamanaka et al. (2005)
E1La	Glyma04g24640	Present on Chr 4, 10,640 kb apart from each other	Xia et al. (2012)
E1Lb	Glyma.04G143300.1		
E2	Glyma.10G221500	Present on Chr 10 with AFLP marker, E60M38, was located in the fifth intron	Watanabe et al. (2011)
E3	Glyma.19G224200	Present on Chr 19 near Satt006	Cregan et al. (1999)
E4	Glyma.20G090000	Present on Chr 20 in vicinity of Satt239-Satt496-E4-Enp-Satt354	Abe et al. (2003), Molnar et al. (2003)
E5	–	Present on Chr 10, located in the close vicinity of E2, or E5 is an allele of the E2 locus	Dissanayaka et al. (2016)
E6	–	Present on Chr 4 near single nucleotide polymorphism marker HRM101	Li et al. (2017)
E7	–	Present on Chr 6, tightly linked to E1	Cober and Voldeng (2001), Molnar et al. (2003)
E8	–	Present on Chr 4 between Sat_404 and Satt136 SSR markers	Cober et al. (2010)
E9	–	Present on Chr 16 fine-mapped to a 245-kb region on Chr 16	Kong et al. (2010)
E10	–	Present at the end of Chr 8	Samanfar et al. (2017)
J	Glyma.04G050200.1	Present on Chr 4	Sijia et al. (2017)
GmAGL1	Gm14g273100	Present on Chr 14	Zeng et al. (2018)

photoperiod-regulated and have been shown to promote flowering in Arabidopsis and soybean (Kong et al. 2010; Sun et al. 2011; Cai et al. 2018). Among the soybean FT functions, GmFT4 has been noted to be strongly induced by LD conditions and was reported to function in delaying flowering when ectopically expressed in Arabidopsis (Zhai et al. 2014; Cao et al. 2016). E1, E2, E3 and E4 delay flowering and maturity under LD by downregulating GmFT2A and GmFT5A (Kong et al. 2010; Watanabe et al. 2011; Xia et al. 2012).

11.3 Quality Traits

Soybean is a unique crop which offers high-quality protein. Soybean is mainly crushed into meal for animal feed production and oil for human consumption producing 79–80% meal, 18–19% oil and 2% waste and hulls. A small quantity of soybean grain is generally used for food products such as roasted soy sprouts, soy

sauce, soy nuts, soy milk, soy curd, bakery products, confectionery and protein bars. The nutritional value of soybean has been reviewed by several researchers (Kumar et al. 2010b; Ahmad et al. 2014). Soybean oil is mainly used for human consumption. It is used for cooking purpose and added to other edible products such as mayonnaise, shortening and margarine. Soybean meal rich in protein content is the most favoured source of animal feed as it leads to rapid weight increase in the animals fed with it. Soybean meal is also used by poultry and swine farmers because of its high protein content. It is also used for cattle, fish and pets. A small quantity of soybean meal is also used for soy protein, soy isolates, soy flour and soy concentrates. Soybean seed is not only rich in high-quality protein and oil; it also contains nutraceutical molecules such as isoflavones, tocopherols, saponins and lecithin. Consumption of soy-based products has been associated with the reduced risk of onset of several lifestyle diseases, such as obesity, cardiovascular diseases (Lichtenstein 1998), diabetes (Nordentoft et al. 2008), osteoporosis (Potter et al. 1998) and oxidative stress complications like breast cancer (Messina et al. 1994), Parkinson's and Alzheimer's in the developed world. It has earned the sobriquet of 'golden bean' due to the various health benefits and its capability to offer the economical nutrition to vegans and poor populace. Despite being economical source of nutrition and offering all the above-mentioned health benefits, this 'golden bean' does suffer from stigmas of beany flavour/off-flavours, the presence of anti-nutritional factors and the susceptibility to oxidation of the oil fraction that limit the utilization of this bean in food uses. Lipoxygenases in soybean seed generate off-flavour producing aldehyde and ketone compounds in the soya products, high PUFA (polyunsaturated fatty acid) content causes poor oxidative stability of soya oil, high phytic acid content affects the bioavailability of essential minerals, while trypsin inhibitor limits the digestibility of protein (Onesti et al. 1991; Brune et al. 2010). Most of the genes responsible for undesirable components in soybean have been mapped and characterized and are being deployed in marker-assisted backcross and forward breeding. A number of specialty soybean genotypes have been developed using these approaches and have been commercialized.

11.3.1 Flavour and Fragrance

Grassy and beany flavour is generated in soyfood products by oxidation of unsaturated fatty acids by lipoxygenases present in the soybean seed (Wilson 1996; Gerde and White 2008). Mature soybean seeds contain primarily three lipoxygenase isozymes, Lox1, Lox2 and Lox3 (Axelrod et al. 1981). The absence of each isozyme 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 present on chromosome 13 (LG F). The *Lox3* locus is present on chromosome 15 (LG E) and segregates independently of *Lox1* and *Lox2* (Kitamura et al. 1985; Davies and Nielsen 1986; Hajika et al. 1992). The repulsion phase linkage present

between *lox1* and *lox2* mutant alleles as they were identified in independent germplasm was the major impediment in development of triple null lipoxygenase soybean desired by soyfood industries (Hildebrand and Hymowitz 1982; Kitamura et al. 1985; Davies and Nielsen 1986). The original repulsion-phase linkage between mutant alleles at *Lox1* and *Lox2* loci was broken by irradiation, resulting in a coupling-phase linkage that eventually led to the development of a triple null lipoxygenase genotype (Hajika et al. 1991; Kitamura 1991). The mutation in null *lox2* genotype is T2849A missense mutation, resulting in the substitution of glutamine for histidine in a highly conserved histidine-rich motif (Wang et al. 1994). This replacement of the iron ligand histidine with glutamine caused structural distortion of the protein, leading to its dysfunction**.** The genetic basis of mutations in *Lox1* and *Lox3* soybean genes was investigated by Lenis et al. (2010) and Reinprecht et al. (2011). Two independent mutations, a 74 bp deletion in exon 8 and a C2880A nonsense mutation, were found to be responsible for the premature truncation of the Lox1 protein in the mutants lacking lipoxygenase activity. The deletion eliminated the highly conserved H531 and the iron ligands H690, N694 and I839, which are necessary for Lox 1 enzymatic activity. A single point mutation (A-G) in exon 6 was detected in *Lox3* of H70, causing a change of amino acid 405 from histidine to arginine. Mapping and characterization of all the three lipoxygenases led to development of gene-specific and linked molecular markers (Lenis et al. 2010; Reinprecht et al. 2011; Kumar et al. 2014; Rani et al. 2013). These molecular markers are being routinely used in several laboratories for development of lipoxygenase-free soybean varieties. The Iowa State University Research Foundation, Inc. (ISURF) and the Committee for Agricultural Development (CAD), USA, have released seven lipoxygenase-free soybean varieties, viz. IA1008LF, IA2053LF, IA2076LF, IA2104LF, IA3027LF, IA3045LF and IA3051LF, for cultivation in the USA. Agriculture and Agri-Food Canada (AAFC)'s 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 for use in high protein and reduced off-flavour soyfood production (Yu et al. 2016). In India, ICAR-Indian Institute of Soybean Research has developed and commercialized lipoxygenase-2-free soybean genotype NRC109 to private soyfood industries using these markers (Kumar et al. 2013a. Many more lipoxygenase-free soybean varieties are in pipeline at ICAR-Indian Institute of Soybean Research.

Young pods harvested at R_6 stage of soybean (when the pod cavity is completely filled, but has not started turning yellow) are cooked and consumed as snack or vegetable (Shanmugasundaram et al. 1991). Such soybean varieties are called as 'vegetable or edamame or green soybean', and these varieties produce highly sweet and fragrant seeds due to higher sucrose content and sweetness-imparting amino acids and bear extra large pods with large seeds (Kumar et al. 2006a, 2011a). The distinct fragrance in the seeds of these varieties is due to the presence of a volatile compound 2-acetyl-1-pyrroline (2AP) (Fushimi and Masuda 2001), which is also found in fragrant rice. A single recessive gene has been reported to control the fragrance in vegetable soybean (AVRDC 2003). This recessive mutation causes elevated 2AP biosynthesis that results in a fragrant aroma in those varieties (Niu et al.

2008). A major QTL for fragrance in soybean was identified by Juwattanasomran et al. (2011), which coincides with the position of betaine aldehyde dehydrogenase 2 (GmBADH2) (Bradbury et al. 2005). Comparison of DNA sequence of gene coding this enzyme in fragrant and non-fragrant soybean varieties revealed a non-synonymous SNP in exon 10, resulting in change of glycine to aspartic acid. PCR-based allele-specific SNP markers have been developed for marker-assisted breeding of fragrance trait in soybean (Juwattanasomran et al. 2011). A new fragrance allele, which has a 2-bp (TT) deletion in exon 10 of GmBADH2, was discovered in another fragrant soybean cultivar Chamame (Juwattanasomran et al. 2012). ICAR-Indian Institute of Soybean Research is using these molecular markers for development of fragrant vegetable-type soybean varieties.

11.3.2 Protein Digestibility

Soybean seeds contain high-quality protein. Paradoxically, trypsin inhibitor present in soybean seeds affects the digestibility of protein (Onesti et al. 1991; Brune et al. 2010). Trypsin inhibitor activity in soybean is dependent upon genotype (Kumar et al. 2001, 2019), the growing location and environment (Kumar et al. 2003, b). Trypsin inhibitor activity is attributed to two polypeptides, namely, Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI). KTI 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) and is considered detrimental to human health (Liener 1994). KTI is heat labile due to the presence of only two disulphide linkages but requires minimum 15–20-min boiling for its complete inactivation in soybean seeds (Chen et al. 2014). However, KTI may remain active in the food and feed products, due to faulty processing and 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).

Presence of KTI in soybean seed is governed by a single gene and controlled by multiple alleles as revealed by genetic studies (Singh et al. 1969; 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 Ta^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 the soybean molecular linkage map (Cregan et al. 1999). Three SSR markers Satt409, Satt228 and Satt429 have been reported to be tightly linked with *ti* locus at a distance of 4.5, 0–3.7 and 5.1 cM, respectively (Kim et al. 2006; Rani et al. 2011). A gene-specific marker has also been designed from the null allele of KTI from genotype PI157440 (de Moraes et al. 2006) and has been deployed in identification of allele of KTI derived from PI542044 (Kumar et al. 2013b). Genotype PI542044, source of null KTI allele, was obtained from US Department of Agriculture and was utilized as a donor of null *ti* allele in

development of KTI-free genotypes NRC101 and NRC102 through marker-assisted forward breeding (Rani et al. 2010), and both the genotypes have been commercialized to private soyfood industries in India. Marker-assisted introgression of the *ti* allele in five elite soybean varieties, viz., NRC7, JS97-52, MACS450, DS93-05 and DS97-12, has been accomplished (Kumar et al. 2011b, 2012, 2015). NRC127 developed through marker-assisted introgression of null *ti* allele in JS97-52 has been released for the farmers of Central India for the states of Madhya Pradesh, Vidhabha and Marathwada region of Maharashtra and Bundelkhund region of Rajatshan, Uttar Pradesh and Gujarat and is the first KTI-free variety available to farmers of India for cultivation. Two KTI-free soybean genotypes, viz. 'Laura' and 'Launa', have been developed in Serbia (Peric et al. 2004).

11.3.3 Mineral Availability

Soybean seeds possess a heat-stable anti-nutritional factor 1,2,3,4,5,6-inositol hexaphosphoric acid commonly referred to as phytic acid. It is the principal source of phosphorus in soybean seeds and is present in much higher quantity in soybeans than in other legumes (Chitra et al. 1995). This compound binds with nutritionally important metals, especially zinc, calcium and magnesium, forming phytic acid-metal complexes (phytin), which are not absorbed readily in the intestine and are largely excreted by humans and non-ruminant animals that have no or limited phytase activity in their digestive apparatus, thereby causing deficiency of these nutrients (O'Dell 1982; Solomon 1982; Forbes et al. 1983). Moreover, phytic acid remains active even after cooking due to its heat-stable nature. Phytic acid also binds with negatively charged protein molecules at alkaline pH and with positively charged protein molecules at pH values below their isoelectric point by charge neutralization. As a result of this non-selective binding to proteins, 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), thereby affecting the quality of soy products. Tofu manufacturing process also requires a relatively large amount of coagulants, namely, $CaSO_4$ and $MgCl_2$, to compensate for the effect of phytic acid on *tofu* quality (Schaefer and Love 1992). The presence of phytic acid is also responsible for hard-to-cook phenomenon in legumes (Jones and Boulter 1983; Bernal-Lugo et al. 1991). Further, the undigested phytin excreted through non-ruminants contribute to soil and water pollution (eutrophication) (Raboy 2001).

In phytic acid biosynthesis during seed development, myoinositol-1-phosphate synthase (MIPS) is the key enzyme catalysing the conversion of Glc-6-P to myoinositol-1-phosphate (MIP), which is converted to 1,2,3,4,5,6 hexakis dihydrogen phosphate (phytic acid) by subsequent phosphorylations. A missense mutation in soybean (*Glycine max*) MIPS structural gene (GmMIPS1) responsible for 50% reduction in seed phytic acid was identified by Hitz et al. (2002). Wilcox et al. (2000) identified a mutant line CX1834 in soybean 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. The identification of a nonsense mutation within a candidate lpa1 homologue present on chromosome 3, Glyma03g32500, suggested that the nonsense mutation at this locus could play a role in the molecular basis for the low phytic acid phenotype in soybean (Maroof et al. 2009). Gillman et al. (2009) identified a novel missense mutation in a conserved portion of the other lpa1 homologue, Glyma19g35230, in CX1834 and developed high-throughput molecular marker assays to directly select for the alleles that control the soybean low phytic acid phenotype. They also reported the identification of the novel lpa2-b allele in M766, another low phytic acid soybean line. Molecular markers developed by Gillman et al. (2009) would help in combining nonsense lpa2-b allele from M766 with the nonsense lpa1-a allele from CX1834 to produce soybeans with even lower levels of phytic acid and increased available phosphate levels. Efforts were also made to reduce phytic acid in soybean seed by downregulation of MIPS by RNA interference (RNAi) technology. But complete RNAi knockdown of GmMIPS1 expression resulted in aborted soybean embryos (Nunes et al. 2006). Transgenic plants appeared normal, while their progeny zygotes inheriting the RNAi construct exhibited early embryo abortion. Bilyeu et al. (2008) generated a soybean line, CAPPA, in which an *Escherichia coli* periplasmic phytase, the product of the appA gene, was expressed in the cytoplasm of developing cotyledons. CAPPA exhibited high levels of phytase expression, resulting in 90% reduction in seed PA, and concomitant increases in total free phosphate.

11.3.4 Oxidative Stability of Soybean Oil

Soybean contributed 60.25% and 27.7% to the worldwide oilseed (600.97 million metric tonne) and edible oil (203.95 million metric tonne) production in 2018–2019, respectively (United States Department of Agriculture 2019). Soybean oil 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 acid are saturated fatty acids, while oleic, linoleic and α-linolenic acid are unsaturated fatty acids. Oleic acid (C18:1) is monounsaturated fatty acid (MUFA) with single unsaturation, while linoleic (C18:2/omega 6) and α-linolenic acid (C18:3/omega 3), 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 causes 10.0- and 21.2-fold faster oxidation in these polyunsaturated fatty acids (PUFA) than oleic acid. The high level of PUFA in the soybean oil renders it susceptible to fast oxidation and development of fishy smell in the stored soybean oil. To avoid the fast oxidation in soya oil, oil industries employ partial hydrogenation. The process of partial hydrogenation employed by oil industries leads to generation of trans fats, which are diabetogenic, atherogenic and carcinogenic (De Souza et al. 2015). Food safety regulatory bodies in several countries

including India 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). Therefore, genetically increasing oleic acid and reducing α-linolenic acid in soybean seed oil is one of the most important breeding objectives for soybean-growing countries (Kumar et al. 2004). The oil with high oleic acid and low α-linolenic acid possesses improved oxidative stability, flavour and storability and, therefore, obviates the need of cost-incurring and health-hazardous process of partial hydrogenation.

Oleate desaturase catalyses the conversion of oleic acid (C18:1) into linoleic acid (C18:2) by inserting a double bond at 12th carbon from the carboxyl end of fatty acid hydrocarbon chain during soybean seed development. 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, FAD 2-1A (Glyma10g42470) and FAD 2-1B (Glyma20g24530) (Schlueter et al. 2007), determines the accumulation of oleic acid. Soybean genotypes carrying mutated alleles of both FAD2-1A and FAD2-1B possess 82–86% oleic acid in the oil extracted from its seeds (Pham et al. 2011). Recently, genomic regions associated with other than candidate genes for the biosynthesis of oleic acid have been recently reported (Rani et al. 2019). Reduction of α-linolenic acid in soybean oil has been accomplished by modulating the desaturase which inserts a double bond at 15th carbon from carboxyl end, thereby converting linoleic to α-linolenic acid. The activity of this desaturase is governed by at least three loci, namely, FAD3A/fan1(Glyma.14g194300), FAD3B/fan2 (Glyma.02g227200) andFAD3C/fan3 (Glyma.18g062000) 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; Chappell and Bilyeu 2006, 2007), FAD3B (Reinprecht et al. 2009) and FAD3C (Bilyeu et al. 2005) have been reported to lower α-linolenic acid content. Bilyeu et al. (2011) was successful in combining mutants of all three FAD genes in producing soybean lines with linolenic acid $< 1\%$ using allele specific markers. 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. Alternatively, transgenic plants homozygous for the cleaved conserved sequences in FAD2-1A and FAD2-1B with elevated levels of oleic acid have also been developed (Haun et al. 2014).

11.3.5 Pyramiding the Desirable Quality Traits

Specialty soybean genotypes which are genetically free from anti-nutritional factor Kunitz trypsin inhibitor and off-flavour-producing lipoxygenases are much sought-after raw material in the soyfood processing. In India, soybean genotype NRC142 carrying null alleles of Kunitz trypsin inhibitor and lipoxygenase-2, which is the

Fig. 11.1 Field view of 'NRC 142,' the genotype pyramided for null alleles of KTI and lipoxygenase-2 gene through marker-assisted selection

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 SSR markers linked to both Ti and Lox2 locus (Oliveira et al. 2007; Rani and Kumar 2018). A field photograph of NRC142 (Fig.11.1), along with the gel depicting the PCR-generated amplicons of null allele-specific markers of lipoxygenase-2 and null Kunitz trypsin inhibitor is presented in Fig. 11.2. Pyramiding of null allele of Kunitz trypsin inhibitor in triple null lipoxygenase would be one of the most desirable commodities for the processing industry.

11.4 Diseases

11.4.1 Rust

Soybean rust (SBR), caused by *Phakopsora pachyrhizi Syd. & P. Syd.*, is a deadly disease of soybean, which can reduce the yield up to 80% (Li et al. 2012). *P. pachyrhizi* was first identified in Japan in 1902 (Hennings 1903). It has gradually spread to soybean-growing countries around the world (Bromfield 1984; Wang and Hartman 1992; Pretorius et al. 2001; Rossi 2003; Yorinori et al. 2005). Though chemical spray containing fungicides can control the disease, it increases the cost of production. Moreover, some of *P. pachyrhizi* races have developed increased

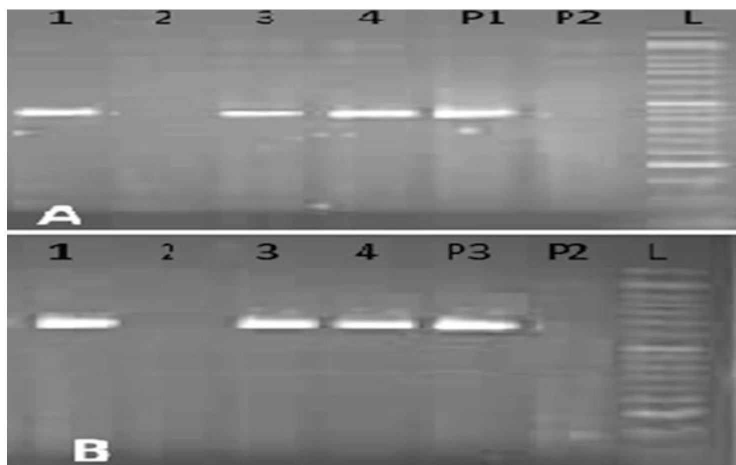

Fig. 11.2 Amplification of null allele of KTI (**a**) and null allele of lipoxygenase-2 gene (**b**) in NRC 142. P1 and P3 represent PI542044, the donor of null allele of KTI (PI542044), and PI596540, the donor of null allele of lipoxygenase-2 (PI596540), respectively. P2 corresponds to recipient genotype JS97-52. L represents 50 bp DNA ladder

tolerance to certain fungicides (Godoy 2009). Development of the varieties genetically resistant to this disease is the most effective measure to control this disease. Scientists worldwide have screened many soybean accessions for resistance or tolerance to soybean rust (Miles et al. 2008; Pham et al. 2010a) and identified 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) (Garcia et al. 2008). Another recessive genes controlling SBR resistance were identified by Calvo et al. (2008). Brogin et al. (2004) identified single sequence repeat (SSR) markers linked to rust resistance present on the variety FT-2 in the linkage group (LG)-C2 of the previous soybean consensus map reported by Cregan et al. (1999). An SBR resistance gene from the variety Hyuuga was mapped at 3 cM interval on LG-C2 between Satt134 and Satt460 (Monteros et al. 2007). Hyten et al. (2007) recently mapped the *Rpp3* locus at the same interval as the report of Monteros et al. (2007). The Rpp1 locus has been mapped to a 1 cM interval on LG-G between Sct_187 and Sat-064. Liu et al. (2016) identified and mapped a new rust-resistant gene in PI 567104B and mapped it on chromosome 18 flanked by the simple sequence repeat (SSR) markers Satt131 and Satt394. 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.

11.4.2 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 USA (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 on 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), and it confers extreme resistance to SMV G1 through G6 (Chen et al. 1991; Hajimorad and Hill 2001). Since then, 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 by Yu et al. in 1994 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 conditions 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, and fine mapping by Shi et al. (2008) led to identification of two closely linked SSR markers: Sat_424 at a distance of 1.5 cM and Satt726 at a distance of 2.0 cM from Rsv3 locus. NBS_C, NBS_D and NBS_E in this genomic region are the likely functional alleles of the Rsv3 locus that confer resistance to soybean mosaic virus (Suh et al. 2011; Redekar et al. 2016; Ma et al. 2017). Rsv4 confers resistance to all seven SMV strains (Chen et al. 1993; Ma et al. 1995). It was identified in soybean cultivar 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 soybean chromosome 2 (Hayes et al. 2000; Maroof et al. 2010; Ilut et al. 2016). This genomic region is devoid of any NBS-LRR gene, and several genes encoding predicted transcription factors and unknown proteins are present within the region (Hwang et al. 2006; 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 on the soybean chromosome number 2 (Wang et al. 2004). Glyma02g13310, 13320, 13400, 13460 and 13470 are the probable candidates genes for Rsc-8 based on their predicted functions and expression patterns (Wang et al. 2011). Another resistance gene Rsc-15 was mapped between Sat_213 and Sat_286 on chromosome 6 (Yang and Gai 2011). The resistance gene Rsc-7 was identified in the soybean cultivar Kefeng No.1 and was mapped to a 2.65-megabase (Mb) region on soybean chromosome 2 (Fu et al. 2006). Yan et al. (2015) identified 15 candidate genes in the 158-kilobase (Kb) region, 1 NBS-LRR-type gene (Glyma02g13600), 1 HSP40 gene (Glyma02g13520) and 1 serine carboxypeptidase-type gene (Glyma02g13620). One of these genes is probable candidates for Rsc-7. 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. This assay consisted of ten SNPs plus one insert/deletion (InDel): two SNPs developed from the candidate gene 3gG2 at Rsv1 locus, two SNPs selected from the clone N11PF linked to Rsv1, one 'BARC' SNP screened from soybean chromosome 13 [linkage group (LG) F] near Rsv1, two 'BARC' SNPs from probe A519 linked to Rsv3, one 'BARC' SNP from chromosome 14 (LG B2) near Rsv3 and two 'BARC' SNPs from chromosome 2 near Rsv4, plus one InDel marker from expressed sequence tag (EST) AW307114 linked to *Rsv4*. The assay developed by them is very useful in marker-assisted development of SMV-resistant soybean varieties. Several breeders have been successful in pyramiding soybean mosaic virus resistance genes using marker-assisted selection (Maroof et al. 2008; Shi et al. 2009).

11.4.3 Yellow Mosaic Disease

Yellow mosaic virus is one of the most destructive and widely distributed plant pathogenic viruses, which causes yellow mosaic disease (YMD) in legumes including soybean (*G. max*), 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 whitefly (*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), were 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. Nucleotide sequences of a virus isolated from soybean plants affected by YMD in India showed 89% similarity with MYMIV; thus, the virus was designated as a soybean isolate of MYMIV (MYMIV-[Sb]) by Usharani et al. (2004). Mungbean yellow mosaic India virus has been reported to infect soybean in India, Vietnam and Indonesia (Nene 1972, 1973; Tsai et al. 2013). YMD resistance genes have been reported in two donors: *G. max* accession PI171443 by Singh and Mallick (1978) and *G. soja* accession PI 393551 by Singh et al. (1974). Yadav et al. (2009) reported accumulation of late viral transcripts and DNA replication in a susceptible cultivar 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 PI 171443. Bhattacharyya et al. (1999) reported a single dominant gene controlling MYMIV resistance in *G. soja* PI 393551, while Rani et al. (2018) reported duplicate dominant gene in this accession. Rani et al. (2017) have mapped MYMIV resistance gene on chromosome number 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. These molecular markers identified by Rani et al. (2017) are being actively deployed for introgression of an MYMIV resistance gene into the predominant cultivars of India. This group has developed NRCSL1, first MYMIV-resistant soybean variety for south zone using marker-assisted forward breeding, and NRCSL2, essentially derived variety (EDV) of JS335, the most popular variety of India, through marker-assisted backcross breeding. Though resistance from the PI171443 accession of *G. max* has been used in several soybean cultivars developed for the northern plains of India even before identification of molecular marker linked to the gene, resistance gene from *G. soja* PI393551 could not be deployed in cultivar development, mainly because of the fact that many backcrosses are required to introgress these genes into *G. max* cultivars, as most of the *G. soja* genome is not useful in cultivated soybean. Backcrossing without a marker linked closely to a desirable trait is very difficult because segregating material that is generated for introgressing an MYMIV resistance gene into high-yielding adapted cultivars must be screened at hot spots or under artificial conditions (Rani et al. 2018). As all MYMIV-resistant soybean varieties of India carry resistance gene from the same donor PI171443, it is important to pyramid resistance gene from other sources for durable resistance to MYMIV in the event of breakdown of resistance to single resistance derived from PI171443. Pyramiding genes conferring resistance to a particular disease would be impossible without molecular markers in the absence of well-characterized strains of causal organisms. 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. These molecular markers linked to MYMIV resistance in *G. soja* are being used to pyramid resistance genes from both *G. soja* and *G. max*.

11.5 Future Prospects

A wide range of tools and genomic resources are available in soybean, which are easily accessible from public databases such as Soybean Knowledge Base (SoyKB) (https://omictools.com/soykb-tool), SoyBase (https://soybase.org) and Phytozome (https://phytozome.jgi.doe.gov/pz/portal). SoyKB is a comprehensive web resource developed for bridging soybean translational genomics and molecular breeding research. It provides information for six entities including genes/proteins, microRNAs/sRNAs, metabolites, single nucleotide polymorphisms and plant introduction lines and traits. It also incorporates many multi-omics datasets including transcriptomics, proteomics, metabolomics and molecular breeding data, such as quantitative trait loci, traits and germplasm information. SoyBase is a database created by the US Department of Agriculture. It contains genetic information about soybeans. It includes genetic maps, information about Mendelian genetics and molecular data regarding genes and sequences. Phytozome is the Plant Comparative Genomics

portal of the Department of Energy's Joint Genome Institute. Families of related genes representing the modern descendants of ancestral genes are constructed at key phylogenetic nodes. These families allow easy access to clade-specific orthology/paralogy relationships as well as insights into clade-specific novelties and expansion.

Multiplexed chip-based technologies like genotyping by next-generation sequencing using SNPs can generate anywhere from 100 to over a million SNPs per run for large-scale studies but are not economical to use in breeding programmes directed at very specific objectives requiring a smaller number of SNPs to be tracked. For analysing a smaller number of SNPs, a cost-effective uniplex assay like Kompetitive allele specific PCR (KASP) can be used. A set of 1082 KASP™ SNP genotyping assay developed from an original set of 1536 SNP markers, the 'Universal Soy Linkage Panel' (USLP 1.0), is now available for soybean breeder. These assays are very helpful in accelerating development of improved varieties by marker-assisted selection (MAS).

Recently developed speed breeding technique that utilizes commonly used fluorescent lamps (220 μmol m^{-2} s^{-1} at the canopy level), a 14 h light (30 °C)/10 h dark (25 °C) cycle and carbon dioxide (CO_2) supplementation at >400 ppm reduced the generation time of the best-characterized elite Japanese soybean cultivar, Enrei, to just 70 d from 102 to 132 d reported in the field, thereby allowing up to five generations per year instead of the 1–2 generations currently possible in the field and/or greenhouse. This method also facilitates the highly efficient and controlled crossing of soybean plants. Appropriate light and temperature conditions reduce the days to flowering, and the reaping and sowing of immature seeds shorten the reproductive period greatly. Speed breeding method and the efficient and cost-effective molecular tools developed for genotyping will accelerate the future soybean breeding programmes.

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Chapter 12
Efficient Improvement in an Orphan Legume: Horsegram, *Macrotyloma uniflorum* (Lam.) Verdi, Using Conventional and Molecular Approaches

Rakesh Kumar Chahota, Nisha Thakur, and Reecha Sharma

12.1 Introduction

Food legumes stand as an important group of crops after the cereals since millennia and have been an essential ingredient of balanced human diet (Bhadana et al. 2013). For human and animal nutrition, it is recognized as the second most valuable plant source (Bhatt and Karim 2009). The ever-increasing demands of vegetable protein and inevitable search for new protein sources have attracted the scientific community towards the underutilized crops (Pugalenthi et al. 2005). A significant contribution to the diet of the rural households particularly during drought, famine and dry season is made by the underutilized crops (Magbagbeola et al. 2010). Besides, in many cases these prove to be the lifesavers for millions of resource-poor people in the regions where ensuring food and nutritional security is one of the main problems, particularly in traditional subsistence farming systems (Haq 2002). Horsegram [*Macrotyloma uniflorum* (Lam.) Verdc] (Fig. 12.1) is an orphan and/or underutilized crop largely grown locally by communities as cultural heritage in special niches in developing countries (Chahota et al. 2013; Uma et al. 2013; Ellis 2016; Mall 2017; Fuller and Murphy 2018). Orphan crops are a diverse set of minor crops that tend to be regionally important but not traded around the world and as such have received little attention from research networks (Bhartiya et al. 2015; Cullis and Kunert 2017). Among various orphan crops, horsegram is an important legume with the potential to be developed into a commercial crop (Cullis and Kunert 2017). However, they play a significant role in many developing countries, providing food security and to alleviate protein and micronutrient deficiencies associated with the predominance of dietary calories from rice and wheat, which are researched heavily by private corporations (IPGRI 2002). They have been largely neglected by both scientific community and industry due to their limited economic importance in the global

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Fig. 12.1 Cultivated horsegram

market when compared to commodities such as rice, corn and wheat (Deodhar 2016; Foyer et al. 2016; Kamei et al. 2016). Among underutilized crops, horsegram has special significance as a legume of indemnity under harsher and more dynamic environmental conditions (Aditaya et al. 2019). Cultivated horsegram is a diploid plant species with chromosome number of $2n$ = 20, 22 and 24. The estimated genome size of horsegram is 400 Mb (Bhardwaj et al. 2013; Hirakawa et al. 2017). Wild members of genus *Macrotyloma* exist in Africa, Australia and India; however, India is regarded as the centre of origin of cultivated horsegram (Vavilov 1951a; Zohary 1970). The origin of cultivated horsegram is not well understood, but the region of maximum genetic diversity is considered to be in the Old World Tropics, especially in India and the Himalayas (Zeven and deWet 1982). The crop as a legume is cultivated mainly in the Indian continent. Genus *Macrotyloma* consists of nearly 25 species indigenous to Africa, Australia and Asia (Verdcourt 1971), and *Macrotyloma uniflorum* var. *uniflorum* is the only cultivated species (Allen and Allen 1981; Lackey 1981). Horsegram is used as an important pulse crop since seeds are rich in protein and consumed in majority by poorest section of the society. Horsegram is an excellent source of protein (17.9–25.3%), carbohydrates (51.9–60.9%), low content of lipid (0.58–2.06%), essential amino acids, energy, iron (Bravo et al. 1999; Sodani et al. 2004), molybdenum (Bravo et al. 1999),

phosphorus, iron and vitamins such as carotene, thiamine, riboflavin, niacin and vitamin C (Sodani et al. 2004). Seeds of horsegram are reported to be high in tannins and polyphenols compared to other legumes (Kadam and Salunkhe 1985), have high antioxidant properties, have many medicinal and therapeutic benefits and are suggested as an Ayurvedic medicine used to treat oedema, piles and renal stones. Seeds of *M. uniflorum* contain insoluble dietary fibres, which are required in normal functioning of lower intestine (Anderson et al. 1994; Kawale et al. 2005; Kang et al. 2006). Horsegram is, however, consumed as sprouts in many parts of India (Ghorpade et al. 1986). The grains are consumed like other pulses for protein requirement. Besides protein, it is also a cheap valuable source of calcium and vitamins (Katiyar 1984).

Owing to its medicinal importance and its capability to thrive under drought-like conditions, the US National Academy of Sciences has identified this legume as a potential food source for the future (National Academy of Sciences 1978). India is the only country cultivating horsegram on a large acreage, where it is used as human food. However, horsegram is a versatile crop and can be grown from near sea level to 1800 m (Asha et al. 2006). It is highly suitable for rainfed and marginal agriculture but does not tolerate frost and waterlogging. It is a drought-tolerant plant and can be grown with rainfall as low as 380 mm. The limited use of dry seeds of horsegram is due to its poor cooking quality. The stems, leaves and husks are used as fodder and being rich in protein are widely used as a feed to milch animals and horses (Blumenthal and Staples 1993). Although horsegram is grown as an important crop of India especially South India, surveys have shown that its cultivation has been drastically reduced which indicates its replacement by other crops which have the higher demands. According to Uma et al. (2013), horsegram has also been called the poor man's pulse in southern India where the seeds are parched and then eaten after boiling or frying, either whole or as a meal.

12.2 Genetic Resources and Distribution

The Germplasm Resources Information Network (GRIN) of the US Department of Agriculture (USDA) has conserved only 35 accessions of horsegram in its gene bank. National Gene Bank of Kenya, Crop Plant Genetic Resources Centre, Kenya Agricultural Research Institute (KARI), Kikuyu, Kenya, has 21 accessions in its gene bank, and 200 accessions are in Ethiopia. The Australian Tropical Crops and Forages Genetic Resources Centre, Biloela, Queensland, has 38 accessions of horsegram germplasm (Brink 2006). National Bureau of Plant Genetic Resources (NBPGR) in New Delhi has the maximum number of systematic collection of this important legume and presently has about 2100 accessions stacked in National Gene Bank. The efforts to collect and conserve the horsegram germplasm started way back in the 1970s with the inception of the PL480 scheme (a scheme under collaboration between Indian Council of Agricultural Research (ICAR) and the USDA project on food security in Haiti, using Public Law 480), and since then

germplasm has been collected from almost all the horsegram-growing areas. Under different exploration and collection programmes, a total of 1627 accessions of horsegram have been collected and maintained at different satellite stations of NBPGR.

The most closely related species of *Macrotyloma uniflorum* is *Macrotyloma axillare* having many desirable traits such as large number of pods/plant, higher seed yield/plant and tolerant to biotic and abiotic stresses. Similarly, *Macrotyloma sargarhwalensis* is another wild species native to northern India having 39.5% protein contents (Yadav et al. 2004). These desirable traits can be introgressed from such wild ancestors to cultivated species to broaden the narrow genetic base of cultivated *M. uniflorum*. The horsegram plant belongs to the kingdom Plantae, subkingdom Tracheobionta, division Magnoliophyta and class Magnoliopsida. The genus *Macrotyloma* (Wight & Arn.) Verdc. – *Macrotyloma* of family Fabaceae – consists of about 25 wild species (Table 12.1) having the chromosome numbers $2n = 2x = 20$ and $2n = 2x = 22$ (Allen and Allen 1981; Lackey 1981; Cook et al. 2005).

Table 12.1 Geographical distribution of *Macrotyloma* species

S. No.	Species name	Area of distribution
1	*Macrotyloma africanum* (Wilczek) Verdc.	Africa
2	*Macrotyloma axillare* (E.Mey.) Verdc.	Africa and Australia
3	*Macrotyloma bieense* (Torre) Verdc.	Africa
4	*Macrotyloma biflorum* (Schum. & Thonn.) Hepper	Africa
5	*Macrotyloma brevicaule* (Baker) Verdc.	Africa
6	*Macrotyloma ciliatum* (Willd.) Verdc.	Asia and Africa
7	*Macrotyloma coddii* Verdc.	Africa
8	*Macrotyloma daltonii* (Webb) Verdc.	Africa
9	*Macrotyloma decipiens* Verdc. Africa	Africa
10	*Macrotyloma densiflorum* (Baker) Verdc	Africa
11	*Macrotyloma dewildemanianum* (Wilczek) Verdc.	Africa
12	*Macrotyloma ellipticum* (R.E.Fr.) Verdc. Africa	Africa
13	*Macrotyloma fimbriatum* (Harms) Verdc. Africa	Africa
14	*Macrotyloma geocarpum* (Harms) Marechal & Baudet	Africa
15	*Macrotyloma hockii* (De Wild.) Verdc. Africa	Africa
16	*Macrotyloma kasaiense* (R. Wilczek) Verdc.	Africa
17	*Macrotyloma maranguense* (Taub.) Verdc.	Africa
18	*Macrotyloma oliganthum* (Brenan) Verdc.	Africa
19	*Macrotyloma prostratum* Verdc. Africa	Africa
20	*Macrotyloma rupestre* (Baker) Verdc. Africa	Africa
21	*Macrotyloma schweinfurthii* Verdc. Africa	Africa
22	*Macrotyloma stenophyllum* (Harms) Verdc.	Africa
23	*Macrotyloma stipulosum* (Baker) Verdc.	Africa
24	*Macrotyloma tenuiflorum* (Micheli) Verdc.	Africa
25	*Macrotyloma uniflorum* (Lam.) Verdc.	Asia, Africa and Australia

Source: Chahota et al. (2013)

Since this crop comes up reasonably well in dry land areas with receding soil moisture conditions and in poor soils where other crops fail to grow, there is also high probability that this plant is also thought to contain a large number of drought resistance genes (Reddy et al. 2008).The crop is generally also not invaded by many pests leading to less yield loss which again makes it a probable gene pool for disease resistance. Due to the varied reasons discussed above, farmers still grow horsegram despite its low yield potential and less attention paid by the policy makers and plant breeders. Over the years, the production and area under this crop has been decreasing tremendously. One of the major bottlenecks is the non-availability of high-yielding and well-adapted genotypes, which can perform better under higher input conditions as this crop is mostly cultivated as intercrop with maize. The high-yielding varieties/lines developed and grown in other parts of the country were found unsuitable and could not perform better when grown in northern hill zones due to photoperiod sensitivity (Chahota et al. 2005). In central India, major districts of Maharashtra such as Ahmednagar, Solapur, Sangli, Nasik, Dhule, Jalgaon, Aurangabad, Jalna, Beed, Chandrapur, Gadchiroli, Thane, Ratnagiri and Sindhudurg cultivate this crop. In Tamil Nadu horsegram is grown mainly during northeast monsoon season (September–January). It is cultivated as a sole crop or as an intercrop in sorghum/ragi or a relay crop. The horsegram cultivars grown in different seasons are adapted to specific day lengths. The types grown in monsoon season are day-neutral, whereas the types in post-monsoon (rabi) season are short-day plants (Balasubramanian 1985). The germplasm accessions of horsegram are being maintained and characterized every year in regional research stations Jodhpur and Thrissur.

12.3 Conventional Breeding Strategies

The conventional variety development programme in horsegram is mainly based on morphological selection for desirable traits either from natural occurring germplasm or from segregating population after hybridization. This may be due to the lack of genetic and genomic information in this crop. Moreover, the presence of many undesirable traits such as twining growth habit and longer days to maturity accompanied by asynchrony, photosensitivity and indeterminate growth habit restricts the commercial cultivation of horsegram. The non-availability of desirable traits in Indian germplasm further aggravated the problem to initiate a successful breeding programme. Furthermore, it is felt that before embarking upon a breeding programme to incorporate desirable traits in different backgrounds to release these modified plant types as a new variety, it is important to know the genetics of different traits. However, lack of genetic information and genetic variability for important agronomic traits in horsegram is a major bottleneck to envisage a scientific breeding programme. Genetic diversity can be created by evaluation of horsegram lines for diversity, by inducing mutations and by hybridization.

A number of studies have been conducted for identifying variability in available natural germplasm. In India, a total of 2100 accessions of horsegram are conserved in the national gene bank, and out of these 1161 accessions were characterized during 1999–2004. While studying on agro-morphological traits in Indian *Dolichos* germplasm, Latha (2006) observed that yield and yield component traits in all promising lines with higher seed yield are of long duration type. Germplasm evaluation for assessment of genetic variability for 12 agro-morphologic characters in 63 horsegram accessions procured from NBPGR, Phagli, Shimla, was carried out by Chahota et al. (2005) at CSK HPKV Palampur (Figs. 12.2 and 12.3). Mahajan et al. (2007) had evaluated ten lines for agro-morphological traits at Vivekananda Parvartiya Krishi Anusandhan Sansthan (VPKAS), Almora. Kulkarni and Mogle (2011) and Kulkarni (2010) identified five high-yielding genotypes by evaluating 22 germplasm lines for different agronomic traits. In addition, a considerable number of attempts have been made on assessing genetic diversity by various research workers (Subba and Sampath 1979; Sharma 1995; Sudha et al. 1995; Tripathi 1999; Prakash et al. 2010; Rana 2010). The efforts are still lacking for harnessing this information for developing varieties and introgressing desirable traits disseminated in different genotypes. Sreenivasan (2003) conducted hybridization studies between photosensitive and day-neutral varieties with black- and brown-coloured seeds and revealed that photoperiod response is a qualitative trait that is controlled by at least two genes. The black seed colour was observed to be dominant over brown, and two genes in polymeric gene action were found to control seed colour. Most of the horsegram varieties released for cultivation in different states in India originated from the local germplasm following their effective and specific evaluation. The varieties developed in different states (Table 12.2) include BR 5, BR 10 and Madhu from Bihar; HPK-2 and HPK-4 from Himachal Pradesh; PDM 1 and VZM 1 from Andhra Pradesh; K82 and Birsa Kulthi from Jharkhand; S27, S8, S39 and S1264 from Orissa;

Fig. 12.2 Germplasm evaluation under field conditions

Fig. 12.3 Germplasm evaluation under polyhouse conditions

Table 12.2 Improved varieties released by different states in India for cultivation

S. No.	Variety	Place of release
1	BR 5, BR 10 and Madhu	Bihar
2	HPK-4 and VLG 1	Himachal Pradesh
3	PDM 1 and VZM 1	Andhra Pradesh
4	K82 and Birsa Kulthi	Jharkhand
5	S27, S8, S39 and S1264	Orissa
6	Co-1, 35-5-122 and 35-5-123	Tamil Nadu
7	Hebbal Hurali, 2 PHG 9 and KBH 1	Karnataka
8	Maru Kulthi, KS2, AK 21 and AK 42	Rajasthan
9	VLG 1	Uttarakhand

Source: Chahota et al. (2013)

Co-1, 35-5-122 and 35-5-123 from Tamil Nadu; Hebbal Hurali 2, PHG 9 and KBH 1 from Karnataka; Maru Kulthi, KS 2, AK 21 and AK 42 from Rajasthan and VLG 1 from Uttarakhand. Some of the improved varieties developed through single plant selection from the bulk collected included Co-1. No 35-5-122 and 123. Hebbal Hurali 1 and 2 were developed by Kumar (2005) by the single plant selection. Many workers have evaluated germplasm, but the results are not encouraging due to the limited genetic variability available for various traits.

Wild forms of horsegram have also been reported in the Western Ghats, especially in the wildlife sanctuaries. *Macrotyloma ciliatum* (Willd.) Verdc. is found in Tamil Nadu (Mathew 1983; Nair and Henry 1983) and Andhra Pradesh (Pullaiah and Chennaiah 1997). *Macrotyloma sar-garhwalensis* is a wild relative of horsegram found in the Central Himalayas of India (Gaur and Dangwal 1997). It is having a high protein content of 38.35% and is a non-twining annual herb, which can be utilized in the breeding programmes for the improvement of protein content (Negi

et al. 2002). Two other species of this genus *Macrotyloma axillare* and *M. Africanum* have also exhibited potential as forage plants.

12.3.1 Mutation Studies

The non-availability of important traits in the germplasm has encouraged many workers to induce desirable traits by using gamma radiation and chemical mutants. Mutation breeding is a best method identified to enhance the genetic variability in crops within short time specifically. In crops which lack the desirable traits in germplasm. Horsegram is also a classical case in which the germplasm is devoid of desirable traits like early maturity, determinate growth habit and dwarf plant type. Many workers have used different types of mutants to induce desirable traits in horsegram. At CSK HPKV Palampur mutation, breeding was started by treating HPKC-2 and VLG-1 varieties of horsegram with gamma radiation and EMS (Chahota 2009). Three doses of gamma radiation, 150, 250 and 350 Gy, were used to irradiate 1000 seeds each. The M_2 generation exhibited three types of mutations, which were morphologically different from the parental lines for one or the other traits. The first group consisted of mutants, namely, M 191, M 193 and M 249, were of semi-dwarf, determinate, photo-insensitive and flowering very early with early and synchronous maturity. The second group of mutants, viz. M 317, M 318, M 319, M 321 and M 322, were dwarf, determinate accompanied by bushy growth habit and photo-insensitive with synchronous medium maturity, Whereas, the third group of mutants, viz. M 150, M 151 and M 201, were only differed for seed colour. All these mutants were of immense practical significance as these can be used directly as a new variety with improved agronomic traits or can be a novel source for these traits in future breeding programmes.

Bolbhat and Dhumal (2012) used gamma radiation (100–600 Gy) and EMS (0.2–0.6%) separately and in combination of both by utilizing the variety Dapoli Kulthi-1 to induce variations and studied the effect on seed germination as seedling damages were assessed in M_1 generation. With increase in doses of mutagenic treatment, there was gradual reduction in seed germination, root length, shoot length and seedling height as doses were increased in M_1. Various viable macro mutations were scored for plant height, primary branches, pods per plant, seeds per pod, pod length, 1000 grain weight and yield per plant in M_2 generations. Other workers such as Gupta et al. (1994), Jamadagani and Birari (1996) and Ramakanth et al. (1979) tried induced variability through mutations for various traits in horsegram. Similarly, CRHG-6 and CRHG-8 lines of horsegram have been registered by Plant Germplasm Registration Committee (PGRC) of ICAR in July 2011 with registration number INGR 11017 and INGR 11018, respectively, and are released in South India. The variability was induced through physical mutagen, γ-ray irradiation of seeds of parental lines. These improved varieties were found to be non-shattering and tolerant to yellow mosaic virus, powdery mildew and mites (Salini et al. 2014).

12.3.2 Wide Hybridization

The major limitation in the improvement of horsegram is the absence of pre-breeding programme, as well as the lack of variability in cultivated horsegram germplasm both at the morphological and molecular level. Hence, wide hybridization could be an important tool to create more variability for broadening its genetic base. Though the genus *Macrotyloma* consists of more than 25 species, there is no account regarding the evaluation of these wild species for desirable traits. The two species of genus macrotyloma namely; *M. uniflorum* and *M. axillare* were compared by Morris (2008), and used a set of descriptors to differentiate these species. The most closely reported related species of cultivated horsegram is *Macrotyloma axillare* having many desirable traits such as large number of pods/plant, higher seed yield/plant and tolerant to biotic and abiotic stresses. Similarly, *M. sar-garhwalensis* is having 39.5% protein contents (Yadav et al. 2004). It is important to conduct evolutionary studies on horsegram so that improved breeding programmes can be carried out for horsegram. Karyotype analysis has been useful in drawing phylogenetic and evolutionary relationships between some related species and species groups. Evaluation of few wild species of *Macrotyloma* has been undertaken at the CSK, Himachal Pradesh Agricultural University, Palampur, India, to initiate a systematic hybridization programme involving cultivated and wild species to transfer desirable traits from *M. axillare* and *Macrotyloma sar-garhwalensis* to cultivated background. *Macrotyloma axillare* has many beneficial traits such as high number of pods per plant, high seed yield per plant and tolerance to cold and drought conditions (Staples 1966, 1982). The cultivated species of *M. uniflorum* is infected by a number of diseases, particularly in high rainfall areas, such as anthracnose, Cercospora leaf spot, Fusarium wilt, Pellicularia root rot and Aschochyta blight. In spite of the fact that *M. axillare* is reported to have resistance against many diseases, hybridization between *M. uniflorum* and *M. axillare* resulted in juvenile flowering in the first year of F_1 plant, hence prolonging the breeding process. Chromosomes were seen to exhibit variation in sizes and secondary constrictions. *M. sar-garhwalensis* manifested larger chromosome followed by *M. axillare* and *M. uniflorum*. This may be the main cause that during meiosis laggards were reported resulting the sterile pollen grain in the cross of *M. axillare* x *M. uniflorum* (Fig. 12.4).

12.4 Conventional Versus Molecular Approach

The ultimate goal of both conventional plant breeding and molecular breeding is to develop crops with improved characteristics by changing their genetic make-up with desirable traits and make it suitable for cultivation in varied agro-climatic conditions. In conventional breeding the main focus is to create the variability by various means and select the desirable one from the segregating populations. The selection process is very crucial it either depends upon the visual observation of the

Fig. 12.4 Sterile F1 plant of *M. axillare x M. uniflorum*

breeder or on various biometrics tools to find suitable one. However, the quantitative traits are mostly influenced by the environment, this type of selection does not always yield results, whereas in molecular approach, this is done by adding a specific new gene or genes to the genome of a crop plant. This can be done either to produce transgenic by recombinant DAN technology or to select desirable plant type based on molecular markers. The most common molecular approach employed in this crop is the use of molecular markers for efficient selection of the desired traits. The practical application of molecular markers is in the form of marker-assisted selection (MAS) is the first step for the tagging or mapping of such traits with the help of molecular markers. It seems that new characteristics can be introduced into lines of our interest using both conventional and molecular approaches. Now, the question arises in the mind of a plant breeder whether to choose a conventional approach or a molecular approach.

With the increasing knowledge about plant systeme and genes, now we understand that many genes contribute to improving sustainable food production. In some cases, conventional breeding will be the best way to deploy these genes that is by cross-breeding with the suitable plant. For example, if a useful gene or gene variant

is discovered in a wild relative, crossing the well-adapted variety with the wild relative may result in mixing together the genomes of the two parents, deploying the carefully selected combination of genes in the high yield line. However, the major limitation in the conventional breeding is the selection of desirable recombination.

To overcome the selection problem in conventional breeding, modern molecular techniques such as 'marker-assisted breeding' can be employed in tandem for desired results. This is possible to reassemble those gene combinations over a relatively small number of generations with relatively less time and resources. Marker-assisted breeding (MAB) is a combined product of traditional genetics and molecular biology (Ribaut and Hoisington 1998) and is one of the simpler methods compared to phenotypic screening especially for traits with laborious screening. We generally prefer selection at seedling stage, which includes the important agronomic traits such as grain quality, resistance factor, abiotic stress, etc.

12.5 Molecular Approach for Genetic Improvement

Within the last two decades, different types of markers have been developed in different crops with their extensive implications in crop breeding (Paux et al. 2012). Of these markers, simple sequence repeats (SSRs) are widely used due to their co-dominant inheritance, multi-allelic nature, high reproducibility and transferability, extensive genome coverage and simple detection (Varshney et al. 2005; Agarwal et al. 2008). These markers have been widely used for genetic mapping, marker-assisted selection, genetic diversity analysis and population genetics. Development of such markers can be easily achieved by using available sequence information, thereby saving enormous resources. Few years ago this crop was considered as the resource-poor crop, but now sufficient marker information has been developed in this crop (Sharma et al. 2015a, b; Chahota et al. 2017; Kaldate et al. 2017). The number of microsatellite/SSR has been shown to be highly variable within and between species and subspecies. Because of their high mutation rate, they constitute the molecular markers with the highest polymorphic information content. To deal with the molecular analysis, first initiative was started with to study the genomic information about the *M. uniflorum* plant. This underutilized warm season orphan crop belongs to subfamily Papilionoideae of Phaseoloids clade, which also includes *Glycine*, *Phaseolus*, *Vigna* and *Cajanus* sp. (Doyle and Luckow 2003; Gept et al. 2005). The complete genomic information is available for the two model legumes, *Medicago truncatula* and *Arabidopsis*, but that may not be very useful in horsegram due to its distance from the warm season grain legumes, as they are in another clade. The recently sequenced *Cajanus cajan* genome can act as the model plant for these orphan warm season legume crops. Sequence information available in *C. cajan* can be crucial in understanding comparative genomics of horsegram. Therefore, the development of genomic resources in horsegram was started with the study to identify translational genomic information available in the well-characterized model plant species. In horsegram this work was started by acquiring the 384 COS markers

developed from *Medicago truncatula* by Cook's Lab, UC Davis, and 200 SSRs of *Cajanus cajan* from ICRISAT 500 *Trifolium pratense* from Kazusa DNA Research Institute, Chiba, Japan, and 200 SSRs of *Lens culinaris* from NIPGR, New Delhi, *Pisum sativum* and *Cicer arietinum*. These marker resources were used for constructing linkage maps and identifying genomic regions linked to traits of agronomic value. Such cross-species genetic information may be very important for 'orphan crops' that have limited or no genomic resources available.

In case of horsegram, plant is considered unsuitable for commercial cultivation due to presence of undesirable traits, viz. longer days to maturity accompanied by asynchrony, photosensitivity and indeterminate growth habit. Some research work on the development of suitable ideotype as well as to study the transferability of genomic simple sequence repeat (SSR) markers of related legume species to prepare a framework genetic linkage map (Fig. 12.5) of horsegram has been initiated at CSK, Himachal Pradesh Agricultural University, Palampur, since 1995 (Chahota et al. 2013).

The first sincere effort to develop the SSR for *Macrotyloma uniflorum* was reported when transcriptome studies were undertaken in the shoot and root tissues of drought-sensitive line (HPKM-191) and drought-tolerant (HPKM-249) genotypes to decipher the response of two genotypes against drought stress at expression of gene at RNA level. A total of 43% of SSRs predominant with mono- and tri-nucleotides with 21,887 unigenes were identified. During drought conditions plants show resistance through various expressions of enzymes, viz. serine/threonine protein kinase, as well as transcription factors NAC, MYB-related and WRKY belonging families which were found to predominate under stress conditions (Bhardwaj et al. 2013). These TFs are found to be improving drought tolerance and pest resistance, reducing water loss by regulating stomatal movement, increasing transpiration efficiency and regulating embryogenesis, chromatin-mediated transcription and systematic acquired resistance (SAR) in plants like soybean, peanut, chickpea and groundnut (Dai et al. 2007; Govind et al. 2009; Wang et al. 2009, 2010; Hiremath et al. 2011; Van-Verk et al. 2011; Gahlan et al. 2012; Guimaraes et al. 2012). We employed the next-generation Illumina sequencing platform to develop a large number of microsatellite markers in this species. Of the total 23,305 potential SSR motifs, 5755 primers were designed (Chahota et al. 2017). Of these, 1425, 1310, 856, 1276 and 888 were of di-, tri-, tetra-, penta- and hexa-nucleotide repeats, respectively. Thirty polymorphic SSR primers and 24 morphological traits were used in 360 horsegram accessions to detect the genetic diversity and population structure. Thirty primers amplified 170 polymorphic alleles with an average of 5.6 alleles per primer having size 80–380 bp. The polymorphism information content (PIC) ranged from 0.15 to 0.76 with an average of 0.50, suggesting that SSR markers used in the study were polymorphic and suitable for characterization of horsegram germplasm. Dendrogram based on Jaccard's similarity coefficient and neighbour-joining tree grouped the horsegram accessions into two major clusters. Similarly, STRUCTURE analysis assigned genotypes into two gene pools, namely, Himalayan origin and southern India. Diversity analysis based on 24 agro-morphological traits also suggested the presence of high

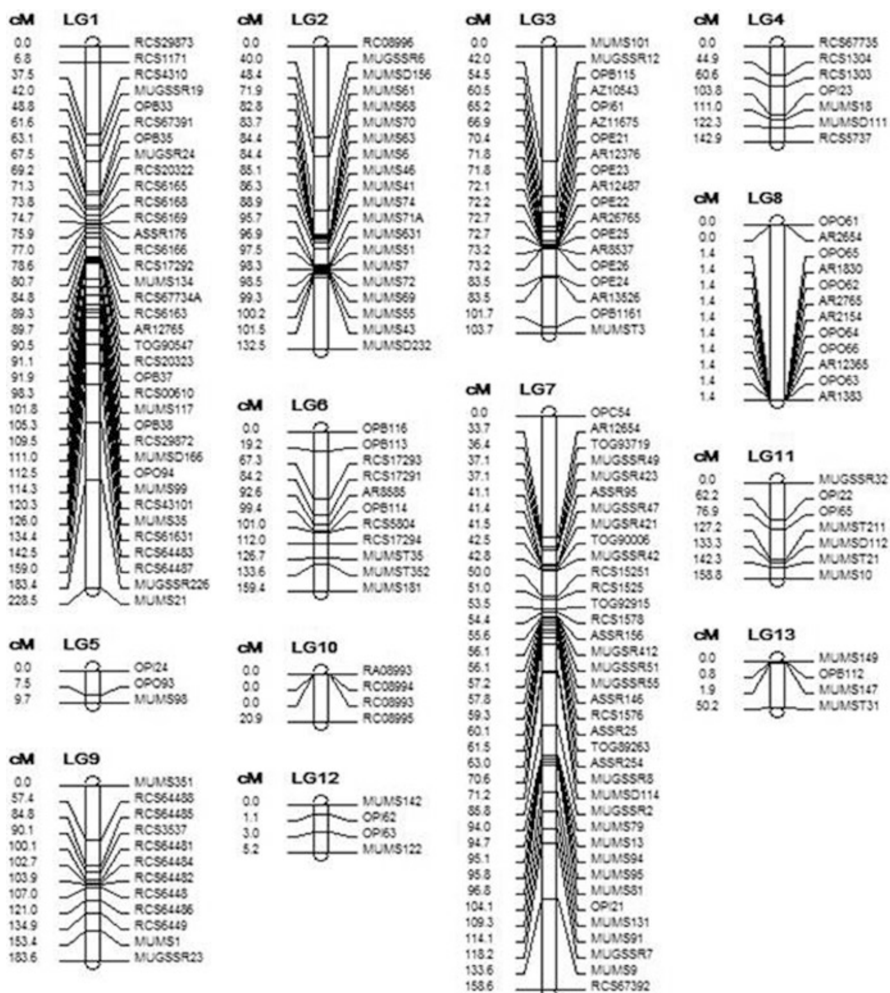

Fig. 12.5 Framework linkage map of horsegram

level of diversity among the accessions. Variation among different cultivars of horsegram (*Macrotyloma uniflorum*) was analysed using different molecular markers. COS, RAPD and SSR markers are generally used to reveal the genetic relationship among horsegram cultivars. A total of 63% variation was reported within the population along with 37% of genetic variation found among horsegram populations (Sharma et al. 2015a).

Furthermore additional SSR and ILP markers in *Macrotyloma uniflorum* were developed at CSK HPKV Palampur and studied the cross transferability, which provide complete genomic architecture for mapping analysis (Sharma et al. 2015b). Authors reported a total of 617 unigenes/contigs in the horsegram in which there were only 84 (14%) having SSR sequences with variable types of

repeat motifs. Maximum numbers of repeat units were tri-repeats (32%) followed by tetra-repeats (28%), di-repeats (23%), penta-repeats (8%) and hexa-repeats (7%). From these 84 SSR-containing sequences, 63 EST-SSR and 13 ILP primers were designed, and 169 primers were synthesized and validated in 20 horsegram accessions. Results attributed that only 218 primer pairs were amplified in which 216 were cross-transferable to other legume species. The primers synthesized showed observed mean values of heterozygosity and PIC (0.518) which revealed high genetic variability in the crop species. These novel SSRs and ILPs developed by the authors can be used in other legume species, which showed at least 69% conservation within genus *Macrotyloma* in which intra-specific transferability level was found higher compared to other cross-transferable markers of different legumes (Peakall et al. 1998; Gupta et al. 2012; Xu et al. 2012). The range of transferability varied from 25.5% in *Glycine max* to 68.0% in *Vigna umbellata* which proved the utility of these novel markers for legume species which has scanty number of SSR markers. These novel SSR and ILP markers developed in the crop species are being utilized in diversity analysis, genetic mapping and QTL analysis of important traits in horsegram. The information generated can also be used in related legume species having limited genome resources. Further, polymorphisms obtained by these markers detect high allelic diversity in horsegram accessions as compared to other legume species, viz. *Lens culinaris* (Liu et al. 2008; Babayeva et al. 2009), *Phaseolus vulgaris* (Blair et al. 2009, 2011; Burle et al. 2010), *Pisum sativum* (Xu et al. 2012), *Cicer arietinum* (Choudhary et al. 2012) and *Cajanus cajan* (Dutta et al. 2011).

Recent advances in next-generation sequencing (NGS) technologies have greatly facilitated the ability to sequence the genome and transcriptomes of several plant species (Thudi et al. 2011). The identification of gene regulatory elements is still a major challenge for molecular biologists. During this post-genomic era, the experimental methods can be complemented with bioinformatic approaches to identify transcription factors (TFs) or factor families responsible for gene expression regulation. Once a regulatory region is delineated experimentally, in silico approach of high-throughput technologies can be used for prediction of gene pattern recognition programmes. Being the underutilized one, but still a ‘future crop’, Kaldate et al. (2017) used next-generation sequencing (NGS) technology for genome-wide development and characterization of novel simple sequence repeat (SSR) markers in horsegram. A total of 2458 SSR primer pairs were designed from NGS data, and 117 SSRs were characterized in 48 diverse lines of horsegram in which they found high mean value of heterozygosity (Ho; 0.64) as compared to expected heterozygosity (He; 0.54). The novel SSR markers developed can be enormously useful for future genetic improvement of horsegram in terms of large-scale diversity analysis, linkage mapping, QTL detection and association mapping studies Chahota et al. 2020. In addition, they can be valuable for conducting similar genetic analyses in other related legume crops devoid of SSR marker resources.

12.6 Conclusion

Horsegram though a lesser known legume but an important source of proteins for underprivileged community of Indian subcontinent. It is being cultivated on the most diverse climatic conditions having very poor soils and input conditions. It can survive under near drought-like situations and give good returns to the farmers when no other legume can be cultivated. It contains several nutritive and anti-nutritive substances like protein, fibres, carbohydrates, phytic acid, phenolic acid, enzymatic inhibitors and other essential diet elements. From the prehistoric period, it is well practised for the treatments of various ailments like curing kidney stones, leucoderma and urinary troubles including heart diseases. Besides, it is known to be protective and promote healing effects on acute gastric ulceration produced by excessive alcohol consumption. Despite the presence of many significant properties in the crop plant, the area and production is declining due to its poor plant architecture. The presence of many weedy characteristics such as indeterminate and twining growth habit, photosensitivity, late flowering and asynchronous maturity resulted in unsuitability for modern farming system. In the past many efforts have been made to improve the existing plant structure, but due to non-availability of desirable traits in the germplasm, there was no headway reported. Development of genomic resources in any crop is the prerequisite to initiate molecular breeding strategies for the development of superior cultivars. In horsegram many strategies were employed to develop genomic resources in this resource-poor crop, and now sufficient number of SSR and SNP markers are available which can be used for marker-assisted selection in this crop. All these genetic and genomic resources can be assessed on www.hillagric.ac.in:1005. In the future we can hope that by using genetic and genomic resources, this crop can be exploited to its full potential.

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Chapter 13
Molecular and Conventional Breeding Strategies for Improving Biotic Stress Resistance in Common Bean

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13.1 Introduction

Common bean (*Phaseolus vulgaris* L. 2n = 22) is the world third most important food legume after soybean and peanut, but first in direct human consumption (Broughton et al. 2003). It has originated in Latin America and is reporting to have two primary centres of origin, one in Mesoamerican and the other in Andean regions, which are easily distinguished by molecular means (Blair et al. 2006). It is a major source of highly valuable seed protein and micronutrients (Broughton et al. 2003; Vaz Patto et al. 2015), and it is an important source of nutrients for more than 300 million people, representing 65% of total protein consumed, 32% of energy and a major source of micronutrients, e.g. iron (Fe), zinc (Zn), thiamin and folic acid (Welch et al. 2000; Broughton et al. 2003; Blair et al. 2010; Petry et al. 2015). Since it is in high nutrient content and commercial potential, it holds great promise for fighting hunger, increasing income and improving soil fertility in sub-Saharan Africa and Latin America. Among the main food legume crops, the common bean shows the greatest variation in growth habit, seed characteristics (size, shape and colour) and maturation time. This variability enables its production in a wide range of cropping systems and environments as diverse as the Americas, Africa, the Middle East, China and Europe (Blair et al. 2010). Despite being cultivated for its fresh pods and grains, beans are produced and consumed mainly as dry grain. Major common bean-producing countries are Brazil and Mexico, while the United States, Canada, Argentina and China are all exporting countries. It is also one of the most potential crops of developing countries of Central America, Andean region of South America and of Eastern and Southern Africa and some parts of India (Singh 1999a). In these regions, beans are grown both for subsistence agriculture and for regional markets where they play an important role in food security and income generation.

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Over the decades global common bean production is affected by major biotic stresses, including fungal, bacterial and viral diseases, insect-pests, nematodes and parasitic weeds. Among the biotic stresses, diseases are notorious agents that significantly affect the common bean production. The majority of bean production occurs under low input agriculture on small-scale farms in developing countries. Beans produced by these resource-poor farmers are highly vulnerable to attack by diseases. High-input farmers have more resources to combat these stresses through the use of pesticides, fertilizers and irrigation. Utilization of such inputs, however, can seriously reduce profitability and threaten the environment, and many pests are not effectively controlled with chemicals. Thus biotic stresses, particularly diseases, continue to represent the major constraints on subsistence production and economic yield of common bean. Hence, incorporating genetic resistance into the elite lines/cultivars through conventional, molecular and genomic-assisted breeding strategies is the most economically efficient way of tackling diseases incidence. Therefore, development of elite cultivars with improved resistance to diseases is a primary goal of bean breeding programmes throughout the world. Cultivars with improved host-plant resistance to multiple diseases can reduce reliance on pesticides in high-input systems, avert risk of yield loss from pests in low- and high-input systems and enable more stable bean production across diverse and adverse environments (low precipitation, high humidity, etc.) and poor soil conditions (low fertility, hillsides, etc.).

13.2 Genetic Resources at World Gene Bank

Common bean is threatened by genetic erosion due to nontraditional farming practices where relatively few genotypes are produced in pure stands and, especially in Latin America, to displacement of common bean by more profitable crops. In situ conservation can be of importance especially in countries like Rwanda, Africa and Brazil, where many landraces are found under diverse conditions and where they are often grown in complex mixtures of as many as 20 seed types. There are a large number of collections of *Phaseolus* germplasm collections, which include the germplasm accessions, landrace, wild and domesticated genotypes of the five domesticated *Phaseolus* species, viz. common bean *(P. vulgaris)*, runner bean (*P. coccineus*), year bean (*P. dumosus*), tepary bean (*P. acutifolius),* lima bean *(P. lunatus)* and other wild *Phaseolus* species are conserved and maintained in different gene banks (Table 13.1). Among them largest ex situ collection of *Phaseolus* is maintained at the International Center for Tropical Agriculture (CIAT) near Cali, Colombia. It holds over 40,000 accessions of which over 37,927 are of *Phaseolus vulgaris*. This was estimated to account for 50–75% of the variability occurring in the centres of diversification for domesticated types but only less than 30% of diversity of wild types (Fig. 13.1). Germplasm collections held in Africa include Bunda Agricultural College, Lilongwe, Malawi (6000 accessions), National Gene Bank of Kenya, KARI, Kikuyu (3000 accessions) and Institute des Sciences Agronomiques

Table 13.1 Common bean collection in different gene banks at global level (FAO 2010)

Gene bank	Accessions (%)	Land race (%)	Wild accessions (%)
CIAT, Colombia	37,927 (14)	30,507 (85)	2153 (6)
USDA, USA	14,674 (6)	9832 (67)	880 (6)
Embrapa, Brazil	14,460 (6)	5784 (40)	–
INIFAP, Mexico	12,752 (5)	7014 (55)	2168 (17)
IPK, Germany	8680 (3)	5729 (66)	87 (1)
NBPGR, India	4353(2)	–	–

Fig. 13.1 Diversity in seed traits, viz. seed size, shape and color within common bean. Similar levels of diversity exist for growth habit, performance and quality traits within common bean

du Rwanda, butare (3000 accessions). African national breeding programmes (e.g. in Uganda) have smaller landrace collections, ICAR-National Bureau of Plant Genetic Resources, New Delhi (India) (4353 accessions).

13.3 Common Bean Production Trends and Gaps

Beans are extremely diverse crops in terms of cultivation methods, uses, the range of environments to which they have been adapted and morphological variability. They are found from sea level up to 3000 m above sea level and are cultivated in monoculture, in associations or in rotations. In global context, India, Brazil, Myanmar and Mexico together account for about 60% of the total area under dry bean, producing a total 11 million tons of dry bean. India has the highest area under dry bean production with 8.3 million ha but produces only 3.4 million tons, whereas Brazil produces nearly the same quantity with 3.8 million ha area (Nedumaran et al.

2015). Latin America, South Asia and Southeast Asia have shown positive trends in production both from a high base with increasing yields. The production in sub-Saharan Africa has increased at the rate of 4.4% per year between 1994 and 2010 largely due to area expansion and slight increase in yield (Nedumaran et al. 2015). Among the grain legume crops, dry bean has the highest area share after soybean at 27 million ha. Its production has grown annually at the rate of 1.9% mostly due to yield gains. However, dry bean had one of the lowest yields at 793 kg/ha in 2008–2010. These low global yield levels are due to the poor performance of developing countries which produce more than 95% of total dry bean, compared to the average yield of bean in developed countries which is 133% higher than that of developing countries. Unlike other legumes, the intensity of diseases is quite high in bean (Beebe et al. 2006). Hence, in addition to developing high-yielding varieties, more research should focus on biotic stress-resistant varieties particularly resistant diseases and demand for specific commercial types. The existing information indicates a huge yield gap between the potential yield of improved varieties and the currently obtained yield by farmers, implying that the realized yield potential is low and the problem lies with other additional inputs to close the gap (Graf et al. 1991).

13.4 Limiting Bean Yield

Diseases are the main biotic factor that limits the amount and the quality of bean production and have been ranked second among biotic and abiotic factors that constrain bean production in Africa (Aggarwal et al. 2004). Currently, more than 50 species of bacteria, fungi and viruses that incite bean diseases have been reported. Of these, eight are economically important (Table 13.2). Constraints to bean productivity vary with region with very different factors likely to concern the subsistence farmer in Latin America and Eastern Africa, and the larger-scale producer is more commonly found in the United States and Europe. Recent studies reported that, among viral diseases, bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV), which are both aphid-vectored *Potyvirus*, occur in most of the bean production regions. Bean golden mosaic virus (BGMV), a whitefly-transmitted Gemini virus, occurs in Argentina, Bolivia and Brazil. Another viral disease, bean golden yellow mosaic virus (BGYMV), also a whitefly-transmitted Gemini virus, may cause severe losses in tropical and subtropical Central America, coastal Mexico (the Caribbean) and Southeastern United States. BCMV and BCMNV are the most common and most destructive viruses that infect common bean as well as a range of other cultivated and wild legumes (Morales 2006). Yield losses due to BCMV and BCMNV can be as high as 100% (Damayanti et al. 2008; Saqib et al. 2010; Singh and Schwartz 2010; Verma and Gupta 2010; Li et al. 2014). In the tropics and subtropics, bean yields are greatly reduced as beans are susceptible to numerous diseases caused by fungal pathogens (Graham and Vance 2003). Anthracnose caused by the fungus *Colletotrichum lindemuthianum* is one of the most economically important diseases of common bean and can cause devastation

Table 13.2 Major diseases of common bean significantly affecting common bean production worldwide (Singh and Schwartz 2010)

Diseases	Causal organism	Transmission mode and survival	Yield loss potential	Distribution pattern
Viral disease				
BCMV	Bean common mosaic virus	Seed, plant debris, aphid	100%	Worldwide
BCMNV	Bean common mosaic necrosis virus	Seed, plant debris, aphid	100%	Worldwide
BGMV	Bean golden mosaic virus	Whitefly (*Bemisia* spp.)	100%	Tropical and subtropical
BGYMV	Bean golden yellow mosaic virus	Whitefly (*Bemisia tabaci*)	100%	Tropical and subtropical
Fungal disease				
Anthracnose (ANT)	*Colletotrichum lindemuthianum*	Seed, wind, plant debris	100%	Worldwide
Angular leaf spot (ALS)	*Phaeoisariopsis griseola*	Seed, wind, plant debris	80%	Worldwide
Powdery mildew (PWM)	*Erysiphe polygoni*	Seed, plant debris	50–80%	Worldwide
Rust	*Uromyces appendiculatus*	Wind, plant debris	50%	Worldwide
Root rots	*Fusarium*, *Pythium*, *Rhizoctonia*	Seed, water, soil, plant debris	100%	Tropical and subtropical
Bacterial disease				
Common bacterial blight (CBB)	*Xanthomonas campestris* pv. phaseoli	Seed, wind, water, plant debris	45%	
Halo blight (HB)	*Pseudomonas syringae pv. syringae*	Seed, wind, water, plant debris	25%	Tropical and subtropical

to farmers' fields resulting in yield losses as high as 95–100% in susceptible cultivars (Guzman et al. 1979; Melotto et al. 2000; Zuiderveen et al. 2016). The fungus *P.griseola* (Sacc.) Crous and Braun (Crous et al. 2006) is the causal agent of angular leaf spot (ALS), a common bean disease with can lead greatest impact in yield leading to losses as high as 80% and reported in more than 60 countries around the world (Stenglein et al. 2003; Miklas et al. 2006). Similarly, the bean rust disease is caused by the biotrophic basidiomycete fungus, *Uromyces appendiculatus*, an obligate parasite of common bean. It causes major production problems in humid tropical and subtropical areas and periodic severe epidemics in humid temperate regions (Souza et al. 2005). Other fungal disease powdery mildew (PWM) caused by *Erysiphe polygoni* DC is causing serious damage to bean crops. Although it has a worldwide distribution, it is considered a secondary disease. However, the incidence of this disease has increased in recent years, mainly due to increased planting of winter crops, where environmental conditions are favourable to the development of pathogens and losses can reach 69%, mainly when the infection occurs before the anthesis. Likewise, common bacterial blight (CBB) is also one of the most serious

diseases of common bean worldwide (Taran et al. 2001). It causes severe yield losses of up to 62%. Genetic resistance is the most effective option for controlling CBB in smallholder common bean production systems. In addition to this, other pathogens are affecting the bean production significantly such as halo blight (HB), white mould (WM) and web blight (WB); these diseases during severe infestation cause yield losses which may reach up to 50–100%. There are greater arrays of disease-causing pathogens important in tropical than in temperate bean production. The warm, often humid environment of the tropics and subtropics favours pathogen development, while the planting of two to three crop cycles per year in some regions provides a continuity of inoculums. Further constraints include the small land area available to individual farmers, limiting the possibilities for crop rotation, and the scarcity and the cost of disease-free seed. However, disease outbreaks generally originate from contaminated seeds or infected plant debris. Because chemical control is expensive and the generation of pathogen-free seeds is often difficult in developing countries, genetic resistance represents the most reliable control strategy.

13.5 Breeding for Disease Resistance

13.5.1 Viral Diseases

13.5.1.1 Bean Common Mosaic and Bean Common Mosaic Necrosis Viruses

The closely related potyviruses, bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV), are major constraints on common bean production (Fig. 13.2). Both viruses are seed-borne and transmitted by several aphid species in a non-persistent manner (Drijfhout 1978). BCMNV and BCMV serotype A has only five identified strains (TN-1, NL-3, NL-3 K, NL-5 and NL-8) (McKern et al. 1992; Mink and Silbernagel 1992; McKern et al. 1994).The genetic control towards both viruses is assured by one dominant I gene or with combination of number of recessive (*bc-u*, *bc-1*, $bc\text{-}1^2$, *bc-2*, $bc\text{-}2^2$ and *bc-3*) genes (Kelly et al. 1995; Strausbaugh et al. 1999). However, each of these recessive genes requires the *bc-u* allele for their expression, and there is an epistatic interaction such that *bc-3* masks the action of *bc-2* and $bc\text{-}2^2$, which in turn mask the action of *bc-1* and $bc\text{-}1^2$. Thus, in the presence of *bc-3*, it is not possible to detect the effects of other recessive resistance alleles irrespective of the BCMV strains used. According to Kelly (1997), the best choice of a partner is the *I* gene because it would appear that each of the two genes has a very different mode of action. Strausbaugh et al. (2003) identified a recessive allele among the host group 3 cultivars (e.g. Olathe, UI 37 and Victor) that imparts better resistance than $bc\text{-}1^2$ to leaf stunting and deformity and plant dwarfing induced by the NL-3 K strain of BCMNV.

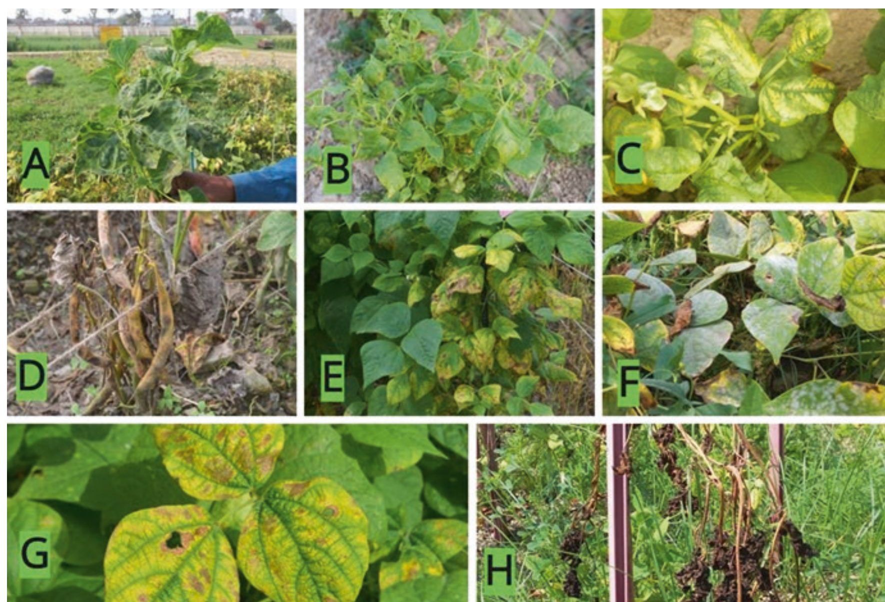

Fig. 13.2 Typical symptoms of viral, fungal and bacterial diseases in common bean A. BCMV

Gene pyramiding in common bean is being utilized to develop more effective resistance to the temperature insensitive-necrosis-inducing (TINT) strains of BCMV present in the United States (Kelly et al. 1995). The independence of the BCMV resistance genes provide opportunities to use gene pyramiding as a strategy in breeding for durable resistance. Bean breeders recognize that the combination of the dominant *I* gene with recessive *bc* resistance genes offers durability over single-gene resistance to BCMV and BCMNV, since the two types of genes have distinctly different mechanisms of resistance (Kelly 1997). Most small-seeded bred cultivars of black carioca (creamstripe), mulatino (cream), navy (white) and red market classes belonging to race Mesoamerica on the American continents carry the dominant *I* resistance gene (Singh 1999b; Miklas et al. 2006). Similarly, most large-seeded alubia (cylindrical white seed), cranberry (cream mottled), light- and dark-red kidney and white kidney cultivars bred in recent years possess the dominant *I* gene (Beaver 1999; Beaver et al. 2003; Miklas et al. 2006). Recently resistance to BCMV disease was established in common bean variety BAT 477, following artificial inoculation with the virus. Crosses were performed between BAT 477 and BCMV susceptible parent Dobroudjanski Ran, a widely spread Bulgarian variety (Vladimir et al. 2014).

13.5.1.2 Golden Mosaic and Bean Golden Yellow Mosaic Virus

BGMV and BGYMV are other most devastating viral diseases of common bean in all tropical and subtropical areas where the crop is grown (Morales and Niessen 1988; Faria et al. 1991). It is transmitted in a persistent manner by the sweet potato whitefly *(Bemisia tabaci* Genn.*)* and the silverleaf whitefly *(Bemisia argentifolii)*, the latter formerly biotype B of *B. tabaci* (Galvez and Morales 1989). Morales and Niessen (1988) screened a wide range of common bean germplasm and reported that resistance to plant dwarfing and pod deformation and tolerance to leaf chlorosis were found in small black-seeded landraces such as Porrillo Sintetico and Turrialba, from Central America, and breeding lines and cultivars derived from them, such as ICA Pijao and Dorado (synonymous with DOR 364). Similarly, large-seeded G 122 and Royal Red, among others, of race Nueva Granada exhibited considerable resistance or tolerance to these same symptoms caused by BGMV and BGYMV. Pyramiding genes from the Durango and Mesoamerica bean races has led to the development of breeding lines with high levels of resistance to leaf chlorosis and pod deformation caused by BGYMV (Singh et al. 2000). The genetic base for BGYMV resistance, however, remains narrow. The emergence of BGYMV strains capable of overcoming the resistance conferred by the recessive resistance gene *bgm-1* could pose a threat to bean production in Central America and the Caribbean. G35172, a scarlet runner bean (*P. coccineus* L.) accession, was identified by CIAT scientists as a source of resistance to BGYMV (Beebe and Pastor-Corrales 1991). Similarly, Osorno et al. (2003) reported that two genes from *P. coccineus* confer resistance to BGYMV.

A common bean line improved for its upright architecture (A429) showed an unexpected high level of BGYMV resistance under field conditions in Guatemala. Mexican common bean genotype, called ‘Garrapato’, combined with the Mesoamerican black-seeded source of BGYMV resistance, Porrillo Sintetico (also a parent of A429), was associated with the high level of BGYMV resistance found in A429 (Morales and Niessen 1988). A429 soon became one of the most widely used sources of *Begomovirus* resistance in common bean breeding programmes in Latin America used to produce a new generation of small red- and black-seeded DOR lines that have been useful as varieties or breeding parents in locations from Southern United States to Northwestern Argentina. Likewise, a red kidney line, DOR 303, was also selected for its apparent high level of BGYMV resistance under field conditions.

13.5.2 Fungal Diseases

13.5.2.1 Anthracnose (ANT)

Anthracnose caused by the fungal pathogen *Colletotrichum lindemuthianum* is a serious seed-borne disease of common bean and can cause devastation to farmers’ fields, resulting in yield losses as high as 95% in susceptible cultivars (Guzman

et al. 1979). This pathogen overwinters in seed and crop residues (primary source of infection) and infects all aerial parts of the bean plant. Typical symptoms are deep, shrunken lesions containing flesh-coloured spores on bean pods that are the most distinctive symptoms of anthracnose, and variability in *lindemuthianum* was first described by Barrus (1911) (Fig. 13.2). When he noticed differences between virulence of two races of anthracnose against 139 bean cultivars eventually these first two races were identified as α and β and laid the foundation for the discovery of greater pathogenic variability. Recent literature shows presence of more than 100 races of pathogen worldwide (Gonzalez et al. 2015), and 1590 isolates of *C. lindemuthianum* inoculated on 12 bean differential cultivars have resulted in the identification of 182 races worldwide. Cultivating resistant varieties is the most economical and effective approach for controlling common bean diseases and is an important goal for common bean breeders. Currently, more than 20 resistance loci are identified against anthracnose disease with a gene symbol *Co* (Kelly and Vallejo 2004; Gonçalves-Vidigal et al. 2012; Coimbra-Gonçalves et al. 2016). Anthracnose resistance genes have been designated with the abbreviation '*Co*' followed by a number for a specific locus (Kelly 2004; Ferreira et al. 2013). Genetic mapping and candidate gene analysis have established genetic map positions for the resistance genes among 7 of the 11 linkage groups (Rodriguez-Suarez et al. 2007; Richard et al. 2014). Previous studies have identified 20 dominant anthracnose resistance genes, which were either identified on Mesoamerican or Andean common bean gene pool. The Mesoamerican genes include *Co-2*, *Co-3* (and its alleles *Co-3*^{2}, *Co-3*^{3}, *Co-3*^{4} and *Co-3*^{5}), Co-4 (and it alleles *Co-4*^{2}, *Co-4*^{3}), *Co-5* (and its allele *Co-5*^{2}), *Co-6*, *Co-11*, *Co-16*, *Co-17*, *Co-u* and *Co-v* (Kelly 2004; Mendez-Vigo et al. 2005; Alzate-Marin et al. 2007; Gonçalves-Vidigal et al. 2007; Rodriguez-Suarez et al. 2008; Vallejo and Kelly 2009; Campa et al. 2011; Gonçalves-Vidigal et al. 2011, 2013; Richard et al. 2014; Sousa et al. 2014; Trabanco et al. 2015; Coimbra-Gonçalves et al. 2016). The Andean genes include Co-1 (and its alleles *Co-1*^{2}, *Co-1*^{3}, *Co-1*^{4} and *Co-1*^{5}), *Co-12*, *Co-13*, *Co-14*, *Co-15*, *Co-x*, *Co-w*, *Co-y*, *Co-z* (Coimbra-Gonçalves et al. 2016; Gonçalves-Vidigal and Kelly 2006; Gonçalves-Vidigal et al. 2008, 2009, 2012, 2016; Lacanallo and Goncalves-Vidigal 2015; Sousa et al. 2015) and *Co-pa* (Sandra et al. 2017).

Incorporating genetic resistance to anthracnose is the area of research and development that holds the most promise for reducing the effects of the pathogen in common bean. Careful selection of genes providing resistance to races producing anthracnose disease is required. The varieties carrying resistance genes provide a short-lasting control over the disease lasting only until new strains of fungus emerge. The major difficulty with the process of compiling the resistance genes is that detection of the resistant plants takes time since it requires systematic inoculation with different strains of the fungus. A literature review indicates that in other countries where cultivation is common, strains of the common fungi have been identified, and the varieties resistant to the identified strains have been developed. MAS have been used successfully to breed for enhanced resistance to anthracnose in the cultivar Perola in Brazil (Ragagnin et al. 2003) and in pinto beans in the United States (Miklas et al. 2003b). But there is a need for caution based on the unsuccessful

attempts to introgress the *Co-4*^{2} gene using marker-assisted backcrossing in two landrace bean cultivars from Ecuador (Ernest and Kelly 2004). Kelly et al. (1994) developed ANT-resistant black-seeded cultivar ‘Raven’ (Co-1 gene) which was then used to develop resistant ‘Phantom. Indirect selection should be periodically verified by direct selection to ensure that the resistance gene is being transferred. Miklas et al. (2003b) using marker-assisted selection introgressed the *Co-4*^{2} resistance allele into a pinto breeding line USPTANT-1. (Kelly et al. 1994) developed ANT resistant black-seeded cultivar ‘Raven’ (*Co-1* gene), which was then used to develop resistant “Phantom”. Genchev et al. (2010) developed anthracnose resistance source using several physiological races of anthracnose identified in Bulgaria which was performed from F1 to F5 generation. The line DG 2-36-58-3 was identified as the most promising by quality complex of growth habit type, vegetation period, type of seeds, yield and presence of *Co-1* and *Co-4* genes that confer resistance to 74 out of 78 worldwide recognized anthracnose races. Achieving durable anthracnose resistance poses a challenge to bean breeders. Due to the high degree of pathogen variability and the continual emergence of new races, single-gene deployment is not an effective strategy to control bean anthracnose. The pyramiding of resistance genes which have complementary spectra of resistance has been suggested as a strategy to circumvent the problem of pathogen variability.

13.5.2.2 Angular Leaf Spot (ALS)

Angular leaf spot (ALS) disease caused by *Pseudocercospora griseola* (Sacc.) is a major disease of common beans in the tropics and subtropics (Stenglein et al. 2003). It is a biotrophic fungus in the early stages of infection which then becomes necrotrophic, when the attack causes the characteristic symptoms of the disease, which are angular necrotic spots limited by the leaf veins (Fig. 13.2). Studies on the variability of *P. griseola* isolates revealed the existence of two major groups of the pathogen, Andean and Mesoamerican, which correspond to and have co-evolved with the Andean and Mesoamerican gene pools of common bean (Guzman et al. 1995; Pastor-Corrales et al. 1998; Crous et al. 2006). Mesoamerican strains of this pathogen are considered more virulent as compared to Andean strains, and they tend to affect both Mesoamerican and Andean beans, while Andean strains are less virulent, affecting mostly Andean genotypes. The disease is of great economic importance in Eastern and Central African countries of Uganda, Kenya, Tanzania, Ethiopia, Rwanda, Burundi and Kivu Province of the Democratic Republic of Congo (Sengooba and Mukiibi 1986; Pastor-Corrales et al. 1998). According to Stenglein et al. (2003), every 10% increase in ALS severity results in 7.9% yield loss. ALS disease is spread within and among fields by wind-blown particles of infested soil and wind-blown and rain-splashed spores. However, the primary source of infection is considered to be infested seed (Cardona-Alvarez and Walker 1956). Resistance to ALS in common bean is controlled by single dominant (Correa et al. 2001; Namayanja et al. 2006) as well as recessive genes (Correa et al. 2001). As the

best form of disease control includes using resistant cultivars, the genetic characterization of resistance sources is very important for the genetic improvement of the crop. In the case of ALS, two dominant resistance genes have been described so far. The first, called *Phg-1*, was identified in the AND 277 variety. While, the second, called *Phg-2*, was identified in the Mexico 54 variety (Sartorato et al. 2000) linked to SCAR OPN02 and RAPDOPE04 markers. Apart from these two genes, dominant monogenic inheritance for resistance to ALS has also been described in the Ouro Negro (Correa et al. 2001) and G10474 varieties. But the relationship of these genes with *Phg-1* and *Phg-2* remains unknown. In addition to qualitative resistance genes, there are also reports of QTLs controlling resistance to ALS. Five QTLs were mapped on linkage group B04, one on B08, one on B09 and three on linkage group B10 (Lopez et al. 2003; Mahuku et al. 2009; Mahuku et al. 2011). The Mesoamerican ALS resistance locus, *Phg-3*, mapped in accession Ouro Negro, is linked to marker G2303 at a distance of 0 cM (Gonçalves-Vidigal et al. 2013).

Breeding for ALS diseases resistance is of considerable importance in common bean breeding. To achieve a rapid progress in such activities, incorporating biotechnological tools in order to increase efficiency and effectiveness is mandatory. Developing bean cultivars with higher yields and with multiple disease resistance will enable farmers to increase bean productivity and achieve greater yield stability. Singh et al. (2003) developed ALS resistant dry bean breeding lines including A 339, MAR 1, MAR 2 and MAR 3 from interracial populations between the Middle-American common bean races. Mahuku et al. (2003) identified 78 interspecific dry bean lines with resistance putatively transferred from the secondary gene pool, which represents important germplasm for future utilization. Traditional breeding at CIAT involving hybridization among resistance sources in single or multiple interracial crosses followed by selection under disease pressure in field nurseries and greenhouse screening trials has resulted in development of germplasm lines MAR1, MAR 2, MAR 3, AND 277 and CAL 143 with improved broad-based resistance to angular leaf spot (Singh et al. 2003; Aggarwal et al. 2004). Other important sources of resistance include BAT 332, MEX 54, Cornell 49–242, Ouro Negro, G 10474 and other *P. vulgaris* landrace accessions and bred lines listed by Pastor-Corrales et al. (1998) and Mahuku et al. (2003). Pyramiding resistance genes into a single genotype is one of the practical approaches through which durable resistance can be achieved. There are several *Phg* genes that have been identified as sources of resistance to *P. griseola* from landraces, secondary and tertiary genes pools. However, utilization of these genes in a breeding programme will depend on the mode of inheritance and the background of the cultivar carrying them. Landrace varieties might be excellent sources for resistance breeding against ALS. Landraces are readily available, adapted to the environments and have been kept by farmers because of their desired traits. In this regard, breeding against multi-races of ALS disease is an overriding consideration which requires gene pyramiding that involves several parents. Therefore, appropriate mating design and genetic analysis that will provide information of the best parent in a combination and best selection methods to identifying superior progenies is important.

13.5.2.3 Powdery Mildew (PWM)

Powdery mildew is a serious disease for many crops worldwide including common bean. Common bean powdery mildew causal agent has been frequently ascribed to Erysiphe poligony DC. But recent studies suggest that it is closer to Erysiphe diffusa (Cooke and Peck) U. Braun and S. Takam, formerly *Microsphaera diffusa* Cke. and Pk. (Almeida et al. 2008). The disease is usually first noted as subtle, small, round, greyish or whitish spots on leaves or stems (Fig. 13.2). Progression of the disease leads to enlargement of the spots and coalescing to a white mass resembling talcum powder on the upper leaf surface, especially in older parts of the plant. In the last years, the incidence of powdery mildew has significantly increased in bean crops at different parts of world. Limited information about resistance sources and nature and inheritance of resistance are available to bean breeders and plant pathologist. The use of resistant bean cultivars can be the most efficient, economic and ecological strategy to provide effective control of this disease. Limited information about sources of resistance to the fungus and the nature and inheritance of resistance are available to bean breeders and plant pathologist. A few sources of resistance to powdery mildew have been described (Schwartz et al. 1981), and a qualitative nature of resistance has been suggested (Bett and Michaels 1995; Ferreira et al. 2001). The response of common bean to powdery mildew was previously reported to be governed by a single dominant gene (Dundas 1936), by one dominant and other recessive resistance genes (Bett and Michaels 1995) or by two complementary dominant genes. Sources of resistance to PWM have also been described (Schwartz et al. 1981) including "Cornel 49, 242, Porrillo Sintetico, Negro San Luis and ESAL 686" cultivars (Rezende et al. 1999; Trabanco et al. 2012; Perez-Vega et al. 2013). Much of these sources are characterized by possessing a few genes involved in the trait with different patterns of action. The genetic positions of resistance genes were first investigated in Xana/Cornell 49,242 recombinant inbred line population. Results showed that the resistance in the genotype Cornell 49,242 was conferred by two independent and epistatic genes: *Pm1*, located in linkage group (LG) Pv11 conferring total resistance in which there is no visible disease symptoms, and *Pm2*, located in LG Pv04, conferring intermediate resistance with a very limited disease development (Perez-Vega et al. 2013). In the same way, Ester Murube et al. (2017) reported that inheritance of resistance to PWM was analysed in three F2:3 populations involving the resistant bean genotypes BelNeb, G19833 and BGE003161. In the three populations, the segregation for PM resistance fit the expected ratio for one dominant gene, and resistance loci were mapped to linkage group Pv04. The physical positions of the flanking markers indicated that the three resistance genes were located between the physical positions 0 and 1.09 Mb. Several analysis of segregating populations revealed that the genetic resistance to PM has a qualitative nature involving major resistance genes with dominant or complementary epistatic relationships (Dundas 1936; Ferreira et al. 2001; Trabanco et al. 2012). From a breeding perspective, molecular-genetic maps and QTL mapping are tools that allow the localization of some genomic regions that control both single and complex

inheritance. This information could be used in breeding programmes for producing new cultivars for resistance to powdery mildew.

13.5.2.4 Rust

Among the fungal diseases, one of the most widespread and important diseases of common bean is rust, caused by a highly variable basidiomycete fungus *Uromyces appendiculatus* (Pers.Pers.) Unger which has a narrow host range – attacks only common bean (Fig. 13.2). This disease is distributed throughout the world, but it effectively causes major production problems in humid tropical and subtropical areas and periodic severe epidemics in humid temperate regions (Stavely et al. 1989; Pastor-Corrales 2003). Severe bean rust epidemics have been reported in Australia, China, the United States and some areas of Europe. Major losses have occurred in Burundi, Ethiopia, Kenya, Malawi, Rwanda, South Africa, Tanzania, Uganda and Zimbabwe. In Latin America, the bean rust is also a serious problem; major losses occurred in Argentina, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Jamaica, Mexico, Nicaragua and Peru (see Stavely and Pastor-Corrales 1989). Disease losses worldwide measured in greenhouse and field conditions can vary from 18 to 100%. The *U. appendiculatus* is a highly variable and is among the most pathogenically variable of all plant pathogens. It has been identified and reported in all bean production areas of the world (Stavely and Pastor-Corrales 1989) and is characterized by highly diverse virulence phenotypes (Harter et al. 1935; Ballantyne 1978; Mmbaga et al. 1996; Souza et al. 2005). According to Lindgren et al. (1995), 1% increase in bean rust severity leads to a yield loss of approximately 19 kg/ha. The major losses in Brazil occur in south, southeast and central areas, including the states of Paraná, Rio Grande do Sul, Santa Catarina, Minas Gerais, São Paulo and Goiás (Souza et al. 2005). Resistance to bean rust is mainly controlled by major single dominant genes (Alzate-Marin et al. 2004; Souza et al. 2007). However, it can be also controlled by single recessive genes (Zaiter et al. 1989), two genes (Finke et al. 1986), two complementary dominant genes (Grafton et al. 1985) or by many genes with minor effect (Edington et al. 1994). According to Souza et al. (2011), 13 dominant rust resistance (RR) genes (*Ur-1* to *Ur-13*) have been identified. In addition to these genes, other important unnamed genes have been identified, such as those present in the common bean cultivars as BAC6, CNC, CSW 643, Dorado, Ouro Negro and PI 260418. The appropriate characterization of RR genes from different origins whether Mesoamerican or Andean is essential for the future for developing breeding for common bean resistance to *U. appendiculatus*. Interestingly, new sources of resistance are always identified and characterized because of the high virulence diversity and variability present in the pathogen population (Araya et al. 2004; Souza et al. 2011). In the common bean breeding programme of the BIOAGRO-UFV, molecular markers were used to assist the transfer of rust and anthracnose resistance genes from the black-seeded cultivar 'Ouro Negro' ('Honduras-35') to the 'carioca-type' cultivar 'Rudá' (Faleiro et al. 2004).

Ragagnin et al. (2005) expanded these efforts and transferred genes for resistance to rust (*Ur-ON*), anthracnose (*Co-4*, *Co-6* and *Co-10*) and angular leaf spot (*Phg-1*) to the 'carioca-type' cultivars 'Rudá' and 'Pérola'.

13.5.3 Bacterial Diseases

13.5.3.1 Common Bacterial Blight (CBB)

Common bacterial blight (CBB) is a significant foliar disease of dry bean caused by the pathogen *Xanthomonas axonopodis* pv. *phaseoli*, a gram-negative bacillus with a genome of approximately 3.9 Mb. This disease is endemic to most regions where common bean is cultivated and is annually responsible for millions of dollars of crop loss worldwide (Fig. 13.2). The bacteria are seed-borne, and under field conditions, dissemination can occur through wind-driven rain or mechanical transfer by insect vectors. In addition to natural methods, the bacteria can be spread by overhead sprinkler systems and the use of infected seeds (Vivader 1993). Studies of the genetic diversity of *X. axonopodis* pv. *phaseoli* have found that the pathogen can be grouped into four genetic lineages. Three of these groups are composed exclusively of *X. axonopodis* pv. *phaseoli*, while the last group contains the *X. fuscans* subsp. *fuscans* strains (Alavi et al. 2008).

Several inheritance studies have been conducted on CBB resistance, and different results were reported depending on various factors such as the pathogenic variability and the genetic background of the parental lines (Fourie et al. 2011). CBB resistance is inherited quantitatively, with largely additive effects, low to moderately high heritability and transgressive segregation (Aggour and Coyne 1989; Arnaud-Santana et al. 1994). Similarly, Miklas et al. (2003a) reported that the inheritance of CBB resistance in Montana No. 5 was polygenic with at least one major gene effect. Likewise, Zapata et al. (2011) reported that CBB resistance was governed by a single dominant gene in resistant lines Wilk-2 and VAX6 and VAX4 and PR 0313-58, respectively. The identification of major quantitative trait loci (QTL) controlling resistance to CBB (Miklas et al. 2006; Viteri et al. 2014b; Singh and Miklas 2015) has facilitated marker-assisted breeding for higher levels of CBB resistance into better-adapted and higher-yielding dry bean lines (Miklas et al. 2000a). CBB-tolerant/resistant dry bean varieties have been developed from germplasm derived from interspecific crosses by various breeding programmes in North America. A great northern variety GN1 is released in 1961 (Coyne 1961). In the same way, OAC-Rex is a white bean developed at the University of Guelph. It was derived from the cross HR20-728 x MBE7 made in 1988. MBE7 was a selection from the cross ICA Pijao (*P. vulgaris*)/PI440795 (*P. acutifolius*)// Ex Rico 23 (*P. vulgaris*). PI440795 was the source of CBB resistance in this cross. HR20-728 is a black bean variety selected from across between Ex Rico and Midnight. OAC-Rex was tested in field trials as OAC 95-4, with full registration occurring in 2002 (Taran et al. 2001). HR67 is a CBB-resistant white bean line developed from a cross

between OAC Rico and XAN 159 with additional crossing with Centralia-3 and HR13-621 (McElroy 1985). Singh and Muoz (1999) and developed CBB-resistant interspecific breeding lines VAX1 and VAX 2 from a multiple-parent interspecific cross between common and tepary bean G 40001. The substantial progress made in molecular marker technology for the common bean holds considerable promise for breeding genetic resistance to CBB. Molecular markers for disease resistance are powerful tools for analysing the genome and are comprehensively applied in mapping genes and MAS (Boyle et al. 2007). To date, 24 QTL conferring resistance to CBB have been identified, distributed across all 11 chromosomes of common bean (Singh and Schwartz 2010; Shi et al. 2011).

13.5.3.2 Halo Blight (HB)

It is an important seed-borne disease of dry beans caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.) Dows. (*Psp*). The disease is a major constraint of dry bean production in moderately cool and wet regions of Africa, Europe, North America and South America (Taylor et al. 1996; Rico et al. 2003). The gene-for-gene interaction between common bean and HB pathogen races was demonstrated using molecular techniques and resistant genotypes (Jenner et al. 1991; Tsiamis et al. 2000). Nine races of the pathogen and five race-specific resistance genes have been previously described. However, a quantitative response to this pathogen has also been described (Yaish et al. 2006). Several researchers studied genetic inheritance of HB resistance. As per the earlier findings, resistance to HB is inherited by single dominant or recessive genes (Asensio et al. 1993; Taylor et al. 1996). Taylor et al. (1996) screened over 1000 accessions of *Phaseolus* spp. and identified both race-specific and non-race-specific resistance to halo blight. For example, CAL 143, great northern Nebraska #1 Sel. 27,'Jules', PI 150414 and Wis HBR 72 among others carry non-race-specific resistance.

Molecular and genetic studies established that the relationship between the race-specific genes and *Psp* races conformed to a gene-for-gene interaction (Jenner et al. 1991). Furthermore, genetic studies have established that inheritance of halo blight resistance depends on the nature of the resistance as well as the genetic background. Chataika et al. (2011) also reported a single dominant resistance gene in the large-seeded (40 g per 100 seeds) Andean breeding line CAL 143. On the other hand, the quantitative race non-specific resistance in PI 150414 is due to a recessive gene (Taylor et al. 1996). Miklas et al. also mapped the dominant *Pse-2* gene, which is derived from the differential genotype ZAA 12 and confers resistance to seven *Psp* races (excluding *Psp* races 1 and 6), to linkage group Pv10. Miklas et al. (2009) identified six random amplification of polymorphic DNA (RAPD) markers tightly linked (0–3.3 cM) with *Pse-1* in a 'CanadianWonder'/UI 3 dry bean population of which three completely linked markers were converted into SCAR markers SH11.800, SR13.1150 and ST8.1350. However, usefulness of these SCAR markers for marker-assisted selection would be limited to populations of large-seeded Nueva

Table 13.3 Summary of bean fungal, bacterial and viral disease distribution, transmission and resistance genes (Teshale et al. 2019)

Disease	Distribution	Seed	Transmission	Resistance
Fungi				
Anthracnose (*Colletotrichum*)	Worldwide (cool = 14–18 °C, humid, and >1000 m in tropics	Yes	Wind and rain; animal and insect	QTL: Pv01, Pv02, Pv04, Pv10, Co-1, Co-2
Angular leaf spot (*Phaeoisariopsis*)	Worldwide (moderate temp.18–25 °C, high moisture)	Yes	Wind	QTL: Pv04, Pv10 (ALS10.1) Phg1, Phg2
Rust (*Uromyces*)	Worldwide	No	Wind, crop and residue	KASP SS68 marker associated with Pv11
Leaf spot (*Ascochyta*)	Tropics (high altitude: >1500 m)	Yes	Splash, contact, crop residue	Quantitative (*P. polyanthus*)
Pythium root rot	Worldwide	Yes	Crop residue, infected soil	QTL: ER3XC (LG3), SV6XC(LG6), Py-1 (LG7)
Fusarium	*Worldwide*	*No*	*Crop residue, infected soil*	*QTL (FRR3.1 km (Pv03), LGs B2 and B3*
Web blight	Worldwide (hot-humid)	Yes	Infected soil, seed	Quantitative
Bacteria				
Common blight (*Xanthomonas*)	Worldwide (cool to moderate temp.)	Yes	Rain, moisture	QTL: BC420 (Pv06), SU91 (Pv08), Xa11.4OV1(Pv11)
Halo blight (*Pseudomonas*)	Worldwide (cool to moderate temp.)	Yes	Rain, moisture	Quantitative: Pse-1, Pse-2, Pse-3, Pse-4, Pse-6, HB5.1 98BB, 98LFA, 96LFA, 96BBS
Bacterial brown spot (*Pseudomonas*)	Worldwide (warm-humid weather)	Yes	Seed, wind	LG02
Viruses				
Bean common mosaic virus and mosaic necrosis virus	Worldwide	Yes	Aphid	Qualitative: bc-1, bc-2, bc-3
Bean golden yellow mosaic virus	Central America, Caribbean	No	Whitefly	Qualitative, quantitative, Bgm-1, RNAi

Granada and small-seeded Mesoamerican races. Significant progress has been made in developing cultivars with resistance to various diseases using conventional breeding. Some important resistance-mapping studies are summarized in Table 13.3. Markers associated with established resistance loci can be used for more efficient breeding to develop resistant cultivars.

13.6 Molecular Breeding

Molecular markers are well-established as powerful tools in plant breeding and genetics for indirect selection of difficult traits at the seedling stage during plant breeding, thus speeding up the process of conventional plant breeding and facilitating the improvement of difficult traits that cannot be improved easily by the conventional methods of plant breeding. In this direction, a large number of genes and QTLs controlling agronomic traits and conferring tolerance to both abiotic and biotic stresses have been identified and tagged using molecular markers in several crop species especially pulses. Despite the importance of common bean in developing countries, its production mostly relies on local cultivars (Gepts and Debouck 1991; Miklas et al. 2006). The local cultivars, however, are commonly known to produce notoriously low yields as they are highly constrained by several biotic factors, including diseases, insect pests, poor seed quality, drought, low soil fertility and poor crop management. Yield losses caused by bean diseases are very significant and devastating in the bean industry (Coyne et al. 2003; Hillocks et al. 2006). However, using classical breeding, significant strides have been made in crop improvement through phenotypic selections for agronomical important traits. In addition to this, bean breeding is complicated by the pathogens variability and different genes conditioning resistances (Wortmann et al. 1998; Kelly 1998). Therefore, classical breeding is limited by the length of screening procedures and reliance on the environmental factors. Hence, deployment of the molecular markers linked to resistance genes could be an alternative, more reliable screening procedure to increase the efficiency of breeding for disease resistance using marker-assisted selection (MAS).

13.6.1 Diagnostic Markers for Disease Resistance Breeding

Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as signs or flags, and they are used as chromosome landmarks to facilitate the introgression of chromosome regions with genes associated with economically important traits. There are three major types of genetic markers: (1) morphological (also 'classical' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA (Winter and Kahl 1995; Jones et al. 1997). Among different genetic markers, DNA markers are the most widely used type of marker predominantly due to their abundance. Various types of DNA markers may be broadly divided into three classes based on the method of their detection: (1) hybridization-based; (2) polymerase chain reaction (PCR)-based; and (3) DNA sequence-based (Winter and Kahl 1995; Jones et al. 1997; Gupta et al. 1999; Joshi et al. 1999).

Nevertheless, in dry bean, early work started in the 1990s with RAPD markers linked to major disease resistance traits, and the tool was deployed in many labs (Kelly 1995; Kelly and Miklas 1998). To expand their versatility, many of these markers were converted to sequence-characterized amplified region (SCAR) markers, and this further expanded their utility throughout the community. A number of these SCAR markers are still being used in breeding programs decades later (Melotto et al. 1996). Among the several diseases in bean, BCMV and BCMNV are the most common and destructive potyviruses known to infect. The independence of the BCMV resistance genes provides opportunities to use gene pyramiding as strategy inbreeding for durable resistance. Bean breeders recognize that the combination of the dominant *I* gene with recessive *bc* resistance genes offers durability over single-gene resistance to BCMV and BCMNV since the two types of genes have distinctly different mechanisms of resistance (Kelly 1997). The molecular methods also facilitate the selection of genotypes with desirable gene combinations. A few markers have been reported as successfully applied for the identification of resistance genes against BCMV and BCMNV. Haley et al. (1994) described an RAPD marker OW13 of 690 bp linked to the *I* gene in coupling. Later, this marker was converted to a sequence-characterized amplified region (SCAR) marker SW13, which was more reliable and reproducible (Melotto et al. 1996). Molecular markers were developed also for the recessive *bc-u* and *bc-1*. These two loci were found to be linked (Strausbaugh et al. 1999). Miklas et al. (2000a) suggested SCAR marker SBD5 for marker-assisted selection (MAS) of $bc1^2$ in snap beans as well as those of Middle-American origin. Recently, Vandemark and Miklas (2005) described codominant interpretation of dominant markers using quantitative PCR to enable discrimination of homozygous and heterozygous individuals for *I* and $bc\text{-}1^2$ genes. These available markers were also used, together with already available framework maps, to estimate the genomic position of disease resistance loci onto the consensus genetic map of common bean.

Over 20 major genes conditioning resistance to anthracnose have been characterized as markers. Molecular markers linked to the majority of major *Co*-genes have been widely reported, and these provide the opportunity to enhance disease resistance through MAS (see Kelly et al. 2003; Kelly and Vallejo 2004). The use of molecular markers to characterize resistant genes to anthracnose has significantly contributed for initial steps of breeding programs by reducing the time and costs involved during the whole process. This occurs because DNA markers are closed linked to genes and are not influenced by environmental factors and they show epistatic or minimum/nonpleiotropic effects. Among the available molecular markers, the ones denominated as SCAR (sequence-characterized amplified regions for amplification of specific band) have been playing great importance on common bean analyses. Until now, there are 14 SCAR markers linked to anthracnose resistance genes, which are SEACT/MCCA, SCAreoli1000, SQ41440, SW12700, SY20830, SC08910, SAS13950, SH181100, SBB141150/1050, SAB3400, SZ20845, SZ04567, SB12350 and SF101072. SCAR markers have been optimized in breeding programmes that search for anthracnose resistant cultivars by implanting assisted backcrosses programmes (Miklas and Kelly 2002).

Several common bean accessions had been identified from Andean and Mesoamerican gene pools, and dominant, monogenic, loci conferring qualitative ALS resistance to specific pathogen races have been mapped in the bean genome. The *Phg-1* locus, identified in AND277, is located on chromosome 1 at a distance of 1.3 cM from marker TGA1.1 (Queiroz et al. 2004; Gonçalves-Vidigal et al. 2011). Mesoamerican resistance locus, *Phg-2*, mapped qualitatively in Mexico54 is located on chromosome 8 at a distance of 5.9 and 11.8 cM from markers SN02 and OPE04, respectively (Sartorato et al. 2000). Two dominant, monogenic, resistance loci were also identified in G10909: PhgG10909A and PhgG10909B (Mahuku et al. 2011). These loci are inherited independently from all other known resistance genes, conferring resistance to ALS race 63-63, which overcomes all other known resistance sources. Quantitative trait locus (QTL) mapping had also been used to identify multiple resistance loci in Andean germplasm. Among many diseases in dry bean, bean rust is also one of the most devastating disease that results in significant loss of seed yield in dry beans and pod quality in snap beans (rust resistance in common bean is conditioned by single and dominant genes identified by the *Ur*- symbol (Kelly et al. 1996). To date, ten genes have been named and tagged, mostly with RAPD or SCAR molecular markers (Miklas et al. 2002). Five genes (*Ur-3*, *Ur-5*, *Ur-7*, *Ur-11* and *Ur-14*) belong to the Middle-American gene pool, while five genes (*Ur-4*, *Ur-6*, *Ur-9*, *Ur-12* and *Ur-13*) belong to the Andean gene pool (Souza et al. 2011). Furthermore, the current molecular markers (mostly RAPD and SCAR markers) linked to rust resistance genes in common bean that were published almost two decades ago yield false-positive and false-negative results, as indicated by the authors that reported the currently available RAPD (OK14620) and SCAR (SK14) markers linked to the *Ur-3* locus (Nemchinova and Stavely 1998). Another most important disease in common bean is CBB is a seed-borne disease that plagues bean production worldwide. SCAR markers BC420, SU91 and SAP6 linked with three major QTL on B6, B8 and B10 (see Kelly et al. 2003), respectively, are being used for MAS of CBB resistance (Yu et al. 2000). To date, 24 QTL conferring resistance to CBB have been identified, distributed across all 11 chromosomes of common bean (Shi et al. 2011). Among these loci, most have been tagged with SCAR markers. Examples include BC420 (Yu et al. 2000), SU91 (Pedraza et al. 1997) and SAP6 (Miklas et al. 2000a), which are linked with three major QTL of particular interest to researchers in CBB resistance (Jifeng et al. 2016).

13.6.2 Tagging and Mapping

Linkage mapping in common beans as in other crops has benefited from a range of molecular technologies that have greatly supplemented the number of genetic markers used in genetic maps for the species. As a result, a large number of markers are in use today for common bean mapping compared to early linkage maps that were based almost entirely on a limited number of morphological markers such as those for flower or seed colour or certain pod traits and growth habit characteristics.

Subsequently, the tagging and mapping of resistance genes in *P. vulgaris* have expanded greatly with the sequencing of the bean genome (Schmutz et al. 2014). The of physical positions for individual genes has generated a map with major resistance gene clusters on eight bean chromosomes. More than 30 individual genes for disease resistance and a similar number of genes for QTL underlying major traits with significant impact to common bean agriculture in the tropics have been successfully linked with markers. Such genes tightly linked with markers are referred to as 'tagged genes'. The primary goal for gene tagging in common bean has been to identify markers tightly linked with disease resistance traits for the purpose of marker-assisted selection. The first linked marker (RAPDA14.1100) was identified for the *Ur-4* rust resistance gene (Miklas et al. 1993) and was used for gene pyramiding and retention of a less effective gene (*Ur-4*) in the presence of an epistatic gene, *Ur-11*, with broad effect against the hypervariable rust pathogen (Stavely et al. 1994). In the past, genetic maps were mostly generated from wide crosses and were not sufficiently dense with markers. In addition, markers linked to a given trait were oftentimes not found in other unrelated populations, restricting their potential use for MAS to certain populations or gene pools in common bean. As an alternative to biparental linkage mapping for the identification of markers tightly linked to monogenic and quantitative disease resistance loci, bean geneticists developed molecular tagging methods utilizing several types of segregating populations, including F2, near isogenic lines (NILs), backcrossed inbred lines (BIL) and recombinant inbred lines (RILs) often in combination with bulked segregant analysis (BSA). The genome-wide SNP coverage of common bean provides an opportunity to discover SNPs underlying genomic regions of disease resistance traits previously identified by QTL mapping. A rapid method to discover or refine markers linked to common bean disease resistance genes would be the application of 'in silico BSA' which consists of inspecting SNP variation among genotyped individuals, with known phenotype, at specific targeted genomic regions. Resistance to BCMV and BCMNV is genetically well-defined and conditioned by a single dominant (*I*) and four recessive (*bc-u*, *bc-1*, *bc-2*, *bc-3*) genes (Marco et al. 2014). The chromosomal position of the *I* gene and *bc-1*^{2} and *bc-3* genes maps independently (Miklas et al. 2006). A SCAR marker (SW13) linked to the *I* gene was developed (Marco et al. 2014) and is widely used by bean breeders. However, in several mapping populations, recombination between the SW13 marker and *I* gene was detected. Since then, many resistance genes have been tagged (see Kelly et al. 2003; Ragagnin et al. 2005; Miklas et al. 2006) including *Ur-3*, *Ur-5*, *Ur-6*, *Ur-7*, *Ur-9*, *Ur-11* and *Ur-13* for resistance to rust; *Co-1*, *Co-12*, *Co-2*, *Co-4*, *Co-4*^{2}, *Co-5*, *Co-6*, *Co-9* and *Co-10*, for anthracnose resistance; and five *Phg* genes (Caixeta et al. 2005) for resistance to angular leaf spot. Most genes to date have been tagged with RAPD markers which have been converted to SCAR markers (Miklas et al. 2006). Further, the discovery of markers linked to CBB, in bean, has revolutionized our understanding of resistance to CBB, caused by *Xanthomonas axonopodis* pv. phaseoli (Xap). The CRSP scientists identified different QTL conditioning resistance in young and adult tissues (Miklas et al. 2006). Five QTL conferring resistance to Xap were identified with four of them located on LGs B2, B5, B7 and B9 in the BJ population. Only one

QTL on B7 was common with four additional QTL that reside on LGs B6–B8 and B10. Some early examples of marker-assisted selection for bean diseases include 23 RAPD markers and 5 SCAR markers associated with 15 different resistance genes, described by Kelly and Miklas (1998). Molecular markers and linkage mapping of rust resistance genes have been reviewed by Miklas et al. (2002). Kelly and Vallejo (2004) provided a summary of markers, MAS, map location and breeding value for anthracnose resistance. Five QTL for angular leaf spot resistance were identified in the DOR 364 × G 19833 population and mapped to linkage groups B4 and B10 (Lopez et al. 2003). All five QTL were located near resistant gene analogs (RGAs) suggesting that they share structural similarities with R genes and perhaps reside within gene clusters because resistance to anthracnose co-located with three of the QTL. The utility of these QTL for breeding purposes has not been fully explored.

More recent reports illustrated that a second bean genotype BAT 93 from the Mesoamerican gene pool has been sequenced by a multinational consortium (Vlasova et al. 2016). Support for the expected gene synteny between the two genomes is displayed by 25,991 protein-coding genes (PCG) mapped in BAT93, where 20,617 were uniquely mapped to 20,618 PCGs in the Andean genome, G19833 (Vlasova et al. 2016). The authors report that 234 of the 852 putative resistance genes identified in the BAT93 genome belonged to the cytoplasmic NBS-LRR class. This compares with 316 NBS-LRR class genes in G19833 that could be mapped to 220 genes in BAT93. However, Vlasova et al. (2016) were unable to find resistance gene clusters that were specific to either of the two genotypes. The tagging and mapping of resistance genes in beans have expanded greatly with the sequencing of the bean genome (Schmutz et al. 2014). The identification of physical positions for individual genes has generated a map with major resistance gene clusters on eight bean chromosomes. Details of the individual clusters are shown in Table 13.4. Genes controlling fungal (*Co*-genes for anthracnose, *Phg* genes for angular leaf spot, *Ur*-genes for rust), bacterial (*Pse*-genes for halo blight).

13.6.3 MAS for Disease Resistance

The main obstacles that limit bean production is diseases such as bean rust, BCMV and BCMNV, angular leaf spot, powdery mildew, halo blight, bacterial blight and anthracnose are most important diseases of common bean throughout the world. Chemical control is expensive, and the generation of pathogen-free seeds is often difficult in developing countries; genetic resistance represents the most reliable control strategy. The use of resistant genotypes is the most economic and ecologically safe management strategy. Therefore, molecular markers have been sought for both simple and complex traits in beans, with an eye to eventual application in MAS. Tagging of genes and QTL in common bean and their application to MAS has been reviewed previously (Kelly et al. 2003; Miklas et al. 2006). In the present chapter, some of the aspects that contribute to the successful use of MAS are considered in greater detail, referring to examples taken from bean breeding for disease

Table 13.4 Linkage group and R-gene clusters in common bean (Kelly and Noaln et al. 2018)

Linkage group	Position (Mb)	Genes	Pathogen
Pv01	50.16–50.30	Co-1	Anthracnose
	49.81–50.51	Co-x	Anthracnose
	ND	Co-w	Anthracnose
	50.26–50.34	Co-1^{HY}	Anthracnose
	50.16–50.55	Co-Pa	Anthracnose
	48.45–50.30	Co-AC	Anthracnose
	50.5	Co-1^2	Anthracnose
	50.51	Co-14	Anthracnose
	50.51	Co-1^4	Anthracnose
	50.51	Phg-1	ALS
	ND	Ur-9	Rust
Pv02	ND	Co-u	Anthracnose
	40.39–40.44	Co-u	Anthracnose
	48.6	NN	Anthracnose
	48.18–48.27	Pse-3	Halo blight
	48.18–48.27	I	BCMV
Pv03	ND	Co-13	Anthracnose
	<0.044	Co-17	Anthracnose
	0.36–0.39	bc-1^2	BCMV
	0.36–0.39	bc-u	BCMV
Pv04	0.59	Pse-6	Halo blight
	ND	Ur-5	Rust
	ND	Ur-D^a	Rust
	1.23–1.26	Ur-14	Rust
	41.88–45.45	Phg-4	ALS
	0.49–0.58	Co-3^4	Anthracnose
	9.08	Co-15	Anthracnose
	1.43	Co-16	Anthracnose
	3.36	Co-3	Anthracnose
	3.36	Co-3^3	Anthracnose
	3.36	Co-10	Anthracnose
	ND	Co-y	Anthracnose
	ND	Co-z	Anthracnose
Pv07	6.85	Co-5^2	Anthracnose
	ND	Co-6	Anthracnose
Pv08	2.38–2.48	Co-4^2	Anthracnose
	ND	Ur-13	Rust
	57.82–58.53	Phg-2	ALS
Pv10	11.18	Pse-1	Halo blight
	3.49	Pse-2	Halo blight
	39.58	Pse-4	Halo blight
	3.82–8.78	Phg-5	ALS

(continued)

Table 13.4 (continued)

Linkage group	Position (Mb)	Genes	Pathogen
Pv11	46.96–47.01	Ur-3	Rust
	51.93	Ur-11	Rust
	ND	Ur-6	Rust
	ND	Ur-D[b]	Rust
	ND	Co-2	Anthracnose

resistance in the tropics at CIAT and within NARS. Recently, a more reliable and specific PCR-based marker known as sequence-characterized amplified region (SCAR) was developed in common bean for molecular breeding. SCAR primers are longer than RAPD primers, and a highly stringent annealing temperature can be employed that prevents mismatching in the priming site during DNA amplification (Melotto et al. 1996). Kelly and Miklas (1998) described the role of RAPDs in MAS and extensively covered efficiency of different linkage orientations for markers linked mainly with specific resistance (SR) genes. Marker-aided selection (MAS) has been used to transfer host-plant resistance to both BCMV and BCMNV into susceptible germplasm. Indirect selection of *I* gene (Haley et al. 1994; Melotto et al. 1996), *bc-3* (Mukeshimana et al. 2005), *bc-1²* (Miklas et al. 2000a), *bc-1* and *bc-u* (Strausbaugh et al. 1999). Genes have been achieved through the use of DNA markers. The feasibility for using SCAR markers in MAS was demonstrated by introducing the *I* gene into susceptible germplasm with the aid of SW13 marker (Kelly et al. 1995; Miklas et al. 2002; Pastor-Corrales et al. 2007; Kelly et al. 2018). Similarly, Sergey et al. (2013) used two SCAR markers (SW13 and SBD5) successfully in marker-aided backcrossing for pyramiding the *I* and *bc-1²* genes, which provide host-plant resistance to BCMV. The identification of anthracnose resistance genes has been facilitated by the development of associated molecular markers. Dongfang et al. (2008) observed that the marker SAS13 (linked to Co-4), which had been widely used in marker-assisted selection, gave anomalous results when applied to Morden003 and a number of other navy bean cultivars. Thus, molecular markers linked to the majority of major *Co*-genes have been widely reported, and these provide the opportunity to enhance disease resistance through MAS (see Kelly et al. 2003; Kelly and Vallejo 2004). Development of common bean lines resistant to angular leaf spot through molecular marker-assisted selection is challenging in common bean breeding programme. RAPD or SCAR markers linked with many of the dominant resistance genes have been obtained (see SCAR list (Miklas 2005). The random amplified polymorphic DNA (RAPD) markers OPN02890, OPE04500 and OPA012950 linked in coupling phase at 5.9, 5.8 and 5.8 cM of the angular leaf spot resistance genes of cultivars 'Mexico 54', 'MAR 2' and 'BAT 332, respectively, were identified. Development of homozygous common bean lines carrying angular leaf spot resistance genes is derived from the cultivars Mexico 54, MAR 2 and BAT 332 through marker-assisted selection by using SCAR molecular markers OPN02890, RAPDOPE04500 and OPAO12950 (De Oliveira et al. 2005). A fine-mapping approach of a major quantitative trait locus (QTL) $ALS4.1^{GS, UC}$ for ALS

resistance in a mapping population derived from the resistant genotype G5686 and the susceptible cultivar Sprite reported by Keller et al. (2015). The substantial progress made in molecular marker technology for the common bean holds considerable promise for breeding genetic resistance to CBB. Previous studies reported that CBB resistances from XAN 159, GN no. 1 Sel 27 and PI 207262 were combined to produce XAN 263 and XAN309 and combined with other sources to produce the VAX dry bean lines with high levels of CBB resistance. A novel resistance QTL with major effect was detected by using SCAR markers such as SAP6 and SU91 on Pv11 linkage group in Othello/VAX 1 (Diego et al. 2014).

13.7 Perspective

For sustained development of improved bean cultivars resistant to many diseases, researchers need to continue identify, share and preserve sources of resistance to the important diseases around the world; develop faster and more reliable screening procedures for both direct selection (phenotypic) and MAS of major disease resistance; better understanding of the inheritance and mechanisms of resistance; and integrate marker-aided breeding to compliment classical breeding on a case-by-case basis. Recently, MAS is being employed principally to bolster phenotypic selection for disease resistance genes in common bean. Disease resistance is often governed by relatively few genes, and phenotypic data are obtained more easily. On the other hand, MAS for more complex traits has yet to find ready application. In this regard, the use of parental surveys of many of the genotypes involved in a given breeding programme is an important first step in implementing MAS. Occasionally the question is raised: Which is better, MAS or conventional selection? This very question betrays a false dichotomy that hinders progress. By itself, MAS is seldom an adequate selection too and therefore must be combined with conventional phenotypic selection. The objective should be to develop the optimal balance between conventional and molecular breeding, and the 'best' balance will be unique to each situation, crop, selection scheme, environment and opportunities for different selection methods. More emphasis is needed on combined selection systems, rather than viewing MAS as a replacement for phenotypic or field selection.

B. BCMNV
C. BGYM
D. Anthracnose
E. Angular leaf spot
F. Powdery mildew
G. Brown spot
H. Root rot

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Index

A
Abiotic stress
  dry pea
    drought stress, 313–316
    evaluations of crop, 309
    frost stress, 316–318
    heat stress, 309–312
    selection, resistance genotypes, 309
  mungbean
    drought stress, 240
    pre-harvest sprouting, 243
    salinity stress, 242
    temperature stress, 241, 242
    water logging, 241
  plant metabolism, 240
  pretreatments, 81
  tolerance in chickpea, 217, 220–221, 223
Advanced backcross quantitative trait loci (AB-QTL), 32
Agronomical traits, 79
Angular leaf spot (ALS), 398, 399
Anthracnose (ANT), 237, 252, 253, 396–398
Antixenosis, 257
Ascochyta blight (AB), 300, 318
Association mapping approaches, 160

B
BAC-end sequences (BESs), 130
Bacterial artificial chromosome (BAC)
  genome research, 262
  molecular markers and QTL, 263
  PCR-based markers, 263
  in *Vigna* species, 262
Bacterial leaf blight (BLB), 253
Bean common mosaic necrosis virus (BCMNV), 392, 394, 395, 406, 408, 409, 411
Bean common mosaic virus (BCMV), 392, 394, 395, 406, 409, 411
Bean golden mosaic virus (BGMV), 392
Bean golden yellow mosaic virus (BGYMV), 392, 393, 396
Begomoviruses, 355
Biotechnological approaches in lentil
  embryo rescue assisted breeding, 80, 81
  HTS (*see* High-throughput sequencing (HTS))
  linkage mapping (*see* Linkage mapping)
  tissue culture, 79, 80
  transcriptomics, 85–87
  transgenic approaches, 81, 82
Biotic stress
  dry pea
    AB, 300–302
    common root rot, 305–308
    Fusarium root rot, 302–304
    fusarium wilt, 304, 305
    powdery mildew, 292, 293, 296, 297
    rust, 297–300
  lentil, 73
  mungbean, 236, 237, 239
Blackgram, *see* Urdbean
Bowman-Birk inhibitor (BBI), 348
Breeding programmes, 79
Bruchid resistance, 257–259
Bulked segregant analysis (BSA) approach, 48
Bulk selection method, 7

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C
Cajanus cajan (L.), *see* Pigeonpea
Cajanus cajan gene expression atlas (CcGEA), 131
Cercospora leaf spot (CLS), 178, 254, 255
Cereal-based cropping systems, 1
Chickpea
  abiotic stresses, 42–43, 223
  biotic stresses, 40–42
  cold stress, 216
  crop yield, 211
  CWRs, 216
  drought stress (*see* Drought stress, chickpea)
  ESTs resources, 222
  GAB (*see* Genomics-assisted breeding (GAB))
  genetic resources, 32
  genetics control, salinity tolerance, 222
  genetic variability, 212, 213, 215
  genomic resources, 32, 43–45
  genotypes, abiotic stress tolerance, 213
  germplasm
    characterization, 52–53
    and genetic resources, 32–33
  heat stress, 214, 215
  herbicide-tolerant varieties, 55
  integrated breeding, 57
  kabuli chickpea varieties, 54
  linkage, physical and functional maps, 46
  machine-harvestable, 55
  molecular breeding approaches, 32
  molecular markers, 32
  novel breeding schemes, 224
  nutrient acquisition efficiency, 56
  nutritionally rich varieties, 57
  physiological traits, 218–219
  regaining chickpea area, 54
  salinity stress, 215, 216
  seed oil, 31
  SNP marker, 212
  speed breeding/RGA, 51
  trait identification, 53
  trait mapping (*see* Trait mapping)
  transcriptomic resources, 43–45
  varietal development, 32–39, 44
  vegetable purpose, 54
  yield improvement, 47, 50
Cicer arietinum L., *see* Chickpea
Clustered regularly interspaced short palindromic repeats-associated system (CRISPR/CAS), 19
Cold stress in chickpea, 216
Cold tolerance
  chickpea germplasm, 216
Common bacterial blight (CBB), 393, 402, 403
Common bean
  bean-producing countries, 389
  biotic stresses, 390
  cultivation methods, 391
  diseases, biotic factor, 392–394
  food legume crops, 389
  genetic resources, 390, 391
  linkage group and R-gene clusters, 410–411
  micronutrients, 389
  nutrient content and commercial potential, 389
  origin, 389
  production, 392
  trends in production, 392
Common bean breeding
  bacterial disease, disease resistance
    CBB, 402, 403
    HB, 403
  fungal disease, disease resistance
    ALS, 398, 399
    ANT, 396–398
    PWM, 400
    rust, 401, 402
  viral disease, disease resistance
    BCMNV and BCMV, 394–395
    BGMV and BGYMV, 396
Common root rot, field pea, 305–308
Composite interval mapping (CIM), 303
Conserved domain database (CDD), 268
Conventional breeding, 33, 122
  abiotic stress-tolerant chickpea, 217
  mungbean breeding, 259
Conventional breeding strategies
  horsegram
    agro-morphological traits, 374
    hybridization, 377
    improved varieties, 375
    vs. molecular approach, 377–379
    mutation, 376
    natural germplasm, 374
    programme, 373
Conventional crossing techniques, 78
Conventional disease management strategies, 305
Conventional linkage approaches, 160
Conventional screening methods, 124
Cool Season Food Legume Crop Database, 85
Cotyledonary nodes, 81
Crop wild relatives (CWRs), 13, 216
Crop yields, lentil, 70

Cross-breeding, 77
Cultivated horsegram, 370
Cytoplasmic genetic nuclear male sterility (CGMS) system, 115
Cytoplasmic male sterility (CMS), 114
Cytoplasmic-nuclear male sterility (CMS), 120

D
Disease resistance breeding, 405–407
Diversity array technology (DArT) markers, 91
DNA-based marker systems, 260
Double haploid (DH), 18, 80
Drought stress, chickpea
  biological processes, 212
  $\Delta^{13}C$ discrimination, 214
  dry pea, 313–316
  escape mechanism, 213
  genetic variability, 213
  genomic resources and QTL mapping, 219, 221
  innovative breeding, 214
  MAGIC breeding scheme, 214
  mungbean, 240
  parameters, 214
  reproductive phase, 212
  water-use efficiency, 214
Drought tolerance, lentil, 72
Dry pea
  area, production and productivity, 284–286
  developmental mutant, 291
  for dwarf type, 291
  earliness, 319
  forms, 283
  genetic resources, 287–289
  for lodging resistance, 289–291
  micronutrients, 283
  multiple disease resistance, 318
  multi-purpose, 319
  nutritional quality, 284
  powdery mildew infected plants, 292
  rust infected plant parts, 298
  seed coat and cotyledon, 283
  systematic, origin and domestication, 286–287
  traits, 318
Dwarfing Mendel's *le-1* mutation, 291

E
Embryo rescue method, lentil, 80, 81
Embryo rescue protocols, 79
Ethyl methanesulfonate (EMS), 77, 78
Expressed sequence tags (ESTs), 45, 131

F
Field pea
  diseases resistance, 292
  drought condition, 313 (*see also* Dry pea)
  frost tolerant, 316
  heat stress, 311
  marrowfat field pea, 287
  pea production, 305
Focused identification of germplasm strategy (FIGS), 6
Food legumes, 369
Frost stress, 316–318
Functional genomic, 222
Fusarium root rot, peas, 302–304
Fusarium wilt (FW), 111, 122, 123, 304, 305

G
GBS-based approach, 132
Gene clusters, common bean chromosomes, 408, 409
Gene tagging/mapping, 407–409
Generation Challenge Program (GCP), 33
Genetic gain, 8–10, 14–17, 20–22
Genetic information, 120
Genetic resources
  common bean, 390, 391
  dry pea, 287–289
Genetic transformation, 185
Gene-transfer methods, 18
Genic male sterility (GMS), 114
Genome-based GBS approach, 45
Genome Survey Sequences (GSS), 268
Genome-wide association (GWA) mapping, 308
Genome-wide association study (GWAS) approach, 47
Genome-wide selection/genomic selection (GS), 16, 49
Genomic estimated breeding values (GEBVs), 17, 51
Genomic resources
  mungbean breeding
    BAC (*see* Bacterial artificial chromosome (BAC))
    EST-SSRs marker, 263, 264
    ILP, 264
    markers, development and use, 260–262
    plant phenomics, 265–267
    RNAi technology, 264, 265

Genomics-assisted breeding (GAB), 32, 39, 44–51, 123, 162
Genomics, chickpea, 219, 221
Genotype and environment (G XE), 259
Genotypic coefficient of variability (GCV), 246
Genotyping by sequencing (GBS), 45, 47
Germplasm resources, 244, 245
Gibberellin (GA) pathway, 291
Grain legumes, 309
Grain yield, 289
Green gram
- anthracnose, 252
- archeological evidences, 145
- area and production, 235
- assessment, genetic diversity, 261
- BAC libraries, 260, 262
- bruchid resistance, 258
- *Cercospora* leaf spot, 237
- CLS resistance, 254
- conventional breeding, 153–157
- description, 235
- diseases, 246
- genetic
  - resources, 151–153
  - variability, 236, 244
- genome sequencing, 157–160, 265
- genome-wide association mapping, 162–163
- genomics-assisted breeding, 160–161
- GSS, 268
- in India, 235
- ISSR markers, 261
- leaf crinkle virus, 238
- moisture stress, 237
- molecular marker, 251
- mungbean (*see* Mungbean)
- pests, 236
- production, 149–151, 235, 244
- RFLP markers, 260
- seed quality and yield, 237
- transgenic technology, 163
- *Vigna* species, 149, 153, 161
- viruses, 247
- yield improvement, 236
- YMV tolerance, 248
- Yum Woon Sen, 144

H

Halo blight (HB), 403
Haplotype assembly, 223
Heat stress
- chickpea, 214, 215
- dry pea, 309–312

Heat tolerance, lentil, 72
High-throughput field phenotyping (HTFP), 12
High-throughput plant phenotyping platforms (HTPPs), 266, 267
High-throughput sequencing (HTS)
- in lentil
  - hybrid heterosis, 83
  - molecular markers, 83
  - NGS, 83–85
  - PacBio, 85
  - QTL, 83
  - RNA-seq, 84
  - SSR markers, 83

Horsegram
- antioxidant properties, 371
- commercial crop, 369
- conventional variety development programme, 373
- cultivars, 370, 373
- description, 369
- dry seeds, 371
- framework linkage map, 381
- genetic resources and distribution, 371, 372
- genome size, 370
- geographical distribution, *Macrotyloma* species, 372
- molecular approach, genetic improvement, 379–382
- production and area, 373
- pulse crop, 370
- source of protein, 370
- as sprouts, 371
- wild forms, 375

Horsegram yellow mosaic virus (HgYMV), 181
Hot spot QTL, 221
Hybridization-based molecular markers, 194

I

Insect resistance, lentil, 73
International Center for Agriculture Research in the Dry Areas (ICARDA), 74, 76
International collection databases, 289
Intron length polymorphism (ILP), 264
Intron spanning regions (ITRs), 131

K

Kunitz trypsin inhibitor (KTI), 348, 349, 352

L
Legume family, 211
Legumes, lentil, 70
Lens culinaris Medik, *see* Lentil
Lentil
adoption, 70
cold and frost tolerance, 72
conventional breeding programs, 193
fungal and bacterial diseases, 70
genetic gain, 193
genomic resources
comparative genome analysis, 201–202
lentil genome sequence, 196
linkage maps, 197–199
mapping populations, 197–199
molecular markers, 194–196
transcriptome analysis, 201
genomics era, 203–204
legumes, 70
marker-trait association studies, 92–93
plate health benefits, 70
pre-breeding, 71–73
productivity, 70, 78
pulses, 70
region-wise targeted traits, 74, 75
salinity tolerance, 72
trait targeted approach, 71
transgenic development, 202
Lentil breeding programme, 74, 77
Lentil spread, 74
Linkage mapping
molecular markers, 87
from multiple mapping populations, 90–91
quantitative trait loci, 87
on single mapping populations, 88–89
Lodging resistance, pea, 289, 290

M
Macrosperma, 71
Macrotyloma sar-garhwalensis, 372
Marker assays, 343
Marker-assisted backcrossing (MABC), 8, 16, 32, 49, 224
Marker-assisted breeding, 379
Marker-assisted recurrent selection (MARS), 16, 32, 49, 224, 226
Marker-assisted selection (MAS), 16–17, 83, 181, 249, 251, 398, 402, 403, 405, 406, 408, 409, 411, 412
Maturity duration, soybean, 344, 345
Mega-environmental design (MED) strategy, 21
Micrografting, 78, 79, 82
Modified pedigree method, 129
Molecular breeding
common bean
diagnostic markers, 405–407
MAS, disease resistance, 409–412
molecular markers, 405
tagging and mapping, 407–409
horsegram, 377–379
Molecular markers, 83, 405
Multilocation environment trials (MET), 9, 12, 17, 20, 21
Multi-parent advanced generation intercross (MAGIC) breeding scheme, 14, 214
Multiple seed descent method, 129
Multiplexed chip-based technologies, 357
Mungbean
abiotic stress (*see* Abiotic stress)
biotic stress, 236, 237, 239
crop improvement strategy
agricultural development programmes, 244
breeding goals, 245
breeding programme, 244
genetic variability, 244
germplasm resources, 244, 245
MYMV (*see* Mungbean yellow mosaic virus (MYMV))
Mungbean breeding
advanced breeding strategies, 269
biotechnological tools, 259
for bruchid resistance, 257–259
goals, 245
mutagenesis, 246
resistance to bacterial diseases
BLB, 253
leaf spot, *Cercospora canescens*, 254, 255
seed-borne, 253
resistance to biotic stresses, 246, 247
resistance to fungal diseases
anthracnose, 252, 253
powdery mildew, 250–252
QTLs/molecular markers, 251
SSR marker-based QTLs, 251
resistance to insects, 255–257
resistance to viral diseases,
MYMV, 247–250
Mungbean yellow mosaic disease (MYMD), 151, 153, 161
Mungbean yellow mosaic India virus (MYMIV), 161, 181, 249, 355, 356

Mungbean yellow mosaic virus (MYMV), 158, 180, 181
- bipartite genome, 248
- causes, 239
- description, 238
- geminiviruses, 247
- ISSR marker, 248
- mutant genotypes, 248
- rate of infection, 248
- susceptibility and resistance, 249
- YMV tolerance, 248

Mutagenesis, 246
Mutation approach, 7
Mutation breeding, 77, 111, 376
Mutation techniques, 77

N

Nested association mapping (NAM), 14
Next-generation sequencing (NGS), 44, 83, 84, 130, 201, 343, 382
NGS-based approaches, 132–133
Novo-based GBS approach, 45

O

Oil quality, soybean, 345, 346, 348
Oleic acid, 350
Orphan crops, 369, 379, 380
Osmoregulation, 240
Oxford Nanopore, 85

P

Pacific Biosciences (PacBio), 85
PCR-based molecular markers, 194, 195
Pea rust, 297, 298
Pest management strategy, 41
Phaseolus vulgaris, *see* Common bean
Phenotypic coefficient of variability (PCV), 246
Phytic acid, 346, 349–350
Phytin, 349
Phytozome, 356
Pigeonpea
- abiotic stresses, 125–128
- biotic stresses, 122–125
- breeding
  - breeding methods, 111–114
  - germplasm, 111
  - heterosis, 114–116
  - hybridization, 114
  - plant habits, 110
  - selection, 114
- genetic improvement program, 110
- genetic resources, 119–121
- genomics and molecular breeding, 130–133
- high protein content, 128–129
- hybrid breeding, 114, 116, 121–122, 133, 134
- molecular markers, 130
- speed breeding, 129–130
- varietal development, 129, 130
- yield improvement, 110, 116–119

Pigeonpea sterility mosaic virus (PPSMV), 123
Pisum sativum, *see* Dry pea
Plant breeding, *see* Dry pea
Plant phenomics, 265–267
Polyunsaturated fatty acids (PUFA), 350
Powdery mildew (PWM), 251, 252
- common bean, 400
- dry pea, 292, 293, 296, 297

Powdery mildew disease (PMD), 178
Pre-breeding, lentil, 71–73
Pre-harvest sprouting, 243
Prioritization of traits, 74
Pulse crops
- agri-food systems, 2
- breeding scheme, 13
- crop–livestock systems, 1
- experimental design, 20–22
- genetic variability, 13–14
- genome editing technologies, 19
- GS, 17
- heritability, 9, 13, 15–17, 20, 21
- high-throughput phenotyping, 12
- indirect selection, 15–16
- MAS (*see* Marker-assisted selection (MAS))
- past breeding efforts, 5–9
- production, 3–5
- rapid generation advancement, 17–18
- recombinant DNA technology, 18, 19
- selection efficiency, 9, 20–21
- selection indices, 14–15
- speed breeding, 17–18
- TPE (*see* Target population of environments (TPE))

Pure line selection, 76

Q

Quantitative trait loci (QTL), 77, 83
- chickpea
  - heat and cold stress tolerance, 221
  - hot spot QTL, 221

mapping in pea, 219, 301
validation, 307

R

Raffinose family of oligosaccharides (RFOs), 57
Random amplified polymorphic DNA (RAPD), 250
Rapid generation advancement (RGA), 51
Resistance gene clusters, bean chromosomes, 408
Restriction amplified polymorphic DNA (RAPD), 130
Restriction fragment length polymorphisms (RFLPs), 130, 176, 251, 258, 260
RNA interference (RNAi), 264, 265
RNA sequencing (RNA-seq), 84
Rust
common bean, 401, 402
soybean (SBR), 352, 353
Rust resistance in pea, 297–300

S

Salinity stress
chickpea, 215, 216
mungbean, 242
Salinity tolerance, lentil, 72
Selection accuracy, 8–10
Sequencing-based markers, 195, 196
Sick plot screening method, 123
Single nucleotide polymorphism (SNP), 44, 131, 163, 195, 343
Single seed descent (SSD) method, 76, 77, 129
Skim sequenced data approach, 47
Soil salinity stress, 215, 216
SoyBase, 356
Soybean
diseases
rust, 352, 353
SMV, 354, 355
YMD, 355
flavour and fragrance, 346–348
flowering and maturity duration, 344, 345
genes, 345
genotype NRC142, 351
lifestyle diseases, 346
lipoxygenases, 346
meal, 346
mineral availability, 349–350
nutritional value, 346
oxidative stability, 350–351
protein digestibility, 348–349
SD plant, 344
tools and genomic resources, 356
traditional methods, 343
trypsin inhibitor activity, 348
Soybean Knowledge Base (SoyKB), 356
Soybean mosaic virus (SMV), 354, 355
Soybean oil, 346
Soybean rust (SBR), 352, 353
Sterility mosaic disease (SMD), 111, 123
Sterility mosaic virus (SMV), 118
Sukjunamul, 144

T

Targeted breeding approaches, 120
Targeting Induced Local Lesions in Genomes (TILLING), 14
Target population of environments (TPE), 11–12
Temperature stress, 241, 242
Tentative unique sequences (TUSs), 44, 45
Theory of quantitative genetics, 10
Thidiazuron (TDZ), 81
Tissue culture, 71, 78–80, 185
Trait mapping, 44, 45, 47–48
Transcript assembly contigs (TACs), 131
Transcriptome sequencing, 84
Transcriptomics, in lentil, 85–87
Transgenic approaches, in lentil, 81, 82
Translational genomics, soybean, 344, 356

U

Urdbean
breeding methods
crop improvement research stations, 187
intra- and interspecific hybridization, 180–184
multiparent-based hybridization, 180
"omic" technologies, 185–186
breeding objectives, 179
crop systematics, 175
floral biology, 176
genetics of disease-pest resistance, 178–179
molecular markers, 176, 181, 185, 186
origin, 175
plant morphology, 176
produces and consumes, 173
producing regions, 174
species relationship, 175–176
trait inheritance, 177–178
Urdbean leaf crinkle virus (ULCV) disease, 179

V
Variability, 246
Vigna radiata L., *see* Green gram
V. mungo L. Hepper, *see* Urdbean

W
Water logging, 241
Whole-genome re-sequencing (WGRS), 109, 130
Whole-genome sequencing (WGS), 84, 130
Window-based bin mapping approach, 47

Y
Yellow mosaic disease (YMD), 181, 184, 355, 356