Satbir Singh Gosal
Shabir Hussain Wani *Editors*

Accelerated Plant Breeding, Volume 4

Oil Crops

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Editors

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Prof. Manjit S. Kang

Dr. Kang is currently an Adjunct Professor in the Department of Plant Pathology at Kansas State University, Manhattan. He served as Vice Chancellor (CEO) of the Punjab Agricultural University (PAU) at Ludhiana, India (April 2007 to April 2011). He previously served for 21 years as Professor of Quantitative Genetics in the School of Plant, Environmental, and Soil Sciences, Louisiana State University in Baton Rouge (USA). Prior to that, he was a Sugarcane Geneticist (Assistant Professor) with the Institute of Food and Agricultural Sciences (IFAS) at the University of Florida

(1981–1986). He acquired industrial experience in hybrid maize breeding when he served as Research Station Manager and Senior Plant Breeder at Cargill, Inc., St. Peter, Minnesota (1977–1979).

Dr. Kang received his Ph.D. degree in Genetics and Plant Breeding from the University of Missouri-Columbia in 1977. He also earned, in 1977, an M.A. (Botany) degree from Southern Illinois University (SIU)-Carbondale.
He received his M.S. (Plant Genetics) degree from SIU-Edwardsville in 1971. He earned a B.Sc. (Agri. & Animal Husbandry) degree with honors from Punjab Agricultural University (PAU), Ludhiana (India) in 1968.

Dr. Kang specializes in quantitative genetics as applied to crop improvement. His expertise is widely recognized and he has received several prestigious honors and awards from various organizations. He is a Fellow of both American Society of Agronomy and Crop Science Society of America. He is also an honorary Fellow of: Indian Society of Life Sciences, Crop

Improvement Society of India, and Punjab Academy of Sciences. He has served as Technical Editor of Crop Science and Editor-in-Chief of Communications in Biometry and Crop Science. He is currently Editor-in-Chief of Journal of Crop Improvement and Technical Editor of Crop Science.

Dr. Kang was recognized by his Alma Mater (PAU) for his significant contributions to plant breeding and genetics at the 36th Foundation Day in 1997. In 1999, he was selected as a Fulbright Senior Scholar (teaching award) to Malaysia. He received from the Association of Agricultural Scientists of Indian Origin 'Outstanding Agricultural Scientist Award for 2007'. He has served as a 'Sigma Xi (Scientific Research Society) Distinguished Lecturer' from July 2007 to June 2009. In February 2010, Amity University, Noida, bestowed on him 'Amity Academic Excellence Award'. In 2011, he received from Guru Nanak Dev University-Amritsar the 'Bishan Singh Samundri Lectureship Award', sponsored by S. Jaswant Singh Rai Memorial Trust. Dr. Kang has served as Chair of the National

*Selection Committee for Fulbright-Nehru Senior Research Scholar awards given by the US-India Educational Foundation (2010 and 2011). In 2013, he was recognized at the Third Jain-Advisor Agro-Dairy Fair in Paragpur (Punjab, India) with 'Excellence Award in Agriculture Development'. In May 2018, the Chief Minister of Punjab (India) conferred on him the "**Pride of Punjab**" award. In November 2019, Punjab Government bestowed on him 'Sri Guru Nanak Dev Ji Achievers Award' during the celebration of 550th Birthday of Sri Guru Nanak Dev Ji.*

Dr. Kang has edited or co-edited and authored or co-authored 15 books and has published 168 peer-reviewed (refereed) journal articles in prestigious international journals, 43 book chapters/encyclopedia essays, and 135 other technical publications.

Dr. Kang has lectured internationally on quantitative genetics as applied to crop improvement – in Hungary under the USDA/OICD sponsorship in 1992; in 1999, in Malaysia (Fulbright program); in 2005 at South Dakota State University ('South

Dakota Crop Improvement Association Lectureship'); at Yunnan Academy of Agricultural Sciences at Kunming in 2006; at International Rice Research Institute in the Philippines (1999); and at International Institute of Tropical Agriculture in Nigeria (2006, 2007). Dr. Kang has organized successful international conferences/ symposia on Genotype-by-environment Interaction in Plant Breeding, Quantitative Genetics and Genomics, Agricultural and Environmental Sustainability, Water Management Strategies for Food Security and Environment Quality, and Preparing Agriculture for Climate Change.

Through a generous donation, Dr. Kang has established, in his father's name, 'S. Gurdit Singh Kang Education and Research Welfare Society (Regd.), Ludhiana, India.

This book is dedicated to Prof. Manjit S. Kang for his enormous contributions in teaching, research, bringing out high quality publications in prestigious international journals, Authorship and Editorship of International Books & Journals relating to crop improvement.

Foreword

Vegetable oils are an important source of calorie for humans and also serve as raw materials for the manufacture of soaps, cosmetics, paints, varnishes, and of late, as biofuels. Besides, the nutrient-rich oilseed meal is widely used as animal feed. The demand for oils and fats is income-elastic and is expected to grow as programmes of the United Nations Sustainable Developmental Goals aimed at eradication of poverty and hunger start yielding dividends. Thus, there is an urgent need to increase productivity of oil-yielding crops through accelerated breeding using conventional and modern tools.

Oil-yielding crops comprise diverse types of plants belonging to different botanical families, and present wide variation with respect to life cycle (annuals, perennials), bearing (seasonal, year round), breeding behaviour (self–pollinating, cross pollinating), ploidy (diploid, polyploid) etc. With corn, rice and cotton contributing significantly to the vegetable oil pool, conventional crop classification as cereals, fibres, oil seeds etc., is no longer tenable. The study of these crops helps gain a comprehensive understanding of various concepts such as plant evolution, domestication and crop improvement. The majority of these crops have a long and rich history of plant breeding, and provide excellent examples of successful applications of both traditional and modern breeding techniques. The success of soybean in India demonstrates the importance of ‘Crop Introduction’. Similarly, the power of micropropagation technology is illustrated in oil palm in Malaysia and Indonesia, who are now major players in vegetable oil trade in the world. Likewise, rapeseed-mustard, soybean and cotton are among the prominent crops where transgenic technology has been widely adopted and has made global impact. Rapeseed breeding shows how haploid technology (anther/microspore culture) can accelerate crop improvement. Genomic resources including draft whole genome sequences have become available for almost all major oil-yielding crops. Hence, applications of genomics, marker-assisted breeding and genetic engineering have also started yielding results. Even neo-domestication through genome editing is being pursued to develop *Camelina sativa* as an industrial biofuel crop.

Given the diverse features, most of these crops have separate, dedicated research institutes, meetings/conferences, and researchers of different oil-yielding crops

rarely interact. Even books and publications tend to be crop-specific leading to isolation. However, the biochemical pathways of fatty acid and triacylglycerol biosynthesis are highly conserved across these crop species. Furthermore, key genes affecting yield such as flowering time, growth habit, seed size etc., also show considerable homology across species. Hence, findings of one crop are potentially transferable to other crops. Therefore, close interactions and cross talks among researchers of these crops would be highly beneficial to all. In this context, the efforts of Drs. Gosal and Wani to put together the latest breeding advances in these crops in a single volume are highly laudable. I hope it will stimulate discussions and promote cross-fertilization of ideas. In particular, it should serve as a handy reference book for students of genetics and plant breeding.

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Preface

Oil crops are considered the second most important determining factor of agricultural economy after the major cereal crops, the world over. Oils extracted from the seeds/fruits of oil yielding plants are being utilized in several ways. Largely used as edible oils which become important constituent of our daily diet. Besides, these are used as raw materials in industries for manufacturing items like hydrogenated oil, paints, varnishes, soaps, lubricants, biodiesel, perfumes, and pet foods. Such oils are also being used in medicines and pharmaceuticals. Oil-cake forms important cattle-feed and organic manure. Oil crop species are highly diverse, including monocots and dicots, growing as short duration annuals and perennials, under temperate, sub-tropical and tropical agro climatic conditions. Vegetable oils are rich in fats, carbohydrates, vitamins such as Vitamin A, Vitamin B1, Vitamin B3 (Niacin), Vitamin B5 (Pantothenic acid), Vitamin E (Thiamin) and minerals including Sodium and Iron. In the current scenario of climate change and global warming, there is rapid emergence of new races of insect-pests and new pathotypes of disease causing agents. Heat, cold and drought stresses are becoming serious threats. Conventional breeding approaches at this juncture seem inadequate to meet the growing demand for superior varieties. Plant improvement has been largely focused on improving higher yield, oil content, and better oil quality, resistance to diseases and insect pests and tolerance to abiotic stresses. Now the growers also demand for high yielding varieties/hybrids possessing; durable and multiple resistance, early maturity, higher harvest index, lodging resistance, varieties with nutrient-use efficiency/water-use efficiency, wider adaptability, salt tolerance, suitable for mechanized harvesting, better processing quality, with unique oil qualities possessing improved minerals, vitamins, fatty acids, and reduced antinutritional factors. During the past decade, significant advances have been made and accelerated methods have been developed for precision breeding and early release of crop varieties. Therefore, Accelerated Plant Breeding, Vol. 4: Oil Crops is state of art compilation and a major reference source for oil crop breeding. This volume will cover chapters dealing with germplasm enhancement and development of improved varieties based on innovative techniques such as Doubled haploidy, Marker Assisted Selection, Marker Assisted Background selection, Genetic mapping, Genomic selection, High-throughput

genotyping, High-throughput phenotyping, Mutation breeding, Transgenic breeding, Genomics-assisted breeding, Speed breeding etc. This Volume includes chapters prepared by specialists and subject experts on different crops/aspects in relation to accelerated breeding. In addition to the general chapter, separate chapters have been included on Soybean, Groundnut, *Brassica* crops, Safflower, Sunflower, Coconut, Castor, Sesame and cotton.

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 & biotechnology at Universities, Research institutes, R&Ds of Agricultural MNC's for conducting research and various Funding Agencies for planning future strategies.

We are highly grateful to all learned contributors, each of who has attempted to update scientific information of their respective area and expertise 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, Late Taja Begum, Sheikh Shazia, Yasir Wani, Muhammad Saad Wani and Maryam Shabir (father, mother, wife, brother, Son and daughter of SHW)} for their continuous support and encouragement throughout the completion of this book.

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Ludhiana, Punjab, India — Satbir Singh Gosal
Srinagar, Jammu and Kashmir, India — Shabir Hussain Wani

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Chapter 1
Breeding Major Oilseed Crops: Prospects and Future Research Needs

A. L. Rathnakumar and M. Sujatha

Abstract Oils obtained from plants have been used primarily for edible purposes and to a greater level in industries. Edible plant oils (EPOs) are extracted mainly from 11 plant sources: 2 are of tree origin, namely, oil palm and coconut; 9 are from annuals like soybean, groundnut, rapeseed-mustard, sunflower, safflower, sesame, cotton seed, and maize and rice (bran); and 2 crops, castor and linseed, are exclusively used for industrial purposes. Although several other sources of oils are also available, their production and use are limited to specific regions. The major objectives in oilseed crop improvement are enhancement of seed and oil yield, quality of oil for edible and industrial purposes, and development of varieties to suit different cropping systems having inbuilt resistance or tolerance to major biotic and abiotic stresses. Achievements in varietal breeding programs of nine annual oil crops and future research needs have been discussed. This chapter also summarizes developments in genomics and other biotechnological tools in seven edible oil crops, namely, *Brassica*, soybean, groundnut, sunflower, sesame, niger, and safflower, and in two industrial crops, viz., castor and linseed, with special emphasis on the prospects of molecular markers in genetic improvement of these crops. Molecular markers reported for genetic diversity assessment and mapping and tagging genes/QTLs for different oil quality traits and their use in marker-assisted selection have also been presented.

Keywords Molecular marker-assisted selection · Genetic resources · Oil quality · Trait breeding · Metabolic engineering

1.1 Introduction

Oils of plant origin have been used since ancient times and have been used in many ways. Predominantly, oils are used for edible purposes. Oils are also used in medicines and pharmaceuticals, industries, biodiesel, and pet foods and as components

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of many other products. During the last three decades, the oil crop production in the world has increased to 240%, while the increase in area and in yield was to the tune of 82% and 48%, respectively (El-Hamidi and Zaher 2018). Over the last few decades, the adoption of these crops has been growing up significantly, cultivated in about 324 million hectares in 2019 worldwide (www.FAOSTAT.org). The prime reason for this phenomenal growth is seed oils are not only a demand for various industries and also for the possibility to use their subproducts (metabolites) in biofuel development (Yadava et al. 2012).

Oilseed crops are very diverse in the plant kingdom and belong to several families, and oils are extracted mainly from their seeds, germs, and/or fruits. About 13 each of herbaceous and woody crops are reported to be important sources of oil (Zhou et al. 2020), but 10 herbaceous and 2 woody (coconut, oil palm) sources are considered important on the basis of their global production and use. Among the different oil-yielding crops, soybean (*Glycine max* L. Merr.), rapeseed/canola (*Brassica rapa* L. var. yellow sarson/brown sarson/toria; *Brassica napus* L.ssp. *oleifera* DC var. *annua* L.; *Eruca sativa* Mill.), mustard (*Brassica juncea* (L.) Czern. & Coss; *Brassica nigra* L. Koch; *Brassica carinata* A. Braun), palm (*Elaeis guineensis* Jacq.), sunflower (*Helianthus annuus* L.), cottonseed (*Gossypium hirsutum* L.), peanut or groundnut (*Arachis hypogaea* L.), sesame (*Sesamum indicum* L.), niger (*Guizotia abyssinica* (Lf). Cass.), and camelina (*Camelina sativa* (L.) Crantz) are commonly used oils, while castor bean (*Ricinus communis* L.), *Jatropha* (*Jatropha curcas* L.), tung tree (*Aleurites fordii* Hemsl.), jojoba (*Simmondsia chinensis* (Link) C. K. Schneid.), *Sachainchi* (*Plukenetia volubilis* L.), and others are used for industrial purposes. Although linseed or flax (*Linum usitatissimum* L.) oil is predominantly used for industrial applications such as oil paint, linoleum, and varnishes, in few pockets seeds of linseed and oil are used for edible purpose. Details of distribution, oil content, and fatty acid composition of important oil crops, chromosome number, genome size, and genomic resources of major oil crops have been provided (Table 1.1).

Analyses of the data of the past three decades on area, production, and productivity (www.FAOSTAT.org) of the eight annual oil crops (soybean, rapeseed-mustard, groundnut, sunflower, sesame, safflower, and linseed) except for niger revealed (Fig. 1.1) that soybean exhibited a phenomenal growth in area over the past three decades from 54.9 million ha in 1991 to more than its double (125.85 million ha) in 2017; production tripled from 102.8 million tons during the year 1991 to 359.5 million tons during 2017. The yield levels of soybean gradually increased from 1873 kg/ha in 1991 to 2857 kg/ha in 2017 with a coefficient of variation of just 11% indicating a slow and steady growth in yield.

In rapeseed-mustard, area, production, and yield witnessed a steady growth. Area increased from 17.6 million ha (1990) to 36.9 million ha (2018) with an average of 28.2 million ha over the three decades. Production varied from 24.4 million tons (1990) to as high as 76.6 million tons (2017), whereas the productivity ranged from 1308 kg/ha (1994) to 2142 kg/ha (2017).

Same is the trend for groundnut which exhibited a steady growth in area from 19. 8 million ha (1990) to 29.7 million ha (2018) with an average of 24.3 million ha

Table 1.1 Basic information on family, chromosome number, genome size, distribution, mode of pollination, oil content, and fatty acid composition of important oil crops

No.	Common name	Species	Family	Chromosome number	Genome size (Mb)	Pollination mechanism	Main producing area	Oil content (%)	Saturated FA (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
1	Soybean	*Glycine max* (Linn.) Merr.	Leguminosae	2n = 4x = 40	1115	Self-pollination	The USA, Brazil, Argentina, China, India, Paraguay, Canada	18–24	15.1	15.0–36.0	42.8–58.1	2.0–14.0
2	Rapeseed	*Brassica napus* L.	Brassicaceae	2n = 4x = 38	1187	Cross-pollination	All over the world	37–46	6.3	56.0–72.0	13.8–24.6	4.3–11.3
3	Sunflower seed	*Helianthus annuus* L.	Asteraceae	2n = 2x = 34	3000	Cross-pollination	All over the world	46–50	12.2	16.4–27.6	60.2–72.1	0.07–1.8
4	Peanut/groundnut	*Arachis hypogaea* L.	Leguminosae	2n = 4x = 40	2800	Self-pollination	Asia, Africa, America	46–57	14.8	37.0–55.6	25.3–39.7	0.40–3.2
5	Sesame	*Sesamum indicum*	Pedaliaceae	2n = 2x = 26	948	Often-cross-pollination	Tropical and temperate regions	43–61	12.4–14.4	36.7–42.0	43.2–48.6	0.2–0.95
6	Linseed/flax	*Linum usitatissimum* L.	Linaceae	2n = 2x = 30	686	Self-pollination	Mediterranean region, Euro-Asian	26–45	9.5	19.9	15.9	52.7

(continued)

Table 1.1 (continued)

No.	Common name	Species	Family	Chromosome number	Genome size (Mb)	Pollination mechanism	Main producing area	Oil content (%)	Saturated FA (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
7	Safflower	*Carthamus tinctorius* L.	Asteraceae	2n = 2x = 24	1350	Predominantly self-pollination; extent of cross-pollination depends on bee activities	India, the USA, Ethiopia China, Russia, Japan, North Korea, Iran	27–25	9.0	13.1	77.7	–
8	Cotton	*Gossypium* spp.	Malvaceae	2n = 2x-52	2200	Often cross-pollination	China, the USA, India, Uzbekistan, Egypt	15–40	27.9	17.6	53.3	0.3
9	Corn	*Zea mays* L.	Gramineae	2n = 2x = 20	2355	Cross-pollination	Tropical and temperate regions of the world	4.5–4.8	14.4	27.5	57.0	0.9
10	Rice	*Oryza sativa* L.	Poaceae	2n = 2x = 24	430	Self-pollination	Almost everywhere, expect Antarctica.	15–23	19.6	43.8	34.0	1.2
11	Hemp	*Cannabis sativa* L. subsp. sativa	Moraceae	2n = 2x = 20	800–900	Cross-pollination	All over the world	25–35	19.0	59.6	3.4	18.0
12	Grape	*Vitis vinifera* L.	Vitaceae	2n = 2x = 38	500	Cross-pollination	All over the world	10–20	10.4	14.3	74.7	0.15

No.	Common name	Species	Family	Chromosome number	Genome size (Mb)	Pollination mechanism	Main producing area	Oil content (%)	Saturated FA (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)
13	Perilla	*Perilla frutescens* (L.) Britt.	Labiatae	2n = 2x = 40	--	Self-pollination	India, Myanmar, Japan, Korea, Indonesia	40–50	17.0	11.0	17.0	55.0
14	Coconut	*Cocos nucifera* L.	Palmae	2n = 2x = 32	2478	Cross-pollination	Indonesia, Philippines, India, Sri Lanka, Brazil, Vietnam, Papua New Guinea	60–64	91.4	6.5	1.5	–
15	Oil palm	*Elaeis guineensis* Jacq.	Palmae	2n = 2x = 32	1800	Cross-pollination	Indonesia, Malaysia, Thailand, Columbia, Niger, Ecuador	50–56	43.8–54.2	37.4–44.1	8.7–12.5	0–0.6
16	Castor	*Ricinus communis* L.	Euphorbiaceae	2n = 2x = 20	320	Cross-pollination	India, China, Brazil, Mozambique, Paraguay, Thailand	40–55	3.0	4.0 (88)[a]	4.0	–
17	Niger	*Guizotia abyssinica* Cass	Asteraceae	2n = 2x = 30	978	Cross-pollination	Africa, Ethiopia, India, West Indies, Bangladesh, Bhutan	30–40	14.0	10.0	76.0	–

[a]In castor, predominant fatty acid is ricinoleic acid (hydroxyl-oleic acid) and constitutes about 88% of the total fatty acids

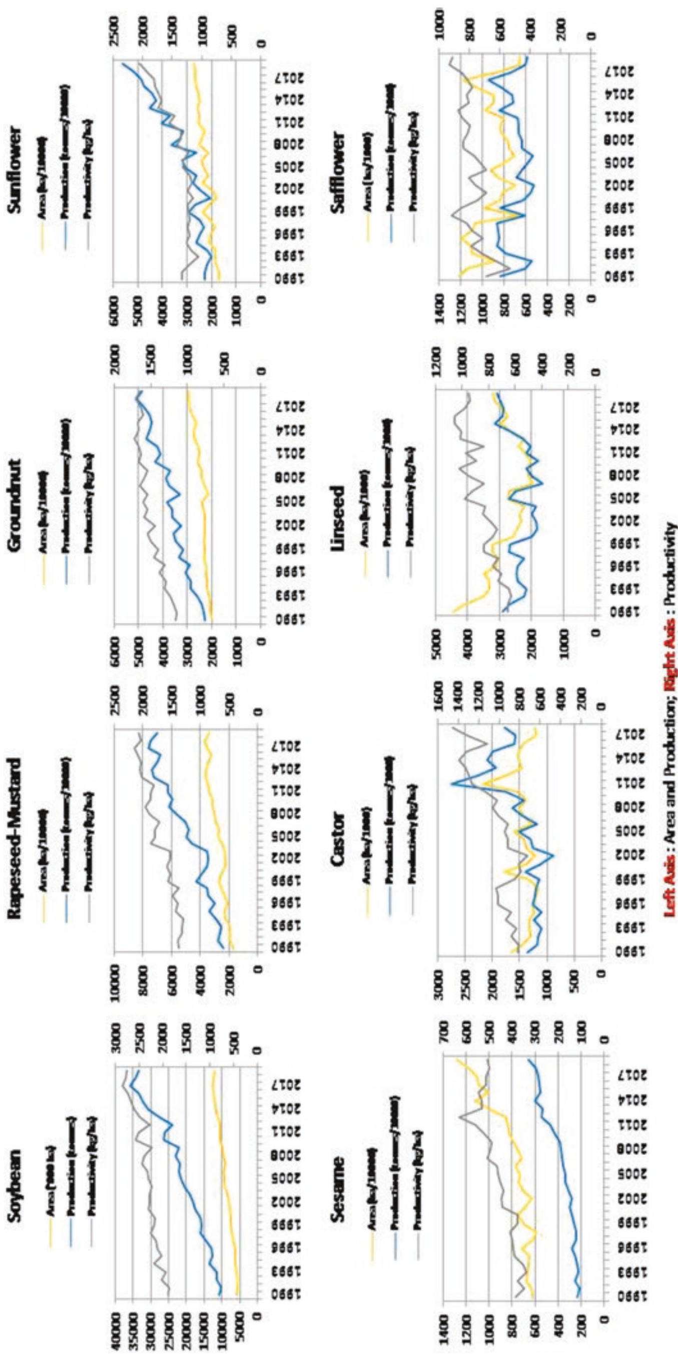

Fig. 1.1 Trends of area, production, and yield of major oil crops (1990–2019)

over the three decades; production increases from 23.0 million tons (1990) to 50.8 million tons (2018); and yields dwindled from 1151 kg ha in the year 1991 to 1722 kg in 2013 and 1713 kg per ha in 2018. The coefficients of variation for both area and yield over the three decades remained very low (11%) indicating a slow growth both in area and yields especially in India and Africa.

Sunflower area was not dramatic during the last three decades, and it varied from 17.03 million ha (1990) to 26.80 million ha (2017). Production dwindled between from 20.02 million tons in 1993 to 51.90 million tons in 2018. The yield levels varied from 1066 kg/ha in 1993 to 1937 kg/ha in 2018.

Sesame area increase was just lower than double its value over 1990, i.e., from 6.13 million ha in 1990 to 11.8 million ha in 2018 despite the demand. Rise in production was from 2.2 million tons during 1991 to 5.9 million tons in 2018, and the increase is mainly registered through increase in area. However, the yield levels ranged from 348 kg/ha in 1990 and reached an all-time high of 633 kg/ha in 2013 and dropped down further to 502 kg/ha in 2018. The increase in yields of sesame over three decades is only 30%.

However, in case of both safflower and linseed, there was a steep decline in area. Safflower area was 1.2 million ha in 1990 and reduced to 0.65 million ha in 2018; production also exhibited a decreasing trend (0.84 million tons in 1990 to 0.61 million tons in 2018), but productivity showed a slight increase (from 690 kg in 1990 to 929 kg in 2018) of about 390 kg/ha over the last three decades. Meanwhile, linseed area declined from 4.4 million ha in 1990 to 3.2 million ha in 2018, but production and yield showed a marginal increase (production: 2.9 million tons in 1990 to 3.1 million tons in 2018; productivity: 658 kg/ha in 1990 to 944 kg/ha in 2018).

Over three decades, average castor area remained at about 1.41 million ha although the area reached an all-time high of 17.4 million ha in the year 2012. Castor productivity witnessed a gradual increase from 800 kg/ha to 1300 kg/ha, the lowest yield being 781 kg/ha in the year 2000 to as high as 1452 kg/ha in the year 2018. Year-to-year and regional variations were not uncommon for area, production, and productivity in all the oilseed crops.

During the last triennium (2016–2019), the extraction of oils from the major sources around the world was 66.18 million tons of palm oil, 7.17 million tons of palm kernel oil, 55.05 million tons of soybean oil, and 24.40 million tons of rapeseed oil. Together these four oils contributed to 88% of total edible oil production of the world. The rest are groundnut or peanut oil (5.59 million tons), cotton seed oil (4.37 million tons), olive oil (3.39 million tons), maize oil (3.15 million tons), coconut oil (3.07million tons), rice bran oil (1.60million tons), sesame oil (1.10 million tons), linseed oil (0.76 million tons), and safflower oil (0.09 million tons) (www.FAOSTAT.org).

1.2 Genetic Resources and International Institutions

Availability of diverse germplasm with heritable variations is very important for continued success in any breeding program. Most of the oil crops currently grown across the globe are spread far away from their primary centers of origin and resulted in adaptation to specific environments/regions where they are being presently cultivated leading to narrow genetic base in these crop species (Jones 1983; Wang et al. 2017). Therefore, the oil crop germplasm of any country would comprise only few accessions from the origin, primary and secondary centers of diversity and more of breeding materials and cultivars developed using these sources, thus further reducing the genetic variation that could be exploited in crop improvement programs.

There are now (by 2019) more than 1750 individual gene banks worldwide, holding a total of around 7.4 million accessions of germplasm, in which about 130 of them hold more than 10,000 accessions each (www.CGIAR.org; https://www.cgiar.org/news-vents/news/guardians-of-diversity-the-network-of-genebanks-helping-to-feed-the-world).

Genebanks are located in all continents, but these are relatively fewer in Africa compared with the rest of the world. Substantial ex situ collections in botanical gardens (2500 around the world) of various plant species are also being maintained. The data of 290 gene banks of different countries, regions, and CGIAR centers indicate that among the different crop species conserved ex situ, oil crops constitute only 3% (FAO 2010), clearly indicating the priority for the oil crop genetic resources in terms of collection, multiplication, evaluation, and conservation has been very low globally. Moreover, these crops have gained economic importance only a couple of decades before, and few of them as secondary sources of oil (rice bran, corn, cotton seed oils) are being exploited only of late. Among the oil crops, only in groundnut and soybean over 15,000 accessions each are currently being maintained by two centers, viz., ICRISAT Asia Centre, Patancheru, India, and SINGER (System-wide Information Network for Genetic Resources) network, respectively. Among the 15,000 groundnut accessions maintained at ICRISAT, only 453 are wild forms and the rest are cultivated forms which exhibit limited morphological variability except for their growth forms (Dwivedi et al. 2007). In case of soybean, most of the accessions maintained by SINGER network are vegetable types. The European Plant Genetic Resources gene banks, the largest network of gene banks numbering 441 (43 national inventories and 398 individual holding institutions), the total number of accessions maintained in the two major oil crops of Europe was only 4879 in case of oil rape and 4444 in case of sunflower against a total collection of 20.19 million accessions of ten important crop species (Vollmann and Rajcan 2009; ECPGR 2019). Notwithstanding these facts, the conservative estimates of FAO indicate that out of about 7.4 million accessions which are currently being maintained in different countries, between 25 and 30 percent of the total holdings (1.9–2.2 million accessions) are only distinct, and the rest are duplicates held either in the same or, more frequently, a different collection (Jaramillo and Baena 2002). Hence, there is an urgent need to augment and enhance the collection of the valuable

genetic resources and evaluate for specific/target traits, and incorporating them in breeding programs remains the foremost activity in genetic enhancement of the oil crops.

1.2.1 Gene Pools

Harlan and de Wet (1971) proposed a three-gene pool concept, primary (GP-1), secondary (GP-2), and tertiary (GP-3), for effective utilization of germplasm resources in crop improvement programs. Genetic resources are identified or developed through multidisciplinary approaches by plant exploration, taxonomy, genetics, cytogenetics, plant breeding, microbiology, plant pathology, entomology, agronomy, physiology, wide hybridization, and molecular biology, including cell and tissue culture, DNA analyses, and genetic transformation. These efforts have produced superior oilseed cultivars with resistance to abiotic and biotic stresses and improved oil quality and quantity. The concept of primary, secondary, and tertiary gene pools and genetic transformation has played a key role in improving oilseed crops.

1.2.2 Primary Gene Pool

The primary gene pool (GP-1), consisting of landraces and biological species, has been identified for most of the oilseed crop species. Wild progenitors of cultivated oilseed crops are identified, postulated, and proposed based on geographical distribution, classical taxonomy, cytogenetics, and molecular methods. For example, the GP-1 for soybean (2n = 40) is only its wild annual progenitor *Glycine soja* Sieb. and Zucc. (2n = 40) (Chung and Singh 2008). Castor belongs to the monotypic genus *Ricinus* of the *Euphorbiaceae*. Although several authors have classified *R. communis* into different species and subspecies on the basis of morphological traits and geographical distribution, none of them are accepted as true species or subspecies and they represent merely the local types or ecotypes adapted to different environmental conditions or human selection (Weiss 2000). For rapeseed, six species depicted in the famous U triangle, viz., *Brassica carinata* (Ethiopian mustard; 2n = 34), *Brassica juncea* (Indian mustard, brown mustard; 2n = 36), *Brassica napus* ssp. *napus* (oilseed rape, fodder rape: 2n = 38), *B. napus* ssp. *napobrassica* (Swede; 2n = 38), and *B. napus* ssp. *napus* var. *pabularia* (leaf rape, kale; 2n = 38), constitute the primary gene pool (Morinaga 1934; U., N 1935). Groundnut is an allotetraploid species (2n = 4x = 40) that evolved from natural doubling of a cross between two diploid progenitors (*A. duranensis* Krapov. and W.C. Gregory and *A. ipaënsis* Krapov. and W.C. Gregory) (Bertioli et al. 2016; Stalker 2017; Levinson et al. 2020). Four *Arachis* gene pools contain 80 species, distributed among 9 sections, and are native to 5 countries of South America. The primary gene pool

consists of landraces and traditional cultivars of groundnut from primary and secondary centers of genetic diversity in South America and other groundnut-growing countries, and one tetraploid wild species *A. monticola* found in northwest Argentina has crossability success with *A. hypogaea* producing normal segregants (Singh and Simpson 1994; Singh and Nigam 2016).

The genus *Helianthus* comprises 53 species within the tribe *Heliantheae* of the family *Asteraceae*, and the cultivated sunflower (*Helianthus annuus* var. *macrocarpus*) has been derived from a widely branched annual plant with many flower heads otherwise called the common sunflower (*H.annuus var. annuus*) (Heiser Jr. 1955). The primary gene pool of the sunflower consists of both cultivated and wild varieties of *Helianthus annuus*, as well as winter's sunflower (*Helianthus winteri* J.C. Stebbins), a perennial species found in the southern Sierra Nevada foothills of California.

Wild species of sesame vary in their habitat, morphological features, and ploidy levels, the latter of which is represented by three chromosome groups: 26, 32, and 64 (Joshi 1961). The progenitor species of cultivated sesame are unknown as no wild species except for *S. malabaricum*, which produces fertile hybrids with *S. indicum*, are known (Weiss 2000). These two species form the primary gene pool of sesame.

The genus *Carthamus* consists of 25 species, distributed worldwide. Among the 25 safflower species, the cultivated safflower grown around the world is only *Carthamus tinctorius* L., containing 12 pairs of chromosomes (Patel and Narayana 1935; Richharia and Kotval 1940). Based upon the chromosome numbers, the genus was categorized into four sections, and the three closely related annual species *C. tinctorius*, *C. palaestinus*, and *C. oxyacantha* together with cultivated types sharing the same chromosome number (2n = 24) are placed in section I. Among these three species, *C. oxyacantha* is proposed to be the wild ancestor of cultivated safflower (Bamber 1916; Ashri and Knowles 1960). Recent DNA sequence-based analyses in four species of safflower revealed that the progenitor species of safflower is most likely *C. palaestinus* which is a self-compatible species native to southern Israel to western Iraq (Chapman and Burke 2007a, b).

Chromosome pairing indicated that cultivated niger, *Guizotia abyssinica* and *G. scabra* subsp. *schimperi*, are morphologically very similar, both annuals, and are attacked by the same pests and diseases. Both species have 2n = 30 chromosomes with a similar karyotype. The hybrid between *G. abyssinica* and *G. scabra* subsp. *schimperi* is fertile and forms 15 bivalents in 95% of the pollen mother cells indicating that *G. scabra* subsp. *schimperi* are the probable progenitor species of niger (Murthy et al. 1993). In both safflower and niger, cytomorphological and molecular phylogeny analyses will throw more light for exploitation of diversity and genetic enhancement in these crop species.

The flax or linseed genus, *Linum*, is a large group with ~230 species (Heywood 1993). The genus is divided into five sections, *Linum*, *Linastrum*, *Cathartolinum*, *Dasylinum*, and *Syllinum*, based on chromosome number, floral morphology, and interspecific compatibility (Gill 1987). Cultivated flax, *L. usitatissimum*, is placed in the section *Linum* and has 30 diploid chromosomes (Tammes 1928). The other

species, *L. angustifolium*, also known as pale flax, is closely related to flax, found mainly in Mediterranean Sea, Iran, and the Canary Islands, and has a similarity to cultivated flax (Diederichsen and Hammer 1995). Both cultivated and pale flax are homostylous, inbreeding species and share similarity in chromosome number (Gill 1987; Tammes 1925). The genetically similar behavior of *L. angustifolium* and *L. usitatissimum* and the ease of hybridization with each other in any direction (male or female) resulting in infertile hybrids (Gill 1966) suggest that *L. angustifolium* is the wild progenitor of flax (Dillman and Goar 1937) and thus form the primary gene pool of flax.

1.2.3 Secondary, Tertiary, and Quaternary Gene Pools

The secondary gene pool (GP-2) includes all species that can be hybridized with GP-1 with at least some fertility in F_1s resulting in gene transfer (Harlan and de Wet 1971). *Glycine max* and castor (*R. communis*) do not have GP-2. The GP-2 for *Brassica* oilseeds includes *B.nigra*, *B.oleracea* (includes crop varieties, *B.alboglabra*, *B. bourgeaui*, *B.cretica*, *B.hilarionis*, *B.incana*, *B.insularis*, *B. macrocarpa*, *B.montana*, *B.rupestris*, *B.villosa* and *B.rapa* (includes wild and cultivated varieties). In case of groundnut, the secondary gene pool consists of diploid species from section *Arachis* which are cross-compatible with cultivated groundnut and produce sterile to partially fertile hybrids despite ploidy differences (Singh and Simpson 1994; Singh and Nigam 2016). Two sesame species namely, *S. alatum* and *S. prostratum*, have been placed under gene pool-2 due to barriers in hybridization with *S. indicum* (Raghavan and Krishnamurthy 1947; Rajeswari and Ramaswamy 2004) although in few reports no seed set has been observed for *S. alatum* during hybridization (Lee et al. 1991;Rajeswari and Ramaswamy 2004).

The tertiary gene pool of soybean comprises 26 wild perennial species of the subgenus *Glycine*. These species are indigenous to Australia and are geographically isolated from *G. max* and *G. soja* (Newell and Hymowitz 1983; Singh 2019). Species that belong to the sections *Procumbentes*, *Erectoides*, and *Rhizomatosae* which are partially cross-compatible with species of section *Arachis* and *A. hypogaea* are grouped under tertiary gene pool in groundnut. The rest of the species of five sections (*Caulorhizae*, *Heteranthae*, *Extranervosae*, *Triseminatae*, *Trierectoides*) of groundnut that are cross-incompatible or very weakly cross-compatible to species of section *Arachis*, form the quaternary gene pool. The gene flow among different gene pools and between different sections and within tertiary and quaternary gene pools is generally limited (Gregory and Gregory 1979; Singh and Nigam 2016). One species of sesame, namely, *S. radiatum*, is placed in gene pool-3 of sesame due to lack of capsule formation, no seed set, and use of embryo rescue methods (Singh et al. 2016) upon hybridization.

1.2.4 Utilization of Genetic Resources in Oil Crops

Despite availability of vast germplasm resources in the oil crops, the genetic base of different cultivars developed in each of these crops is very narrow (Hyten et al. 2016; Holbrook et al. 2014; Wang et al. 2017; Khedikar et al. 2020). For example, in soybean, it has been reported that for northern and southern North America breeding pools, there were only 19 ancestors with 17 of them common to both regions of the USA. The 19 ancestors contributed to 85% of the genes to each region (Gizlice et al. 1994; Hyten et al. 2016; Cober et al. 2009). The same is true for oilseed rape as well, and the major reason can be attributed to geographical constraints, selection bottle necks during origin of species, and subsequent domestication. More specifically, the modern canola varieties with zero erucic acid and low glucosinolate originate from only two varieties: “Liho” and “Bronowski” (Hasan et al. 2006; Friedt and Snowdon 2009). Of the canola varieties released in Australia from 1995 to 2002, 11 ancestral varieties contributed 98.7% of the pedigree composition, and 2 ancestors (Canadian low erucic spring variety “Zephyr” and Polish low glucosinolate spring variety “Bronowski”) were present in the pedigrees of every variety (Cowling 2007).

In groundnut, although large number of accessions have been evaluated for desirable traits either at USDA or ICRISAT, relatively few accessions only have been utilized in breeding programs for cultivar development in the USA and ICRISAT (Isleib et al. 2001; Dwivedi et al. 2007) leading to narrow genetic base of the cultivars. In spite of the large number of cultivars available to growers, the US groundnut crop has been characterized as being genetically vulnerable to diseases and insect pests (Hammons 1972; Hammons 1976; Knauft and Gorbet 1989). This has been due to the commercial success of specific cultivars grown in particular production areas. For example, in the three major production regions of North America, the runner-type cultivar ‘Florunner’ dominated the southeastern US. (Georgia, Florida, and Alabama which produces approximately 65 percent of all USA-grown groundnut) from 1972 to 1993 and in the Virginia-Carolina (VC) production area (which accounts for nearly 13 percent of all USA-grown groundnut),the most dominant cultivar over 40 years in the VC areawas ‘Florigiant’. Even in India, a single variety, “GG 20” (released and notified in 1992), developed by Gujarat State Agricultural University is grown in almost 60–70% of area (about 1.2 million ha) under groundnut in the state and has become popular in other states as well. The narrow genetic base of cultivars in castor and coconut owing to their monotypic species nature is also an impediment for further genetic improvement in these crops.

One of the ways that plant breeders can increase the genetic diversity of a crop is to incorporate diverse germplasm into the breeding populations from which thousands of accessions and cultivars can be derived. Besides the variability available in primary gene pool of different oil crop species, introgression of useful genes from wild species into the cultivated species has attracted the oil crop breeders because of their resistance to diseases and insect pests for which the genetic variation in primary gene pool is limited. The most accessible variability of primary and secondary

gene pools has been successfully utilized in few crop species like soybean and groundnut. Further success in introgression of the novel genes like resistance to major insect pests and diseases and drought and cold and heat tolerance into the cultivated background has been limited due to poor understanding of genome relationships, cross-incompatibility, and nonavailability of true progenitor species. The exploitation of tertiary and quaternary gene pools awaits advancement in the biotechnological techniques/interventions and policy decision with regard to release of transgenic varieties and genome edited lines at global level (Singh and Nigam 2016).

1.3 Mode of Pollination and Breeding Behavior in Oil Crops

Many of the edible oil crops exhibit a wide range of pollination mode/mechanism(s) like self- and cross-fertilization, self-incompatibility, etc. notably seed cotton, coconut, sunflower, rapeseed, and niger. Sunflowers have one of the two pollination systems; in most oil-producing cultivars, the flower switches between the male and female phases, whereas in hybrid production, specifically bred male and female lines are planted within the same field. Both benefit from insect visitation to optimize pollen transfer to female plants (Free 1993). Rapeseed and canola are highly self-compatible and readily set pods with wind and self-pollination; further, their high nectar concentration makes them attractive to insects which can increase pollen transfer and increase the total yield by 20% (Bommarco et al. 2012; FAO 2018). In seed cotton, biotic pollination resulted in a 20% increase in seed weight and a 16% increase in lint production (Rhodes 2002; Potts et al. 2014).

Some edible oil crops gain very little benefit from pollination, such as soybean, groundnut, and linseed (Williams 1991; Palmer et al. 2001a, b), whereas olive is entirely wind pollinated (Klein et al. 2007). Safflower and sesame are basically self-pollinated but certain degree of cross-pollination does occur in sesame (Ashri and Knowles 1960; Andrade et al. 2014) due to bee activities, while bees, butterflies, and other flies aid in cross-pollination in safflower.

In oil palm, male and female inflorescences are borne in the same tree separated by time and space. Cross-pollination through the weevil, *Elaeidobius kamerunicus* Faust (*Curculionidae*), is predominant (Syed et al. 1982; Abrol and Shankar 2012). Coconut is monoecious with protandrous staminate flowers, and hence, it is highly cross-pollinated aided by bees.

Although the breeding systems of the oilseed crops together with inheritance of the targeted trait(s) primarily decide the breeding method to be adopted, it has been observed that in self-fertilized oil seed crops like soybean, groundnut, and flax, yield improvement per se remains restricted in comparison with the cross-fertilized oil crops.

1.4 Major Goals of Oil Crop Breeding, Achievements and Strategies

Different breeding methodologies have been adopted in oil crops depending upon their breeding systems. Pollination mode in oilseed crops ranges from highly self-pollinated (soybean, groundnut, linseed) to often cross-pollinated (cotton, sunflower, safflower, *Brassica*, sesame, coconut) plants. Hybrid sunflower, safflower, and rapeseed are also produced using cytoplasmic male sterility. Conventional breeding methods (selection, pedigree, bulk, backcross, single-seed descent) have produced oilseed crops with high seed yield, oil content, and quality coupled with resistance or tolerance to major biotic and abiotic stresses. As it would be beyond the scope of this chapter to discuss the genetic enhancement accomplished in each of these traits, the discussions will be restricted to improvement made in seed yield, oil content, seed oil quality, and anti-nutritional factors.

1.4.1 High Seed Yield

To improve productivity of any crop plant, it is essential to increase seed yields. However, the agricultural area worldwide has been flat for over 40 years (FAO 2017). Therefore, improving seed yield per plant has become increasingly important. Since increasing seed yield is one of the major issues in plant science, effective strategies for increasing yield have been explored by many oil crop breeders.

Soybean began its transition from a forage crop to a valuable source of protein and oil with the establishment of the US Regional Soybean Industrial Products Lab at Urbana in 1936. Breeding soybean largely remained with the public sector breeders until the passage of the Plant Variety Protection Act (PVP) in 1970. Government protection of intellectual property in the form of cultivars encouraged private industry to heavily invest in soybean breeding, and today the bulk of research is conducted by industry rather than public institutions. However, public sector breeders still play an important role in soybean breeding and release of improved cultivars.

The main reason for the slow phase of increase in soybean yield is mainly due to stagnation in productivity in Asian continent mainly comprising China (remained at 1.8 tons/ha) and India (remained at 1.1 tons/ha). In India, production of soybean is confined to the states of Maharashtra and Madhya Pradesh which contribute 89% of the total production, while Rajasthan, Andhra Pradesh, Karnataka, Chhattisgarh, and Gujarat contribute the remaining 11% production, mostly grown as a rainfed crop. Soybean is highly sensitive to environment, most importantly to moisture stress, thus restricting the productivity in these regions.

Soybean yield potential has been increased by increasing the number of pods per plant, which has been achieved by increasing the number of nodes per plant while decreasing internode length to prevent lodging due to excessive height. In addition, number of seeds per plant and seed weight also contributed to yield improvement

(Sharma et al. 2016; Xu et al. 2020). Large number of cultivars in China (651), the USA (258), Brazil (69), and India (107) have been developed and released for cultivation.

In an effort to discover the genetic variability for seed yield in soybean, a genome wide association study (GWAS) was performed on 451 diverse lines from the USDA core collection for height, internode length, and the number of nodes. The QTL signifying Dt1 was found correlating to height and number of nodes, but no significant QTLs for internode length were uncovered. This suggests that genomic selection for variation in plant height is feasible (Moreira et al. 2019). Further improvement should come from identification of traits associated with yield, understanding the genetic mechanisms underlying their inheritance in addition to developing photo-thermo-insensitive cultivars and other stress-tolerant cultivars.

Genetic improvement of seed yield of rapeseed and mustard in the Indian subcontinent is the primary breeding objective, while in western world breeding for quality assumed priority. In case of Europe and Canada, breeding for oil for human consumption and oil cake (meal) quality for animal nutrition received the top priority than the other countries (Gupta 2012). In case of winter oilseed rape, the increase in cultivated area is responsible for only a 20% rise in global crop biomass production, whereas the intensification of the production process, mainly through breeding, accounts for the remaining 80% increase in seed yield (Swiecicki et al. 2011). The morphological traits responsible for superior performance of oilseed rape can be considerably modified by breeders. Intensive breeding efforts conducted in the 1960s have contributed to the economic significance of this species (low levels of erucic acid and glucosinolates). The yield limiting factors identified were number of siliques per unit area, number of seeds per silique, and the 1000-seed weight (Diepenbrock 2000) which can form a suitable selection criterion for increasing seed yield. Dry matter accumulation at rosette stage and leaf area index (LAI) have also been reported to be associated with seed yield (Olsson 1990). Hybrid breeding by exploiting the heterosis through the two sources, viz., male sterility Lembke of Germany (MSL; genic male sterile system) in *B. napus* and Ogura CMS system of France from radish, should further help in increasing seed yields of oil rapeseed.

In India, rapeseed-mustard is the second most important source of edible oil. Under the umbrella of All India Coordinated Research Project on Rapeseed Mustard (AICRP-RM), a total of 248 varieties of rapeseed-mustard have been released till 2018, and out of them, 185 varieties released and notified comprise of Indian mustard, 113; toria, 25; yellow sarson, 17; gobhi sarson, 11; brown sarson, 5; karan rai, 5; taramira, 8; and black mustard, 1. These include six hybrids and varieties having tolerance to biotic (white rust, *Alternaria* blight, powdery mildew) and abiotic stresses (salinity, high temperature) and quality traits and have been recommended for specific growing conditions. In 2019, three more hybrids, "Kesari Gold (31J3403)," "Kesari 5111 (PCJ03-401)," and one private sector's hybrid "Bayer Mustard 5222 (Pro 5222)," have also been released and notified for cultivation in India. However, lack of stable fertility restorers for different male sterile systems has hampered the exploitation of these CMS systems for producing commercial hybrid seed.

Groundnut being largely a rain-dependent crop, wide variations in production and productivity, across and within the regions/countries around the world, are quite frequent. The crop is grown in two distinct production systems – low- and high-input production systems. Low-input production system, predominant in Asia and Africa, is characterized by rainfed cultivation and, with little inputs, manual labor and low yields (700–1000 kg/ha). However, in high-input production system coupled with mechanization, as prevalent in the USA, Australia, Argentina, Brazil, China, and South Africa, the groundnut yields are as high as 2.0–4.0 tons/ha. High pod and seed yields are the ultimate goals of a groundnut breeder. In *kharif*, yield levels up to 3.0 tons/ha and in *rabi*-summer up to 9.0 tons/ha have been reported under farm conditions in specific locations even in India (Rathnakumar et al. 2015). However, the average yields in India hover around 1.0 to 1.5 tons/ha depending on rainfall (quantum and distribution) in *kharif* and during *rabi*-summer,1.5 to 2.0 tons/ha. Thus, there exists a wide gap between the potential and realized yields. In addition, few biotic factors reduce yield of groundnut in *kharif* season. Therefore, any further increase in yield of *kharif* groundnut should be possible by developing stress-tolerant varieties which respond to low inputs. For *rabi*-summer cultivation, the varieties should respond to high nutrient and management conditions with high water use efficiency as the crop is raised totally under irrigated conditions.

The important yield components of groundnut are pod number, seed mass (weight of 100 kernels), and shelling outturn. However, it appears that yield improvement in most groundnut-growing states in India was brought about through a progressive improvement in pod size of the new varieties (Reddy 1988) and number of pods, size of pods, and seed size (Nigam et al. 1991; Janila et al. 2013, 2016). However, shelling outturn could not be improved substantially in the modern-day cultivars which ranges from 68% to 70% (Rathnakumar et al. 2010). For example, the pod weight increased from 68 g in PG-1 (1953) to 100 g in c-501 (1961), to 120 g in M-13 (1972), and to 119 g in M-37 (1982) in Punjab; from 76 g in RS-1 (1953) to 103g in RSB-87 (1961) in Rajasthan; from 80 g in T-28 (1960) to 118 g in Chandra (1977) in Uttar Pradesh; from 72 g in AK-12-24 (1940) to 75 g in SB-XI (1965), to 119 g in JL-24 (1978), to 120 g in TG-17 (1982), and to 127g in UF-70-103 (1984) in Maharashtra; from 52 g in Kadiri-71-1 (1971) to 91 g in Kadiri-2 (1978) and Kadiri-3 in Andhra Pradesh; from 77 g in s 206 (1969) to 88 g in Dh-3-30 (1975) in Karnataka; and from 76 g in TMV-2 (1940) to 91 g in TMV-7 (1967) and 92 g in TMV-9 (1970) in Tamil Nadu (Reddy 1988).

Over the years, 220 public bred varieties have been released as of 2020 in India, and in these varieties the yield improvement has also been achieved through progressive increase in seed size. For example, during 1940–1950, the varieties had small seeds in the range of 29.4 g/100 seeds (AK-12-24) to 36.6 g/100 seeds (TMV 3 and TMV 4). However, after four decades, the average seed size of the varieties was medium (44.8 g/100 seeds) with a range of 27 g/100 seeds (Pragathi) to 90 g/100 seeds (B-95). During the previous decade (2001–2010), the average seed size of the varieties remained medium (47.9 g/100 seeds) (Rathnakumar et al. 2013).

Further improvement in this crop can be achieved through inter subspecific crosses between Virginia types with more fruiting nodes, and large seeds with

Spanish bunch types with early maturity may simultaneously increase the number of pods and seed mass. Most of the high yielding groundnut varieties released globally have resulted from the higher harvest index brought about by reduction in the total biomass. Breeding for high biomass coupled with high harvest index can be one of the strategies to further increase yield in many groundnut-growing countries.

Sunflower was used by the American Indians around 3000 BC. The native Americans were the first "sunflower breeders" to improve and select types that varied widely for length of growing season, degree of branching, and the size and color of achenes. Later it was introduced into Europe during the sixteenth century, gradually spreading to Russia where it became widely recognized as an oilseed crop. Breeding and selection to improve sunflower at experimental stations was initiated in Russia as early as 1910 in Kharkov station and at Kruglik and Saratov stations in 1912 and 1913, respectively. Major objectives in sunflower breeding include improved seed yield, early maturity, shorter plant height, uniformity of plant type, and resistance to major diseases and insect pests. The introduction of hybrid cultivars exploiting the heterosis created a major breakthrough in increasing the seed yield of sunflower by around 25% across different growing regions (Fernández-Martínez et al. 2009). Further significant improvement in grain yield has not been reported on a large scale before or after this point (Lopez Pereira et al. 2008). However, several studies have identified specific traits associated with seed yield improvement in sunflower, namely, head size and number of seeds per head, seed weight (Miller et al. 1982; Connor and Hall 1997), and indirect and adaptive traits like improving the combining ability of parental lines, shorter plant stature in areas associated with lodging risk (Schneiter 1992), high degree of fertility in regions with limited or nil pollinator populations (Miller et al. 1992), or pronounced head inclination in high temperature and intense sunlight or high risk of bird predation areas (Hanzel 1992; Linz and Hanzel 2015) and disease resistance in case of hybrid sunflowers. However, almost all the sunflower hybrids currently cultivated are derived from a single CMS source, i.e., *H. petiolaris* (PET1), and hence, diversification of CMS sources and fertility restorers under agronomically superior genetic backgrounds will further enhance yields.

Although almost all the oil crops are grown under marginal and submarginal lands having poor soil fertility in developing nations including India, sesame, niger, and safflower are almost neglected crops grown purely under rainfed conditions and under input starved conditions. In case of sesame, seed yield failed to show any marked increase for over five to six decades across the world, although sesame oil is used largely in Asia and Africa. Previous studies of various sesame breeders indicated that plant height, number of branches per plant, capsules per plant, seeds per capsules, and 1000-seed weight are the traits which have shown significant and positive correlations with yield (Ashri 1998; Singh et al. 2016). The capsules per plant had highest direct effect on seed yield followed by 1000-seed weight. Hence, these traits may be used as selection criteria in breeding programs for the improvement of seed yield of sesame (Mustafa et al. 2015). In addition to the above, the physiological attributes such as harvest index and crop growth rate (CGR) which exhibit positive relationship with seed yield (Chauhan et al. 1996; Ruchi 2008)

should be included in the selection criteria for breeding high yielding varieties of sesame. Early senescence of lower leaves, seed shattering from lower and matured capsules, and indeterminate growth habit resulting in differential maturity of capsules and seeds are the major bottlenecks in the improvement of seed yield in sesame (Rao et al. 2002; Cagirgan 2006). Breeding for improved/ideal plant types for different production regions, determinate habit, and non-shattering types would increase further the seed yield in sesame.

In safflower, studies on development of the sequential traits of seed yield (heads/plant, seeds/head, and seed weight) indicated that genotype had a large effect on seed weight and smaller effects on seeds/head and heads/plant. Location effects were generally highly significant for each trait. The sequential traits showed independence in a correlation analysis. Together the traits accounted for 97% of the variation in yield, with head numbers and seeds in the head accounting for most of the variation (Golkar et al. 2011). Seed weight accounted for most of the variation in yield, followed by seeds/head and head numbers. Regression analyses indicate that for selection, one should give more weight to head numbers and seeds/head when all these traits are considered simultaneously and to head numbers when one trait is considered at a time. In general, head numbers or seeds/head or both traits could be responsible for high yielding lines. Seed weight was generally inflexible in different environments, but heads/plants and seeds/head were more flexible (Abel and Driscoll 1976; Arslan 2007). Though the crop has tremendous potential to be grown under varied conditions and to be exploited for various purposes, the area under safflower around the world is limited largely due to the lack of information on its crop management and product development (Singh and Nimbkar 2007). It has remained as a neglected crop due to its low seed oil content (28–36%), spininess (in some genotypes), and vulnerability to number of diseases and pests (Sujatha 2008). However, further investigations on physiological traits associated with yield components and their manipulations through breeding can increase safflower seed yield.

In niger, number of branches, capitulum/plant, seeds/capitulum, and 1000-seed weight are the major yield contributing traits. For niger to be competitive with other oilseed crops, its seed yield must be significantly improved. To achieve this objective, single-headed, dwarf types must be developed with uniform maturity resulting in reduced shattering losses. The Ethiopian germplasm collection contains short-stature plants which could be used for the development of dwarf types. Genetic variation exists for number of heads per plant that could be utilized in breeding programs to select single headed types (Getinet and Sharma 1996). The presently used normal-height niger accessions have many leaves and a low harvest index (Belayneh et al. 1986). Reducing plant height would decrease the number of leaves per plant and result in a better harvest index. Shorter plants would be capable of utilizing fertilizer more efficiently in that seed yields could be increased through the application of fertilizers. Standard niger types respond to fertilizer application by increasing vegetative growth, which promotes lodging of the crop and decreases seed yield.

Seed yield is a quantitative trait that is the most important in an oilseed flax breeding program. The number of improved cultivars has been released in different

countries, but the yields remained low in many developing countries. Although numerous crop characteristics and environmental factors have been reported to influence seed yield, little is published on basic crop characteristics of flax that affect yield, such as canopy expansion and light interception, dry matter production, and partitioning. During the reproductive phase, light use efficiency and harvest index are correlated with grain production under favorable growing conditions (D'Antuono and Rossini 1995). The factor which increased the amount of dry matter was reported to be the air temperature during the period of plant emergence – budding and large amount of rainfall during vegetative stage reduce average seed yield by about 40%. Hence, breeding for improved seed yield of flax needs to consider these physiological traits before formulation of suitable breeding strategies.

The world castor productivity has increased 146% in the last five decades with 4.0% compound growth rate (Anjani 2014). The tremendous improvement in castor productivity was mainly because of development of number of high-yielding hybrids, especially in India. In the world, the castor production and productivity are high in India (more than 80% of the worldwide production) along with Mozambique, China, Brazil, Myanmar, Ethiopia, Paraguay, and Vietnam. The development and popularization of castor hybrids led to rapid increase in productivity and production in India. Prior to cultivation of castor hybrids, castor production was less than 300 kg/ha, which has now escalated to 1593 kg/ha in 2018–2019 (https://eands.dacnet.nic.in). In Brazil, seed yields averaged around 667 kg/ha over the last 10 years, and yields of up to 1600 kg/ha under better soil fertility and agronomic practices have also been reported (Anjani 2014). Presently the main objectives of the breeding programs around the world are earliness of seed maturation, plant architecture amenable for mechanized harvest, and disease resistance (root not and gray mold). These should be combined with superior productivity of cultivars and at least of 48% oil content of seed. Most breeding programs target genotypes/hybrids with short height (less than 1.5 m), height of primary raceme between 20 and 40 cm, less than 150 days for harvesting, erect plant, and non-shattering fruits (Milani and Nóbrega 2013; Lavanya et al. 2018). Using genome-wide association analysis, candidate genes associated with nine agronomically important traits including the candidate genes encoding a glycosyltransferase related to cellulose and lignin biosynthesis have been associated with both capsule dehiscence and endocarp thickness. It has been hypothesized that the abundance of cellulose or lignin in endocarp is an important factor for capsule dehiscence (Fan et al. 2019). This finding can provide a lead for castor breeding and genetic study, especially in preventing capsule dehiscence and thereby preventing yield losses.

1.4.2 Increasing Seed Oil Content

Since oils of plant origin are commercially important, improving oil content in several crop species has long been a major focus by the breeders of several countries. Planned breeding efforts have led to the improvement of oil content in several crops.

The oil content of the seeds of modern cultivars is significantly higher than those of wild species (Škorić 1992; Zheng et al. 2008) barring few exceptions. The oil content in the most prevalent oil crops ranges from 20% in soybean to more than 60% in candlenut, sesame, *Oiticica* and *Ucuhuba* (Murphy 1996). Therefore, there is a potential to increase oil content in other oil crops.

Increasing oil content in the seeds has been a major objective in soybean breeding programs across the globe. Domestication of soybean from land races with low oil and high protein content has resulted in an adaptive balance of these two maxima. Relationship between oil concentration and seed yield and between oil and protein content is more intrinsic and negative (Brim and Burton 1979; Burton 1987), and hence, breeding for high oil concentration results in lower seed yield and protein contents. A balanced approach for modest gain in oil concentration and yield needs to be targeted without compromising seed protein content (Cober et al. 2009). Through mutagenesis, Bhatnagar et al. (1992) were able to break this negative association and obtained stable genotypes with high protein and oil content. Oil content in soybean has been reported to be maternally influenced (Brim et al. 1968) with additive gene action (Singh and Hadley 1968; Raut et al. 2000). The QTLs associated with seed oil and fatty acids in soybean have been extensively investigated, and more than 322 oil QTLs and 228 fatty acid QTLs have been reported in all 20 chromosomes in the SoyBase database. However, most of these identified QTLs have low selection accuracy and have not been effectively used in marker-assisted selection (MAS) in soybean for seed oil due to insufficient linkage disequilibrium with desirable QTL alleles and the genetic complexity of the trait (Yao et al. 2020).

Some predictions state that the oil content of rapeseed, which is currently 45–48% in Canada and around 42% in China and Australia, might even reach 65% (Wang et al. 2010a, b; Seberry et al. 2011; Wang et al. 2018). Recently, a ultrahigh oil content rapeseed line, “YN171,” with 64.8% oil content in *B. napus* has been developed, and the structural analysis of its seeds indicated a high positive correlation between the oil body organelles to seed ratio and oil content of the seed, and it has been estimated that rapeseed oil content could even reach 75% through breeding (Hu et al. 2013).

Wide variation exists for oil content in groundnut germplasm. It ranges from 46.5% to 63.1% in cultivated types, while the range observed in wild species was from 43.6% to 55.5% (Norden et al. 1987). In few wild *Arachis* species, oil content up to 60% has also been reported (Wang et al. 2010a, b). Oil content and yield has been reported to be independent, thus suggesting possibilities of breeding varieties with high yield and oil content. Narrow-sense heritability has been worked out to be high (Martin 1967) for oil content. Inheritance of oil is governed by two pair of alleles with nonadditive genetic component being predominant (Basu et al. 1988). Hence, selection should be postponed to later generations to eliminate the undesirable recombinants. Following hybridization and wide-scale screening efforts, several high oil lines (>50%) were identified, but the stability for the trait could not be obtained. However, extensive multilocation testing identified four high oil-yielding lines ICGV 05155, ICGV 06420, ICGV 03042, and ICGV 03043 for release in India (Janila et al.2016).

Sunflower is mainly grown for its oil; crushing factories offer premium price for types with more than 40% oil. The ornamental value of sunflower was turned to an important oil source, and over a span of two to three decades, oil content has been enhanced from 30–33% to 43–46% and even up to 50% in certain cases in Russia following Pustovoit method of reserves (Fick and Miller 1997a, b). The kernel to hull ratio is one of the main features that decides the oil content in sunflower. This ratio varies between 10 and 60% in sunflower germplasm (Fick and Miller 1997a, b), and it has been reported that two-thirds of enhancement in oil content came through the reduction in hull content while one-third came from actual increase in oil content (Alexander 1966). However, there exists a negative correlation between husk content and between seed yield and oil content (Kaya et al. 2007), and hence, the breeding strategies should be balanced to achieve higher values for yield and oil content while reducing the hull content. Thus, ease of hulling or its removal automatically forms a criterion while selection. Genetics of hull content has also been worked out which indicates that the trait is controlled by polygenes with minor effects but acting on additive manner with a high heritability (Kovacik and Skaloud 1990) whereas oil content per se has been reported to be sporophytically controlled (Pawlowski 1964).

Sesame has a relatively superior oil quantity and quality than major oil crops. The oil content ranges from 34.4% to 59.8% but is mostly around 50% (Ashri 1998; Dossa et al. 2017), and values up to 69.8% have also been reported in some cultivars (Baydar et al. 1999). Both genetic and environmental factors affect oil content in sesame. Late maturing cultivars have been reported to have higher oil content than early maturing ones. Indeterminate cultivars have also been observed to possess higher oil content than the semi- or partially determinate types. Variations also occur between the capsules located at different positions of the same plant such that seeds obtained from basal capsules of the main stem possess higher oil content than those located toward the apex and on side branches (Mosjidis and Yermanos 1985). Black seeded cultivars were also found to have lower oil content than brown and white seeded types, thus complicating the breeding and selection scheme for improving the oil content. However, phenotypic correlation between oil content and seed yield is also reported to be weak suggesting that it would be possible to develop sesame varieties with both high yield and high oil content. A recent study on GWAS in sesame identified 46 candidate causative genes, including genes related to oil content, fatty acid biosynthesis, and yield. Several of the candidate genes reported in the study for oil content encode enzymes involved in oil metabolism. Two major genes were also found to be associated with lignification and black pigmentation in the seed coat and were also observed to be associated with large variation in oil content. The genes identified in sesame for oil production and quality probably play important roles in other closely related oilseed species (e.g., sunflower) as well, offering the opportunity to look for genes with common function (Wei et al. 2015).

Over the decades, one of the major breeding goals in safflower has been and continues to be to increase seed oil content. Safflower seeds are usually white or creamy in color, and their typical composition is 55–65% kernel and 33–45% hull (Singh and Nimbkar 2007). In normal hull types, the whole seed contains 25–37%

(Weiss 2000), but in very thin hull types, this ratio increases to 46–47% (Golkar 2014). Number of seed coat phenotypes with their genetic control have been identified: partial hull (*par par*), which is recessive to normal hull, inherited independently of thin hull (*th th)* and striped hull *(stp stp)* (Urie 1981), grey-striped hull (*stp2*) (Abel and Lorance 1975) and reduced hull (*rh rh)* (small dark blotches on the seed*)*. Partial hull plants produce achenes which are predominantly dark with high oil and protein levels, and the partial hull character is recessive to reduced hull (Urie 1986). In California, genetic variations for hull content have been developed with a resultant increase in oil content of 42–50%, and hence seed/hull ratio assumes importance. With its simple inheritance, this ratio can be modulated through suitable breeding schemes for increasing oil content in safflower seeds. The same holds good for niger as well. The oil content of niger seed varied from 30% to 50% (Seegeler 1983). The oil, protein, and crude fiber contents of niger are affected by the hull thickness, and thick-hulled seeds tend to have less oil and protein and more crude fiber. In Ethiopia, where the crop is mainly used for edible oil purpose, medium to late maturing types were found to possess high oil content (Abat) types. With the available genetic variations in niger germplasm, oil content can be increased by 5% through selection of genotypes with less hull content (Getinet and Teklewold 1995; Getinet and Sharma 1996).

Oil content in seeds of castor germplasm ranges from 42% to 58% with conflicting reports of its inheritance: polygenic control (Zimmerman 1958), additive gene action (Rojas-Barros 2001), dominance gene action (Okha et al. 2007), and under sporophytic control (Rojas-Barros 2001). Similar to sunflower, safflower, and niger, there exists a negative correlation between seed oil and hull content, with the low hull content reported to be partially recessive over normal hull (Moshkin and Dvoryadkina 1986). It has been demonstrated recently that recurrent selection through screening single seed is an effective method to improve oil content in castor (Grace et al. 2016). Two cycles of recurrent selection increased the mean oil content from 50.33% to 54.47%. Consequently seed weight also increased after two selection cycles, thus establishing a positive relationship between seed oil content and seed weight which allows further improvement of oil content by screening for larger seeds in a population (Grace et al. 2016). However, the role of environment needs to be ascertained in confirming the results obtained in other castor growing regions of the world.

Unfortunately, some studies reported an inverse relationship between oil and protein accumulation in the seeds of some species, such as rapeseed and soybean (Chung et al., 2003; Cober and Voldeng 2000; Hu et al. 2013). Additionally, Vollmann and Rajcan (2009) noted other growth traits also correlated with oil content, such as time to flowering, seed weight, and fatty acid concentrations, which complicate the process of breeding for oil. Recent studies using quantitative trait loci analyses revealed that seed oil contents are controlled by many genes with additive effects (Li et al. 2011; Jiang et al. 2014) indicating that it would be a challenge for the crop breeders to improve oil content through conventional/traditional breeding methods. Biotechnological interventions, genomic tools, and gene editing techniques may be useful in obtaining desired levels of oil content in these crops.

1.4.3 Breeding for Improvement of Quality Traits in Oil Crops

The quality traits in oil crops include both physical and chemical attributes. Nutritional traits include oil, protein, sugar, iron and zinc content, fatty acid profile, and freedom from toxins, while the other quality parameters include visual and sensory attributes (consumer and trader preferred traits) and traits desirable in food/oil processing industries. Similarly, desirable traits for confectionery uses fetch higher price in the market because of its export value which includes seed traits like uniformity of seed shape, intact testa and its color, flavor components, high sugar and protein contents, low oil, and freedom from toxic principles like phytic acid or allergens as in case of groundnut. Depending on the nature of use, low oil and high protein contents (for food use), high oil content (for oil use), and high-oleic/high-linoleic fatty acid ratio (for longer shelf life) are important targeted traits in oil crop breeding programs. The other important quality consideration in assessing the utility of the produce of oil crops includes the quality meal or cake which remains after extraction of oil. Protein and fiber contents and their digestibility and freedom from toxic substances determine their value. Covering all the aspects of quality of each of the oil crop is beyond the scope of this chapter, and hence, functional and nutritional quality improvements are dealt here. For a better understanding of the subject, few earlier reviews on this subject may be consulted (Fernández-Martínez et al. 2004; Yadava et al. 2012; Vollmann and Rajcan 2009; Singh and Nimbkar 2007; Golkar 2014).

1.4.3.1 Genetic Improvement of Fatty Acid Composition

Initially, focus was in increasing oil content, but efforts of the present day are directed toward modification of fatty acid composition of seed oil for food and non-food purposes which has gained much attention during the last decade mainly due to the identification of sources and molecular markers associated with the fatty acids whose composition decides the quality and functionality of oils. Most of the edible oils are rich (>65%) in polyunsaturated fatty acids mainly linoleic and linolenic fatty acids which are unstable oxidatively resulting in rapid spoilage of oil and the food. Hence, to improve the oil quality in crops, lowering the levels of poly unsaturation and increasing the contents of monounsaturated fatty acid, i.e., oleic acid which has relatively higher oxidative stability and higher shelf life, have been aimed, thereby increasing the functional use of the oils.

In soybean, three genes, *fan*1, *fan*2, and *fan*3, were identified that individually reduce the linolenic acid to 2.9–4.9% and in combination were able to reduce it to 1% from different germplasm accessions (Hammond and Fehr 1983; Ross et al. 2000). Using these genes, breeders have successfully developed high yielding lines and cultivars with more than 80% high-oleic acid (HO) soybeans which occupy most of the soybean areas in the USA. These research efforts lasted over 40 years employing conventional pedigree breeding and backcrossing followed by selection

and fatty acid profiling. Targeted perturbation of fatty acid desaturase-2 (FAD2) alleles not only resulted in HO (75–80%) soybeans but concomitantly reduced palmitic acid by 7–8%, which is 20% reduction over original palmitic acid content (Kinney and Knowlton 1997).

In rapeseed and mustard, identification of naturally occurring zero erucic acid mutants in both *B. napus* and *B. rapa* marked a new era of oil quality improvement through mutagenesis in any crop (Downey and Craig 1964). The first low erucic acid spontaneous mutant was obtained from the German spring rapeseed "Liho" and released for cultivation in the 1970s. The Polish spring rapeseed variety "Bronowski" was identified in 1969 as a low glucosinolate type, and these two varieties formed the basis for developing high yielding "00" types (low erucic acid and low glucosinolate or canola) internationally through backcross breeding approach. The first "00" canola variety "Tower" was released in 1974, and thus canola became the most important oil crop of the temperate region of the world (Friedt and Snowdon 2009). Further, the variety "Splendor" or "Nexera" having "high-oleic and low linoleic (HOLL or HOLLi)" with more than 75% oleic acid and 3% linolenic acid has been developed through experimental mutagenesis followed by selection. These varieties fetch premium price in the international market both for human consumption (low erucic acid and high-oleic acid types) and animal feed (low glucosinolates types) (Friedt and Snowdon 2009).

Indian rapeseed-mustard breeding program was also reoriented to accommodate quality parameters and lay emphasis to develop "canola" varieties. Initial efforts concentrated on the development of genetic stocks for low erucic acid in the indigenous background using exotic sources. Sustained efforts at Punjab Agricultural University (PAU), Ludhiana; Tata Energy Research Institute (TERI), New Delhi; Indian Agricultural Research Institute (IARI), New Delhi; G.B. Govind Ballabh Pant University of Agriculture and Technology (GBPUA& T), Pantnagar; and Indian Council of Agricultural Research-Directorate of Rapeseed and Mustard Research (ICAR-DRMR), Bharatpur have resulted in the development of zero erucic mustard lines (LEB 15, LES 39 CRL 1359–19, YSRL 9-18-2, TERI (OE) M 9901, TERI (OE) M 9902, PRQ 9701, BPR6-205-10 and BPR 91–6). Several "0"/"00" strains of rapeseed-mustard have been registered with the National Bureau of Plant Genetic Resources (ICAR-NBPGR) New Delhi, viz., INGR 98001 (0), INGR 98002 (0), INGR 98005 (0), INGR 99007 (00), INGR 99008 (00) (Chauhan et al. 2002). Work is in progress and efforts have been underway to improve the agronomic base of low yielding zero erucic lines and to recombine low erucic acid and low glucosinolate to develop "00" varieties.

Oil quality in groundnut refers to oil content, fatty acid composition, iodine value, ratio of oleic to linoleic acid (O/L), and stability or shelf life. Genetic manipulation of fatty acid composition has been reported by few workers. The Virginia types generally have higher oleic acid content while Spanish-Valencia's have higher linoleic acid. This results in a lower iodine value for oil of Virginia types and indicates that these types will become rancid through autoxidation more slowly than the Spanish-Valencia's. The groundnut breeder is faced with a paradox when breeding for oil quality. Consumers prefer to have oils both with low iodine (long shelf life)

and high iodine value (to have high level of unsaturation from the health point of view). Crosses between all the four habit groups have shown that a wide range of iodine values can be obtained through recombination of genes from different parents and that the iodine value in groundnuts is highly heritable (Bovi 1982).

The oleic/linoleic (O:L) acid ratio, which is an indicator of oil stability and shelf life of groundnut products, varied between 1 and 3 in different cultivars. However, in two Florida breeding lines in the USA, O:L ratio of 40 was reported (Norden et al. 1987). Moore and Knauft (1989) followed up this work further and reported that the high O:L ratio in these lines was governed by two recessive genes. Genomics-assisted breeding (GAB) approaches including marker-assisted selection (MAS) and marker-assisted backcross (MABC) breeding schemes were used successfully in the development of high-oleic cultivars (Janila et al. 2016). Initially linked markers for mutant FAD2 alleles were deployed for improving the nematode-resistant variety "Tifguard" by transferring mutant alleles using MABC, leading to the development of the improved breeding line 'Tifguard' high O/L (Chu et al. 2011). Subsequently, these linked markers were used in MABC and MAS approaches for converting three elite varieties, ICGV 06110, ICGV 06142, and ICGV 06420, into high-oleic lines. These high-oleic lines contained up to 80% oleic and reduced palmitic and linoleic acid, a perfect combination for industry and cooking oil use. Recently, two high-oleic varieties, namely, ICGV 15083 (Girnar 4) and ICGV 15090 (Girnar 5), derived from the cultivar ICGV 05141 using MAS were released for the first time in India after multilocational validation of their performance for yield and stability of high-oleic acid. Substantial progress has also been obtained in developing foliar disease (rust and LLS) resistant cultivars under high-oleic background (Janila et al. 2016; Bera et al. 2018; Shasidhar et al. 2020).

As in other oil crops, high-oleic trait has been explored in sunflower. Monogenic (designated as "ol") dominance of the gene controlling this trait with several modifiers has been reported (Miller et al. 1987; Fernández-Martínez et al. 2009; Pérez-Vich et al. 2002). Three recessive alleles each, P1, P2, P3, for high palmitic acid and three (Es 1, Es2, Es3) for high stearic acid have been reported (Pérez-Vich et al. 2006) and determined by the genotype of the developing embryo, thereby complicating the selection scheme. All the alleles for the target trait need to be introgressed into both the parents in case of hybrid development (Fernández-Martínez et al. 2004).

Sesame is primarily grown for its oil-bearing seed in different countries. Beside the high oil content, sesame seeds contain almost 18% protein, and among the fatty acids, oleic acid (39.6%) and linoleic acid (46%) are the two main components with the ideal ratio of almost 1:1 (Anilakumar et al. 2010). Until 2013, the molecular mechanisms of the high oil content and quality in sesame seeds were unclear. An association mapping of oil and protein contents and oleic and linoleic acid concentrations based on multi-environment trials was conducted using 79 simple sequence repeats (SSR), sequence-related amplified polymorphism (SRAP), and amplified fragment length polymorphism (AFLP) markers in 216 Chinese sesame accessions (Wei et al. 2013). Only one associated marker (M15E10-3) was identified for oil content in two environments suggesting inadequate molecular markers and/or germplasm resources. On the basis of reference genome sequence, the sesame genome

was found to harbor low copy of lipid-related genes (708) compared to soybean (1298). In a comprehensive GWAS for oil and quality traits in 705 sesame accessions under 4 environments, 13 significant associations were unraveled for oil, protein, sesamin, sesamolin, saturated fatty acids (SFA), unsaturated fatty acid (USFA), and their ratio SFA/USFA (Wei et al. 2013). Several causative genes were uncovered for oil content, sesamin, and sesamolin, but none were identified for oil quality indicating that still some more studies are required to unravel the genetic control of these valuable traits.

Safflower has remained as a neglected crop due to its low seed oil content (28–36%). The nutritional value of safflower oil is related to its high level of polyunsaturated oils (Weiss 2000). Safflower oil contains about 75% linoleic acid that is essential for human nutrition (Weiss 2000). Knowles (1968) registered the first safflower accession UC-1 (PI 572434) having high-oleic acid (78%). Safflower cultivars with high-oleic acid content (>70%) have been developed and commercially successful and, two lines, CR-50 with high palmitic acid and CR-13 with high stearic acid, were developed (Hamdan et al. 2009). Incorporation of the high-oleic trait through conventional breeding techniques has been a slow process due to recessive inheritance and difficulties associated with phenotyping by biochemical methods. DNA-based marker-assisted selection (MAS) for high-oleic trait would accelerate the breeding efforts in safflower. A recent study with a set of high-oleic varieties were found to carry the same mutation in the fatty acid desaturase 2-1 gene, CtFAD2-1, which is presumed to be the "ol" allele associated with high-oleic acid content in safflower. Genotypic assays, namely, Kompetitive Allele Specific PCR (KASP) and the Amplifluor™ SNPs Genotyping System (Amplifluor®), were designed for the prediction of high-oleic trait based on the mutation in the CtFAD2–1. The assays were thoroughly validated in segregating populations derived from crosses between low- and high-oleic parents. Through marker-assisted backcrossing scheme, the high-oleic allele, "ol" from the exotic variety, Montola-2000 was incorporated into the background of popular Indian linoleic type variety, "Bhima," and a set of promising high-oleic lines (75.2–81.8%) were developed (Kadirvel et al. 2020). These MAS-derived lines showed consistent expression of high-oleic acid content over seasons and comparable seed and oil yield performance with the local check varieties. The genotypic assays reported in this study were robust, nondestructive, and codominant and accurately predicted the high-oleic trait in segregating populations, thus recommending for fast-track breeding of high-oleic cultivars in safflower.

In niger, the fatty acid composition of oil from the accessions characterized at Ghinchi, Ethiopia, was analyzed using gas chromatography. Linoleic acid ranged from 74.8% to 79.1% with a mean of 76.6%. Contents of other fatty acids were palmitic acid (7.8–8.7%), stearic acid (5.8–7.4%), and oleic acid (trace amounts, 0.5–1.5%). Further evaluation of germplasm to identify genes for high-oleic traits as observed in other oil crops would help furtherance of oil quality for both consumption and industrial purposes in niger (Getinet and Sharma 1996).

Castor seed contains about 50% oil which is composed of 80–90% ricinoleic acid. This hydroxyl fatty acid is unique and cannot be synthesized outside of the

castor seed. A number of chemicals and polymers are synthesized and used in bio-based fuels and industrial products from castor oil. Reduction of ricinoleic acid in castor oil will reduce the importance of this oil functionally, but its high viscosity reduces its use as a biofuel. A mutant USDA accession (PI 179729) has been identified in which conversion of oleic to ricinoleic acid has been partially blocked resulting in HO types (78% oleic acid against the normal of 4%). The trait is controlled by two independent major genes (ol Ml) exhibiting dominant-recessive epistasis (Rojas Barros et al. 2005). Understanding further the genetic regulation of this trait through molecular tools can help in developing suitable varieties.

Dry seed of linseed contains 35–45% oil and around 60% of linolenic acid. Due to its high iodine value, linseed oil has been used primarily for industrial purposes, such as linoleum floor covering, with a high level of unsaturated fatty acids making the oil very reactive and resulting in a short shelf life. Mutation breeding in flax led to the development of a new type of edible flax seed oil that has nearly eliminated the α-linolenic acid (ALA) (Green and Marshall 1984; Rowland 1991). The deficient ALA trait is known to be controlled by two recessive genes (ln1 and ln2) at independent loci (Green and Marshall 1984; Rowland 1991; Ntiamoah and Rowland 1997). Low linolenic acid cultivars have introduced linseed to the edible food market. In 1994, the Flax Council of Canada developed the term “Solin” to describe linseed with less than 5% linolenic acid. The original hybridization work was carried out by CSIRO in Australia with the release of two Linola cultivars in 1992 under the Plant Varieties Rights Scheme. “Linola 947” was the first Solin cultivar registered in Canada. Solin cv. “Linola™ 989” has been reported to have 46% oil (dry basis) and 34% protein. Few more varieties (“Linola™ 1084,” “Linola™ 2047,” “Linola™ 2090,” “Linola™ 2126,” “Linola™ 2149”) have also been developed (with <5% linolenic acid) and released subsequently in Canada (Dribnenki and Green 1995; Dribneki et al. 2007), and in India “TL 99” (an induced mutant with <5% linolenic acid) has also been released during 2018–2019.

1.4.4 Genetic Engineering in Oil Crops and Identification of Genes for Novel Traits

The oil crops are usually grown under rain-dependent production systems in developing countries mainly in Asia and Africa, while in countries like the USA and in Europe, they are grown under well-managed growing conditions. The oil crops grown under these situations are challenged by both biotic and abiotic stresses and further complicated by the recent climate change scenario. Although advances in oil crop breeding and management have resulted in substantial improvement in seed yield and oil content and quality, for further improving the seed yield, oil content, nutritional quality, and industrial needs, newer techniques like genetic engineering through exploitation of the available plant genetic resources in combination with

modern molecular tools for genome-wide association studies (GWAS) and application of genomic selection are very much essential.

In soybean, 809 worldwide accessions were assembled and phenotyped for 2 years at 3 locations for 84 agronomic traits. Genome-wide association studies identified 245 significant genetic loci, among which 95 genetically interacted with other loci. It has been determined that 14 oil synthesis-related genes are responsible for fatty acid accumulation in soybean and function in line with an additive model (Fang et al. 2017). Genome-wide association studies conducted on 249 soybean accessions from China, the USA, Japan, and South Korea for 15 seed amino acid contents by following genotype by sequencing (GBS) indicated presence of genetic variation for amino acids among the accessions. Among the 231 single nucleotide polymorphisms (SNPs) significantly associated with variations in amino acid contents, 15 SNPs were localized near 14 candidate genes involved in amino acid metabolism. Twenty-five SNP markers were observed to associate with multiple amino acids which can be used to simultaneously improve multi-amino acid concentration in soybean. Genomic selection analysis of amino acid concentration showed that selection efficiency of amino acids based on the markers significantly associated with all 15 amino acids was higher than that based on random markers or markers only associated with individual amino acid. The identified markers could facilitate selection of soybean varieties with improved protein quality (Qin et al. 2019).

GWAS was performed for three seed-quality traits, including erucic acid content (EAC), glucosinolate content (GSC), and seed oil content (SOC) using 3.82 million polymorphisms in an association panel in rapeseed-mustard. Six, 49, and 17 loci were found associated with EAC, GSC, and SOC in multiple environments, respectively. The mean total contribution of these loci in each environment was 94.1% for EAC, 87.9% for GSC, and 40.1% for SOC. A high correlation was observed between phenotypic variance and number of favorable alleles for associated loci, which will contribute to breeding improvement by pyramiding these loci. Four novel candidate genes were detected by correlation between GSC and SOC and sequence variations. The study also validated detection of well-characterized *FAE1* genes at each of two major loci for EAC on chromosomes A8 and C3, along with *MYB28* genes at each of three major loci for GSC on chromosomes A9, C2, and C9 which would be useful for genetic improvement of *B. napus* (Wang et al. 2018). In a similar genome-wide association study (GWAS), using an association panel comprising 92 diverse genotypes, GBS identified 66,835 loci, covering 18 chromosomes in Indian mustard (*Brassica juncea*). Different loci (16, 23, and 27) were found associated with oil (16), protein (23), and glucosinolates (27), respectively, including common SNPs for oil and protein contents. Annotation of the genomic region around the identified SNPs led to the prediction of 21 orthologs of the functional candidate genes related to the biosynthesis of oil, protein, and glucosinolates. The identified loci will be very useful for marker-aided breeding for seed-quality modifications in *B. juncea* (Javed et al. 2020).

In groundnut, a genome-wide association study was conducted to investigate the genetics basis of oil, protein, 8 fatty acid concentrations, and O/L ratio using a

diverse panel of 120 accessions of the US mini core collection with 13,382 single nucleotide polymorphisms (SNPs) grown over 2 years. A total of 178 significant quantitative trait loci (QTLs) associated with seed composition traits were identified. RNA-Seq analysis identified 282 DEGs (differentially expressed genes) within the 1 Mb of the significant QTLs for seed composition traits. Among those 282 genes, 16 candidate genes for seed fatty acid metabolism and protein synthesis were screened according to the gene functions. Quantitative trait locus (QTL) analysis using genotyping and phenotyping data identified 8 QTLs for oil content including 2 major (QTLs, qOc-A10, and qOc-A02) ones and 21 QTLs for 7 different fatty acids (Zhang et al. 2021). The QTLs identified in this study could be further dissected for candidate gene discovery, and development of diagnostic markers for breeding improved groundnut varieties with high oil content and desirable oil quality.

In sunflower, commercial hybrid seed production currently relies on a single cytoplasmic male sterility (CMS) source, PET1, and the major fertility restoration gene, Rf1, leaving the crop genetically vulnerable to various pests and diseases. A new fertility restoration gene, *Rf7*, which is tightly linked to a new downy mildew (DM) resistance gene, *Pl34*, in the USDA sunflower inbred line, RHA 428, was identified. To identify markers associated with the fertility restoration trait in a panel of 333 sunflower lines, 8723 SNP markers were used for genotyping. Twenty-four SNP markers were significantly associated with the trait, and these markers were validated in a world collection panel of 548 sunflower lines and observed to be associated with the Rf1 gene (Talukder et al. 2019). The SNP and SSR markers tightly flank the *Rf7* gene, and the *Pl34* gene would benefit the sunflower breeders in facilitating marker-assisted selection (MAS) of *Rf* and *Pl* genes.

GWAS was performed on 705 diverse sesame varieties for 56 agronomic traits in 4 environments, and 549 associated loci were identified. Examination of the major loci resulted in identification of 46 candidate causative genes, including genes related to oil content, fatty acid biosynthesis, and yield. Two major genes associated with lignification and black pigmentation in the seed coat were also observed to be associated with large variation in oil content which may accelerate selection efficiency in sesame breeding and to formulate improvement strategies for a broad range of oilseed crops (Wei et al. 2015). Yet another study on GWAS in sesame for 39 seed yield-related traits including capsule size, capsule number, and seed size at 3 different environments using 705 diverse lines identified novel candidate genes, such as *SiLPT3* and *SiACS8*, which may control capsule length and capsule number traits, thus forming the basis for research on genetics and functional genomics toward seed yield improvement in sesame (Zhou et al. 2018).

1.5 Future Research Strategies

Oil crops are grown mainly in the USA, Europe, Russian Federation, Australia, Africa, China, India, Malaysia, Indonesia, Canada, Argentina, Brazil, Paraguay, and Uruguay apart from Middle Eastern regions. The growing conditions vary in each of these countries mainly of irrigated, high input, and well-managed situations, and in countries like China, India, and Africa, cultivation of oil crops is restricted to rain-dependent, low input, and management conditions. Hence, oil crop breeding is dichotomous suiting to these two situations although the major breeding goal is to improve seed yield, oil, and protein contents. Hence, clear-cut breeding strategies should be worked out on cropping system perspective suiting to the above needs rather than improving the yield per se of the individual crops.

Most of the oil crops especially in Asian and African continent are grown in marginal lands under rainfed conditions. Frequent drought spells varying in intensity and duration coupled with attack by various biotic and abiotic stresses impede with the genetic enhancement of these crops. Hence, development of stress-tolerant varieties with higher seed and oil yield is the need of the hour.

Seeds are the basic unit of crop production, human nutrition, and food security in any crop. A key trait which determines the performance of seeds is the seedling vigour which is a complex trait but very essential especially in rain-dependent production regions of the world where soil moisture availability immediately after the rains will be for a limited period of time, and hence seed/seedling vigour and rapid and uniform establishment and nutrient use efficiency using the available soil moisture are critical for crop productivity. Improving seedling vigour to enhance the critical and yield defining stage of crop establishment remains a primary objective of the agricultural industry and the seed/breeding companies that support it (Finch-Savage and Bassel 2015). Knowledge of the regulation of seed germination has developed greatly in recent times, yet understanding the basis of variation in vigour and therefore seed performance during the establishment of crops remains limited. Hence, understanding of seed vigour at ecophysiological, molecular, and biomechanical level is paramount in these crops. Alongside, seed viability during storage of oil crops especially in orphan crops like safflower, niger, and sesame is an important researchable issue as the seeds of these crops are stored by the resource-poor farmers under poor or suboptimal storage conditions. Soybean crop needs special attention in this area as its seed contains higher concentrations of protein and oil than cereal crop seeds, and oxidation of these biomolecules significantly reduces seed longevity and decreases germination ability in addition to its greater sensitivity to environment, and hence, seeds easily get deteriorated. Generally, soybean seed vigor can be maintained for less than a year and, hence, needs to be multiplied every year. Varieties with good seed vigor are essential for maintaining optimum plant population and stable high yields. Hence, along with seed size, viability of seeds needs to be addressed for maintaining plant population as well as stable yield. Certain landraces of China exhibited better seed vigor than cultivars (Hao et al.

2020), and hence, a fresh relook into the available genetic resources for these two traits is very important for increasing the yields and seed quality in oil crops.

In case of groundnut, fresh seed dormancy is an important trait as the pods are subterranean, and unseasonal rains at crop harvest stage will spoil the produce leading to huge economic loss of the resource-poor semiarid farmers especially in India where more than 70% groundnut area is occupied by erect-type cultivars where fresh seed dormancy is absent. Hence, assembling the vast genetic resources available in these regions and evaluation for yield and other related traits including the resistance/tolerance to prevailing major biotic and abiotic stress factors at diverse environments and utilizing them in breeding programs would further enhance the genetic potentials of this crop. In orphan crops like sesame, safflower, linseed, and niger, there is a reduction in area under cultivation in different countries including India mainly due to the stringent competition from other economically profitable crops and cropping systems. Hence, high yielding and short duration varieties suiting to the profitable cropping pattern/systems need to be developed. One area where most of the oil crops suffer is due to lack of clear-cut studies on management strategies for irrigation water and nutrient use efficiencies. Although genetic variation for nutrient and water use efficiency has been reported in few crops, traits associated with these parameters further need to be addressed.

Oil crops are rich in energy but have C_3 mode of photosynthesis and, hence, are photosynthetically less efficient in partitioning of assimilates when compared with C_4 plants. Since large amount of biomass is still locked up in the vegetative parts of the plants, remobilization of photosynthates from vegetative parts to their respective reproductive parts also improves HI and, thus, seed yield. Although several studies are available on biomass accumulation, the physio- and biochemical mechanisms regulating assimilate partitioning and their genetics are yet to be elucidated in detail. The target traits include expanding and optimizing light capture by the leaf canopy, inducing a more rapid relaxation of non-photochemical quenching at photosystem II, increasing the carboxylation capacity of the Rubisco enzyme as well as minimizing oxygenation and photorespiration, enhancing the regenerative capacity of the carbon reduction cycle, optimizing the electron transport chain, and adding components of cyanobacterial or algal systems to pump CO_2 or compartmentalize Rubisco (Bailey-Serres et al. 2019).

Improving photosynthetic efficiency is neither a new nor a universally accepted idea. Some have argued that the selection pressures endured by photosynthesis render it unamenable to improvement. Despite decades of research, the challenge of engineering Rubisco for improved specificity and carboxylation rate remains unmet. However, some recent successes in engineering photosynthetic enzymes and introducing novel pathways into chloroplasts may lead to substantial gains in crop performance including oil crops.

The current trajectory for crop yields is insufficient to nourish the world's population by 2050. Greater and more consistent crop production must be achieved against a backdrop of climatic stress that limits yields, owing to shifts in pests and pathogens, precipitation, heat waves, and other weather extremes. Above all temperature extremes, frequent floods and drought may increase consequently. Genetic

variations available in the reservoirs of germplasm resources in each of the crops need to be captured for use in future breeding programs.

The increasing demand for proteins worldwide as human nutrition and animal feed leads to a growing interest on other protein sources. Moreover, with increasing urbanization and income rise in many developing countries, per capita consumption of animal products would rise, and consequently the demand for feed grains would increase by 3% annually in these countries (FAO 2017). The oil meal or cake which remains after extraction of oil from the seed is a good source of protein both for ruminant and nonruminant animals. However, certain toxic compounds like phytate, erucic acid, glucosinolates, aflatoxins, etc. need to be eliminated/reduced through recent genetic and genomic tools and gene editing techniques.

Among the oil crops, groundnut crop has the unique advantage of a good source of fodder especially in Asian countries. About 40% is the underground pod biomass, while the aerial vegetative portion contributes 60% of the total biomass. They are rich in protein (14–21%) even at harvest stage, and hence any improvement in nitrogen content of the haulms would qualitatively improve the animal performance in terms of meat and milk yields. Only very few reports (Omokanye et al. 2001; Nigam and Blümmel 2010) are available on genetic variation for fodder quality traits like nitrogen/protein content, in vitro dry matter digestibility (IVDMD), metabolizable energy values, lignin, and fiber fractions. However, inheritance and strategies and selection schemes for evaluation of these important fodder quality traits in segregation generations are missing in the literature which is an important grey area in groundnut research.

Seeds of oil crops like sunflower, groundnut, and sesame are also used in confectioneries, and hence, high protein and sugar contents and low oil along with flavor compounds need to be addressed. Although there are few reports on these areas, further studies on flavor compounds and their genetic control, molecular and biochemical mechanisms regulating these compounds, and breeding strategies to exploit them need to be designed.

Studies on oil QTLs and candidate genes for oil content and oil quality traits through molecular approaches including GWAS are being accumulated in the literature during the last decade, and their validation in different genetic resources and breeding populations need immediate attention for genetic improvement of the oil crops.

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Chapter 2
Accelerating Soybean Improvement Through Genomics-Assisted Breeding

Sonali Mundhe, Ravindra Patil, Manoj Oak, and Santosh Jaybhay

Abstract Soybean [*Glycine max* (L.) Merr.] is considered as a wonder crop as it contributes to about 28% of the vegetable oil and 70% of the protein meal useful for food and feed preparations. Soybean ranks first among the oilseed crops with the production of more than 340 million metric tons for the last 5 years. The increasing trend in soybean production during the last few decades is mainly attributed to an increase in the area under soybean cultivation. Further increase in global soybean production is heavily dependent on an increase in productivity by developing high-yielding climate-resilient varieties that can fulfill the ever-increasing demand for soybean in the global market. The major constraints that limit the productivity of soybean include limited genetic diversity available for breeders and several biotic and abiotic stresses, which pose a severe threat to the crop. In this scenario, the conventional breeding approaches appear insufficient to achieve high productivity and genetic gain. Recent advances in mutation breeding and genome editing have provided new tools to generate targeted novel genetic variations. Simultaneously, molecular breeding techniques such as high throughput genotyping, marker-assisted breeding, speed breeding, and genomic selection have shown the potential to develop improved breeding lines with greater precision and higher genetic gain per unit time.

Keywords Soybean · *Glycine max* (L.) Merr · Accelerated breeding · Marker-assisted breeding · Genomic selection · Genome editing

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2.1 Introduction

With the production of 339 million tons in 2020, soybean ranked first among the major oilseed crops, such as sunflower, cottonseed, groundnut (peanut), rapeseed, and palm, in the international trade market. During the past decades, collective efforts have been made by soybean growers and crop scientists, leading to an increase in global soybean production from 155.1 million metric tons in 1999 to 339 million metric tons in 2020 (Fig. 2.1). Over the last decade, its production has increased at an average rate of over 5% per annum. Brazil grows soybean over the largest area and holds a share of about 23% of the world's soybean production, followed by the USA (18.5%), China (10.9%), Argentina (9.3%), and India (6.3%) (Fig. 2.2a). Soybean contributes about 55 to 58% of global oilseed production (Wilson 2008) (Fig. 2.2b) and about two-thirds of the world's protein concentrate for livestock feeding. It is the most important legume contributing to about 70% (244.39 million metric tons) of the world's protein meals (Fig. 2.2c) and about 28% (58.25 million metric tons) of the global oil production (Fig. 2.2d) for food and livestock feeding (Oilseeds: World Markets and Trade 2021, https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf). Soybean is regarded as the most important protein source than wheat and maize. Soybean is rich in seed protein content (~40%) and oil content (~20%); hence it is useful for feed and food products. Soybean protein is called a complete protein because of its amino acid composition. It is used as a raw material for health drinks, food, and animal feed all over the world. Soybean

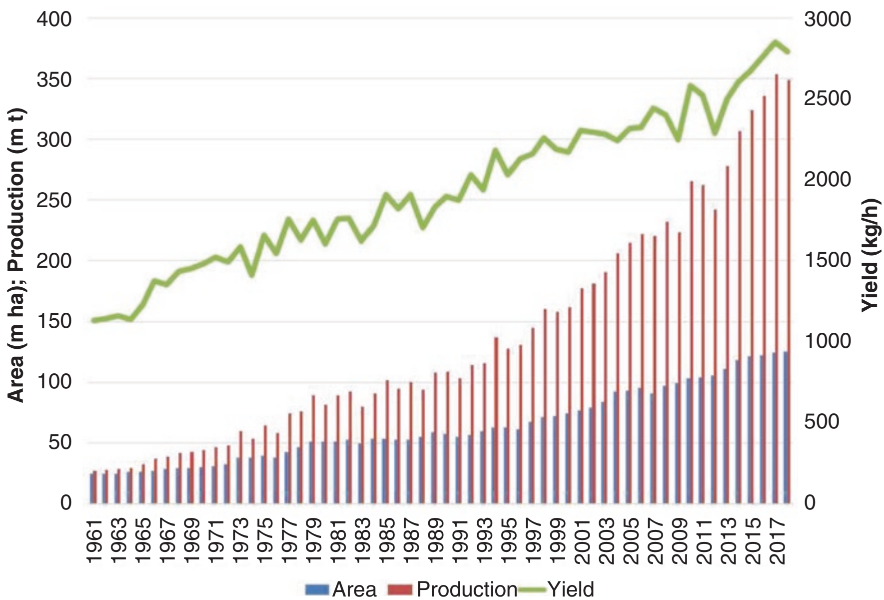

Fig. 2.1 Soybean: global area, production and productivity trends. (Source: Food and Agriculture Organization, 2019, www.fao.org)

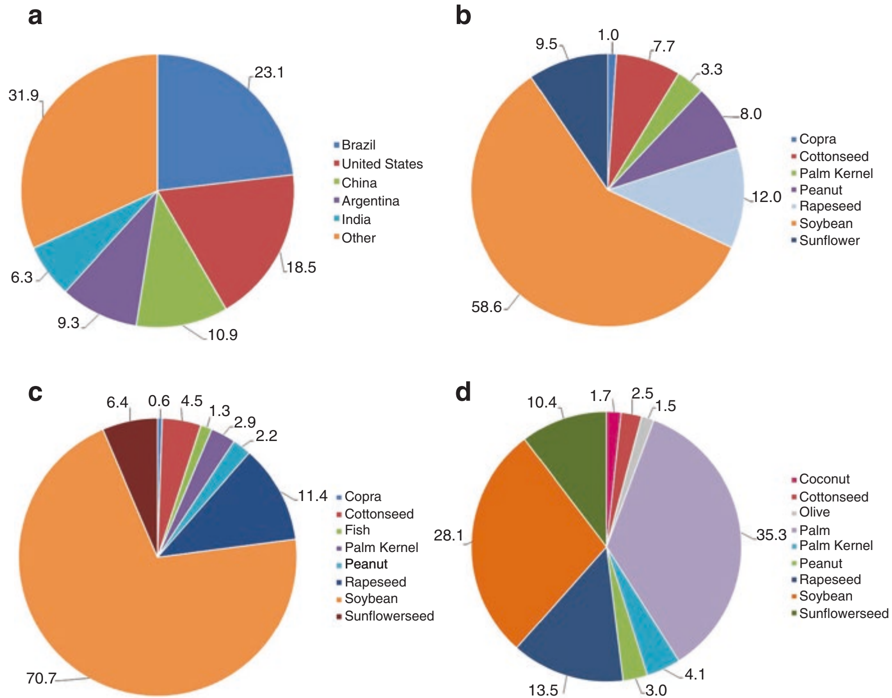

Fig. 2.2 Worldwide production and utilization of soybean during the year 2019–2020. Percent share of major soybean producing countries (**a**), percent contribution of soybean to global oilseed production (**b**), percent contribution to protein meal (**c**), percent contribution to global vegetable oil production (**d**). (Source: USDA, FAS, March 2021)

as a crop is useful in improving soil fertility due to its ability to fix atmospheric nitrogen. Nitrogen fixation in soybean is brought about by a mutualistic relationship between the soybean roots and *Bradyrhizobium japonicum* bacterium, which forms nodules (swellings) in the roots. The bacterium aids the plant to fix or convert atmospheric nitrogen into a more usable form. It was shown that soybean crops could fix 44 to 238 kg of nitrogen per hectare (Peoples et al. 1995).

The progress made in the field of soybean improvement is impressive despite various bottlenecks faced by breeders and researchers. An average growth rate of about 5% in annual production is achieved so far across the world. Significant improvement through conventional breeding has been witnessed for important traits such as grain yield, oil content, protein content, and biotic stress tolerance. Mutation breeding has been instrumental in providing new variations for the target traits during the last six to seven decades (Kharkwal and Shu 2009; Nakagawa 2009). However, breeding soybean with improved grain yield and abiotic stress tolerances has met limited success, mainly due to (1) narrow genetic diversity in cultivated soybean; (2) the difficulties in breeding for tolerance traits, which include complexities introduced by genotype × environment interactions and the relatively

infrequent use of simple physiological traits as measures of tolerance; (3) desired traits which can only be introduced from closely related species.

Recent advances in the field of DNA-based markers have allowed mapping and characterizing the genetic components underlying complex traits such as grain yield, domestication-related traits, seed composition, nutrient-use efficiency, resistance to biotic stress, and abiotic stress tolerance in soybean (Sebastian et al. 2010; Kumawat et al. 2016). DNA markers provide enhanced selection efficiency with the prediction of phenotype at an early generation stage. Molecular marker-based genomic selection helps in the rapid selection of the desired genotype and accelerates the breeding cycle. The objective of the present chapter is to provide an overview of breeder-friendly genomic tools and techniques such as marker-assisted breeding, speed breeding, TILLING, and genome editing, which can accelerate the flow of desired alleles from germplasm to the advanced breeding lines with precise selection.

2.2 Genetic Resources in Soybean

Genetic resources play a key role in the development of new cultivars. Cultivated soybean, *Glycine max* (L.) Merr. belongs to the family Fabaceae (Leguminosae), the subfamily Papilionoideae, the tribe Phaseoleae, the genus *Glycine* Willd, and the subgenus *Soja* (Moench). Soybean includes two genera, cultivated soybean (*Glycine max*) and wild annual soybean (*Glycine soja*). Based on linguistic, geographical, and historical literature, it was proposed that the domestication of the soybean occurred in the eastern half of North China (primary center of origin) during 1700–1100 BC (Hymowitz 1970). Due to its importance in Chinese civilization, soybean was treated as the sacred grains along with barley, wheat, rice, and millet (Morse et al. 1949). Soybean was distributed to Japan, Vietnam, Indonesia, Thailand, the Philippines, Myanmar, Nepal, and North India from the first to sixteenth centuries. These regions were further recognized as the secondary center of origin of the soybean (Hymowitz 1990). In sixteenth and seventeenth centuries, soybean was introduced to Europe, followed by North America in the eighteenth century (Morse et al. 1949).

2.2.1 Wild and Cultivated Species of Soybean

The genus *Glycine* contains two subgenera *Soja* and *Glycine*. Subgenus *Soja* contains two species, viz., *Glycine max*, a cultivated species, and *Glycine soja*, a wild annual species. The wild perennial species belonging to subgenus *Glycine* carry diverse genome and phenotypic traits; hence, it may prove as a source of important traits such as biotic and abiotic stress tolerance. However, these wild relatives belong to the tertiary gene pool as per the concept of the gene pool in soybean

(Harlan and de Wet 1971), which could not develop fertile hybrids with the cultivated soybean; therefore, the genetic diversity present in wilds remained unexplored (Singh and Hymowitz 1999). This may be one of the reasons to have narrow genetic diversity in cultivated soybean. These wild species, mainly confined to Australia, are being maintained in Canberra, Australia, and are recognized by the International Plant Genetic Resources Institute as the world base collection for perennial *Glycine*. All of these species generally carry 2n = 40 chromosomes, except for *G. hirticaulis*, *G. tabacina*, and *G. tomentella* (Vaughan and Hymowitz 1983; Brown et al. 1987; Hymowitz et al. 1997). The subgenus *Soja* is most diverse in the eastern half of North China, whereas maximum diversity for the subgenus *Glycine* occurs in Australia.

2.2.2 Global Soybean Germplasm Collections

According to an estimate, soybean germplasm comprises more than 1 lakh accessions of *G. max*, about 10,000 accessions of *G. soja*, and about 3500 accessions of wild *Glycine* species (Palmer et al. 1995). Germplasm maintained at USDA contains about 16,962 accessions of soybean belonging to species *G. soja* and *G. max* (Hill and Nelson 1997). The details of the soybean accessions are available in the International Legume Database and Information Service, USDA-Germplasm Resources Information Network (www.ars-grin.gov). China has the world's largest collection of soybean germplasm, containing more than 40,000 accessions, which have been preserved and maintained at the National Gene Bank of China (Li et al. 2020). This entire collection was divided into three subcollections, i.e., core collection, mini core collection, and integrated applied core collection (Qiu et al. 2013). Similarly, around 11,300 soybean accessions are conserved at the National Institute of Agrobiological Sciences (NIAS) Genebank in Japan. They include local landraces collected in Japan and overseas and improved varieties and breeding lines developed by regional Japanese agricultural research institutes or introduced from overseas agricultural research institutes and wild soybeans (Kaga et al. 2012). In Korea, the Rural Development Administration Gene Bank has maintained about 700 landraces (Yoon et al. 2003; Li et al. 2020).

In India, 1400 soybean germplasm lines were assembled in the 1950s to start the soybean improvement work under the All India Coordinated Research Project on Soybean in collaboration with the University of Illinois, Urbana-Champaign, USA. Nearly 130 varieties have now been bred and released by Indian soybean breeders using exotic/indigenous germplasm through hybridization at various centers; furthermore, eight varieties have been released as direct introductions (Mishra and Verma 2010). Indian soybean germplasm collections comprise about 3000 accessions at ICAR National Bureau of Plant Genetic Resources, New Delhi, and about 2500 accessions at ICAT Indian Institute of Soybean Research, Indore (Gupta et al. 2018). Recently, the genetic diversity of 277 soybean accessions was explored using a novel biotechnological tool termed as multi-trait allele-specific genic marker

assay. This high throughput genotyping assay was designed for 22-plex and 7-plex alleles at 15 genes governing 10 different agronomic and quality traits in soybean (Kumawat et al. 2021). The above-listed gene pools, along with exotic germplasm, can be utilized in the future for the development of soybean varieties with different important qualitative and quantitative traits to achieve food security.

2.3 Speed Breeding

Conventional breeding takes about 12 years to generate a stable, improved soybean cultivar. It is possible to shorten the time required to obtain stable homozygous breeding lines of soybean using summer nurseries in South Asia. However, water scarcity during the summer season poses a major limitation to conduct summer nurseries. Therefore, an alternative approach that shortens the breeding cycle is warranted. Speed breeding has emerged as a simple, flexible, and efficient tool to reduce generation time that enables to accelerate the breeding program. The speed breeding approach was successfully used to advance up to six generations per year of spring wheat, durum wheat, barley, chickpea, and pea and four generations of canola (Ghosh et al. 2018; Watson et al. 2018). This approach mainly uses

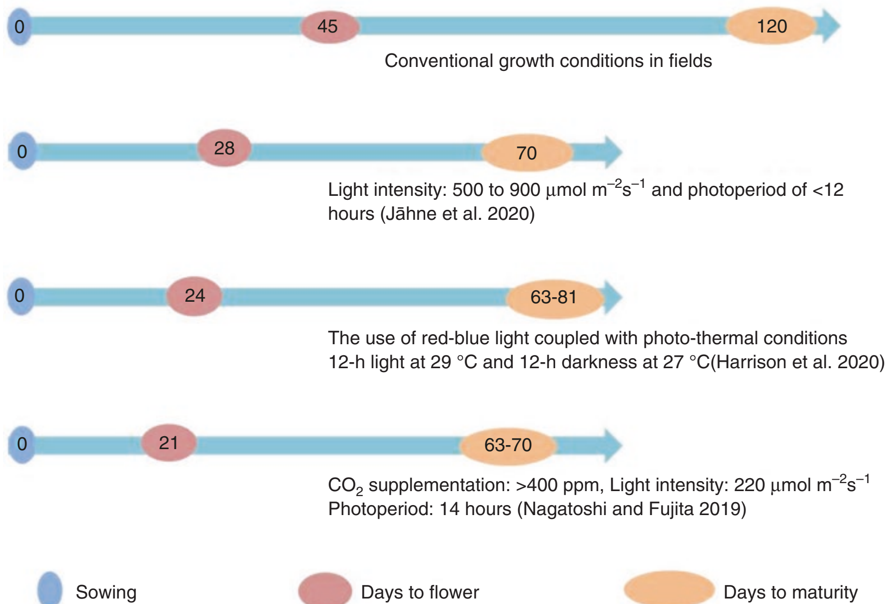

Fig. 2.3 Schematic presentation of effect of various speed breeding protocols on generation time in soybean

light-emitting diode (LED)-based prolonged photoperiod to achieve a faster developmental rate of the plant in long-day and day-neutral crops. However, prolonged photoperiod hinders initiation of flowering in photoperiod-sensitive short-day crops. Speed breeding protocol was, therefore, further optimized using an LED system that allows modifying light quality and intensity suitable for a short-day crop such as soybean (Jähne et al. 2020). A combination of light intensity of 500–900μmol m^{-2} s^{-1} and photoperiod of 10 to 12 h was found to hasten flowering time to about 24 days after planting of soybean (Fig. 2.3), thus allowing up to five generations of soybean in 1 year. Further, about 2 days earlier flowering (~21.8 days) could be achieved by increasing light intensity above 1000μmol m^{-2} s^{-1}. This short photoperiod resulted in homogeneous early flowering in soybean genotypes with late maturity. A blue-light enriched, far-red-deprived light spectrum was found promising to obtain shorter and sturdier plants amenable to compact, multi-storey high throughput speed breeding protocol for soybean (Hitz et al. 2019; Jähne et al. 2020). The use of red-blue light coupled with photothermal conditions (12-h light at 29 °C and 12-h darkness at 27 °C) also showed hasten maturity period in soybean ranging from 63 to 81 days versus 120 days observed in field conditions (Harrison et al. 2020).

Carbon dioxide is reported as another factor that affects days to flower in plants. CO_2 supplementation in the growth chamber was reported to reduce the number of days to flower in rice (Ohnishi et al. 2011; Tanaka et al. 2016), whereas a low concentration of CO_2 was found to be associated with delayed flowering in *Arabidopsis* (Li et al. 2014). The effect of CO_2 concentration on soybean growth was tested recently. It was observed that the CO_2 supplementation at >400 ppm along with the light intensity of 220 mmol m^{-2} s^{-1} at the canopy level and photoperiod of 14 h could reduce the generation time of soybean to just 70 days against 102–132 days required in field conditions (Nagatoshi and Fujita 2019), thus allowing up to five generations of soybean per year instead of one to two generations currently possible in the field conditions. Moreover, the authors have observed that the soybean plants with CO_2 supplementation showed a significantly higher number of healthy flowers and much-improved crossing efficiency than plants without CO_2 supplementation.

Harvesting early or immature seeds to shorten the reproductive period is another way to reduce the generation time, provided that the dormancy is broken immediately after harvesting. It can be done through cold stratification or by applying gibberellins which promote seed germination (Hickey et al. 2019). Cold stratification was used to break the dormancy of immature wheat and barley seeds that helped to reduce generation time by 15 days (Watson et al. 2018). Gibberellin was used to improve the germination of soybean seeds harvested early, which showed a marginal improvement in the rate of germination; however, it resulted in elongated hypo- and epicotyls of soybean seedlings which are undesirable attribute in the case of multi-storey growth chambers (Jähne et al. 2020). Therefore, one should be cautious while selecting the method to reduce generation time.

2.4 Mutagenesis in Soybean

Cultivated soybean showed very low genetic diversity due to several bottlenecks such as domestication, selection pressure during repeated breeding cycles, founding events to introduce soybean crop with very few varieties to a new geographical region, etc. Domestication of cultivated soybean from its wild relative [*Glycine soja* (Sieb. and Zucc.)] was the bottleneck with the most impact on genetic diversity. It resulted in a reduction of sequence diversity present in wild species to half, loss of 81% of the rare alleles, and significant change in allele frequencies of 60% of the genes (Hyten et al. 2006). Induced mutagenesis can increase the genetic diversity in a shorter time than the naturally occurring spontaneous mutations. Physical mutagen such as X-ray was initially used to develop mutant populations and identification of seed coat mutant in soybean (Rode and Bernard 1975). Since then, X-ray mutagenesis has been used to generate various mutants for fatty acid composition in soybean seeds (Takagi et al. 1989; Rahman et al. 1994, 1995; Anai et al. 2012; Gillman et al. 2014). Soybean mutants with null Kunitz trypsin inhibitor activity reduced phytate content, and lipoxygenase-free seeds were developed using gamma irradiations (Kim et al. 2010; Lee et al. 2011, 2014; Yuan et al. 2012). Several of the soybean mutants generated through gamma irradiations and their derivatives with improved agronomic traits were released for cultivation in Japan, China, and India (Kharkwal and Shu 2009; Nakagawa 2009).

Although mutation breeding using ionizing radiations has proven successful, there are some limitations imposed by tetraploidy in soybean, gene duplications, and identification of small number of desired mutants from a large population (Parry et al. 2009). In polyploid crops such as soybean and wheat, most of the genes are present in two to three similar but redundant homoeologs; therefore, a random mutation in one of these copies seldom results in phenotypic changes (Slade et al. 2005), and this makes soybean one of the most challenging crops to implement mutation breeding approach. Chemical mutagen such as ethyl methanesulfonate (EMS) causes G/C to A/T transitions at the DNA level. These single nucleotide changes caused by EMS can be detected by a reverse genetic tool termed as Target Induced Local Lesions IN Genome (TILLING). TILLING has been successfully used to detect novel variations at fatty acid desaturase gene *GmFAD2–1A* and *GmFAD2–1B* of soybean, which resulted in improvement of cooking quality of soybean oil with increased oleic acid content (Dierking and Bilyeu 2009; Hoshino et al. 2010; Lakhssassi et al. 2017) (Table 2.1). Similarly, a mutation at *GmFAD3-2a* was isolated by TILLING and used to reduce levels of α-linolenic acid, a highly unstable fatty acid component associated with an unpleasant odor and reduced shelf life of soybean oil (Hoshino et al. 2014). Recently, TILLING-by-target captured sequencing technique was used to detect EMS-induced mutations at stearoyl-acyl carrier protein desaturase genes *GmSACPD-A*, *GmSACPD-B*, and *GmSACPD-D* (Lakhssassi et al. 2020). These mutants showed enhanced nutritional value with significant increase in the stearic acid component without affecting nodule development and growth. This work highlighted the successful application of TILLING and

Table 2.1 Summary of TILLING used for development of novel variation in soybean

Trait	Gene	Mutagen	Detection method	Reference
Fatty acid biosynthesis	Stearoyl-acyl carrier protein desaturase (*GmSACPD-A*, *GmSACPD-B*, *GmSACPD-D*)	Ethyl methanesulfonate (EMS)	TILLING-by-sequencing	Lakhssassi et al. (2020)
Fatty acid biosynthesis	*GmFAD2-1A*, *GmFAD2-1B*	Ethyl methanesulfonate (EMS)	Cel 1	Dierking and Bilyeu (2009) and Hoshino et al. (2010)
			Li-Cor, targeted sequencing	Lakhssassi et al. (2017)
			TILLING-by-sequencing	Millas et al. (2019)
Reduced α-linolenic acid content	Glyma18g06950 (*GmFAD3-2a*)	Ethyl methanesulfonate (EMS), X-ray	Cel 1	Hoshino et al. (2014)
Chlorophyll biosynthesis	Mg-chelatase subunit gene (*ChlI1a*)	Ethyl methanesulfonate (EMS)	TILLING-by-sequencing	Li et al. (2017)
Shoot architecture and nodulation	*GmCLV1A* and *GmNARK* (CLAVATA1-like receptor kinase genes)	Ethyl methanesulfonate (EMS)	Targeted sequencing	Mirzaei et al. (2017)
Raffinose family oligosaccharides	*RS2* (Raffinose synthase gene)	Ethyl methanesulfonate (EMS)	Cel 1	Dierking and Bilyeu (2009)
	RS2 and *RS3* (Raffinose synthase gene)	N-nitroso-N-methylurea (NMU)	Cel 1	Thapa et al. (2019)
Soybean cyst nematode (SCN) resistance	*Rhg4* (resistance to *Heterodera glycines* 4)	Ethyl methanesulfonate (EMS)	Targeted sequencing	Liu et al. (2012)

next-generation sequencing to help breeders and biotechnologists to improve the nutritional quality of soybean without affecting agronomic traits (Alfonso 2020). TILLING was also used to induce mutations at *RS2*, a gene encoding raffinose synthase in soybean, which resulted in reduced antinutritional factors such as raffinose and stachyose and improvement of seed meal quality (Dierking and Bilyeu 2009). Further, combining the mutants for *RS2* and *RS3* eliminated nearly 90% of the raffinose family oligosaccharides in soybean (Thapa et al. 2019). Besides quality improvement, TILLING was also used to generate novel mutants resistant to soybean cyst nematode (SCN), the most economically important pathogen of soybean (Liu et al. 2012). The study reported map-based cloning of the candidate gene SHMT (serine hydroxymethyltransferase) underlying *Rhg4* locus that confers

resistance to SCN. Although TILLING has been successfully used to detect point mutations in the target gene, one should be cautious while using the gel-based detection method as it may give some false-positive mutations due to nonspecific hybridization (Lakhssassi et al. 2017). It may be due to the high copy number of the target genes and similarity with the soybean genome. Therefore, the authors have further recommended using TILLING-by-sequencing method to identify mutations.

2.5 Marker-Assisted Breeding

Genetic markers used in plant breeding can be classified into classical markers and DNA markers (Xu 2010). Classical markers include morphological markers, biochemical markers, and cytological markers. DNA markers include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), and single nucleotide polymorphism (SNP). Among the multiple applications of DNA markers in plant science, the most promising for cultivar development is marker-assisted breeding (MAB). DNA markers that are tightly linked to important genes or loci have enormous potential to improve conventional

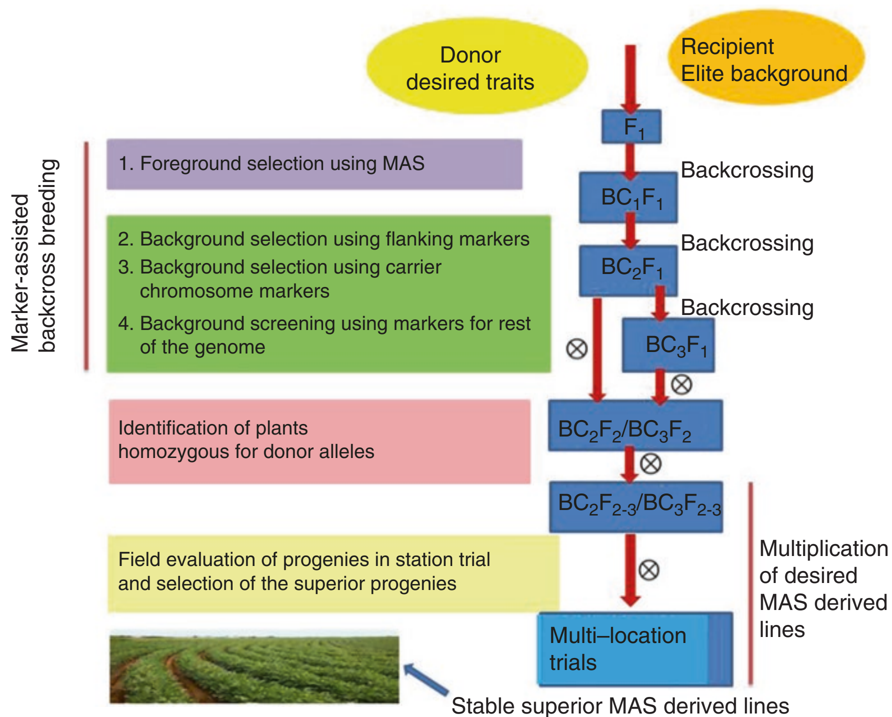

Fig. 2.4 Flowchart showing protocol of marker-assisted breeding in soybean

plant breeding efficiency and precision via MAB. Recently, several allele-specific functional markers have been reported for various important traits in soybean such as flowering and maturity, pod dehiscence, fragrance, salt tolerance, soybean cyst nematode oleic acid content, raffinose content, and Kunitz trypsin inhibitor (Kumawat et al. 2016). Similarly, tightly linked markers were also identified for the nutritional value of seeds (phytic acid content, glycinin, β-conglycinin content, aroma, lipoxygenase), which may facilitate a more efficient selection of new varieties free of antinutritional compounds. The schematic presentation of the marker-assisted backcross breeding method is as shown in Fig.2.4.

MAB approach dramatically accelerates precise and efficient introgression of desired genes in recipient variety, as well as rapid recovery of the genetic background. In wheat, marker-assisted background selection could achieve transfer *Yr15*, a stripe rust resistance gene in a recurrent variety and recovery of 97% of the genetic background of the recurrent parent with just two backcrosses ($BC_2F_{2:3}$), whereas phenotypic selection could recover only 82% of the background in BC_4F_7 plants (Randhawa et al. 2009). This example suggested that the MAB successfully reduces the time required to obtain advanced breeding lines to half compared to conventional methods.

Marker-assisted selection (MAS) and marker-assisted backcrossing have been widely adopted to improve resistance to diseases and other relatively simple traits (Tuberosa 2012). In soybean, MAB has been successfully deployed in few soybean breeding programs for introgression of single genic as well as polygenic traits in the

Table 2.2 Details of marker-assisted breeding conducted for improvement of soybean for various important traits

Target trait	Gene/locus	Marker type	Reference
Low raffinose family oligosaccharides content	*RS3*	Gene-specific SimpleProbe	Hagely et al. (2020)
Elimination of Kunitz trypsin inhibitor (kti)	*Ti3*	SSR	Bulatova et al. (2019), Maranna et al. (2016) and Kumar et al. (2015)
Elimination of off-flavor and improvement of seed longevity	*lox2*	lox2 specific	Rawal et al. (2020)
High oleic acid content	*FAD2–1A*, *FAD2–1B*	Gene-specific SimpleProbe	Pham et al. (2010, 2011)
Resistance to soybean mosaic virus	*Rsv1*, *Rsv3*, and *Rsv4*	SSR	Saghai Maroof et al. (2008) and Shi et al. (2009)
	R_{SC4}, R_{SC8}, and R_{SC14Q}	SSR	Wang et al. (2017)
Resistance to soybean cyst nematode	*rhg1*, *Rhg4*	SSR	Santana et al. (2014)
Grain yield	Yield QTL	SSR	Sebastian et al. (2010)
Seed protein content (SPC)	QTL (*Prot-08-1*)	SSR	Zhang et al. 2015
Salt tolerance	*GmSALT3*	SSR	Liu et al. (2016)

desired genetic background (Table 2.2). MAB was found to be efficient to improve quantitative traits contributing to the nutritive value of soybean, such as seed protein content and oil quality in soybean. Two cycles of MAB for seed protein content (SPC) in soybean using SSR markers could fetch up to 9% of transgressive segregation in the trait (Zhang et al. 2015). Improvement in oil quality in terms of elevated oleic acid content (up to 86%) was achieved by combining *FAD2–1A* and *FAD2–1B* alleles (Pham et al. 2010, 2011). MAB was also successfully used to improve disease resistance and abiotic stress tolerance in soybean. Simple sequence repeat (SSR) markers linked to three soybean mosaic virus (SMV) resistance loci (R_{SC4}, R_{SC8}, and R_{SC14Q}) were used to assist pyramided breeding for the disease resistance in soybean (Wang et al. 2017). Similarly, three independent SMV resistance loci (Rsv1, Rsv3, and Rsv4) have been identified in soybean and pyramided using molecular markers (Shi et al. 2009). Improvement in salt tolerance in soybean was demonstrated by MAS for *GmSALT3* gene in cultivated varieties (Liu et al. 2016). The study has shown that the MAS could accelerate breeding for improved yield components under saline stress in the field.

In India, marker-assisted backcross breeding in soybean was initiated under the "Accelerated Programme on Crop Improvement" funded by the Department of Biotechnology, New Delhi. The target was to introgress the null allele of Kunitz trypsin inhibitor (*ti3*) to elite soybean varieties for nutritional quality improvement (Kumar et al. 2011). The development of Kunitz trypsin inhibitor (KTI)-free soybean is crucial for the soy-food industry as the heat inactivation incurs extra cost. Null allele *ti3* of KTI from PI542044 was introgressed into the cultivar JS97–52 and MACS 450 (recurrent parents) through marker-assisted backcrossing (Kumar et al. 2015; Oak et al. unpublished) (Fig. 2.5a). A similar approach was adopted to

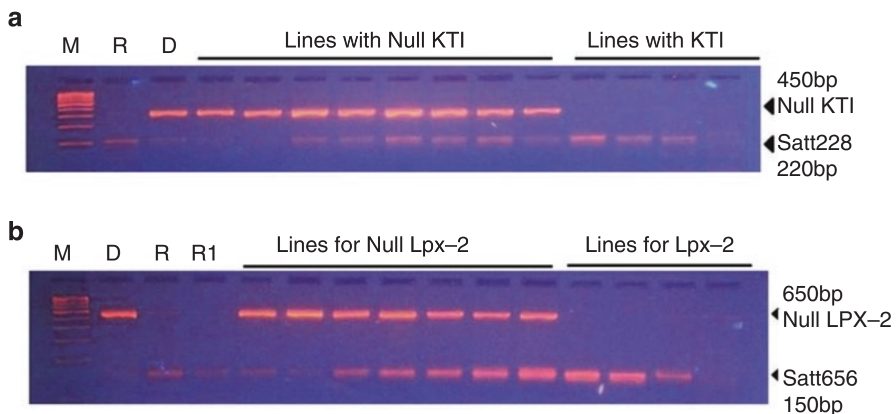

Fig. 2.5 Marker-assisted foreground selection in soybean using multiplex PCR with gene-specific marker and a linked SSR marker. (**a**) PCR profile of Kunitz trypsin inhibitor null allele, M: 100 bps Ladder, D: PI542044, R: MACS 450, linked SSR (Satt228) was used to confirm working of PCR reaction. (**b**) Lipoxygenase-2 null allele-specific marker, M: 100 bps Ladder, D: NRC109 R: MACS 450, R1: JS 93–05, linked SSR (Satt656) was used to confirm working of PCR reaction

eliminate KTI from two popular soybean genotypes, DS9712 and DS9814 (Maranna et al. 2016). Recently, two marker-assisted *ti3* introgression lines, NRC127 and MACSNRC1667 (Kunitz trypsin inhibitor-free), were released for cultivation. This program was further extended to marker-assisted pyramiding of *ti3* with a null allele of lipoxygenase-2 (*lox-2*) to eliminate beany flavor in soybean end products. Functional DNA markers were used for foreground selection, and SSR/STS were used for background selections (Fig.2.5b). Null lipoxygenase-2 (*lox-2*) was introduced in different varieties using the marker-assisted backcross breeding method (Rawal et al. 2020), which resulted in the release of India's first lipoxygenase-2-free soybean variety NRC132. Similarly, the program has been successful in delivering the first-ever Kunitz trypsin inhibitor-free and lipoxygenase-2-free variety NRC 142 in the year 2021. These varieties can be directly used by processing industries as a raw material for food products as well as feed without preheat treatment. Moreover, these products will be preferred by the consumers due to reduced beany flavor. Therefore, the program is one of the best examples of MAB delivering improved varieties at an accelerated rate.

The available literature showed that two to three backcrosses are preferred in many MAS breeding programs. In few cases, background recovery was carried out using SSR markers. At present, markers for almost all the major traits in soybean are available; their deployment in soybean improvement programs requires close collaboration between the breeders and molecular biologists, availability of the infrastructure, validation of markers, and availability of donor genotypes from the gene pool.

2.6 Genomic Selection

Marker-assisted breeding was found to be less effective in achieving significant gain in complex quantitative traits such as grain yield. It may be due to the influence of several genetic (several minor genes, QTL × QTL interactions) and nongenetic factors (genotype × environment interactions) on the detection of the QTL governing such traits (Sebastian et al. 2010). Genomic selection (GS), on the other hand, uses several markers across the entire genome to predict the breeding value of the breeding line for selection. Genome-wide dense markers allow GS to quantify Mendelian sampling without extensive phenotyping of the entire population. It reduces cycle length to save time and also enhances genetic gain per unit time (Crossa et al. 2017). In soybean, GS was compared with the conventional phenotype selection to test its advantages in terms of accuracy and time gains in selection. Genotype and phenotype data of 324 soybean accessions were used in the analysis, and it was observed that the GS provide higher accuracy for grain yield (0.72), days to maturity (0.83), and plant height (0.68); moreover, it reduces selection time by 50% (Matei et al. 2018). In another study, 1284 soybean breeding lines were used to evaluate the accuracy of GS for grain yield and protein content. The authors could predict about 32 and 39% of phenotypic variation for seed protein content and grain yield,

respectively, suggesting the efficiency of GS in breeding programs (Duhnen et al. 2017). Xavier et al. (2016) used 5555 RILs from soybean nested association mapping panel SoyNAM to carry out GS for yield components such as grain yield, number of reproductive nodes, pods per node, number of pods, days to maturity, and plant height. They found that the training population size of 2000 lines showed the greatest improvement in genome predictions. Also, the training population size was the most promising factor to get precise predictions, whereas increasing marker density marginally improved the accuracy. Similarly, Stewart-Brown et al. 2019 also highlighted that the success of the prediction model depends on the size of the training set than the marker density. The authors have reported predictive abilities of 0.81, 0.71, and 0.26 for seed protein content, oil content, and grain yield. Since the lower predictive ability was observed for grain yield, the authors further suggested a combination of a larger training set and increased genetic relatedness among the individuals to improve prediction abilities.

2.7 Genome Editing for Precision Breeding

Recently, site-directed nucleases (SDNs) or site-specific nucleases (SSNs) have enabled unprecedented genome editing, allowing precise mutagenesis at the target gene. Such a precise genome editing involves the application of zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), or the more recent clustered regularly interspaced short palindromic repeat/Cas9 (CRISPR/Cas9). ZFNs and TALENs provide site specificity by protein-DNA interactions, while CRISPR/Cas9 system relies on the complementarity between guide RNA and the target DNA sequence. SDN-mediated genome editing can induce all types of mutations expected in crop improvement program; therefore, it may be implemented in breeding programs to generate transgene-free edited plants with the desired phenotype (Chen et al. 2019; Xu et al. 2020). In soybean, genome editing is currently focused on the phenotype mainly governed by a major gene such as oleic acid content (*GmFAD2*), beany flavor (*GmLox1*, *GmLox2*, *GmLox3*), or altered flowering phenotype (*GmFT2*, *GmFT4*); however, the technology has potential to tackle complex traits such as yield, protein content, and biotic and abiotic stress tolerance.

Genome editing has been used in several functional genomics studies in soybean to evaluate the functions of target genes. ZFNs were used to obtain mutations in *DICER-LIKE* (*DCL*) genes (*GmDCL1a*, *GmDCL4a*, Gm*DCL4b*), RNA-DEPENDENT RNA POLYMERASE (*GmRDR6a, GmRDR6b*), and HUA ENHANCER1 (*GmHEN1a*) (Curtin et al. 2011; Sander et al. 2011), whereas TALENs were used to induce mutations in *GmDCL2b* and *Phytoene desaturase* (*GmPDS11*, *GmPDS18*) in soybean (Curtin et al. 2018; Du et al. 2016). CRISPR/Cas9 has been extensively used in the development of several genome editing platforms and functional genomic studies in soybean (Bao et al. 2019; Campbell et al. 2019; Cai et al. 2015; Du et al. 2016; Jacobs et al. 2015; Kanazashi et al. 2018; Li

et al. 2015; Michno et al. 2015; Sun et al. 2015; Wang et al. 2019a, b). Recently, Bai et al. 2020 demonstrated an advanced strategy to develop multiplexed mutant population by optimizing key steps in screening protocol. The authors have constructed 70 CRISPR/Cas9 vectors to target 102 candidate genes and their paralogs. CRISPR/Cas9 system is further modified to obtain targeted single base substitution at FLOWERING LOCUS T (*GmFT2a* and *GmFT4*) in soybean (Cai et al. 2020). It showed that the system could be successfully used to generate functional SNPs associated with important agronomic traits of crops.

Besides developing genome editing platforms and optimizing protocols, the technique is now being used for targeted manipulations in the important agronomic traits, nitrogen fixation, nutritional and quality traits, disease resistance, and abiotic stress tolerance in soybean and other crops. Oil quality of soybean has been improved by increasing monounsaturated oleic acid and decreasing polyunsaturated fats through TALEN- and CRISPR/Cas9-mediated mutagenesis of fatty acid desaturase-2 genes (*FAD2–1A* and *FAD2–1B*) (al Amin et al. 2019; Do et al. 2019; Haun et al. 2014; Wu et al. 2020). Beany flavor to soybean products is an undesirable trait that limits the use of soybean in food preparations. Knocking out *GmLox1*, *GmLox2*, and *GmLox3* encoding seed lipoxygenase by CRISPR/Cas9 could reduce beany flavor (Wang et al. 2020). Mutations at *FLOWERING locus T* (GmFT2a/5a), *LUX ARRHYTHMO*, and *LATE ELONGATED HYPOCOTYL* (*GmLHY*) showed early flowering, flowering time adaptation, and reduced plant height, respectively, in soybean (Han et al. 2019; Bu et al. 2021; Cheng et al. 2019). The CRISPR/Cas9 system has become more popular among researchers because of its simpler protocol, lower cost, and more flexibility to use than ZFNs and TALENs (Xu et al. 2020). The available literature showed that genome editing has made it possible to introduce novel variation in the available soybean gene pool, which otherwise has a narrow genetic base. The induced variation will allow breeders to deploy newer alleles in the soybean breeding program.

2.8 Challenges in Soybean Improvement and Future Directions

Accelerated soybean breeding is possible with the reduction in generation time, which may be achieved through the rapid development of homozygous lines using doubled haploid (DH) production protocol. The development of a high throughput DH production program in soybean would be extremely valuable to obtain a desired genetic gain of the crop. There have been minor advances in soybean androgenesis, root formation, and rare shoot induction. However, an efficient reproducible method to produce doubled haploids in soybean is lacking so far. Recalcitrance to in vitro regeneration exhibited by most soybean tissue may be one of the major constraints to the development of commercial DH production protocol in soybean (Croser et al. 2006).

Although a significant increase is achieved in potential productivity of soybean through painstaking efforts by the breeders, the actual average productivity attained at farmers' fields is merely about 40% of the potential productivity (Venkateswarlu and Prasad 2012). Constraints to optimum productivity of soybean include the rainfed area under cultivation coupled with the erratic behavior of rain (Agarwal et al. 2013). Precipitation is one of the major climatic factors which determine the yield of rainfed crops like soybean. Insufficient, erratic or irregular, and uneven rains received during the soybean crop growth period hinder the yield due to the unavailability of soil moisture during critical growth, development, and reproductive stages. The occurrence of drought at one or the other stage of crop growth is attributed as one of the major factors responsible for the low productivity of soybean in India (Bhatia et al. 2014). Therefore, identification of genetic resources in the form of soybean genotype resilient to water-stress and genomics-assisted techniques to mitigate water-stress is essential to obtain sustainable yield. Several efforts are on to identify the genetic basis for water-stress tolerance in soybean through various approaches such as QTL mapping, genome-wide association mapping, and comparative transcriptomic studies (Valliyodan et al. 2017). However, the trait is governed by several genetic components and highly influenced by environmental factors, which restricts the identification of a robust system to select the potential breeding line. GS with a precise prediction model for traits contributing to water-stress tolerance could be explored to select suitable candidates.

A lot of genetic diversity available in wild soybean species remained unexplored due to incompatibility for hybridization of these species with the cultivated soybean. These species carry a useful gene pool for biotic and abiotic stress tolerance. With the advances in genome sequencing, transcriptome sequencing, and comparative genomics, orthologs of novel genes from the wild species can be explored for their variation in the cultivated soybean. Since a narrow genetic base is present in cultivated soybean, genome editing and TILLING can be useful to induce a range of variations, including knockdown to knockout alleles, in these orthologs. It should be coupled with the speed breeding facility for rapid deployment of these alleles in breeding programs.

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Chapter 3
Genetic Enhancement of Groundnut: Current Status and Future Prospects

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Abstract About 94% of the world groundnut (*Arachis hypogaea* L.) production comes from the rainfed crop grown largely by resource-poor farmers. Several biotic and abiotic stresses limit groundnut productivity, together causing annual yield losses of over US $ 3.2 billion, and probably half of this could be recovered through genetic enhancement in groundnut. Cultivated species and the wild *Arachis* species do carry novel genes which could be employed for improve-

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ment of both seed yield and quality in addition to imparting resistance to diseases and insect pests. Many of the wild *Arachis* species are not cross compatible with the cultivated groundnut. However, the efforts to overcome incompatibility in wide crosses have been successful in transferring the novel genes through interspecific progenies. The conventional breeding procedures employ hybridization and phenotype-based selection followed by selection of promising breeding lines through yield evaluation trials. In the past, these were achieved mainly through mass selection and pure-line selections; subsequently backcross and pedigree approaches were largely employed followed by inter- and intraspecific hybridization. Simultaneously, the induced mutagenesis played a significant role in the development of multiple stress-tolerant high-yielding varieties. However, these methods of genetic enhancement suffer from linkage drag and hybridization barrier apart from difficulty in delimiting the genomic regions to be transferred. The recent developments in biotechnology (genetic engineering and marker-assisted breeding) have immense potential for improving the efficiency and precision of genetic enhancement in groundnut. Overall progress made so far with respect to genetic enhancement of groundnut for productivity, tolerance to biotic and abiotic stresses, quality, etc. through various methods have been reviewed in this chapter.

Keywords Genetic enhancement · Groundnut · Productivity · Tolerance to biotic and abiotic stresses · Conventional breeding · Marker-assisted breeding · Genetic engineering

3.1 Introduction

Cultivated groundnut, also known as peanut (*Arachis hypogaea* L.), is cultivated in 108 countries worldwide on an area of 29.6 million hectares with a total global production of 48.86 million tons and 1.61 tons/ha productivity (FAOSTAT 2019; http://www.fao.org/faostat/en/#data/QC). Although originating in South America, the vast majority of groundnut is produced in Asia and Africa: Asia 55.7% (27.2 Mt) and Africa 33.9% (16.6 Mt). The remaining 10% (5.06 Mt) comes from North America, the Caribbean, Europe and Oceania. Approximately 94% of groundnut is produced in the developing world, mostly under rainfed conditions. The major groundnut-producing countries are China, India, Indonesia, Myanmar and Vietnam in Asia; Nigeria, Sudan, Democratic Republic of Congo, Chad, Mozambique, Zimbabwe, Burkina Faso, Uganda and Mali in Africa; the USA in North America; and Argentina, Brazil and Mexico in Latin America and the Caribbean.

The average (FAOSTAT 2019) yield of groundnut in Africa is 0.97 t ha^{-1} which is markedly lower than groundnut yields in Asia (2.45 t ha^{-1}) and in Latin America and the Caribbean (1.02 t ha^{-1}), while yields are by far the highest in North America

(4.42 t ha^{-1}) and China (3.89 t ha^{-1}). The largest groundnut acreage in Asia occurs in India (4.7 m ha) followed by China (4.5 m ha). However, India ranks below China in total production with 6.73 Mt in India as against 17.5 Mt in china, as its average yield is 1.42 t ha^{-1}. The key factors contributing to higher yields in China are (1) introduction of improved varieties presently covering 90% of the total groundnut area, (2) adoption of improved cultural practices including crop rotation and polythene film mulching, (3) rewards to groundnut growers for producing higher yields and (4) national policies for price support systems and marketing opportunities (Shuren et al. 1996). In contrast, groundnut yields in Africa are very low with many countries reporting yields as low as 0.5–0.8 t ha^{-1}. Although the Latin American and the Caribbean regions contribute only 10% of the world groundnut production, high yields of 3.4 t ha^{-1} in Argentina and 3.3 t ha^{-1} in Brazil have been reported.

3.2 Constraints to Groundnut Production

Groundnut is a vital source of proteins and nutrient-rich fodder for livestock and is considered globally as a major oilseed crop. Being a segmental allopolyploid with AABB genome conformation, the cultivated peanut is considered to have evolved through single interspecific hybridization amid two diploid species. A number of biotic and abiotic forces restrict the production and productivity of peanut. Drought and temperature among abiotic stresses and rust, early leaf spot (ELS), late leaf spot (LLS) and aflatoxin among biotic stresses are the global constraints to groundnut production and adversely influence seed quality. Regionally, groundnut rosette disease (GRD) in Africa; bacterial wilt, leaf miner, *Spodoptera* and peanut bud necrosis disease (PBND) in South and/or South East Asia; corn earworm, lesser cornstalk borer, southern corn rootworm, *Sclerotium*, nematodes and tomato spotted wilt virus (TSWV) in North America; and low calcium and phosphorus availability in acidic soils in Latin America and Caribbean are important constraints to groundnut production. These stresses together cause annual yield losses exceeding US $ 3.2 billion, and probably half of this could be recovered through genetic enhancement in groundnut (ICRISAT 1994; Dwivedi et al. 2003).

3.3 Status of Groundnut Breeding

Intensive attempts to develop superior peanut varieties with inherent tolerance/resistance and enriched nutritional components were executed to combat stress factors in fulfilling the requirements of farmers and consumers. Assessment of genetic diversity and development of a saturated genetic linkage map are important steps in the development of molecular marker-assisted breeding programmes.

3.3.1 Wealth of Groundnut Genetic Resources

3.3.1.1 Cultivated Genetic Resources

Over 15,000 accessions of cultivated groundnut, including 6351 landraces, from 92 countries are housed at ICRISAT (India). They differ for many vegetative, reproductive, physiological and biochemical traits including their reactions to abiotic and biotic stresses (Upadhyaya et al. 2003). The *Arachis* gene pool includes sources of resistance to rust, ELS, LLS, GRD, PBNV, *A. flavus*, bacterial wilt, leaf miner, *Spodoptera*, jassids, thrips and iron chlorosis and tolerance to low and high temperature and drought as well as sources of photoperiod insensitivity and variation in total sugars, oil and protein contents and O/L ratio and for flavour attributes. However, much of this variability remains poorly understood and underutilized in genetic enhancement efforts mainly because of the large number of accessions in the ex situ collections, lack of data on the extent of the diversity present in them for specific characteristics and high genotype (G) X environment (E) interactions for traits of economic importance. Upadhyaya et al. (2011) developed a core collection of 1704 groundnut accessions consisting of 584 (34.3%) accessions from subsp. *fastigiata* var. *vulgaris*, 299 (17.5%) from subsp. *fastigiata* var. *fastigiata*, 27 (1.6%) from subsp. *fastigiata* var. *peruviana*, 6 (0.4%) from subsp. *fastigiata* var. *aequitoriana*, 784 (46.0%) from subsp. *hypogaea* var. *hypogaea* and 4 (0.2%) from subsp. *hypogaea* var. *hirsuta* and arrayed these accessions in 23 clusters, and this core when further evaluated could provide new sources of variation for use in breeding programmes (Upadhyaya et al. 2003).

Plant breeders in the USA have registered 62 *Arachis* germplasm lines possessing genes for resistance to biotic and abiotic stresses and for seed quality traits for use in breeding programmes (Isleib and Wynne 1992). Of these, 27 were introduced germplasm. However, because of stringent industry and market demands, US plant breeders use only those accessions that conform to market and industry standards. This has resulted in a narrowing of the genetic base of released cultivars there. ICRISAT breeders have used 78 plant introductions to develop 73 elite germplasm lines. Of these, 41 have been released for cultivation in 19 countries, and the remainder possesses genes for early maturity, seed dormancy, seed quality, photoperiod insensitivity and resistance to rust, ELS, LLS, thrips, jassids, leaf miner, *Spodoptera*, PBNV, iron chlorosis and aflatoxin and tolerance to drought, and these elite germplasm accessions/lines are widely used by NARS breeding programmes to transfer these traits into locally adapted cultivars (Dwivedi et al. 2003).

3.3.1.2 Wild *Arachis* Genetic Resources

The cultivated peanut (*Arachis hypogaea* L.) is an allotetraploid species with a very large and complex genome. This species is susceptible to numerous foliar and soil-borne diseases for which only moderate levels of resistance have been identified in

the germplasm collection, but several of the 81 wild species are extremely resistant to many destructive peanut diseases. Peanut species were grouped into nine sections, but only taxa in section *Arachis* will hybridize with *A. hypogaea*. Most of these species are diploid, but two aneuploids and two tetraploids also exist in the section. The first peanut cultivars released after interspecific hybridization were 'Spancross' and 'Tamnut 74' during the 1970s from a cross between *A. hypogaea* and its tetraploid progenitor. However, introgression of useful genes from diploids has been difficult due to sterility barriers resulting from genomic and ploidy differences. To utilize diploids in section *Arachis*, direct hybrids have been made between *A. hypogaea* and diploid species, the chromosome number doubled to the hexaploid level, and then tetraploids recovered with resistances to nematodes, leaf spots, rust and numerous insect pests. 'Bailey', a widely grown Virginia-type peanut, was released from these materials, and other cultivars are grown in Asia and South America. Alternatively, hybrids between diploid A and B genome species have been made, the chromosome number doubled, and cultivars released with nematode resistance derived from *Arachis* species. Introgression from *Arachis* species to *A. hypogaea* appears to be in large blocks rather than as single genes, and new genotyping strategies should enhance utilization of wild peanut genetic resources (Stalker 2017).

3.4 Desirable Traits in *Arachis* Species for Crop Improvement

As compared with accessions in the *A. hypogaea* collection, extremely high levels of resistance have been identified in *Arachis* species for many important peanut pathogens and insects (Stalker and Moss 1987; Dwivedi et al. 2007). In addition, Upadhyaya et al. (2011) identified superior accessions of wild peanuts for both agronomic and nutritional quality traits, including days to flowering and high levels of percentage of oil, protein and sugars. Important for crop improvement are the 29 diploid ($2n = 2x = 20$) species in section *Arachis*, because these materials will hybridize with the cultivated peanut. Fortunately, many disease and insect resistances have been identified within this group for the most severe problems of peanut production. Introgressing wild species alleles from diploid species to the cultivated peanut has proven successful for developing improved cultivars with pest and disease resistances. However, utilization of alleles from wild species has had limited impact in many peanut-producing areas due to difficulties producing hybrids, sterility in hybrids and the lack of molecular tools to follow traits of interest in introgression lines. *Arachis cardenasii* has been one of the most useful sources of genes from wild species to date, especially for nematode, leaf spot and rust resistances, but crosses involving other species have also been used in breeding programmes. As new interspecific hybrids are created with an array of diploid species and

genotyping strategies become more user-friendly for the peanut breeder, wild peanut species will become more routinely used across production regions.

Utilization of wild *Arachis* species following interspecific hybridization has resulted in the development to many elite germplasm lines and cultivars with improved level of resistance to diseases and insect pests (Singh and Nigam 2016; Stalker 2017). At ICRISAT, several elite lines have been developed with desirable characters transferred from wild *Arachis* species such as ICGV86699 (Reddy et al. 1996) with multiple pest resistance, ICGV87165 (Moss et al. 1998) with multiple disease and insect resistance, ICGV99001and 99004 with resistance to LLS and ICGV99003 and 99005 resistant to rust (Singh et al. 2003). Varieties such as Spancross (Hammons 1970), Tamnut 74 (Simpson and Smith 1975), Coan (Simpson and Starr 2001), NemaTAM (Simpson et al. 2003), ICGV-SM 85048 (Nigam et al. 1998a, b) and ICGV-SM86715 (Moss et al. 1998), having a genetic base from wild *Arachis* species, were released for cultivation, mostly in the USA. The development and utilization of synthetic amphiploids such as TAG-6 with large genetic variation (Simpson et al. 1993) has made possible the transfer of resistance genes from wild species into cultivated groundnut. TAG-6 is a synthetic amphiploid derived from crossing an AA genome donor hybrid (*A. cardenasii* × *A. diogoi*) with a BB genome species, *A. batizocoi*, followed by colchicines treatment of the sterile triploid to produce fertile hexaploid, TxAG-6 (Simpson et al. 1993). Using this amphiploid in crossing programmes with cultivated groundnut has resulted in the release of two cultivars, Coan and NemaTAM, carrying genes for root-knot nematode (*M. arenaria*) resistance from *A. cardenasii* (Simpson and Starr 2001; Simpson et al. 2003). The development of a fertile, cross-compatible synthetic amphidiploid, TxAG-6 ([A. batizocoi (A. cardenasii _ A. diogoi)]4x), opened novel opportunities for the introgression of wild alleles for disease and pest resistance into commercial cultivars (Denwar et al. 2021).

Two fertile artificially induced allotetraploids (also known as amphidiploids or neotetraploids), viz. GA-BatSten1 (Reg. no. GP-239, PI 695418) and GA-MagSten1 (GP-240, PI 695417) derived from crosses between wild diploid species of peanut, *A. batizocoi* × *A. stenosperma* and *A. magna* × *A. stenosperma*, respectively, are compatible with cultivated peanut, carry resistance to early and late leaf spot and root-knot nematode and are being used in breeding programmes in the USA for the production of resistant cultivars but also for widening the genetic base of the cultigen and improving yield, seed size, vigour and other traits. The four allotetraploid interspecific hybrids IpaCor4x (*A. ipaensis* × *A. correntina*), IpaDur4x (*A. ipaensis* × *A. duranensis*), IpaSten4x (*A. ipaensis* × *A. stenosperma*) and ValSten4x (*A. valida* × *A. stenosperma*) are reported to be cross compatible to cultivated peanut, and therefore, they can be readily used for peanut cultivar improvement. The documentation of the morphological and reproductive characterization of these materials allows phenotypic traits such as plant vigour (demonstrated by increased plant biomass, plant height, flower production, among others) to be introgressed into peanut breeding lines (Levinson et al. 2021). Further, a new source of root-knot nematode resistance from *Arachis stenosperma* is incorporated into allotetraploid peanut (*Arachis hypogaea*) and being used in breeding programmes for introgressing the

new source of nematode resistance and to widen the genetic basis of agronomically adapted peanut lines (Ballén-Taborda et al. 2019).

3.5 Conventional Breeding Approaches

Groundnut breeding objectives in the past were achieved mainly through mass and pure-line selections. Subsequently to accomplish breeding objectives, peanut breeders employed backcross and pedigree approaches followed by inter- and intra-specific hybridization in a considerable way. Simultaneously, peanut breeding through the mutagenic approach played a noteworthy part during the development of multiple propitious high-yielding varieties (Badigannavar et al. 2007). Traditional breeding approaches helped in identification and advancement of cultivars with inherent resistant traits, but such resistance traits are tightly linked with inferior pod and kernel characteristics that are extremely challenging to break. However, efforts to overcome incompatibility in wide crosses, by using non-conventional techniques, have started to liberate interspecific progenies with high levels of resistance to leaf spots, nematodes, *Spodoptera* and leaf miner. Marker-assisted backcross breeding should minimize the linkage drag as it greatly facilitates monitoring of introgressed chromosome segments carrying beneficial genes from wild *Arachis* to cultivated groundnut. An efficient tissue culture and transformation system has been developed, and transgenic groundnut plants with *IPCVcp* or *replicase*, *GRAVcp* and rice *chitinase* genes have been produced that are in various stages of characterization under containment glasshouse and/or field conditions at ICRISAT (2001). Transgenic approach may be the best option to introduce genes for resistance to aflatoxin as conventional breeding has failed to enhance the level of resistance beyond that present in cultivated groundnut germplasm. For traits such as GRAV, PBNV and TSWV, the use of wide hybridization and/or genetic transformation may be the most efficient strategy to introduce resistance genes into cultivated groundnut. Once favourable genes are introduced into cultivated groundnut through wide crossing and/or genetic transformation techniques, these genes will become ideal candidates for marker-accelerated introgression. DNA marker-based genetic linkage map should enable breeders to effectively pyramid genes for good seed quality (high O/L ratio and resistance to aflatoxin) and resistance to ELS, LLS, aflatoxin, nematodes, leaf miner and *Spodoptera* and tolerance to drought into agronomically enhanced breeding populations in a much shorter time than would be possible by conventional techniques.

In the recent past, substantial efforts are being made to develop sufficient PCR-based markers (particularly SSR and SNP markers) for the construction of high-density genetic linkage map and for the routine application in the molecular breeding of abiotic stress tolerance, biotic stress resistance, yield and seed quality in groundnut. A number of reproducible molecular markers were developed that are associated with salinity and drought tolerance, as well as resistance to biotic stresses like rust, and leaf spots, and to a certain extent *Sclerotinia* blight, etc.

Agrobacterium-mediated genetic transformations, via in planta or particle-bombardment approaches, have resulted in development of transgenic peanuts with enhanced yield attributes and inherent resistance against a few biotic and abiotic stresses. Such genetically transformed peanut populations could also be employed as donor parents in traditional breeding system to develop fungal and a few virus disease-tolerant varieties. Nevertheless, it could be suggested that a combination of breeding and biotechnological tools and approaches might deliver an inherent, cost-effective as well as eco-friendly solution in developing better peanut varieties globally (Gantait et al. 2019).

3.6 Yield Gap Analysis and Impact of Improved Technologies in Groundnut

Groundnut is primarily cultivated over an area of 5.00 million ha and the production stands at 7.00 million tonnes. The average yield levels are 14.29 q/ha (QE 2015–2016) in the states of Gujarat, Andhra Pradesh, Karnataka, Rajasthan and Tamil Nadu. Kharif is the predominant cultivation season for oilseeds accounting for 84% of the gross cropped area under oilseeds. The productivity levels under *kharif* season range from 5.28 q/ha in Andhra Pradesh to 20 q/ha in Tamil Nadu. Low SRR, high seed requirement, bud necrosis, leaf spot diseases, root grub and leaf miner are important reasons for low productivity. Groundnut productivity ranges from 1.8 to 2.8 t ha^{-1} with a yield gap ranging from 3% (Tamil Nadu) to 176% (Andhra Pradesh), average the yield gap of 71% nationally, where yield gaps can be bridged by focusing on technology transfer and thereby minimizing the national yield gap average. In this direction efforts are being made to bridge the groundnut yield gap through Front Line Demonstrations (FLDs) of improved varieties and production technologies in the farmers' field; release of improved groundnut varieties with multiple biotic/abiotic stress tolerance for cultivation and their breeder seed production (BSP) in the last two decades for enhancing the genetic gains in the farmers' field in groundnut are detailed below.

3.6.1 Impact of Improved Varieties and Production Technologies on Productivity of Groundnut

At various AICRPG centres in India, a total of 12,039 demonstrations were conducted in rainy (*kharif*) and post-rainy (*rabi*-summer) seasons in the last 20 years (2000–2020) (Table 3.1) with demonstration on improved package of practices including improved varieties, integrated disease and pest management, integrated nutrient management, integrated weed management, water management, biofertilizers, biocontrol agents and whole package. The Front Line Demonstration (FLD)

Table 3.1 Impact of improved package of practices on productivity of groundnut in the last 20 years

Year	Total no. of FLDs conducted	Improved practice pod yield (Kg/ha)	Farmers' old practice pod yield (Kg/ha)	Increase in yield over farmers' practice (%)
2000–2001	259	2112	1519	39
2001–2002	262	1774	1380	29
2002–2003	375	1859	1389	34
2003–2004	449	1917	1560	23
2004–2005	640	2082	1665	25
2005–2006	673	1806	1379	31
2006–2007	630	2000	1658	21
2007–2008	568	2043	1649	24
2008–2009	674	2182	1698	29
2009–2010	852	2206	1743	27
2010–2011	879	2242	1773	26
2011–2012	705	2217	1827	21
2012–2013	774	2454	2004	22
2013–2014	722	2624	2149	22
2014–2015	594	2247	1843	21
2015–2016	609	2162	1737	24
2016–2017	497	2247	1793	25
2017–2018	557	2391	1943	23
2018–2019	518	2389	1923	24
2019–2020	430	2536	2028	26
2020–2021	372	2206	1829	20.8
Total/mean	12,039	2176	1738	25.6

Source: AICRPG Annual Reports 2000–2001 to 2020–2021

results indicated that the improved variety alone could increase the pod yield by 20–35% over local varieties, while, by adopting integrated nutrient management (INM) practices, the pod yield increased by 15–25%, and by adopting IPM practices, pod yield increased by 10–15%. A 20% advantage in yield could be obtained by adopting the integrated disease management practices. With the application of PGPR, the pod yield increased by 10–20%. Adoption of integrated weed management (IWM) practices helped in enhancing pod yield by 10%. The whole package of management practices (excluding variety) could bring about increase in pod yield by 20–30%.

3.6.2 Genetic Enhancement Through Release and Cultivation of Improved Groundnut Varieties with Multiple Biotic/Abiotic Stress Tolerance

Improved groundnut varieties resulting from genetic improvement have contributed to enhanced production and productivity and meet the needs of the producers, processors and consumers. The yield productivity increase varied across different growing regions. Wide ranges of varieties of groundnut are cultivated to meet the food, oil and industrial needs. Groundnut breeding programmes have extensively used phenotyping tools for selecting plants/progenies with desirable traits (Janila et al. 2013). The conventional breeding procedures employ hybridization and phenotype-based selection followed by selection of promising breeding lines through yield evaluation trials. With the advent of genomic tools, marker-assisted breeding (MAB) was deployed to enhance efficiency of selection of target traits in groundnut (Pandey et al. 2012; Varshney et al. 2014; Janila et al. 2016).

Over 276 groundnut cultivars were released between 1920 and 2000 for cultivation in various countries in Asia, Africa and the Americas. Each has specific adaptation to its respective region of production and cropping system (Isleib et al. 1994). Breeding for high seed yield has caused changes in dry matter allocation. More recently developed cultivars have reduced vegetative mass, shorter main stem length and greater reproductive allocation (partition more of their daily assimilate to fruit) than those developed previously (as predicted by Duncan et al. 1978). Further studies on reproductive efficiency (RE) revealed that high yield in more recently released cultivars appears to be related more to total flower production than to RE, and therefore, future increases in seed yield might be accomplished by developing cultivars with a combination of high RE, harvest index and total flower count (Coffelt et al. 1989). A yearly genetic gain of nearly 15 kg per hectare has been reported for large-seeded Virginia-type cultivars released from the 1950s to the 1970s in the USA (Mozingo et al. 1987). The highest-yielding cultivars developed during the 1950s, 1960s and 1970s had an average yield increase of 3.4%, 10.2% and 18.5%, respectively, over the standard NC 4. However, since the 1970s there has been increased emphasis on improving pest resistance and quality traits so that the yield potential

of cultivars released since that time has not surpassed those of the highest-yielding cultivars released during the 1970.

Likewise, in India, with the establishment of the All India Coordinated Research Project on Oilseeds (AICRPO) by the ICAR in 1967, the systematic work on groundnut breeding was given yet another impetus. Subsequently in 1992, the groundnut component was delineated from AICRPO and given independent status by formation of 'All India Coordinated Research Project on Groundnut (AICRP-G)'. Since then, many niche-specific, stress-tolerant and high-yielding varieties developed by various SAUs and other ICAR institutions have been released. By 2012, 194 varieties (108 Spanish bunch, 5 Valencia, 50 Virginia bunch and 31 Virginia runner) have been released, and their compendium has been published by Directorate of Groundnut Research, Junagadh (Rathanakumar et al. 2013). Groundnut varieties (78 Spanish bunch, 29 Virginia bunch and 11 Virginia runner) released in the last two decades {2000–2020} under aegis of AICRP-G that are in active seed chain and have contributed significantly in genetic enhancement of groundnut in India are listed in Table 3.2. Further, 54 trait-specific groundnut germplasm registered with NBPGR, New Delhi, till date are enlisted with their identity, pedigree, salient features, developers and developing institute, etc. (Table 3.3).

3.6.3 Breeder Seed Production of Improved Groundnut Varieties in India

Groundnut is a high-seed volume crop requiring a seed rate of 150–160 kg seed pods/ha. Production of breeder seed in adequate quantity is a real challenge due to its low seed multiplication ratio (1:10). During the X plan period, the total groundnut breeder production was 23467.2 q. Subsequently, in XI plan, the production shoots up to a huge total of 67874.9 q. The number of varieties in these plan periods in the seed chain was 50–60. Altogether in the last 15 years (2006–2007 onwards) where Directorate of Groundnut Research (DGR) arranged the breeder seed production programme, there has been a total production of 1,74,543 q of breeder seed with bulk of the production (about 80%) coming from 6–8 varieties that are being produced in large quantity (> 500 q/annum), viz. Kadiri 6, Kadiri 9, Dharani, G 2-52, GPBD 4, ICGV 91114, ICGV 00350 and TAG 24 (Table 3.4). Such a high production was possible by the concerted efforts of the scientists of 22 AICRP-G centres and also for undertaking major production in rabi-summer season under assured breeder seed production. Though there are few instances of setback in meeting the indented/allocated targets in few varieties, by and large, in most of the cases, the allocated targets were fulfilled (AICRPG Annual Reports; Chauhan et al. 2016).

In groundnut, seed replacement rate (SRR) is a measure of how much of the total cropped area was sown with certified seeds (improved varieties) in comparison to farm-saved seeds (old/absolute varieties/landraces) which has a strong positive relationship with the crop productivity. Hence, to achieve desired productivity levels

Table 3.2 Groundnut varieties released in the last 20 years (2000–2020) for their cultivation in different agro-climatic zones of India

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
Habit group: Spanish bunch (SB)														
1	Phule Vyas (JL 220)	JL-80 × VG-77	MPKV, Jalgaon	2000	821 (E) 13.9.2000	State; kharif	Maharashtra	2000	90–95	68	52	–	–	Early maturity, high oil content (53%)
2	Co (Gn) 4 (TNAU 269)	TMV-10 × ICGS-82	TNAU, Coimbatore	2001	1134 (E) 15.11.2001	State (kharif/ *rabi*-summer)	Tamil Nadu	1500 (kharif) 1950 (rabi-summer)	105–110	73	53	–	LLS, rust	Drought tolerant, high oil content (53%)
3	GG 7 (J 38)	S-206 × FESR-8	JAU, Junagadh	2001	1134 (E) 15.11.2001	Central (kharif)	Gujarat, Southern Rajasthan	2150	100	76	49	–	LLS	Early maturity
4	VRI(Gn) 5	CG-26 × ICGS-44	TNAU, Virudhachalam	2001	1134 (E) 15.11.2001	State (kharif/ *rabi*-summer)	Tamil Nadu	2130 (kharif) 2380 (rabi-summer)	105–110	74	50	–	LLS, rust	–
5	AK 159	JL-24 × CGC-4018	PKV, Akola	2002	937 (E) 04.09.2002	Central (kharif)	Madhya Pradesh, Maharashtra	1600 (kharif)	106	68	51.0	–	–	Early maturity, high oil content (51%)

6	GG 6	CGC-3 ×	GAU, Junagadh	2003	283 (E) 12.03.2003	State (*rabi*-summer)	Gujarat	2780	100	73	50.0	–	–	Early maturity, high shelling outturn
7	TG 37A	TG-25 × TG-26	BARC, Mumbai	2004	161 (E) 04.02.2004	Central (kharif/ *rabi*-summer) Zones I, II and Zone IV	All India (Rajasthan, UP, Punjab, Gujarat, Orissa, West Bengal, Bihar and NEH states	2080 (kharif) 2840 (*rabi*-summer)	105–110	64	48	–	Collar rots, LLs, rust	–
8	TPG 41	TG-28A × TG-22	BARC, Mumbai	2004	161 (E) 04.02.2004	Central (*rabi*-summer)	All India	2080	122	69	49		Rust	–
9	Vikas (GPBD 4)	KRG-1 × ICGV-86855	UAS, Dharwad	2004	642 (E) 31.05.2004	Central (kharif) Zone V Zone IV	Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu; Jharkhand, Orissa, West Bengal	2200	107	68	49		LLS, rust	–
10	Kadiri 5	JL-24 × VG-55-7	ANGRAU, Kadiri	2005	122 (E) 02.02.2005	State (kharif)	Andhra Pradesh	2200 (K)	100–110	70	48–49	–	LLS, ELS	Drought tolerant
11	Kadiri 6 (K 1240)	JL-24 × Ah-316/S	ANGRAU, Kadiri	2005	122 (E) 02.02.2005	State (kharif)	Andhra Pradesh	2200 (K) 3100–3800 (R/S)	100–110	70	48–49	–	LLS, ELS	–

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
12	Pratap Mungphali-1 (ICGV 92035)	ICGV-86033 × ICG-2214	MPAU&T, Udaipur	2005	1566 (E) 05.11.2005	State (kharif)	Rajasthan	2500–3000	108	62	48–50	Leaf miner, *Spodoptera*, thrips	ELS, LLS, PBND	–
13	Prutha (Dh-86)	Dh-40 × Dh-8	UAS, Dharwad	2005	122 (E) 02.02.2005	Central (*rabi*-summer)	Gujarat, Maharashtra, Orissa, Southern Rajasthan, West Bengal	4020	125–127	68	48	Jassids, thrips,	ELS, LLS	High harvest index
14	Ratneshwar (LGN 1)	JL-24 × NCAc-17090	MAU, Latur	2005	122 (E) 06.02.2007	State; kharif	Maharashtra	1490	105	69	51.2	–	LLS, stem rots, rust, PBND	High oil content (51.2%)
15	Abhaya (TPT 25)	K-134 × TAG-24	ANGRAU, Tirupati	2006	1178 (E) 20.07.2007	State (kharif/ *rabi*-summer)	Andhra Pradesh	2300 (kharif) 3760 (rabi-summer)	106	76	52.3	Jassids, *Spodoptera*, thrips	LLS	Drought tolerant
16	GG 8 (J 53)	27-5-1 × JL-24	JAU, Junagadh	2006	1572 (E) 20.09.2006	Central (kharif)	Madhya Pradesh, Northern Maharashtra	1710	104–107	69	46	–	Collar and stem rots, PBND	–
17	Prasuna (TCGS 341)	TCG-1717 × TCG-1518	ANGRAU, Tirupati	2006	1178 (E) 20.07.2007	State (kharif/ *rabi*-summer)	Andhra Pradesh	2000 (kharif) 4000 (*rabi*-summer)	110	74–76	50.0	Kalahasti Malady	PBND	–

18	TG 38B (TG-38)	Gamma ray mutant of F1 seeds of Girnar-1 × TG-26	BARC, Mumbai	2006	1572 (E) 20.09.2006	Central (*rabi*-summer)	Bihar, Orissa, West Bengal, NE states	2770	115–125	71	48	–	Stem rots	–
19	Phule Unap (JL 286)	JL-86 × NCAC-343	MPKV, Jalgaon	2007	1178 (E) 20.07.2007	State (*rabi*-summer)	Maharashtra	2230	93–95	72–75	49–50	Leaf miner, *Spodoptera*, thrips	LLS, stem rots, rust,	Early maturity
20	SG 99	ICGV-86829 × ICGV-87160 {ICG(FDRS) -10}	PAU, Ludhiana	2007	1178 (E) 20.07.2007	State (kharif/ *rabi*-summer)	Punjab	2200 (kharif) 2500 (*rabi*-summer)	102	65	52.3	–	–	Mid-season moisture stress tolerant, early maturity, high oil content (52.5%)
21	TLG 45	TG-19 × TAG-24	MAU, Latur	2007	122 (E) 06.02.2007	State; kharif	Maharashtra	1500	114	66	51	–	–	High oil content (51%)
22	TMV (Gn)-13 (TNAU-325)	Selection from Pollachi red local	TNAU, Coimbatore	2007	1178 (E) 20.07.2007	State (kharif)	Tamil Nadu	2580	105–110	75	50	–	LLS, PBND, rust	Drought tolerant, red kernel
23	ICGV 91114	ICGV 86055 × ICGV 86533	ICRISAT, Hyderabad	2007	1178 (E) 20.07.2007	State (kharif)	Andhra Pradesh	2000 (kharif)	90–95	75	48	–	LLS, rust	Early maturity, drought tolerant

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
24	Kalahasthi (TCGS 320)	TCG-1709 × TCG-1518	ANGRAU, Tirupati	2007	1703 (E) 05.10.2007	State (kharif/ *rabi*-summer)	Andhra Pradesh	1400 (kharif) 3760 (*rabi*-summer)	110	74–76	52.3	Jassids	PBND	–
25	Narayani (TCGS 29)	JL-24 × Ah-316/S	ANGRAU, Tirupati	2007	1703 (E) 05.10.2007	State (kharif/ *rabi*-summer)	Andhra Pradesh	2250 (kharif) 3764 (*rabi*-summer)	100	72–76	47.5	–	–	Drought tolerant
26	Vasundhara (Dh-101)	R-9241 × Dh-51-2	UAS, Dharwad	2007	122 (E) 06.02.2007	Central (*rabi*-summer)	Assam, Jharkhand, Orissa, West Bengal	2870	120–130	67	50	*Spodoptera*, thrips	PBND, stem rots	–
27	Ajeta (R 2001-3)	ICGS-11 × ICG-4728	UAS, Raichur	2008	2458 (E) 16.10.2008	Central (kharif)	Andhra Pradesh, Karnataka, Southern Maharashtra, Tamil Nadu	2440	105–120	68	46–48	–	PBND	Drought tolerant
28	TG-51	TG-26 × Chico	BARC, Mumbai	2008	2458 (E) 16.10.2008	Central (*rabi*-summer)	Assam, Jharkhand, Orissa, West Bengal	2675	124	68	49	–	Root rots, stem rots	–
29	VL-Moongphali -1	Selection from ICGV-86590	VPKAS, Almora	2008	211 (E) 29.01.2010	State (kharif)	Uttarakhand	1943	125–130	72.6	42.2	–	LLS, root rots	–

30	Greeshma	TIR-46 × JUG-37	ANGRAU, Tirupati	2009	2187 (E) 27.08.2009	State (kharif/ *rabi*-summer)	Andhra Pradesh	2100 (kharif) 4000 (*rabi*-summer)	95–100	75–76	48–50	–	LLS, aflatoxin,	Early maturity, drought and heat tolerant
31	Jawahar Groundnut 23 (JGN-23)	JL-24 × ICGV-7886	JNKVV, Khargaone	2009	449 (E) 11.02.2009	State (kharif)	Madhya Pradesh	1630	104	70	49	–	ELS, LLS	Drought tolerant
32	Kadiri 9	Kadiri-4 × Vemana	ANGRAU, Kadiri	2009	2187 (E) 27.08.2009	State (kharif)	Andhra Pradesh	2500–3000	105–110	65–70	50	–	–	Drought tolerant
33	VRI(Gn)6 (VG 9816)	ALR-2 × VG-9513	TNAU, Virudhachalam	2009	449 (E) 11.02.2009	Central (kharif)	Andhra Pradesh, Karnataka, Southern Maharashtra, Tamil Nadu	2260	100–106	66	47	–	LLS, PBND, rust	–
34	Girnar 3 (PBS 12160)	Girnar-1 × ICGS-11	DGR, Junagadh	2010	733 (E) 01.04.2010	Central (kharif)	Manipur, Orissa, West Bengal	1520	104–111	70	45	Leaf miner, thrips	–	–
35	GPBD 5	TG-49 x GPBD-4	UAS, Dharwad	2010	2137 (E) 31.08.2010	Central; Karnataka (kharif)	Jharkhand, Manipur Karnataka	1500	105–110	68	46	–	LLS, rust	–
36	JL 501	Selection from TAG-24	MPKV, Jalgaon	2010	211 (E) 29.01.2010	Central (kharif)	Gujrat	1660	102	67	48	–	–	Early maturity
37	K 1319 (Kadiri Harithandra)	91/57-2 × PI-476177	ANGRAU, Kadiri	2010	733 (E) 01.04.2010	Central (*rabi*-summer)	Andhra Pradesh, Karnataka, Maharashtra	3720	122	68	48	*Spodoptera*, thrips, jassids	ELS, LLS, PBND, rust, stem rots	Fresh seed dormancy

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
38	Vijetha (R 2001-2)	ICGS-11 × ICG-4728	UAS, Raichur	2010	211 (E) 29.01.2010	Central (kharif)	Andhra Pradesh, Assam, Jharkhand, Karnataka, Maharashtra, Orrisa, Tamil Nadu, West Bengal	1630–2522	105–120	68	46–48	–	PBND	–
39	Pratap Raj Mungphali	Selection from ICGV 98223	MPAU&T, Udaipur	2011	283 (E) 07.02.2011	State (kharif/ *rabi*-summer)	Andhra Pradesh, Rajasthan	1432 (kharif) 2280 (*rabi*-summer)	97	70	48	*Spodoptera*, jassids, thrips, leaf miner	ELS, LLS, PBND,	Early maturity
40	RARS-T-1	TAG-24 × TG-19	ANGRAU, Tirupati	2011	283 (E) 07.02.2011	State (kharif/*rabi*-summer)	Andhra Pradesh	2500 (kharif) 4000 (*rabi*-summer)	115	70	44	–	–	Large seeded
41	RARS-T-2	Tirupati-4 × TIR-45	ANGRAU, Tirupati	2011	283 (E) 07.02.2011	State (kharif/*rabi*-summer)	Andhra Pradesh	3734 (kharif) 4200 (*rabi*-summer)	90–95	70	48	–	–	Drought tolerance, early maturity
42	GJG-31 (J-71)	GG-5 × ICGV-90116	JAU, Junagadh	2012	1708 (E) 26.07.2012	State (kharif)	Gujrat	1632	103	72	48	–	Stem rots	–
43	GJG-31 (J-71)		JAU, Junagadh	2012	1708 (E) 26.07.2012	State (kharif)	Gujrat	1632	103	72	48	–	Stem rots	–

44	GJG-9 (J-69)	GG-2 × PBS-21065	JAU, Junagadh	2012	1708 (E) 26.07.2012	State (summer)	Gujrat	3483	117	70.6	49	–	–	–
45	ICGV 00350	ICGV 87290 × ICGV 87846	ANGRAU, Tirupati	2012	456 (E) 16.03.2012	Central (*rabi*-summer)	Central (Tamil Nadu and Andhra Pradesh)	2988	114	65	50	–	LLS, rust, stem rots	–
46	Dharani	TCGS 1043	ANGRAU, Tirupati	2013	2817 (E) 19.09.2013	State (kharif/*rabi*-summer)	Andhra Pradesh	1887 (kharif) 2666 (*rabi*-summer)	105–110	75–77	48–50		Stem rots, dry root rots	Drought tolerance
47	G 2-52	Gamma ray-induced mutant of GPBD 4	UAS, Dharwad	2015	S.O. 2680(E)/ 01.10.2015	State (kharif)	Karnataka	2000–2500	105–110	71	48		LLS, rust	–
48	Groundnut Co 7	ICGV 87290 × ICGV 87846	TNAU, Coimbatore	2015	S.O. 2680(E)/ 01.28.01.2015	State (kharif/*rabi*-summer)	Tamil Nadu	2300 (kharif) 2806 (*rabi*-summer)	100–105	71	50.5–51.0	–	Rust	Drought tolerant
49	Phule Bharati (JL 776)	Derivative of a complex cross [(ICGV 92069 × ICGV 93184)SIL4 x ICGV 98300]	MPKV, Jalgaon	2015	S.O.1228(E)/ 07.05.2015	Central (kharif) Zone III	Maharashtra, Madhya Pradesh	2100	108	69	50	–	–	–

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
50	GKVK 5		UAS, GKVK, Bangaluru	2016	S.O. 3540(E)/ 22.11.2016	State (kharif)	Karnataka	2500–2800	115–120	74	50	–	LLS	–
51	KCG 6	CTMG 6	UAS, Bangaluru	2016	S.O. 3540(E)/ 22.11.2016	State (*rabi*-summer)	Karnataka	3663	116	67	51	–	Rust, LLS	–
52	AGL-06-320	(J 11 × CG 52) × ICGV 86015	TNAU, Tindivanam	2017	S.O. 1007(E)/ 30.03.2017	Central (Zone IIIB) (*rabi*-summer)	Tamil Nadu, Andhra Pradesh	2741	115	70.7	50.3	–	–	–
53	Kadiri Amaravathi	K 1535	ANGRAU, Kadiri	2017	S.O. 1007(E)/ 30.03.2017	State (kharif)	Andhra Pradesh	1600–1800	110–115	65	50	Jassids, thrips	–	–
54	VRI 8 (VG 09220)	ALR 3/AK 303	TNAU, Vridhachalam	2017	S.O. 2805(E)/ 25.08.2017	State (kharif/*rabi*-summer)	Tamil Nadu	2130 (kharif) 2700 (*rabi*-summer)	105–110	70	49	Jassids, thrips	LLS, rust	Medium bold seeds
55	Avtar	ICGV 93468	CSAUA&T, Kanpur	2018	S.O. 6318 (E) 26.12.2018	State (*rabi*-summer)	Uttar Pradesh	2400	85–95	70.6	51.1	Pod borer	PBND	Early maturity
56	Dh-232	GPBD 4 × TG 37A	UAS, Dharwad	2019	S.O. 6318 (E) 26.12.2018	State (kharif)	Karnataka	2500–3000	105–110	78.7	46.9		LLS, rust	–
57	Dh-245	Gamma ray-induced mutant of GPBD 4	UAS, Dharwad	2019	S.O. 6318 (E) 26.12.2018	State (kharif)	Karnataka	2500–2900	105–108	73	45.9		LLS, rust	High oleic (72%)

58	GJG 32	ICGV 03043	JAU, Junagadh	2018	S.O 399(E) 24.1.2018 S.O. 6318 (E) 26.12.2018 (area expansion)	Central; Zone V (kharif)	Andhra Pradesh, Karnataka, Tamil Nadu, Southern Maharashtra, Telangana; area expansion in Gujarat	1947	109	66	50	–	–	High oil content (50%)
59	GJG 33	ICGV 07222	JAU, Junagadh	2018	S.O 1379(E) 27.3.2018	Central (*rabi*-summer)	Tamil Nadu, Andhra Pradesh, Telangana	3064	113	67	51	–	–	High oil content (51%)
60	TMV-14	VRIgn 6 × R 2001-2	TNAU, Tindivanam	2019	S.O. 1498 (E) 01.04.2019	State (kharif)	Tamil Nadu	2124	95–100	70.6	48	*Spodoptera*, thrips, leaf miner	LLS, rust	Early maturity
61	Phule Unnati	RHRG-6083	MPKV, Rahuri	2019	S.O. 3220 (E) 05.09.2019	State (kharif/*rabi*-summer)	Maharashtra	2854 (kharif) 3990 (*rabi*-summer)	111–128	68.0	52	*Spodoptera*, thrips	LLS, stem rot, rust	High oil content (52%)
62	AK 335 (PDKVG-335)	Selection from TG 36B	PDKV, Akola	2019	S.O. 1498 (E) 01.04.2019	State; kharif	Maharashtra	2200–2400	110–113	68.7	48–49	Jassids, thrips, aphids	Tikka, collar rot, stem rot	–
63	BSR-2 (BSG 0912)	VRI 2 × TVG 0004	TNAU, Bhavanisagar	2019	S.O. 3220 (E) 05.09.2019	State: kharif, rabi-summer	Tamil Nadu	2222 (kharif) 2360 (rabi-summer)	105–110	70.2	45	Jassids, thrips, aphids	Rust, LLS	–

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
64	Pragathi (TCGS-894)	TCGS-894	ANGRAU, Tirupati	2019	S.O. 99 (E) 06/01/2020	Central; *rabi*-summer	Tamil Nadu, Telangana, Andhra Pradesh	2816	115	70	48	Leaf miner, thrips	–	–
65	Dh 256	R-2001-2 × GM 4-3-12	UAS, Dharwad	2019	S.O. 99 (E) 06/01/2020	Central (Zone V); kharif/*rabi*-summer	Andhra Pradesh, Karnataka, Tamil Nadu, Telangana	3258 (kharif) 3500 (*rabi*-summer)	110–115	70	50	Leaf miner, thrips	Tolerance to foliar diseases	Mid-season drought tolerance
66	Dheraj	TCGS-1073	ANGRAU, Tirupati	2019	S.O. 3220 (E) 05.09.2019	State; kharif, *rabi*-summer	Andhra Pradesh	2547 (kharif) 3690 (*rabi*-summer)	105–115	73–80	48–49	–	–	Heat tolerance, high WUE
67	Gujrat Groundnut-34 (GG 34)	AG-2012-06	AAU, Anand	2019	S.O. 3220 (E) 05.09.2019	State; summer	Gujarat	3715	111–125	67.9	52.8	–	–	High oil content (52.8%)
68	Phule Chaitanya	Central-KDG-160	MPKV, Digraj	2019	S.O. 1498 (E) 01.04.2019	Central; *rabi*-summer	Tamil Nadu, Andhra Pradesh, Telangana	2184	116	66.7	51.6	–	LLS	–
69	Phule Dhani	JL 1085	MPKV, Rahuri	2019	S.O. 3220 (E) 05.09.2019	Central; kharif	Tamil Nadu, Andhra Pradesh, Karnataka	3333	109	68	50	–	LLS	–
70	GG 41 (Padma)	JPS 65	JAU, Junagadh	2020	S.O. 99 (E) 06/01/2020	State, kharif	Gujarat							

71	ICGV 06189	{[BPIPn9 × ICGV 95172) × {[ICGV 88414 × USA630 × ICGV 951720]}	UAS, Dharwad and ICRISAT	2020	S.O. 99 (E) 06/01/2020	State, kharif	Karnataka	2100–2500	105–108	73	43	–	–	Large seeded
72	Jagtial Palli 1	JCG 2141	RARS, Jagtial (PJTSAU, Hyderabad)	2020	S.O. 99 (E) 06/01/2020	State, kharif/*rabi*-summer	Telangana	3220	105–110	70	49	–	–	–
73	Pratap Mungfali-3	UG-116	MPUAT, Udaipur	2020	S.O. 99 (E) 06/01/2020	State, kharif	Rajasthan	3388	108	70	47	Jassid, leafhopper, *Spodoptera*, thrips	ELS, LLS, rust	–
74	Chhattisgarh Mungfali-1 (CGM-1)	ICGV 87846 × ICGV 99240	IGKV, Chhattisgarh	2020	SVRC, 12.06.2020	State Kharif	Chhattisgarh	4200	114	70	45	–	Rust, LLS	–
75	Kadiri Lepakshi	K 1812	ANGRAU, Kadiri	2020	S.O. 500 (E) 02.02.2021	Central (Zone V); kharif	Andhra Pradesh, Telangana, Tamil Nadu, Karnataka	3397	120–125	72	48	*Spodoptera*	Rust	–
76	K 1719	Kadiri Chitravathi	ANGRAU, Kadiri	2020	S.O. 500 (E) 02.02.2021	Central; *rabi*-summer	Andhra Pradesh, Telangana, Tamil Nadu	3110	110–113	70	49	Thrips	Collar rots, PBND	Large seeded
77	J 87	GG 36	JAU, Junagadh	2020	S.O. 500 (E) 02.02.2021	Central; *rabi*-summer	Punjab, Uttar Pradesh	4165	108	71	50	Leaf miner	ELS	–

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
78	Dh 257	ICGV 07211 × ICG 2381	UAS, Dharwad	2020	S.O. 500 (E) 02.02.2021	Central (Zone IIIA); *rabi*-summer	Karnataka, Maharashtra	3514	110	70	51	Leaf miner	ELS, PBND	Mid-season drought tolerance
Habit group: Virginia bunch (VB)														
1	Manjra (LGN 2)	R-33-1 × TMV-10	MAU, Latur	2001	92 (E) 02.02.2001	Central (kharif)	Gujarat	1750	115–120	65–70	49	–	–	Suitable for rainfed condition
2	Sneha	IES-883 × JL-24	–	2002	937 (E) 04.09.2002	State (kharif)	Kerala	3820	95–100	70	50	–	–	Early maturity
3	Snigdha	DH-3-32 ×JL-24	KAU, Vellayani	2002	937 (E) 04.09.2002	State (kharif)	Kerala	3550	95–100	70	50	Thrips, jassids, *Spodoptera*, leaf miner	ELS, LLS, rust	Early maturity
4	Co (Gn) 5	(Co-2 xICGS-86010) × (Co-2 × VG-119 × ICGS-50)	TNAU, Coimbatore	2005	1177 (E) 25.08.2005	State (kharif)	Tamil Nadu	1580	125	73.8	53.8	Leaf miner, *Spodoptera*	Rust, PBND	High oil content (53%)
5	GG 21 (JSSP 15)	Somnath × NCAc-2232	JAU, Junagadh	2005	449 (E) 11.02.2009	Central (kharif)	Uttar Pradesh, Punjab, Northern Rajasthan	1840	123	64	53	–	LLS	High oil content (53%)
6	AK 265	ICGS-11 × US-63	PDKV, Akola	2007	1703 (E) 05.10.2007	Central (kharif)	Andhra Pradesh, Karnataka, Southern Maharashtra	1900	120	68	47	–	LLS, rust	Drought tolerant

7	AK 303	(ICGV-88384 × JL-24) × (ICGV-88438 × ICG-5240)	PDKV, Akola	2007	449 (E) 11.02.2009	State (kharif)	Maharashtra	2100	125	72	49	–	–	Large seeded (HSW 80 g)
8	TBG 39 (TG 39)	TAG-24 × TG-19	RAU, Bikaner	2007	2458 (E) 16.10.2008	State (kharif)	Rajasthan	3150	118	64.5	50	–	–	Large seeded (HSW 67 g)
9	Girnar 2 (PBS-24030)	Selection from M-13 × R-33-1	NRCG, Junagadh	2008	72 (E) 10.01.2008	Central (kharif)	Punjab, Northern Rajasthan, Uttar Pradesh	2910	130	69	51	Jassids, thrips	LLS, PBND, rust	Large seeded (HSW 62 g)
10	ICGV 00348	ICGV-87290 × ICGV-87846	TNAU, Virudhachalam	2008	2458 (E) 16.10.2008	Central (kharif)	Andhra Pradesh, Karnataka, Southern Maharashtra, Tamil Nadu	2010	1324	66	47	–	ELS, LLS, rust	–
11	VRI (Gn) 7	TMV-1 × JL-24	TNAU, Virudhachalam	2008	2187 (E) 27.08.2009	State (kharif)	Tamil Nadu	1860	120–125	72	48	Leaf miner	LLS, rust	Suitable for rainfed condition
12	Kadiri 7	{ICGV-86522 × ICG(FDRS) 10} × ICGV-91172	ANGRAU, Kadiri	2009	2187 (E) 16.10.2008	State (kharif)	Andhra Pradesh	1640	120–125	68	47	Jassids, thrips	ELS, LLS	Large seeded (HSW 65–75 g)
13	Kadiri 8	{ICGV-86522 × ICG (FDRS) 10} × ICGV-91172	ANGRAU, Kadiri	2009	2187 (E) 16.10.2008	State (kharif)	Andhra Pradesh	1520	120–125	69	47	Jassids, thrips	ELS, LLS	Large seeded (HSW 65–75 g)

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
14	Mallika (ICGV 00440)	(ICGV 88386 ×ASHFORD) × ICGV-95178	RAU, Hanumangarh	2009	2187 (E) 27.08.2009	Central (kharif)	All India	2580	125–130	66–70	48	–	Collar rots, PBND	Drought tolerant, large seeded (HSW 73 g)
15	TGLPS-3 (TDG 39)	TAG-24 × TG-19	UAS, Dharwad	2009	449 (E) 11.02.2009	State (kharif)	Karnataka	2500–3000	115–120	70	46	–	–	Large seeded (HSW 67 g)
16	HNG 69	CSMG-84-1 × PG-1	RAU, Hanumangarh	2010	211 (E) 29.01.2010	Central (kharif)	Punjab, Northern Rajasthan, Uttar Pradesh	2800	131	66	50	–	Collar ELS, stem rots	–
17	Raj Durga (RG 425)	ICG-5013 × RG-340	RAU, Durgapur	2011	283 (E) 07.02.2011	State (kharif)	Rajasthan	1788	128	70	50	–	–	–
18	Co 6 (ICGV 87846)	Derivative from Cross CS 9 × ICGS 5	TNAU, Coimbatore	2012	1708 (E) 26.07.2012	State (kharif)	Tamil Nadu	1914	125–130	73.5	49.5–51.0	–	LLS, rust	Drought tolerant
19	HNG 123	Chandra × RSB-87	RAU, Hanumangarh	2012	456 (E) 16.03.2012	Central (kharif)	Punjab, Rajasthan, Uttar Pradesh	2648	124	68	49	*Spodoptera*, leaf miner	Collar LLS, stem rots	–
20	GJG-22 (JSSP-36)	JSSP-17 × GG-20	JAU, Junagadh	2013	312 (E) 01.02.2013	State (kharif)	Gujrat	1770	118	72	51.6	–	Collar rots	–
21	Birsa Groundnut 4	BAU 25	BAU, Kanke	2015	S.O. 268(E)/ 28.01.2015	State (kharif)	Jharkhand	3000–3500	115–120	70–74	50.78	–	LLS	–

22	Raj Mungfali-2	RG 578	SKRAU, Durgapura	2015	S.O. 268(E)/ 28.01.2015	Central (kharif)	Odisha, West Bengal, Manipur	1480	120	72	46	Thrips, jassids, *Spodoptera*, leaf miner	LLS, dry root rots, ELS, rust	–
23	Phule Morna (KDG 123)	Selection from ICGV 04168 (ICGV 96050 × ICGV 96239)	MPKV, Rahuri	2016	S.O. 3540(E)/ 22.11.2016 and S.O 399(E) 24.1.2018 (area extended)	Central (kharif)	Gujrat, Rajasthan, Odisha, WB, Jharkhand, Manipur, Tamil Nadu, Andhra Pradesh, Karnataka, Southern Maharashtra	2212	114	70	44	–	–	–
24	Phule Warna (KDG 128)	Derivative of a complex cross (ICGV 87121 × ICGV 87853) × ICGV 92023) × ICGV 98300)]	MPKV, Rahuri	2016	S.O. 3540(E)/ 22.11.2016 and S.O 399(E)24. 1.2018 (area extended)	Central (kharif) Zone II Zone V	Andhra Pradesh, Karnataka, Tamil Nadu, Southern Maharashtra, Gujrat, Rajasthan	2425	113	65	50.9	–	LLS, rust	–
25	Raj Mungfali-3 (RG 559-3)	TKG-19A × Kadiri-3	SKNAU, Durgapura	2016	S.O. 3540(E)/ 22.11.2016	Central Zone I (kharif)	Rajasthan, UP, Punjab	3173	125	69	49	Thrips	–	–
26	Gujrat Groundnut HPS-2	GG HPS-2	JAU, Junagadh	2019	S.O. 1498 (E) 01.04.2019	State; kharif	Gujarat	2835	111–124	68.6	48.8	–	–	Large seeded

(continued)

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
27	Konkan Bhuratna	RTNG-29	DBSKKV, Dapoli	2019	S.O. 1498 (E) 01.04.2019	State; kharif	Maharashtra	2500–3000	110–115	74	50.01	Thrips, jassids, leafhopper	LLS, ELS, rust, PBND	High oil content (50.1%)
28	Girnar 4	ICGV 15083	DGR, Junagadh	2020	S.O. 99 (E) 06/01/2020 S.O. 500 (E) 02.02.2021	Central (Zone V); kharif	Andhra Pradesh, Gujrat, Karnataka, Rajasthan, Tamil Nadu, Telangana	3218	110–112	67	53	Leafhopper, leaf miner, *Spodoptera*, thrips	LLS, PBND, rust, stem rots	High oleic acid (78.5%)
29	Girnar 5	ICGV 15090	DGR, Junagadh	2020	S.O. 99 (E) 06/01/2020 S.O. 500 (E) 02.02.2021	Central (Zone V); kharif	Andhra Pradesh, Gujrat, Karnataka, Rajasthan, Tamil Nadu, Telangana	3124	113	67	53	Leafhopper, leaf miner, *Spodoptera*, thrips	Collar rots, LLS, rust, stem rots	High oleic acid (78.5%)
Habit group: Virginia runner (VR)														
1	GG 14 (JSP 28)	GG-11 × R-33-1	JAU, Junagadh	2003	283 (E) 12.03.2003	Central (kharif)	Northern Rajasthan, Punjab, Haryana, Uttar Pradesh	2159	123	65	52	Leaf miner, *Spodoptera*, thrips	–	High oil content (52%)

2	RG 382 (Durga)	BG-2 × Durgapura	RAU, Durgapur	2005	122 (E) 02.02.2005	State (kharif)	Rajasthan	2200	125	64	49	Thrips, leaf miner	ELS, rust	Fresh seed dormancy
3	Utkarsh (CSMG 9510)	MA-10 × ICG-7894	CSAU&T, Mainpuri	2005	449 (E) 11.02.2009	Central (kharif)	Uttar Pradesh, Punjab, Northern Rajasthan	2190	125	66	49	–	Rust	–
4	GG 16(JSP 39)	JSP-14 × JSSP-4	JAU, Junagadh	2006	1572 (E) 20.09.2006	Central (kharif)	Andhra Pradesh, Kerala, Tamil Nadu	2058	119	63	46	Leaf miner, *Spodoptera*, thrips	PBND, root rots	–
5	M-548	M-37 × Blanco Puro White	PAU, Ludhiana	2007	1178 (E) 20.07.2007	State (kharif)	Punjab	2180	123	69	51.4	–	Collar rots, ELS, LLS	High protein content
6	Gujrat Groundnut HPS-1 (JHP HPS-44)	JSP-21 × VG-5	JAU, Junagadh	2010	2137 (E) 31.08.2010	State; kharif	Gujarat	2125	110–120	68	49	–	–	Drought tolerant, large seeded (HSW 76 g)
7	Divya (CSMG 2003-19)	Amber × ICG-1697	CSAU&T, Mainpuri	2011	2326 (E) 10.10.2011	Central (kharif)	Uttar Pradesh, Rajasthan	2760	129	69	47	–	PBND	–
8	GJG-17 (JSSP-48)	JSP-11 × GG-6	JAU, Junagadh	2013	312 (E) 01.02.2013	State (kharif)	Gujrat	1798	121	66	48	–	Stem rots	–

Table 3.2 (continued)

Sl No	Variety	Pedigree/ other name	Developing centre	Year of release	Notification number	Released for state/central/ season	Area of adoption	Pod yield (kg/ha)	Days to maturity	Shelling %	Oil content	Reaction to pests	Reaction to diseases	Special traits
9	Raj Mungfali-1 (RG 510)	RG-318 × RG-340)	RAU, Durgapur	2012	456 (E) 16.03.2012	Central (kharif)	Punjab, Rajasthan	2558	112–138	68	49	Grasshopper, thrips, jassids,	Collar rots, LLS, PBND, stem rots	Large seeded (HSW 76 g)
10	GJG 18	JSP 49	JAU, Junagadh	2015	S.O. 268(E)/ 28.01.2015	Central (kharif)	Odisha, West Bengal, Jharkhand, Manipur	1450	121	69	48	–	–	–
11	GJG 19	JSP 51	JAU, Junagadh	2016	S.O. 2238(E)/ 29.06.2016	Central (kharif)	Odisha, West Bengal, Jharkhand, Manipur	1876	122	69	47	–	–	–

Table 3.3 Novel trait-specific groundnut germplasm registered with NBPGR

Sl No	Donor identity	National identity	INGR No.	Year	Pedigree	Novel unique features
1	Mutant 28-2	IC296686	INGR98003	1998	VL-1	Leaf spots, army worn and thrips resistant
2	GPBD 4	IC296810	INGR01031	2001	KRGI × CS16 (ICGV86855)	Resistant to late leaf spot (LLS) and rust
3	PBS 24004	IC296811	INGR01032	2001	Latur 33 and Tifrun	Iron chlorosis tolerant
4	PBS 30008 (Ginar 1 nlm)	IC296812	INGR01033	2001	Cultivar Girnar 1	Narrow leaf mutant
5	PBS-30017 (Ginar 1 lym)	IC296813	INGR01034	2001	Cultivar Girnar 1	Lemon yellow colour leaf mutant
6	PBS 29031	IC296913	INGR03096	2003	M13 × NC Ac 17278 (selection in F6)	Large seeded, high yield and high oil content with low O/L ratio
7	PBS 30138	IC296915	INGR03097	2003	Mutant of PBS 30138	Curly leaf character with field resistance to rust and LLS
8	TCGS-635	IC296917	INGR03098	2003	Tirupati-1 × ICGV 86398	Pentafoliate leaf mutant
9	TG-18 AM	IC296610	INGR04039	2004	Mutant of TG 18 A	Disease lesion mimic leaf trait
10	TGE-1	IC296612	INGR04040	2004	Tall mutant × TG 9 1981	Early (95 days) foliaceous stipule and high shelling (80%)
11	Small leaf mutant	IC296613	INGR04041	2004	Mutant from Spanish Improved (1968)	Dwarf with small leaf size
12	MH 34	IC401583	INGR04076	2004	Sel TG-9	High oil content (54%)
13	CS 19	IC415060	INGR04096	2004	Clipper/PL 172	Multiple resistance and high harvest index
14	Imparipinnate mutant	IC323372	INGR04097	2004	BCU 73/DL 88/ Clipper	Imparipinnate leaves with small leaflets
15	Suppressed branched mutant	IC323373	INGR04098	2004	BCU 73/PL 172/ ALFA 93	Suppressed primary branches and large basal leaflets
16	TG-18A	IC553271	INGR07032	2007	Mutant of TG18	Large pod and seed
17	TGM-167	IC0595257	INGR13011	2013	Mutant of TFDRG 5	Gibberellin-insensitive dominant dwarf mutant

(continued)

Table 3.3 (continued)

Sl No	Donor identity	National identity	INGR No.	Year	Pedigree	Novel unique features
18	NRCG 12431	IC0595258	INGR13012	2013	Collection	Low level of infection (7%) and free from colonization of *Aspergillus flavus* in kernels
19	NRCG 14380	IC0595259	INGR13013	2013	Collection	Fresh seed dormancy up to 40 days
20	NRCG 14368	IC0595260	INGR13014	2013	Collection	Fresh seed dormancy up to 40 days
21	TGM-38	IC0591394	INGR13025	2013	Mutant of TAG 24	Sub-orbicular leaflet, erect, compact and dwarf plant type
22	TGM-51	IC0591393	INGR13026	2013	Mutant of TAG 24	Funnel leaflet, dwarf plant type
23	PKVG 8	IC570070	INGR09038	2009	JL24x Nc Ac17127	Tolerance to iron chlorosis
24	NRCG 09-1	IC567685	INGR09127	2009	Normal leaf with white testa germplasm x crinkle leaf shape with rose testa mutant	Multiple phenotypic marker stock (crinkle leaf, white testa colour), NIL of crinkle leaf with rose testa mutant
25	NRCG 09-2	IC567686	INGR09128	2009	Normal leaf with red testa germplasm x crinkle leaf shape with rose testa mutant	Multiple phenotypic marker stock (crinkle leaf, red testa colour), NIL of crinkle leaf with rose testa mutant
26	NRCGCS-77	IC0582472	INGR10029	2010	(CT 7-1 × SB 11)× *A. kretschmeri*	Resistance to PBND, stem rot, LLS, ELS, rust and *Alternaria* leaf blight
27	NRCGCS-85	IC0582473	INGR10030	2010	(CT 7-1 × SB 11)× *A. diogoi*	Resistance to PBND, stem rot, LLS, ELS, rust and *Alternaria* leaf blight
28	NRCGS-86	IC0582474	INGR10031	2010	(CT 7-1 × SB 11)× *A. correntina*	Resistance to PBND, stem rot, LLS, ELS, rust and *Alternaria* leaf blight
29	NRCG-14326	EC0548192	INGR10032	2010	NRGC working collection	Source of fresh seed dormancy (40 days), oil content 50%

(continued)

Table 3.3 (continued)

Sl No	Donor identity	National identity	INGR No.	Year	Pedigree	Novel unique features
30	NRCG-14336	IC0582477	INGR10033	2010	NRGC working collection	Source of fresh seed dormancy (40 days), oil content 51.2%
31	NRCG-14350	IC0582478	INGR10034	2010	NRGC working collection	Source of fresh seed dormancy (60 days), oil content 50.5%
32	NRCG-14409	IC0582479	INGR10035	2010	NRGC working collection	Source of fresh seed dormancy (60 days), oil content 49.8%
33	NRCGCS-21	IC0583387	INGR10036	2010	(CT 7-1 × SB 11) *A. diogoi*	Resistant to PBND, stem rot; tolerant to LLS, ELS
34	NRCGCS-83	IC583388	INGR10037	2010	(CT 7-1 × SB 11) *A. diogoi*	Resistant to PBND, stem rot and *Alternaria* leaf blight; tolerant to LLS
35	NRCGCS-124	IC0583389	INGR10038	2010	(CT 7-1 × *A. kretschmeri*	Resistant to PBND, stem rot and *Alternaria* leaf blight; tolerant to LLS, ELS
36	NRCGCS-180	IC0583390	INGR10039	2010	J 11 × *A. cardenansii*	Resistant to PBND, stem rot, *Alternaria* leaf blight; tolerant to LLS, ELS
37	NRCGCS-222	IC0583391	INGR10040	2010	(C.364 × PBDR 25) × *A. kemfinercadoi*	Resistant to PBND, stem rot and *Alternaria* leaf blight; tolerant to LLS, ELS
38	NRCG-11846	IC0583392	INGR10041	2010	NRGC working collection	High fodder biomass (2.4 ton/ha/year), perennial, for pasture development, high crude fibre (31.2%) and ash (11.7%) contents
39	NRCG-11847	IC0583393	INGR10042	2010	NRGC working collection	High fodder biomass (3.8 ton/ha/year), perennial, for pasture development, high protein content (16.9%)
40	NRCG-12035	IC0583394	INGR10043	2010	NRGC working collection	High fodder biomass (1.8 ton/ha/year), seed forming, semiperennial; for pasture development, protein content (14.8%)

(continued)

Table 3.3 (continued)

Sl No	Donor identity	National identity	INGR No.	Year	Pedigree	Novel unique features
41	NRCG-12990	IC0583395	INGR10044	2010	NRGC working collection	High fodder biomass (2.7 ton/ha/year), perennial, for pasture development, protein content (12.1%) and iron content (0.5%), binds soil through tough rhizomes
42	NRCG-17205	IC0583396	INGR10045	2010	NRGC working collection	High fodder biomass (3.6 ton/ha/year), perennial, for pasture development, binds soil through tough rhizomes, high protein content (14.2%) and iron content (0.7%)
43	NRCG-17206	IC0583397	INGR10046	2010	NRGC working collection	High fodder biomass (3.2 ton/ha/year), perennial, for pasture development, binds soil through tough rhizomes, high protein content (16.8%) and iron content (0.7%)
44	AKG 18-1	IC0587384	INGR11022	2011	Isolated from the cross Jyoti × EC76446 (292)	Multi-foliate leaves, five to eight leaflets in 30% of leaves, reticulated two seeded pods
45	NRCGCS-15	IC0589174	INGR11054	2011	(CT7-1 × SBXI) × *A. pusilla*	Highly resistant to PBND, resistant to stem rot, rust; tolerant to LS
46	NRCGCS-74	NA	INGR11055	2011	(CT7-1 × SBXI) × *A. pusilla*	For better resistance to diseases
47	NRCGCS-186	NA	INGR11056	2011	(C-364 × PBDR-25) × *A. oteroi*	For better resistance to diseases
48	NRCGCS-196	IC0589180	INGR11057	2011	(GUAG-10 × CGC-4018) × *A. correntina*	For better resistance to diseases
49	TG M-112	IC0585932	INGR11058	2011	Mutant of TAG 24	White to light orange flower colour mutation
50	NRCG CS 281	IC0616376	INGR16019	2016	DR × PV × *A. duranansis*	Spanish bunch genotype with extra-large kernel size (HPS type)

Table 3.3 (continued)

Sl No	Donor identity	National identity	INGR No.	Year	Pedigree	Novel unique features
51	NRCGCS-602 (HOS-130 or HOP_IL_130)	IC630593	INGR19080	2019	ICGV06100 × SunOleic 95R	High oleic acid (80%) content
52	NRCGCS-605 (HOS-145 or HOS-IL_ MAS_145)	IC630594	INGR19081	2019	ICGV06100 × SunOleic 95R	High oleic acid (80%) content
53	NRCGCS 636 (HOS-89)	IC635044	INGR20049	2020	ICGV 06100 × SunOleic 95R	High oil content (56%)
54	NRCGCS-635 (HOS-30)	IC635045	INGR20050	2020	ICGV 06100 × SunOleic 95R	High oil (56%)

Source: http://www.nbpgr.ernet.in:8080/registration/InventoryofGermplasm.aspx

and for attaining sustained food security, anomalies, viz. skewed SRR and low varietal replacement rates (VRR), should be addressed appropriately. Therefore, there is need for strengthening the quality seed production programme and induction of recently released varieties into the seed chain through concerted efforts involving plant breeders/sponsoring organizations and state departments of agriculture. However, current breeder seed indent/production is inadequate for maintaining the requisite SRR. The major reasons for deficit could be attributed to their low seed multiplication ratio (SMR), high seed rate/unit area, less efficient seed production chain and aberrant climatic conditions. The level of indents is declining in many crops. Further, issues of non-lifting of seed need to be seriously addressed as it can be the most important factor of demotivation for breeder seed producing agencies for taking up such privileged activity. They should prepare at least 5-year seed rolling plan (2017–2022) phasing out old and obsolete varieties with latest released varieties. Then they should come up with crop-wise/variety-wise realistic indents to the concerned organization considering the expected gross cropped area, ideal seed replacement rate and gradual annual increase, at least 3 years in advance. Appropriate MoUs should be developed with the different stakeholders for firm commitments of procuring the seed, thus mitigating the problem of non-lifting. Introducing bar/QR code is desirable for traceability of breeder seed source in multiplication chain for quality seed production. Development of variety-specific molecular markers to enable rapid genetic purity testing, management of nucleus seed and its maintenance to either replace or supplement grow-out test and a network on developing national database of crop varietal DNA profile (fingerprinting) which should be created to facilitate quality breeder seed production is foremost.

In recent years, the climate changes had adversely affected agricultural production in the country, and the seed production programme is not an exception; soybean and chickpea seed production were severely affected. Therefore, there is an urgent need to identify alternate areas or new niches in non-traditional season/areas for

Table 3.4 Status of groundnut improved varieties breeder seed production during the last 15 years in India

Year	No of varieties	Major varieties (>500 q production per annum)	Indent (q)	Production (q)
2006–2007	48	TMV 2, JL 24, TAG 24, GPBD 4	8489.22	4506.65
2007–2008	61	TMV 2, JL 24, TAG 24, GPBD 4	8043.61	8756.65
2008–2009	57	TMV 2, JL 24, TAG 24, GPBD 4 (Vikas), Dh 86	9191.75	7544.45
2009–2010	54	TMV 2, GPBD 4, Dh 86, JL 24, TAG 24	22886.95	16406.5
2010–2011	53	TMV 2, JL 24, GPBD 4, Dh 86,TAG 24, Kadiri 6	19679	15091.6
2011–2012	69	Kadiri 6, Kadiri 9, Narayani (TCGS 29), GPBD 4, JL 24, ICGV 91114, TAG 24	25501.6	20075.65
2012–2013	47	Kadiri 6, GPBD 4, ICGV 91114, TAG 24, JL 24	13075.3	12013.84
2013–2014	53	Kadiri 6, Kadiri 9, GPBD 4, ICGV 91114, TAG 24	12463.35	12995.82
2014–2015	50	Kadiri 6, Kadiri 9, GPBD 4, ICGV 91114, TAG 24	11309.85	10458.91
2015–2016	34	Kadiri 6, Kadiri 9, ICGV 91114, GPBD 4	6726.8	9823.13
2016–2017	42	Kadiri 6, Kadiri 7, Kadiri 9, Kadiri Harithandhra, GPBD 4, TG 37A	11376.23	13952.63
2017–2018	45	Kadiri 6, Kadiri 9, G 2-52, Kadiri Harithandhra, Dharani, GPBD 4, TAG 24	10168.41	12513.36
2018–2019	47	Kadiri 6, Dharani, G 2-52, GPBD 4, ICGV 00350	10458.91	9323.05
2019–2020	50	Kadiri 6, Dharani, GPBD 4, G 2-52	9343.31	8809.23
2020–2021	60	Kadiri 6, Dharani, Kadiri Amravati, Kadiri 9, GPBD 4, G 2-52	13299.55	12271.56
Grand total			192013.84	174543.03

Source: AICRPG Annual Reports 2000–2001 to 2020–2021

compensatory seed production. Institution of 'National Seed Grid' and identification of provenances for off-season seed production in oilseed and pulses will help in meeting the seed requirement and mitigating effects of climatic vagaries for groundnut – Gujarat, Andhra Pradesh and Karnataka. The focus should be on quality seed production of short-duration drought-escaping varieties of groundnut. Unemployed youths can be trained in the field of seed quality assurance, and with financial support and seed quality assurance laboratories, *seed clinic* may be established in major seed-growing areas (Rajendra Prasad et al. 2017).

3.6.4 A Success Story of GPBD 4 from UAS, Dharwad: Model for Adoption of Improved Groundnut Varieties in Farmer's Field in India

An important milestone in the history of groundnut crop improvement was created with development and release of GPBD 4 (Vikas), a first foliar disease (LLS and rust)-resistant variety with acceptable pod and kernel features through interspecific hybridization (KRG-1 × ICGV 86855) at UAS, Dharwad (Gowda et al. 2002). It has been released for cultivation for both Southern and Eastern India comprising of Karnataka, Andhra Pradesh, Tamil Nadu, Maharashtra, West Bengal and Orissa states through AICRPG system where foliar diseases are the major threat for groundnut production. It has high yield potential (2800–3000 kg/ha) with high shelling outturn (76%) and oil content (48%) coupled with better oleic acid (50%) among the Spanish bunch varieties grown in India. Due to its resistance to foliar diseases, it gives higher fodder (haulm) yield also. The yield potential, good-quality haulm and desirable pod and kernel features of GPBD 4 groundnut variety realized in the farmers' fields are depicted in Fig. 3.1 that will serve as *model for adoption of improved groundnut varieties in farmer's field in India.*

Further, GPBD 4 has been widely used as a donor for foliar disease resistance in India and across the globe. Further, many productive, foliar disease-resistant MABC lines have been developed in India through UASD-DGR-ICRISAT-BARC collaborative efforts under JL 24, TAG 24, ICGV 91114, TMV 2, GJG 9, GG20 and GJGHPS 1 genotypes adoptive background (Varshney et al. 2014; Kolekar et al. 2017; Shashidhar et al. 2020), and such derived NILs are being tested under AICRPG system in the recent years (AICRPG Annual Reports and AGM Proceedings, 2020–2021).

Fig. 3.1 A success story of GPBD 4 from UAS, Dharwad; model for adoption of improved groundnut varieties in farmer's field in India

GPBD 4 is the major variety that has spread to farmer's field in Karnataka (> 1.5 lakh ha) and other southern and eastern states of India (>5 lakh ha) leading to long-lasting impact of enhancing area, production and productivity of groundnut in India with an average annual breeder seed indent of 1000 q totalling to 17,500 quintals of breeder seed production from UAS, Dharwad (Table 3.4), since its notification in 2004 with a monitory value to the tune of INR 600 crores.

3.7 Rapid Generation Advancement and Speed Breeding in Groundnut

The current rate of varietal development and replacement in farmer's field is still very low. More than often it takes more than a decade to develop improved varieties that are subsequently commercialized in farmers' field. Therefore, the new technology 'rapid generation advancement' or 'speed breeding' has come to the rescue by shortening the life cycle of a crop species and, therefore, allowing researchers to make more generations in a year. Although this concept is not new for groundnut (O'Çonnor et al. 2013), the recent emphasis on it has brought more awareness and realization for this technology (Hickey et al. 2019). Studies on effects of temperature and photoperiod on vegetative and reproductive growth in groundnut provided basic information on the possibility of shortening the life cycle under controlled conditions (Nigam et al. 1994). The study performed experiments under controlled environment conditions in growth chambers using three temperature regimes (22/18, 26/22 and 30/26 °C, day/night) to assess performance of genotypes under long-day (12 h) and short-day (9 h) photoperiods and suggested that the pod-to-peg ratio (PPR) could be used as indicator of genotypic sensitivity to assess photoperiod effect in groundnut. The speed breeding technology was then optimized and used in groundnut to make at least one more generation per calendar year to increase generation advancements (O'Connor et al. 2013). These studies initially performed optimized ideal plant population in large pots and examined the impact of 24-h light system to determine genotypic variation on photoperiod sensitivity. One of these studies successfully deployed speed breeding techniques in breeding rust-resistant groundnut lines (O'Connor 2012) and another study for rapid generation of a population starting from F2 to F5 generation under controlled greenhouse conditions (O'Connor et al. 2013) showing that four generations/year are possible in groundnut.

Realizing the importance of speed breeding in rapid generation advancement in groundnut, a fresh momentum can be seen to fine-tune this technology for using it in different genomics and breeding applications. Today this technology is possible for hundreds of plants, which may transform further to handle hundreds of thousands of plants at one go. Among major applications of speed breeding, the major possible applications in groundnut include (a) faster development of genetic populations such RILs, NAM, MAGIC and NILs for trait mapping, (b) accelerated domestication and faster generation advancements for synthetic groundnuts, (c)

integration with MABC/MAS/pyramiding for faster development of molecular breeding products and (d) fast-forwarding genomic selection breeding through rapid generation advancement. In summary, the speed breeding has great potential in speeding up the process of genetic population development, accelerated domestication, trait mapping, MAS/MABC and genomic selection breeding in groundnut (Pandey et al. 2020b).

3.8 Genomic-Assisted Breeding in Groundnut

The last decade witnessed rapid development of genomic resources such as large-scale molecular markers, genetic maps and genome sequences and their deployment in genomic-assisted breeding (GAB) in groundnut (see Pandey et al. 2016, 2020a, b; Varshney 2016; Varshney et al. 2019). There are three GAB approaches, namely, marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and genomic selection (GS). MABC and MARS require trait association, while the GS does not need such analysis. Realizing the limitation associated with MABC and MARS to capture small-effect genetic factors, GS has emerged as the most promising, efficient and cost-effective breeding approach which captures both small- and large-effect genetic factors. GS promises to achieve higher genetic gains to improve complex traits such as yield and oil content in groundnut (Pandey et al. 2020a, b). If integrated with rapid generation advancement technology such as speed breeding, the GS can make remarkable achievement and positive impact on breeding programmes in groundnut (Pandey et al. 2020a).

3.9 Genomics of Biotic Stress Tolerance

Genomic resources and the tools are assisting genetic enhancement in peanut. The genome sequences of the progenitor diploids (Bertioli et al. 2016; Chen et al. 2016; Lu et al. 2018), primitive tetraploid (Yin et al. 2018, 2020) and the cultivated tetraploid (Bertioli et al. 2019; Chen et al. 2019; Zhuang et al. 2019) are available. Currently, the Sequence Read Archive (SRA) of National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/sra) has the whole-genome re-sequencing (WGRS) data of 231 genotypes comprising wild diploids, tetraploids and botanical varieties. Transcriptomes and methylomes are also available in peanut (Bhat et al. 2021a). Various types of markers have been developed even at genome-wide scale [chapter by Bhat et al. (2021b) in this book]. Trait mapping efforts could successfully map resistance to early leaf spot, late leaf spot and rust [chapter by Bhat et al. (2021b) in this book]. Other biotic stress-related traits mapped till date include stem rot resistance (Dodia et al. 2019), aflatoxin production (Yu et al. 2020), tomato spotted wilt virus (TSWV) resistance (Agarwal et al., 2018, 2019), bacterial wilt resistance (Luo et al. 2019) and resistance to aphid vector of

groundnut rosette disease (Herselman et al. 2004). Identification of genomic regions for these traits would certainly help enhancing genetic potential of peanut through molecular breeding as it has been demonstrated for late leaf spot and rust resistance [chapter by Bhat et al. (2021b) in this book].

3.10 Genomics of Abiotic Stress Tolerance

Linkage mapping-based QTL analysis was performed in a groundnut mapping population TAG 24 × ICGV 86031, and some main-effect QTLs and many epistatic QTLs were identified (Ravi et al. 2011). Further, a consensus map with three mapping populations, viz. TAG 24 × ICGV 86031, ICGS 76 × CSMG 84-1 and ICGS 44 × ICGS 76, was developed and utilized for identification of 153 main QTLs and 25 epistatic QTLs with low to moderate phenotypic variance for drought tolerance-related traits (Gautami et al. 2012). This study suggested the utilization of marker-assisted recurrent selection (MARS) and genomic selection (GS) for crop improvement. However, for other abiotic stresses like high temperature, efforts are currently being made to detect genomic regions using linkage mapping (JL 24 × 55-437) and bulk segregant transcriptome mapping approaches. Recently, about 19 main-effect QTLs were identified for drought tolerance and Fe chlorosis and identified several transcription factors like bHLH, MyB and NAM at the QTL region (Pandey et al. 2021). Fe chlorosis is often believed to occur in soils with higher pH and calcareous soils, limiting the crop productivity during drought conditions (Naidu et al. 2017). So far, not much emphasis has been paid on the mapping salinity and cold tolerance in groundnut. The reference collection of groundnut comprising of 300 genotypes was used in genotyping with DArT and SSR markers and phenotyping for 50 important agronomic, disease, quality traits and drought tolerance-related traits. The genotypes were phenotyped in well-watered and water-stressed conditions, and about 152 MTAs were detected in both conditions (Pandey et al. 2014).

The gene expression atlas has provided the information on network of genes expressed during different developmental stages of groundnut plant in *A. hypogaea* (Clevenger et al. 2016) and *A. fastigiata* (Sinha et al. 2020) subspecies. A report on the interpretation of the transcriptome profile of two wild species, *A. duranensis* and *A. magna*, identified eight candidate genes that shared identical expression profiles in response to drought conditions and recovery at multiple stages. The genes such as *NAC* and *bZIP1* were annotated to be involved in signalling in response to drought in *A. duranensis* roots. Other genes that are involved in primary metabolism (*CA* or *NIT*) and cell protection/adaptation mechanisms (*CDSP*, *DiP* or *EXLB*) were also reported in both *A. duranensis* and *A. magna* (Brasileiro et al. 2015).

Transcriptomics approaches to understand the mechanism of drought and the genes expressed during drought stress were identified (Quan et al. 2007; Bhogi Ding et al. 2014). Drought-induced transcription factors were identified using transcriptomics-based approach (Govind et al. 2009; Guimaraes et al. 2012).

Genetic engineering approaches have been utilized to introduce tolerant genes especially for drought and salinity into peanut. The genes like *AtDREB1A*, *AtNHX1*, *mtlD*, *AtNAC2*, *AtDREB2A*, *AtHB7* and *AtABF3* were introduced into groundnut from different sources like *Arabidopsis*, *Agrobacterium*, etc. (Bhatnagar-Mathur et al. 2007; Vadez et al. 2007; Asif et al. 2011, Patil et al. 2014; Pruthvi et al. 2014).

Though there are efforts to understand the mechanisms and identification of genomic regions by QTL or association mapping approaches for drought tolerance in groundnut, the information is not successfully utilized in molecular breeding to improve the cultivars for better water use efficiency and provide more yield in drought conditions. There is lot of potential to use genomics strategies and trait prediction using genomic selection to study abiotic stress tolerance and improve cultivars for the changing climate conditions due to global warming. The studies related to high-temperature tolerance, cold tolerance and salinity tolerance are still in infancy, and efforts are being made globally.

3.11 Transformation

Genetic engineering using transformation of foreign genes, either directly (biolistic) or via *Agrobacterium*, could significantly enhance the genetic potential of peanut for various traits (see Gantait and Mondal 2018). These efforts are favoured by the development of genotype-independent and enhanced *in planta Agrobacterium tumefaciens*-mediated genetic transformation method (Karthik et al. 2018). Several genes imparting resistance to biotic and abiotic stresses have been transferred to peanut, and the phenotypic changes were observed. Several studies reported engineering *AhFAD* genes to alter oil quality, depressing *AhFAD2* gene (Xu et al. 2018), HpRNA-mediated gene silencing of oleate desaturase (Yin et al. 2007), production of eicosapentaenoic acid (EPA, 20:5n-3) in transgenic peanut through the alternative Delta8-desaturase pathway (Wang et al. 2019) and increased oil content and altered fatty acid composition in seeds of peanut (Tang et al. 2018).

Ectopic expression of MYB repressor *GmMYB3a* improved drought tolerance and productivity of transgenic peanuts under water-deficit conditions (He et al. 2020). A novel salt-inducible WRKY transcription factor gene, *AhWRKY75*, conferred salt tolerance in transgenic peanut (Zhu et al. 2021). Bhalani et al. (2019) reported improved tolerance to soil-moisture-deficit stress among the transgenics expressing *AtDREB1A* which regulates the antioxidant mechanisms. Expression of *Escherichia coli*-derived mannitol-1-phosphate dehydrogenase (mtlD) in peanut improved photosynthetic, physio-biochemical and yield parameters under soil-moisture-deficit stress (Patel et al. 2017). RNA interference (RNAi) could prevent aflatoxin accumulation in transformed peanuts (Arias et al. 2015). Aflatoxin control by exogenous delivery of double-strand RNA (dsRNA) was also demonstrated (Power et al. 2020). These efforts indicate the possibilities of enhancing the genetic potential of peanut in the future.

Other genetic engineering methods (see Krishna et al. 2015) might also contribute for the genetic enhancement of peanut. Gene editing using CRISPR/Cas9 technology (Yuan et al. 2019) and TALEN-mediated targeted mutagenesis (Wen et al. 2018) has been reported for enhancing the oleate content by targeting *AhFAD2* gene. These successful examples along with the advancement in the genomics would certainly attract more efforts to improve peanut for various traits. Shu et al. (2020) applied the CRISPR/Cas9 tool in peanut hairy root transformation system to explore the function of nod factor receptor (NFR) genes which initiate peanut plant response to rhizobia. With the advancements in pangenomics and population genomics for exploring the allelic differences underlying the phenotypic variations, gene editing could be more promising in peanut.

3.12 Conclusion and Future Perspective

Apart from domestication and evolution, the cultivated groundnut has been subjected for genetic enhancement for improved agronomic traits, productivity, tolerance to biotic and abiotic stresses and quality. Improved varieties with significant genetic gains have been bred worldwide. Efforts to utilize the vast wild relatives in groundnut breeding have also contributed immensely in exploiting the novel genes/alleles. New avenues encompassing the biotechnological and omics approaches are promising. Genomics, transcriptomics and proteomics of groundnut are now expanding towards pangenome, super-pangenome and population genome to understand the basis and the extent of variability for the important traits. Genetic engineering including overexpression, gene suppression and gene editing is also in progress for the genetic enhancement of groundnut. Overall, these technical advancements might supplement the conventional methods to realize genetic enhancement of groundnut in the future.

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Chapter 4
Recent Advances in Genetics, Genomics, and Breeding for Nutritional Quality in Groundnut

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Abstract Groundnut is an essential oilseed legume primarily cultivated in Asia, Africa, and the Americas. It is referred to as the "poor person's almond/protein" which serves essential amino acids and nutrients required for good health. Besides its health benefits, it is affordable and easily cultivated in semi-arid tropics, and the idea of further enrichment of micronutrient and protein content may help in resolv-

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ing the issue of hidden hunger especially in Asian and African countries. Even the World Health Organization has recommended the use of high-protein ready-to-use therapeutic food product, PlumpyNut, from groundnut. The availability of high oleic groundnut further provides opportunity for consumers to have affordable cooking oil with comparable quality benefits to olive oil. The current emphasis on varietal development is yield and oil content in addition to disease resistance, which should now also put emphasis in developing nutrition-rich groundnut varieties. Genomics-assisted breeding can accelerate the process of developing nutrition-rich groundnut; however, identification of genes and associated markers is the prerequisite genomic information. This chapter presents the current status on breeding, genetics, and genomics studies on nutritional traits in addition to some successful examples such as high oleic varieties wherein the marker application helped in breeding high oleic varieties faster and with more precision.

Keywords Malnutrition · Vitamins · Minerals · Groundnut · Antioxidants · Marker-assisted selection · Genomics-assisted breeding

4.1 Introduction

Malnutrition or "hidden hunger" is a serious health issue in developed as well as developing countries, and the major cause of malnutrition is unbalanced diet. For instance, a person is malnourished when he is taking very large quantity or too small quantity of nutrients in the diet. The green revolution has addressed the food demand of a large growing population, and now there is a need to increase the quality of food with adequate amount of nutrients. Different forms of malnutrition are affecting different age groups. Around 462 million adults are underweight, whereas 1.9 billion are overweight or obese. Among children, one in ten is born with low birth weight. Approximately 45% of deaths among under 5-year-old children are due to undernutrition (WHO 2018).The mortality rate in these different age groups has skyrocketed due to nutrition deficiency leading to becoming immuno-compressive and more prone to diseases such as pneumonia and tuberculosis (Behl 2017). In rural and tribal areas, due to their low socioeconomic status, high numbers of undernutrition are noted. The human body performs complex functions which require energy in the form of proteins and carbohydrates and supplements such as vitamins and micronutrients for normal functioning. These nutrients are mainly sourced

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through daily dietary food and not synthesized by the body. Therefore, when their intake is insufficient for longer time, micronutrient deficiency disorders are the consequence. No single food contains all vitamins and minerals, so consumers have to balance and diversify their diet to ensure optimum nutrient intake. Malnutrition remains to be an important public health problem in India even with having several major programs to report the concern, viz., the Integrated Child Development Scheme ICDS, Mid-Day Meal (MDM), and also the Food Security Act (Viswanathan 2014; Arumugam 2015). To address this serious issue, functional foods rich in carbohydrate, fat, protein, minerals, and vitamins can be added in the diet.

Groundnut or peanut (*Arachis hypogaea* L.) is a nutritious food popularly known as poor persons' almond because of its nutritional value and availability in ample quantity accessible in cheaper price to the rural and tribal areas of the world. It is generally cultivated in semiarid tropic regions in over 100 countries of Asia, Africa, and America (Fig. 4.1). Presently, groundnut is cultivated globally in over 29.6 million hectares with a yield of 48.8 million tons of unshelled pods during 2019 (http://www.fao.org/faostat/en/#data/QC). China is the top producer of groundnut in the world with 17.5 million tons of unshelled pods during 2019, while India is the second biggest producer of groundnut with yield of 6.7 million tons of unshelled pods during 2019. Groundnut is the richest source of energy component like oil (fat), protein, and carbohydrates. Additionally, it contains vitamins, minerals, and antioxidant. It is an abundant source of protein with the capability of meeting 46% of recommended daily allowance. Essential vitamins such as vitamin B, C, E, and K are important for normal body growth, boosting the immune system, and improving metabolism. Furthermore, minerals such as iron, zinc, copper, calcium, magnesium, manganese, and selenium are important for the cardiac disease, enhance the immunity system, and have important anti aging property.

Groundnut with all the above essential vitamins and minerals also represents a high-calorie diet with 884 calories per 100 g of oil. In Africa, a ready-to-use therapeutic food PlumpyNut prepared from groundnut is a popular protein source used to reduce malnutrition in children because of its high protein content (25%) which is

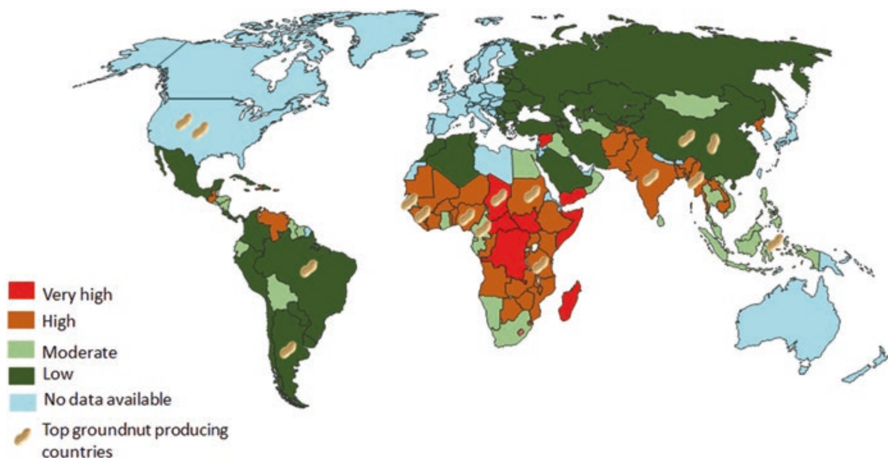

Fig. 4.1 Global malnutrition status in different countries from very high, high, moderate, and low severity and top 13 groundnut producing countries

higher than any true nut (UNICEF 2007). In western countries, groundnuts are mainly used in industries for making confectionery products, butter, chocolates, etc. Bold seeded groundnuts are consumed directly in roasted or boiled form as table purpose. However, in Asian countries like India and China, groundnuts are primarily crushed for extracting edible oil which is used for cooking and deep frying. Groundnut oleic acid is a monounsaturated fatty acid which lowers cholesterol in the diet and protects from cardiovascular diseases. Recently, two high oleic groundnut varieties are developed and released in India for commercial cultivation. The cake derived after extracting oil is largely used in animal feed industry because of its high protein content. After harvesting groundnut haulm, shells offer protein rich forage and silage for livestock feed especially for milking cattle. Consumption of small quantity of groundnut can take care of major percentage of recommended daily intake, which makes this crop of great importance in combating malnutrition across the world. However, there are several anti-nutrients or contaminants such as allergens and aflatoxin contamination, respectively, affecting nutritional quality of groundnut. Groundnut allergy above 24 ppm is a severe health hazard particularly in western countries of the world. The ELISA-based protocol for Ara h1, Ara h2, Ara h3, Ara h6, and Ara h8 (Pandey et al. 2019a) and its deployment in screening large number of genotypes identified groundnut lines with very low allergen content as compared to the varieties that are prevalent in current seed and food chain (Pandey et al. 2019b).

Molecular markers and speed breeding are the important genomic resources which have accelerated the breeding process making them faster and more precise (Pandey et al. 2020). In addition to conventional breeding approaches, the development of diagnostic markers (*FAD2A* and *FAD2B*) linked with high oleic acid trait in groundnut has been successfully used in the development of several high oleic groundnut varieties across the world. Currently, such diagnostic markers are being discovered for various traits using different marker systems to use in molecular breeding programs (Chu et al. 2011; Janila et al. 2016; Pandey et al. 2016; Kumar et al. 2020). Deployment of a combination of diagnostic markers for pyramiding quality and nutritional traits provided cost-effective way in performing marker-based early generation selection of desired lines (Deshmukh et al. 2020; Shasidhar et al. 2020).

4.2 Ready-to-Use Therapeutic Foods (RUTF) Made from Groundnut

Recently, the World Health Organization (WHO) recognized the importance of RUTF for malnourished children due to high nutrient content. Groundnut and its products are rich in nutrients such as protein, edible fats, fibers, vitamins, and minerals, making them a practical and handy option for improving the nutritional status by providing critical nutrients essential for growth, development, metabolism, and

boosting immunity (Geulein 2010). PlumpyNut is a crucial RUTF product produced by Nutriset, which is specifically designed to treat acute malnutrition without any complications (Ali et al. 2013). PlumpyNut is a groundnut-based paste composed of vegetable fat, groundnut butter, skimmed milk powder, sugar, malt dextrin, lactose rum, mineral, and vitamin complex. One sachet of it has an energy value of 500 Kcal. It is nutritionally dense and equivalent to F-100 therapeutic milk and can be eaten by babies who aren't yet ready for solid foods. It is portable and nonperishable as it is served in a foil pouch. PlumpyNut has a long shelf life and can be stored even without refrigeration for longer periods of time (max. 24 months).

Furthermore, groundnut butter is high in calories and fat; two tablespoons daily of peanut butter can deliver more than a quarter of recommended daily intake of fat. According to USDA, two tablespoons (32 g) of smooth groundnut butter with added salt contain 7 g of protein, 8 g of carbohydrates, 16 g of fat, 140 mg of sodium, 2 g of fiber, and 3 g of sugars along with several vitamins and which provide a total of 190 calories. Peanut butter can be eaten as a bread spread or as a dip for fruits and veggies, which is a delicious way to meet daily amount of fruits and veggies. It can also be blended into a smoothie or a protein shake or swirled into vanilla yogurt for a healthy breakfast. Though groundnut allergies are one of the most severe food allergies, nutritional benefits of Peanut butter countervail the consequences, especially for people who are nutritionally deprived or who don't get enough protein in their daily intake.

Additionally, groundnut chikki is also delicious and a traditional Indian sweet dish. It is prepared from roasted groundnuts, jaggery, and ghee. It is a combination of vital vitamins and proteins of groundnuts and good amount of iron from jaggery and ghee which is a powerhouse for multivitamins and omega fatty acids. One piece of groundnut chikki provides 79 calories, out of which proteins comprise of 8 calories, and carbohydrates account for 39 calories and the remaining 32 calories come from fats and fibers. Groundnuts in chikki which possess monounsaturated fatty acids especially oleic acid can help in maintaining the cholesterol levels in the blood. Masala groundnut is a crispy and tempting popular snack made from salted roasted groundnuts coated with thick paste of besan prepared with gram flour and rice flour in 2:1 ratio, red chili powder, salt, ginger garlic paste, turmeric, and water. The roasted groundnut seeds coated with gram flour provide ample amount of protein, fats, and carbohydrates along with good amounts of iron, thiamine, and folate. Besides protein, corn flour provides adequate amounts of fiber. Ginger garlic paste helps in digestion and prevents bloating or gas formation. Red chilli powder, turmeric, and chat masala added in this recipe not only intensify the flavor but also provide requisite nutrition to the body, thereby avoiding untimely hunger pangs.

Snacking on dry-roasted groundnuts between meals provides ample amount of nutrients for sustaining energy levels and maintaining active lifestyle. Roasting increases anti-oxidant levels such as p-Coumaric acid, removes toxic aflatoxins, and enhances the taste. These are high-protein low cal snack where each ounce provides 160 calories within a healthy range. Roasted groundnuts can also be used as toppings on salads, sundaes, desserts, other dairy-based preparations, and pasta dishes, which makes it versatile. However, boiling groundnuts enriches their nutritional and

anti-oxidant profile and offers unique flavor and taste. Boiled groundnuts can be directly enjoyed as a snack or can be made into soup or sauces. In rural areas, the freshly harvested groundnut (shelled) boiled in saltwater is used as delicious food which is an important protein source. Bumbu kacang (groundnut sauce), groundnut "chutney" or paste, and groundnut milk (lactose-free healthy drink) are some of the healthy groundnut products. In India, groundnut oil has highest consumption rate with 30%, 25% for snacks, 12.12% is exported after allergens and aflatoxin estimation, 12% for planting, 8% used for chikki production, 6% in daily routine foods, 5% for salted groundnuts, 1.26% shelled boiled and roasted,0.44% for export quality butter and 0.03% for chocolate production (Fig. 4.2). A jar of groundnut butter and a bag of roasted groundnuts can last up to a year in the refrigerator, which makes them sustainable and available year-round.

4.3 Nutritional Value of Groundnut

Groundnuts are inexpensive and valuable source of nutrients mostly for the people with low economic status. It is an admirable source of amino acids present in protein and lipids such as saturated and unsaturated fatty acids and other most healthy component such as dietary fibers and polysaccharides present in carbohydrates. The proximate composition of groundnut nutrient constituent recently reported was 31–46% fat, 20.7–25.3% protein, and 21–37% carbohydrate (Bonku and Yu 2020). The constituent of Indian raw groundnut kernel was stated as 47.27% fat, 25.48% protein, and 17.43% carbohydrate (Bonku and Yu 2020). The nutrient composition in 100 g of raw groundnuts is presented in Table 4.1.

4.3.1 Protein

Groundnut is an excellent source of proteins compared to other nuts. Consumption of 100 g of groundnut kernel can provide 46% daily protein requirement of our body. It has all amino acid in different proportions (Table 4.2). Groundnut kernel proteins have various protein subunits such as arachin, conarachin I, and conarachin II (Yamada et al. 1979). There is no difference between the subunits of conarchin and arachin. Arachin is divided into four classes: class I, constituting three acidic subunits with 47.5 kDa, 45.1 kDa, and 42.6 kDa molecular weight and one basic subunit with 21.4 kDa MW; class II, constituting three acidic subunits with 47.5 kDa, 45.1 kDa, and41.2 kDa MW and one basic subunit with 21.4 kDa MW; class III which is an additive pattern of class I and class II; and class IV constituting two acidic polypeptides with 47.5 kDa, 45.1 kDa MW and one basic peptide with 21.4 kDa MW (Krishna et al. 1986). Conarchin class I and II have comparatively

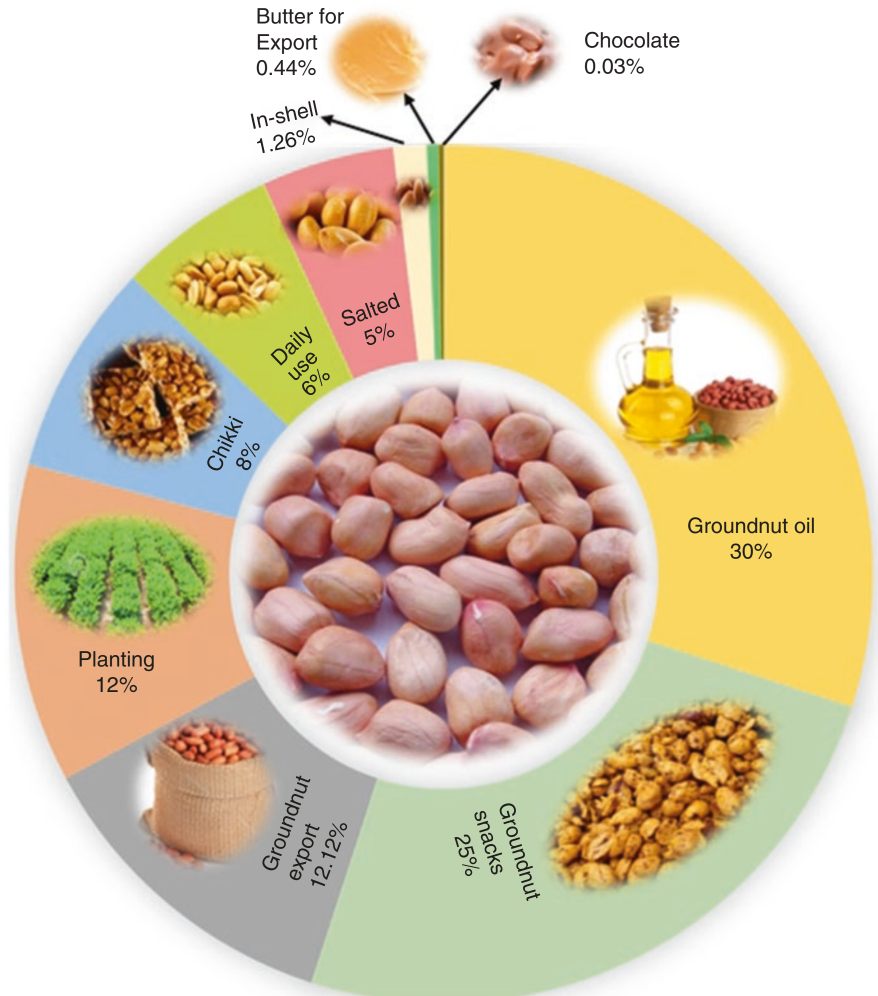

Fig. 4.2 Groundnut consumption rate in India by category
Source: Presentation by http://www.kanbifoods.com/duringthe ICAR DGR Industry Interface Meet 2020 (7th Dec 2020), at ICAR-DGR, Junagadh, Gujarat, India

higher lysine and methionine than arachin (Savage and Keenan 1994). Therefore, the nutritive value of groundnut can be increased more by increasing the conarachin proportion which accounts around 33% of total protein in groundnut kernel. Groundnut is a rich source of glutamic acid, aspartic acid, leucine, and arginine (Adeyeye 2010). Industrially, groundnut proteins are very useful for the formulation of new high protein product and for protein preparation.

Table 4.1 Summary of the nutrition compound and its phenotyping platforms and health benefits in groundnut

Compounds	Raw peanut (100 g)	% RDA (recommend daily allowance)	Health benefits	Phenotyping tools
Carbohydrates	16 g	12	Energy releasing molecules	NMR
Fat	49 g	165	Lowers the risk of heart disease and stroke	NIRS
Protein	26 g	46	Good emulsifying activity, good emulsifying stability, and good water storage capacity	NMR, NIRS
Dietary fibers	9 g	22	It may reduce the risk of cardiac disease, gastric problems and cancer and reduce the risk of metabolic disorder such as diabetes, cardiac disease, cancer, and disorder of the immune system	NMR
Vitamins				
Folates	240 µg	60	Significant particularly in early stages and pregnancy since it helps in the production and support of cells	GC-MS
Niacin	12.066	75	Reduced the risk of heart disease	GC-MS
Pantothenic acid	1.76 mg	35	Pantothenic acid aids in the digestion and combination of unsaturated fats	GC-MS
Pyridoxine	0.34 mg	27	It works as cofactor	GC-MS
Riboflavin	0.13 mg	10	It is important for the metabolism of fats, carbohydrates, and proteins and is needed for skin well-being and normal vision	GC-MS
Thiamin	0.64 mg	53	Thiamine (B1) having thiamine pyrophosphate as the coenzyme, assuming a significant function in oxidative decarboxylation and co-carboxylation responses related with sugar and amino acid digestion that is needed for energy metabolism, and it is significant for the nerve and brain	GC-MS
Vitamin E	8.33 mg	55.5	Antioxidative vitamin, preventing the oxidation of hemoglobin and prevents the oxidation of unsaturated fatty acids present in consumed foods	GC-MS
Minerals				
Calcium	92 mg	9	Important for the normal function of the visual cycle and in the mechanism of blood coagulation also associated with muscle physiology	ICP-OES

(continued)

Table 4.1 (continued)

Compounds	Raw peanut (100 g)	% RDA (recommend daily allowance)	Health benefits	Phenotyping tools
Copper	1.14 mg	127	Synthesized the key proteins such as collagen and hemoglobin	ICP-OES
Iron	4.58 mg	57	Chelating agent, involved in oxygen transport, regulate cell growth and differentiation	ICP-OES
Zinc	3.27 mg	30	Zinc is a basic mineral for typical development and improvement during pregnancy, youth, and immaturity	ICP-OES
Magnesium	168 mg	42	Helps in digestion by relieving constipation, increases energy level, and regulates the level of calcium, potassium, and sodium	ICP-OES
Manganese	1.934 mg	84	A trace element	ICP-OES
Phosphorus	76 mg	54	Important for metabolism, balances body PH, maintains energy levels, maintains strong bones, detoxes the body through urination and excretion	ICP-OES
Selenium	7.6 μg	13	It prevents the cancer and also has antiaging effects	ICP-OES
Sodium	18 mg	1		ICP-OES
Potassium	705 mg	15	It plays an important role in the brain and nerve functions and for muscle development	ICP-OES

Source: USDA National nutrient database; GC-MS, gas chromatography and mass spectroscopy; ICP-OES, inductively coupled plasma atomic emission spectroscopy; NMR, nuclear magnetic resonance; NIRS, near-infrared spectroscopy

4.3.2 Fatty Acids

Groundnut is the main source of edible oil with high concentration of monounsaturated fatty acid. The oil content slightly varies between different growth habits. In Spanish bunch, the oil content ranges between 42.0 and 53.8%, in Virginia bunch (spreading) it ranges between 45.0 and 58.6%, and in runner type it ranges between 41.2 and 53.6% on dry weight basis. Groundnut kernels constitute about 50% oil, of which 80% of groundnut oil contains oleic acid and linoleic acid and remaining 20% oil is made from the six saturated fatty acids such as palmitic acid, arachidic acid, steric acid, gadoleic acid, behenic acid, and lignoceric acid (Moore 1999; Janila et al. 2016) (Fig. 4.3). Hence, consumers are more benefited by high oleic acid as it enhances the shelf life of groundnut (Pandey et al. 2014a). Oleic acid plays an important role in health related issues such as suppressing the tumorigenesis and inflammatory disease. The autoxidative stability of oleic acid is higher than linoleic acid; therefore, products prepared from high oleic groundnut have longer shelf life

Table 4.2 Amino acid composition of dry-roasted groundnut and groundnut butter

Amino acid	Dry-roasted groundnut (100 g)	Groundnut butter (100 g)	Phenotyping tools
Essential			
Histidine	599	176	LC-MS
Isoleucine	833	195	LC-MS
Leucine	1535	489	LC-MS
Lysine	850	215	LC-MS
Methionine	291	84	LC-MS
Phenylalanine	1227	380	LC-MS
Threonine	811	166	LC-MS
Tryptophan	230	73	LC-MS
Valine	993	247	LC-MS
Non-essential			
Alanine	941	290	LC-MS
Arginine	2832	875	LC-MS
Glutamic acid	4949	1609	LC-MS
Aspartic acid	2888	965	LC-MS
Glycine	1427	454	LC-MS
Proline	1045	445	LC-MS
Tyrosine	963	262	LC-MS
Serine	1167	468	LC-MS

Source: USDA data base
https://tools.myfooddata.com/protein-calculator/173806/100g/1

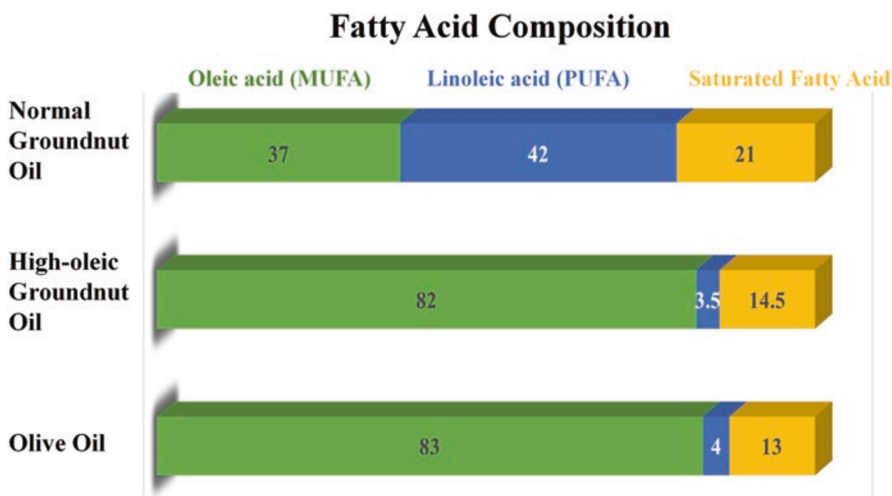

Fig. 4.3 Fatty acid composition in normal and high oleic groundnut as compared to olive oil

than linoleic acid. An ideal groundnut variety should have linoleic acid content less than 1%, because higher linoleic acid content contributes to oxidative rancidity (Janila et al. 2016). The alteration of oleic acid to linoleic acid through adding a

double bond is catalyzed by a unique enzyme fatty acid desaturase (*FAD*) which plays a necessary role in digestion of fatty acid and maintains the cell membranes. Oil content and oil quality can be estimated in very low cost by using the NIRS spectroscopy and gas chromatography. However, GLC (gas liquid chromatography) is an ideal method for determining the oil quality. The reason behind this is sometimes infrareds have difficulty in distinguishing the linoleic acid and eicosanoic acid (20:1). Different ranges (2.4–4.0%) for arachidonic are reported, but usual range of arachidonic acid is 0.9–3.5% (Hoffpauir 1953).

4.3.3 Dietary Fibers and Micronutrients

Usually we think that fruits and vegetables are rich source of fibers, but legumes are also chief source of dietary fibers. High fiber content of groundnut makes it a low glycemic index (GI) food with 14 GI and glycemic loads of 1% (USDA 2019). Low glycemic index foods slowly release sugar in the blood and therefore maintain blood sugar levels (Foster et al. 2002). Among all legumes, groundnut has likewise good quantity of fibers. The dietary fiber contributes 8.4 g per 100 g of raw groundnut with 22% RDA. Dietary fibers are distinguished into two parts, soluble and insoluble. Insoluble polysaccharides are cellulose and hemicellulose, and soluble oligosaccharides are raffinose, stachyose, and verbacose. Insoluble fiber has the ability to bind with toxic chemicals made through digestion of food allowing their consequent exclusion through the feces. Soluble fiber has also important property as it may decrease the risk of cardiac disease, gastric problems, and cancer. Therefore, the collective belongings of soluble and insoluble fibers reduce the risk of metabolic disorder such as diabetes, cardiac disease, cancer, and disorder of immune system (Bonku and Yu 2020).

Groundnuts are rich source of vitamins and minerals in measurable quantities (Table 4.1). Considering recommended daily values of vitamins such as folate, vitamin E, riboflavin, thiamine, biotin, and niacin as well as minerals such as iron, zinc, calcium, magnesium, copper, and phosphorous illuminates the beneficial role of groundnut in a well-balanced diet. Moreover, some of the highest quantities is that folate has a 60% RDA. Folate is an important vitamin required during pregnancy, as it helps in the production and support of cells (Whitney and Rolfes 2018). Niacin (75% RDA) reduces the risk of heart diseases, while thiamine (53% RDA) works as a cofactor for several metabolic enzymes. Manganese with 84% RDA is also a cofactor for metabolic enzymes, while copper with 127% RDA is producer of key proteins such as hemoglobin and collagen. Similarly, phosphorous has a 54% RDA and is important for metabolism which maintains strong bones. Minerals can be estimated using atomic absorption spectroscopy (AAS) or inductively coupled plasma atomic emission spectroscopy (ICP-OES), and vitamins can be estimated using the gas chromatography or mass spectroscopy (GC-MS).

4.3.4 Resveratrol

Reseveratrol is a part of polyphenol group of compounds with antioxidant properties. Resveratrol can protect from risk of cancer and heart diseases. Red colored grapes are rich in resveratrol, but now groundnuts are also emerging as good source of resveratrol in kernel and other parts of the plant, such as roots shell, leaves, etc. (Hasan et al. 2013). A stillbean resveratrol is a phytoalexin and secondary metabolite with low molecular weight (Sales and Resurreccion 2014). It also has anti-oxidant, antifungal, and anti-inflammatory properties playing important roles in defense against pre- and post-harvest aflatoxin contamination. Resveratrol consists of two forms: cis-resveratrol and trans-resveratrol. Trans-resveratrol is a more biologically active form of cis-resveratrol and has more proliferative property than cis-resveratrol. Trans-resveratrol holds potential health benefits against cardiovascular-related diseases, prevents the formation of tumor, prevents the neurodegenerative disease such as Alzheimer's disease, and has anti-aging properties (Sales and Resurreccion 2014). Recent report suggested that resveratrol content ranged between 58 and 619 μg/kg in raw groundnut kernel in which upon processing such as roasting, boiling, and peeling, resveratrol activity reduced 6–88%, 27–94%, and 46–100%, respectively (Bagade et al. 2020).

4.4 Genomics of Nutritional Quality Traits in Groundnut

4.4.1 Linkage Mapping

Molecular markers have been utilized for the improvement of nutritional quality-associated traits in groundnut (Sarvamangala et al. 2011; Wilson et al. 2017; Deshmukh et al. 2020; Shasidhar et al. 2020). The availability of groundnut reference genome for diploid progenitors (Bertioli et al. 2016; Chen et al. 2016) and tetraploid (Bertioli et al. 2019; Chen et al. 2019; Zhuang et al. 2019) has made it easy for genome-wide SNP variant genetic mapping and genome-wide association studies (GWAS) in groundnut. Groundnut germplasm holds high level of variation for oil content which provides an opportunity to perform GWAS to identify genomic regions associated with nutritional traits in groundnut (Yol et al. 2017). Several quantitative trait loci (QTLs) controlling grain oil content, fatty acid composition, and protein content have been reported (Pandey et al. 2014a; Shasidhar et al. 2017; Wilson et al. 2017; Liu et al. 2020a). Pandey et al. (2014a) used a RIL population (Sun Oleic 97R × NC94022) and identified six QTLs for oil content accounting 3.0–10.2 PVE% and nine QTLs for oil content with 3.9–14.0 PVE% in Tifrunner × GT-C20 RIL population. A major QTL (*qOCB3*) with 14.36% PVE with 3.9 LOD was detected on chromosome B03 for oil content (Huang et al. 2015). Subsequently, three major QTLs were identified with 18.0–25.0% PVE in advanced backcross population (Wilson et al. 2017); and eight QTLs were detected with 5.67–22.11

PVE% for RIL population (ICGV07368 × ICGV06420) for oil content (Shasidhar et al. 2017). Several studies reported oil content exhibits additive inheritance which provides an opportunity in pyramiding associated loci in the groundnut breeding program (Fu et al. 2017; Shasidhar et al. 2017; Zhaoming et al. 2017). Another three QTLs explaining 1.5–10.2% PVE were identified in a RIL population (Sarvamangala et al. 2011). Recently, seven QTLs were identified on five chromosomes (A04, A05, A08, B05, B06) accounting 6.07–27.19% PVE (Xuhua 13 × Zhonghua 6) including one major and stable QTL (*qOCA08.1*) has been identified on A08 with 10.14–27.19% PVE (Liu et al. 2020a). Similarly, 27 QTLs for oil content including A major and stable *qA05.1* QTL, with a LOD range of 13.62–26.94 and 9.62–22.74% PVE, were identified using whole-genome resequencing approach (Sun et al. 2021). A more recent study identified two major and stable QTLs *qOCB06* with 22.59% PVE and *qOCB10.1* with PVE range 9.18–12.55% across three environments in a RIL population Zhonghua10 × ICG12625 (Guo et al. 2021). Apart from oil content, the fatty acid components are also the important quality traits which are associated with groundnut product shelf life and benefit human health (O'bKeefe et al. 1993). Earlier study reported two mutants of fatty acid desaturase (FAD) gene *FAD2A* and *FAD2B* genes from A- and B-subgenomes, respectively, which were identified from high O/L genotypes and responsible to encode enzymes that allow the transition of oleic acid into linoleic acid in groundnut (Lopez et al. 2000; Chu et al. 2011).

Further, QTL analysis study was done in two RIL populations to analyze the relative contribution of FAD2 alleles in oil quality (Pandey et al. 2014a). This study reported 21 major effect QTLs with 1.04–42.33% PVE in SunOleic 97R × NC94022 population for oleic acid, linoleic acid, and oleic/linoleic acid ratio and 23 (M-QTLs) with 3.63–28.98% PVE in Tifrunner × GT-C20 population for oleic acid, linoleic acid, and oleic/linoleic acid ratio. Recently, (Hu et al. 2018) studied the effect of FAD2 alleles on oleic acid and linoleic acid content which was also validated in various genetic backgrounds utilizing high density genetic map. Sarvamangala et al. (2011) identified four QTLs associated with oleic acid, linoleic acid, and O/L ratio accounting to 1.4–9.7% PVE in RIL population (TG 26 × GPBD 4). A total of ten QTLs (seven major) accounting to 1.72–20.20% PVE were identified in RIL population (Zhonghua 10 × ICG12625) for six fatty acids (Huang et al. 2015). Another 20 major QTLs have been recently detected with 10.3–78.6 PVE % and LOD range from 3.7 to 191 in F_2 population (ICGV 06420 × SunOleic 95R) (Shasidhar et al. 2017). Another study identified a major QTL explaining a 15.1% PVE for oleic acid on chromosome A09 in a RIL population TMV 2 × TMV 2-NLM (Hake et al. 2017). A more recent study identified four QTL clusters for saturated fatty acid (palmitic, stearic, arachidic, behenic, and lignoceric acid) (Liu et al. 2019). Twenty major QTLs were detected on three QTL clusters (CLB04-1, CLB04-2, and CLB04-3) on chromosome B04 accounting 10.77–41.89% PVE. Another QTL cluster (CLB06) on chromosome B06 contained six QTLs for stearic, arachidic, and behenic acid with up to 20.32% phenotypic variation. Further research on these QTL clusters will help to understand fatty acid metabolism and will assist in the identification of diagnostic markers which can be utilized in improving groundnut cultivars using marker-assisted selection. Protein content is

also one of the important traits which enhances the nutritional quality of groundnut in case of both food and feed. Previous study reported six QTLs for protein content with 1.50–10.70% PVE and 2.87–3.63 LOD in RIL population (Sarvamangala et al. 2011), while a recent study identified one major QTL (AhTE0003-AhTE0332) for protein content accounting for 26.4% PVE and 11.2 LOD on chromosome A10 (Hake et al. 2017). A very recent study identified nine additive QTLs for resveratrol content with 5.07–8.19% PVE and LOD 2.50–3.64 in RIL population (Luo et al. 2021).

4.4.2 Association Mapping

Association studies allow us to unravel the trait of interest in diverse panel with high precision; therefore, this approach can be useful for studying the genetics of nutritional quality traits in groundnut. Recently, a panel of 292 lines enabled identification of 12 associated markers for oil content including one highly stable association (*AGGS1014_2*) with 9.94% PVE (Liu et al. 2020b). Similarly, genetic basis of nutritional quality traits was examined using GWAS in reference set comprised of 300 diverse global collection of groundnut and enabled the detection of 24 marker trait associations (MTAs) in which 2 MTAs were identified for oleic acid content with 16.42–20.8% PVE%, 22 MTAs for O/L ratio with 13.7–47.45% PVE, 25 MTAs for oil content with 5.84% (gnPt-714399) to 40.37% (TC4G10) PVE, 11 MTAs for protein content with 11.63–36.1% PVE, and 1 MTA for zinc content with 15.63% PVE (Pandey et al. 2014b). Similarly, a GWAS analysis conducted in 120 genotypes from the US minicore collection led to identification of 24 QTLs for boron (B), 2 QTLs for copper (Cu), 6 QTLs for sodium (Na), 3 QTLs for sulfur (S), and 1 QTL for zinc (Zn) with 18.35–27.56% PVE. In addition, mining of genomic regions further discovered 110 casual candidate genes. Interestingly, *arahy.KQD4NT* (position 5,413,913−5,417,353) has been detected as the important elemental/metal transporter gene identified on chromosome B04 (Zhang et al. 2019). The list of QTLs identified for nutritional traits in groundnut is provided in Table 4.3.

Further such studies are required using high density genotyping and sequencing-based mapping leading to fine mapping the genomic regions and candidate gene discovery for their deployment in breeding nutrition-rich groundnut varieties faster and with more precision.

4.5 Breeding Biofortified Groundnut Varieties

Biofortification can reasonably combat malnutrition in more cost-efficient manner. It is a process of enriching or enhancing the nutritional value of crops with the help of breeding (Bouis and Saltzman 2017). High UFA to SFA ratio in groundnut makes it a healthy cooking oil when compared to palm and coconut oil (O'Byrne et al.

Table 4.3 Summary of the genetic mapping and genomic regions for nutritional trait in groundnut

Genotyping platforms/ mapping approach	Mapping population/diverse panel	Significant outcome	Reference
Linoleic acid, oleic acid, oleic/linoleic acid ratio (O/L), and other fatty acids			
1. SSR markers (genetic mapping)	Zhonghua 10 × ICG 12625	59 QTLs identified for saturated fatty acid with 3.63–43.4% PVE	Liu et al. (2019)
2. ddRAD-Seq and AhTE markers (genetic mapping)	TMV 2 × TMV 2-NLM	Detected one major QTL for oleic acid with 15.1 PVE % PVE on chromosome A09	Hake et al. (2017)
3. SSR, DArT, and DArTseq markers (genetic mapping)	ICGV 06420 × SunOleic 95R	Identified 20 major QTLs with 10.3–78.6 PVE % and LOD range from 3.7–191	Shasidhar et al. (2017)
4. SSR and SNP (genetic mapping)	Florunner × TxAG-6	17 QTLs were detected for fatty acid concentration with 12.00–32.00% PVE	Wilson et al. (2017)
5. SSR markers (genetic mapping)	Zhonghua 10 × ICG 12625	Identified seven major QTLs with 12.80–20.20% PVE	Huang et al. (2015)
6. SSR markers (genetic mapping)	SunOleic 97R × NC94022,Tifrunner × GT-C20	Identified 34 major QTLs on 5 linkage group for fatty acids	Wang et al. (2015)
7. SSR markers (genetic mapping)	SunOleic 97R × NC94022 (S-population), Tifrunner × GT-C20 (T-population)	Identified 21 (M-QTLs) with 1.04–42.33% PVE in S-population and 23 (M-QTLs) with 3.63–28.98% PVE in T-population	Pandey et al. (2014a)
8. SSR markers (genetic mapping)	TG 26 × GPBD 4	Identified four QTLs, with 1.40–9.70% PVE	Sarvamangala et al. (2011)
9. SSR markers (GWAS)	Diverse panel (300 genotypes)	24 marker trait associations (MTAs) were identified in which two MTAs were detected for oleic acid with 16.42–20.8% PVE and 22 MTAs were detected for OLR with 13.7–47.45% PVE	Pandey et al. (2014b)

(continued)

Table 4.3 (continued)

Genotyping platforms/ mapping approach	Mapping population/diverse panel	Significant outcome	Reference
Oil content			
10. ddRAD-seq (genetic mapping)	Xuhua 13 × Zhonghua 6	Seven QTLs have been detected on five chromosome (A04, A05, A08, B05, B06) with 6.07–27.19% PVE. One major and stable QTL (*qOCA08.1*) has been detected on A08 governing 10.14–27.19% PVE	Liu et al. (2020a)
11. SSR markers (GWAS)	292 association panel	Identified 12 associated markers for oil content including 1 highly stable association (*AGGS1014_2*) with 9.94% PVE	Liu et al. (2020b)
12. Whole-genome resequencing (genetic mapping)	Yuhua15 × W1202	Identified 27 QTLs for oil content including 1 major and stable qA05.1, with LOD range from 13.62 to 26.94 and 9.62–22.74% PVE	Sun et al. (2021)
13. SSR markers	Florunner × TxAG-6	Identified three QTLs for oil content with 18.00–25 PVE %	Wilson et al. (2017)
14. SSR, DArT, and DArTseq markers	ICGV 07368 × ICGV 06420	Identified two major QTLs (*qOc-A10* and *qOc-A02*), for oil content with 22.11 and 10.37% PVE and LOD score of 13.2 and 4.8	Shasidhar et al. (2017)
15. SSR markers (genetic mapping)	Zhonghua 10 × ICG 12625	Detected one major QTL (*qOCB3*) with 14.36 PVE % and 3.9 LOD on chromosome B3	Huang et al. (2015)
16. SSR markers (genetic mapping)	TG 26 × GPBD 4	Identified three QTLs, for oil content accounting for 1.50–10.2% PVE, and LOD 3.0–5.2	Sarvamangala et al. (2011)
17. SSR markers (bulked segregant analysis)	TG 26 × GPBD 4	Identified one major QTL with 11.03 PVE %	Gomez et al. (2009)
18. SSR markers (GWAS)	Diverse panel (300 genotypes)	Identified 25 MTAs for oil content with 5.84% (gnPt-714,399) to 40.37% (TC4G10) % PVE	Pandey et al. (2014b)

(continued)

Table 4.3 (continued)

Genotyping platforms/ mapping approach	Mapping population/diverse panel	Significant outcome	Reference
19. SSR markers (genetic mapping)	Zhonghua10 × ICG12625	Identified two major and stable QTLs (*qOCB06* accounting for 22.59% PVE and *qOCB10.1*accounting for 9.18–12.55%)	Guo et al. (2021)
Protein content			
20. SNP array-Affymetrix (GWAS)	Diverse panel (120 genotypes)	Identified 36 significant quantitative trait loci associated for boron, copper, sodium, sulfur, and zinc with 18.35% – 27.56% PVE	Zhang et al. (2019)
21. ddRAD-Seq and AhTE markers (genetic mapping)	TMV 2 × TMV 2-NLM	Identified one major QTL for protein content with 26.4% PVE and 11.2 LOD on chromosome A10	Hake et al. (2017)
22. SSR markers (genetic mapping)	TG 26 × GPBD 4	Identified six QTLs, for protein content with 1.50–10.70% PVE and 2.87–3.63 LOD	Sarvamangala et al. (2011)
23. SSR markers (GWAS)	Diverse panel (300 genotypes)	Identified 11 MTAs for protein content with 11.63–36.1% PVE and 1 MTA for zinc content with 15.63% PVE	Pandey et al. (2014b)
Resveratrol content			
24. DDRAD seq (genetic mapping)	Zhongua 6× Xuhua 13	Identified nine QTLs for resveratrol content with 5.07–8.19% PVE and 2.50–3.64 LOD	Luo et al. (2021)

1997). High oleic acid (HOA) in groundnut oil helps in lowering low density lipoprotein cholesterol (LDLC) levels and reduces the chances of cardiovascular diseases (CVD) (Bolton and Sanders 2002; Yamaki et al. 2005). So, breeding for improved groundnut lines with HOA is essential to make it a healthier option for eradication of malnutrition in rural and tribal areas. An attempt was made by Florida Agricultural Experiment Station, USA, and developed a multiline cultivar SunOleic 97R with 81.8% oleic acid content (Gorbet and Knauft 2000). It is comprised of three breeding lines originating from F435- 2-2-E-2-l-b4-E-b2-b3-l-E (high oleic) and "Sunrunner" (F519-9) cross, where the latter was harnessed as the recurrent parent (Norden et al. 1985;Gorbet and Knauft 2000). With the development of diagnostic markers for HOA, marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) have flattered as the fruitful approaches in groundnut for attaining crop improvement (Pandey et al. 2020). Accordingly, an attempt was made

to enhance oleic acid level in Tifguard (nematode-resistant cultivar) using linked markers for HOA and nematode resistance by two simultaneous backcross programs where Florida-07 and Georgia-02C were used as donors for HOA (Chu et al. 2011). To supplement the accuracy of MAS, phenotyping was performed on progenies of BC_3F_2 which showed homozygosity for HOA and nematode resistance alleles. By using MABC and MAS, mutant alleles of *FAD2A* and *FAD2B* were transferred from SunOleic 95R (HOA line) into ICGV 06420, ICGV 06142, and ICGV 06110 (Janila et al. 2016). As a result, 27 lines were improved with 53–58% of oil content and ~ 80% of oleic acid alongside 28 lines with 42–50% of oil content and ~ 80% of oleic acid. Thus, backcross lines with HOA were produced and advanced to multilocation yield trials. As a consequence, “Girnar 4” and “Girnar 5” were identified and released as best performing varieties with HOA content in India in India 2020 (Nawade et al. 2016, 2019; Bera et al. 2018a, 2018b, 2019; Kamdar et al. 2020). In an effort, KASP assay-based MABC was deployed for detection of *FAD2A* mutations to improve the oleic acid content in four groundnut cultivars (Yuanza 9102, Yuhua 9326, Yuhua 9327, and Yuhua 15) of China (Huang et al. 2019). As a result, 24 HOA lines with similar agro-morphological features as that of recurrent parents (with 79.49–92.31% genome recovery) were developed and are going through multilocation trials for potential release. GJGHPS 1, GJG 9, and GG 20 were deployed using MABC approach to improve both HOA content and foliar disease resistance (FDR) (Shasidhar et al. 2020). As a consequence, >50 FDR ILs (introgression lines) and > 80 high oleic ILs were developed in BC_3F_7 generation and carried forward for seed multiplication. Similarly, >200 ILs (BC_3F_4) have been generated by using Kadiri 6, Dh86, ICGV 00351, and ICGV 87846 for HOA and FDR (**ICRISAT unpublished**). >200 pyramided lines were also developed by using the above ILs by integrating HOA and FDR into all of the six varieties and are carried out for further testing, evaluation, and release. Besides, >300 HOA breeding lines (F3-F7 and BC3F3-BC3F7) have been developed in the background of high yielding varieties like GG22, GG20, GJG32, Kadiri-6, DH86, DH256, DH257, Kadiri Lepakshi, TG37A, TKG19A, TG51, TG81, JL 501, Girnar 2, NRCGCS268, and NRCGCS257 and are in different stages of testing (**ICAR-Directorate of Groundnut Research**). Almost all HOA groundnut cultivars resulted from natural mutations in *ahFAD2A* and *ahFAD2B* genes until Minhua 8 and Minhua 6 were subjected to gamma-ray and EMS-based mutagenesis, respectively, to create new high oleate lines in groundnut (Zhuang et al. 2019). As a result, three varieties from Minhua 8 and four from Minhua 6 were developed with HOA and better agronomic performance. Two of them are apparently going through multilocation trials for further testing.

Recently, two elite varieties, GPBD 4 and G-252, with high productivity, oil content, resistance to late leaf spot (LLS), and rust diseases were improved for oleic acid content using MABC. Since both the recurrent parents already possessed the mutant allele at *AhFAD2A*, only mutant allele at *AhFAD2B* was transferred from the donor SunOleic 95R (oleate of 80.6%). Three rounds of backcrossing with foreground selection using allele-specific PCR and Kompetitive allele-specific PCR (KASP) assay identified a large number of plants homozygous for the mutant allele

at *AhFAD2B* in BC_nF_2 generations. Evaluation of the advanced generations could identify six and ten lines with significantly higher oleate than GPBD 4 and G-252, respectively. Considering the yield, shelling percentage, and oil and oleate content, the most promising lines $HOBC_2GPS_7$ and $HOBC_2G2S_5$ were selected with 112% and 142% oleate recovery over GPBD 4 and G-252, respectively (Jadhav et al. 2021). All the high oleic groundnut varieties are summarized in Table 4.4. With the advancement in genome-editing techniques, targeted mutations can be created to address human requirements in the foreseeable future (Pandey et al. 2020).

Table 4.4 Summary of the high oleic cultivars in groundnut

Institute/organization	Agronomic type	High oleic cultivars
University of Florida, USA	Runner	Andru II, Florida-07, FloRun™ 107, SunOleic95R, SunOleic97R, TUFRunner™ 511, TUFRunner™ 297, and York
	Virginia	Florida, Fancy, and Spain
Florida Agricultural Experiment Station, USA	Runner	Anorden, GP-1, and Hull
University of Georgia, USA	Runner	Georgia-14N, Georgia-02C, Georgia- 09B, Georgia-13M, Georgia-16HO, Tifguard High O/L, and GA-T2636M
	Virginia	Georgia-05E, Georgia-11J, Georgia Hi-O/L, and Georgia-08V
Lubrizol Crop, Ohio, USA	Runner	M2-225
	Virginia	Mycogen-Flavorunner
Texas A & M University, USA	Runner	TAMrun OL01, TAMrun OL02, and TAMrun OL07
	Spanish	Olin and TAMrun OL06
Texas Agri Life Research, USA	Runner	Tamrun OL11, TAMrun OL12, and Webb
	Spanish	Schubert
Oklahoma State University, USA	Runner	Lariat
	Virginia	VENUS
US Department of Agriculture – Agriculture Research Service, Oklahoma Agril Exp Station, Texas Agri Life Research, USA	Runner	TIFNV-High O/L
	Virginia	Red River Runner
US Department of Agriculture – Agriculture Research Service and North Carolina Agric. Res. Service, USA	Virginia	Brantley
North Carolina State University, USA	Virginia	Sullivan, Wynne (N08081olJC)
US Department of Agriculture – Agriculture Research Service, Texas Agri Life Research, and Oklahoma State University, USA	Spanish	ARSOK-S1 (TX996784)
Coastal Plain Experiment Station in Tifton, Georgia, USA	Spanish	Georgia-04S

(continued)

Table 4.4 (continued)

Institute/organization	Agronomic type	High oleic cultivars
New Mexico Agricultural State University, USA	Valencia	NuMex 01
Queensland Department of Employment, Economic Development and Innovation, Kingaroy, Grains Research and Development Corporation, Australia	Runner	Holt, Menzies, Page, Farnsfield, Redvale, and Tingoora
	Virginia	Middleton and Wheeler
Queensland Department of Employment, Economic Development and Innovation, Kingaroy, Australia	Virginia	Fisher
ICAR – Directorate of Groundnut Research, Junagadh, India	Virginia	Girnar 4 (ICGV 15083) and Girnar 5 (ICGV 15090)
Bhabha Atomic Research Centre, India	Spanish	TGM 192M
University of Agricultural Sciences, Dharwad and Bhabha Atomic Research Centre, India	Spanish	GM 4-3 and GM 6-1
Instituto Agronômico, Campinas, Brazil	Runner	IAC 503, IAC 505, IAC OL3, IAC OL4, and IAC OL 5
El Carmen, Argentina	Runner	EC-98 (AO) and Granoleico
	Virginia	GUASU (AO)
Cash Crops Research Institute, Guangxi Academy of Agricultural Sciences, Manning, China	Spanish	Guihua 37
Agricultural Research Council Grain Crops Institute, Potchefstroom, South Africa	Spanish	SA Juweel
Department of Southern Area Crop Science, Milyang, Korea	Spanish	K-Ol

Source: updated table from Nawade et al. 2018

4.6 Anti-nutritional Compounds

The availability of nutritious and safe food is severely influenced by the occurrence of toxin contamination produced by fungus along the food chain. It has been reported that mycotoxin affects one-fourth of the food crops in the world from standing in the field to postharvest processing and storage (Wu 2007). This fungal toxin is concerned with mainly three genera, namely, *Aspergillus*, *Fusarium*, and *Penicillium* (Reddy et al. 2010). Between them, the aflatoxin produced by the genus *Aspergillus* is widespread in major food crop such as maize, groundnut, dried fruits, spices, milk, and its product (Cheraghali et al. 2007; Romagnoli et al. 2007; Mutegi et al. 2009; Perrone et al. 2014; Iqbal et al. 2015; Pandey et al. 2019; Soni et al. 2020). The consumption of aflatoxin contaminated food has caused serious impact on human welfare (Sherif et al. 2009; Atherstone et al. 2016). Therefore, various nations have made severe safety rules and explicit guidelines with ideal edge to

control the exposure of aflatoxin to human health and welfare. The strict standard set by the European Union (EU) on the consumption of aflatoxin affected food is 4 μg/kg (European Commission-EC 2010). Similarly, the USA has made regulation in which aflatoxin contamination acceptable limit is 20 μg/kg (Wu, 2007). Nevertheless, standards set by the EU are not feasible to adopt worldwide because numbers of countries with lower GDP and poor economy especially those of Africa and Asia continents cannot follow these regulations due to additional cost of cultivation to meet those standard. Therefore, taken into consideration human health and wealth, alternatives are required to ensure the aflatoxin level below safe limit. Understanding of the biology of *Aspergillus* and the toxin produced is one step toward the improvement of crops with minimum aflatoxin contamination. Novel approaches are required for breeding groundnut varieties with low aflatoxin contamination which will ensure beneficial exports and healthy life.

Groundnut allergy is a severe food allergy and the most likely food to cause anaphylaxis or death which affects 1–2% of the world populations (Pandey et al. 2019a). Australia is the highly affected country across the world. Further, majorly affected countries include the USA, Canada, Denmark, the UK, and France. Recently, there was no available vaccine to resist allergy or method to reducing allergenicity from the groundnut food stuffs. Groundnut kernel comprises 32 various types of seed storage proteins, and among them 18 are allergenic proteins (Pele 2010). Groundnut allergens are distinguished into two classes such as major allergens and minor allergens within them Arah1, Arah2, Arah3, and Arah6 are classified as major allergens because of their lethal reactions recognized through IgE leading to anaphylaxis or death. In the previous study by the technique of bacterial artificial chromosome sequencing, around 617 kb from the cultivated groundnut genome (cv. Florunner UF-439-16-1003-2) and 215 kb from a wild relative (*Arachis duranensis*; A genome) were sequenced, and three Ara h 1, one Ara h 2, eight Ara h 3, and two Ara h 6 allergen coding genes were identified (Ratnaparkhe et al. 2014). Furthermore, 21 allergen coding genes were identified in the A genome. Among them, nine have already been identified in groundnut, and the remaining were homologous of the other crops (Chen et al. 2016). Lately, monoclonal antibody-based sandwich ELISA procedure has successfully been standardized on various sets of groundnut accessions and identified major allergens such as Ara h 1, Ara h 2, Ara h 3, Ara h 6, and Ara h 8 (Pandey et al. 2019a). Lower allergen containing lines were identified to design future breeding programs for development of low allergen containing groundnut varieties (Pandey et al. 2019b). Just a while ago, US FDA (Food and Drug Administration) studies showed that the groundnut consumption in 4–10 months (infancy) of age reduced the fear of groundnut allergy as it is specified that early consumption of groundnuts is one of the path to decrease the severe groundnut allergy (https://www.fda.gov/food/cfsan-constituentupdates/fda-acknowledges-qualified-health-claim-linking-earlygroundnut-introduction-and-reduced-risk). According to health claim with respect to allergic reactions on consuming groundnut, the connection between the utilization of food sources containing ground groundnuts, and a diminished danger of creating allergy, the FDA found the logical proof suitable as well as recommended to realizing offices to give

pure data for nourishments to try not to cheat consumers. Moreover, FDA would be analysed groundnut containing food accordingly qualified health certificate with respect to it decrease the fear of groundnut allergy (https://www.fda.gov/media/107357/download). With the help of low allergen content groundnut lines comes the opportunity to develop vaccine or therapeutic products and decrease the risk of groundnut allergies.

4.7 Summary

Groundnut is cultivated in semi-arid zone covering Asia and Africa continents where malnutrition is alarming. Conventional breeding approaches helped in breeding several high oleic varieties across the world, but not much emphasis has been paid for other nutritional compounds. The groundnut crop has huge potential to deliver highly nutrition-rich products to the consumers which not only will help in addressing the issue of malnutrition but also will help in providing high energy and nutrition by consuming less quantity food products. Next-generation genetic improvement approaches including genomic selection and genome-editing should be explored for breeding more nutritious groundnut varieties. Most importantly, deeper understanding on nutritional traits through precise phenotyping and sequencing will help in pinpointing the causal genes that make this crop so nutrition-rich. Also the promotion and adoption of nutrition dense groundnut varieties should be on priority in order to ensure the benefits reaching to the farmers, industry, and consumers.

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Chapter 5
Accelerated Breeding for *Brassica* Crops

Alison M. R. Ferrie and Patricia L. Polowick

Abstract Canola *(Brassica napus*, *Brassica rapa*, *or Brassica juncea)* is a major crop in Canada generating billions of dollars for the economy every year. However, to sustain production, there is a continued need to increase yield potential under adverse conditions (environmental, disease/pest pressures) as well as better utilize the products of the crop. Plant breeders are under pressure to develop cultivars that have traits than can adapt to the ever-changing growing conditions. Acceleration of the breeding program is one vital step of the process. In this chapter, we discuss and provide detailed protocols for doubled haploidy, speed breeding, and genetic transformation/gene-editing methods that can be and have been used in canola breeding programs to incorporate traits of interest and accelerate the development of new canola varieties for Canada.

Keywords *Brassica* · Canola · Doubled haploidy · Microspore embryogenesis · Speed breeding · Transformation

5.1 Introduction

The Brassicaceae family is an economically important group of plants which includes vegetable and oilseed crops. *Brassica napus* L. and other oilseed *Brassica* species are major crops grown around the world. The primary canola/oilseed *Brassica*-growing regions are Canada, Europe, China, India, and Australia (FAO December 2020). Generally, around 32–36 million ha are seeded with a production of 70–75 mMT; however, production does fluctuate due to climatic conditions (FAO.org). In Canada, the canola acreage seeded in 2020 was 8.4 m ha (2020) (Statistics Canada) with a production of about 16 mMT. Except for that produced under contract, there is very little rapeseed (HEAR – high erucic acid rapeseed) grown in Canada. Canola is a major crop in Canada used domestically as well as

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being exported internationally. The total value of canola exports is $9.3 billion Cdn, which includes seed, oil, and meal exports.

In Canada, "canola" is defined as "Seeds of the genus Brassica (*Brassica napus*, *Brassica rapa*, or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenylglucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Internationally regulated standard, Canola Council of Canada website). Canola is the number one edible oil in Canada and is marketed as a "heart-healthy oil" (Canola Council of Canada). Canola oil is a good source of vitamins E and K as well as omega-3, omega-6, and omega-9 fatty acids. There is 7% or less saturated fat and no transfatty acids.

In addition to being a healthy oil for human consumption, canola is being touted as a healthy oil for the environment. Biofuel production is increasing in Europe and North America, and canola oil can be used as it is a low-carbon, sustainable, and renewable resource. There is also increasing interest and usage in canola meal for livestock feeds and aquaculture.

To meet the edible oil and biofuel demands of a growing world population, there is a need to increase canola production without an associated increase in the number of hectares. This can be achieved through improved genetics and agronomics. To take advantage of these genetic improvements, methods that rapidly bring these new varieties to market are required.

5.2 *Brassica* Breeding Programs

As in most crop breeding programs, the objectives are improving yield and the agronomics (stress tolerance, disease resistance, insect resistance, and lodging resistance) of the crop. The yield and quality of the end products (oil, meal) are also important aspects of the breeding program whether it is for human consumption, aquaculture and livestock, or biofuels. Improvements in oil content, fatty acid composition, and protein quality are evaluated throughout the breeding process.

Conventional breeding programs can be time-consuming, taking 10+ years to develop new cultivars. Incorporating doubled haploidy, counter-season nurseries, and other technologies, new cultivars can be produced in approximately 6 years (Ulrich et al. 1984; Obermeier and Friedt 2015). Using DH methods, blackleg-resistant lines were identified within 4 years of the initial cross, and the cultivar was registered 6 years after the initial cross (Stringam et al. 1995a, b). Winter canola varieties have also been developed using DH methodologies, reducing the breeding cycle by 4 years (Cegielska-Taras et al. 2015). Genetic transformation permits the introduction not just of foreign genes for enhancement of agronomics and adjustments to seed composition, but the introgression of alleles from wild relatives or wide crosses without the requirement of backcrossing to remove the other undesired

alleles that may come with the cross. The advent of gene editing has the potential to make these changes even more directly. At the same time, where necessary, accelerated generation time, in combination with marker selection, can shorten the time required for backcrossing to obtain cultivars with the desired background.

5.3 Doubled Haploidy

The production of doubled haploid (DH) plants is an important technology which can be used for both research and plant breeding applications. Recent reviews have described the in vitro and in vivo methods used to generate DH plants (Niazian and Shariatpanahi 2020; Kalinowska et al. 2019). For the *Brassica* species, androgenesis, culture of the male gametophyte, is the preferred method, and much of the early work was focused on the culture of anthers (Keller and Armstrong 1978, 1979; Dunwell et al. 1983). Anther culture protocols are available and have been successfully used for cultivar development. However, to increase efficiency of the DH process, it was necessary to remove the anther wall and only culture the microspores. In 1982, Lichter (1982) first reported the production of haploid plants from isolated microspores of *B. napus*. The development of embryos from microspores is very similar to the development of zygotic embryos; therefore, the microspore culture system can also be used in biochemical, physiological, and genomic studies (Shahmir and Pauls 2021).

For the *Brassica* species, isolated microspore culture protocols are well established and are routinely used in breeding programs for developing new cultivars. Most of the canola breeding programs in Canada use DH methods. Although DH protocols have been used for many years, improvements and modifications are continually being published. There are a number of factors that influence microspore embryogenesis; this includes pre-isolation conditions (genotype, donor plant conditions, pretreatments, developmental stage of the microspore) and post-isolation conditions (media components, culture temperature). These factors, which are stress treatments or combinations of stress treatments, are a trigger for embryogenesis (Shariatpanahi et al. 2006: Islam and Tuteja 2012: Testillano 2019).

5.3.1 Pre-isolation Conditions

Generally, *B. napus* genotypes respond well to microspore embryogenesis protocols although there are some lines that do not respond, and fine-tuning of the protocol is required. Topas 4079, a DH line derived from the cultivar Topas, is used as the model line for microspore culture experiments (Pechan and Keller 1988). The cultivar Topas does not respond as well to microspore culture techniques (personal observation). For *B. rapa oleifera*, CV-2 is a highly responsive

line and has been used in many studies (Ferrie et al. 1995). Genotype screening studies have also been carried out with *B. carinata* (Barro and Martín 1999) and *B. juncea* (Hiramatsu et al. 1995; Lionneton et al. 2001) with the identification of embryogenic lines.

Healthy plants are essential for successful production of microspore-derived embryos. Although donor plants grown in the field or greenhouse can produce embryos, a controlled environmental unit is preferred as temperature can be adjusted and insects can be easily controlled. Most protocols require a cool temperature (10/5 °C) just prior to bolting. This slows the growth of the plant, which allows a longer period to select the microspores at the appropriate developmental stage. Similar growing conditions have also been reported for *B. rapa*, *B. juncea*, and *B. carinata* (Ferrie 2003). We have observed that the flowering plants can be kept for several months without a reduction in embryogenic response.

Pretreatment of the buds either chemically or physically is usually not required in the *Brassica* species especially when the donor plants are grown under cold conditions. Some studies have shown that storing the buds in a refrigerator for several days can substitute for the whole plant cold pretreatment. The best developmental stage of the microspore for DH culture is typically the mid-late uninucleate stage to early binucleate stage.

5.3.2 Post-isolation Conditions

Culture medium is a critical component influencing microspore embryogenesis response. The most commonly used basal medium is NLN, which was developed by Lichter (1982) and modified by others (Huang and Keller 1989). Over the years, additives have been used to enhance embryo quantity, quality, and regeneration to plants. As mentioned, stress treatments are a trigger for embryogenesis, but this can lead to autophagy, programmed cell death, and the production of reactive oxygen species (ROS), which are not conducive to embryogenesis. Additives are used to increase tolerance to these stress-inducing processes and products and allow for embryo development. A few of the more recent additives (antibiotics, growth regulators, polyamines, epigenetic chemicals) are listed in Table 5.1.

Other media additives have also been used but more so with a focus on the vegetable *Brassica* species. There is potential that these additives may have a beneficial effect on the oilseed *brassicas*. Examples include methylene blue (Chen et al. 2019b), reduced ascorbate, reduced glutathione (Zeng et al. 2017), activated charcoal (Pilih et al. 2018; Shumilina et al. 2020), nonionic surfactants (i.e., Pluronic F-68, Triton X-100, Tween 20) (Gao et al. 2020), thidiazuron, brassinolide (Jia et al. 2019a, b), L-ascorbic sodium salt (Niu et al. 2019), and suberolylanilidehydroxamic acid (Zhang et al. 2016).

The environmental conditions in which the microspores are maintained after isolation are also a factor influencing embryogenesis. With the *Brassica* species, a heat shock is generally required. For *B. napus* this is 32 °C for 72 h, for *B. rapa* 32 °C

Table 5.1 Media components that enhance the production of microspore-derived embryos and/or regeneration in *Brassica* species

Brassica species	Treatment	References
	Antibiotics	
B. napus	Cefotaxime, 50 mg/l,24 h	Ahmadi et al. (2014b)
B. napus	Vancomycin, 100 mg/l, 24–48 h	Ahmadi et al. (2014b)
B. napus	Ampicillin, 50–100 mg/l, continuous culture	Mineykina et al. (2020)
	Growth regulators	
B. napus	Abscisic acid, 0.5 mg/l, 12 h	Ahmadi et al. (2014b)
B. napus	Jasmonic acid, 1 mg/l, 24 h	Ahmadi et al. (2014a)
B. napus	Salicylic acid, 0.2 or 0.5 mg/l, 6 h	Ahmadi et al. (2014a)
	Polyamines	
B. napus	Putrescine, 0.2 mg/l, 48 h	Ahmadi et al. (2014a)
	Epigenetic chemicals	
B. napus	5-Azacytidine, 2.5 μM, 4 days	Solís et al. (2015)
B. napus	Trichostatin A, 0.5 μM, 20 h	Li et al. (2014)
B. napus	BIX-01294, 1–2.5 μM, 4 days	Berenguer et al. (2017)
	Other treatments	
B. napus	Chitosan, 10 mg/l, 2 days	Ahmadi and Shariatpanahi (2015)
B. napus	Proline, 100 mg/l, 2–5 days	Ahmadi and Shariatpanahi (2015)
B. napus	MnCl2, leupeptin, Ac-DEVD-CHO, concanamycin A, E64	Pérez-Pérez et al. (2019)
B. napus	H_3BO_3, 1162–2162 μM, continuous culture	Mahasuk et al. (2017)
B. napus	Iron starvation for 3 days	Leroux et al. (2016)

for 48 h, and for *B. juncea* 35 °C for 48 h (Ferrie 2003). Lower temperatures (18 °C) have also been shown to induce embryogenesis (Prem et al. 2012), and with the addition of polyethylene glycol, microspore-derived embryos could be induced at 4, 15, 18, and 24 °C (Ferrie and Keller 2007).

The basic *B. napus* protocol is presented here. For other oilseed *Brassica* species such as *B. rapa* and *B. juncea*, the protocols are similar except for slight modifications, which will be described.

Doubled haploidy protocol:

5.3.3 Donor Plant Conditions

- Grow donor plants in growth cabinets where environmental conditions and insect or disease problems can be easily controlled. Plants should be healthy, robust, and free of pests. Plants can be grown under greenhouse or field conditions, but we have found that there can be a decrease in embryogenesis under these conditions.

- Fill plant pots (15 cm) with a commercial mix (e.g., Redi-Earth soil mix). Add approximately 5 ml slow-release fertilizer (14-14-14 Nutricote 100) to each pot.
- Plant two seeds per pot which can be reduced to one plant/pot at the two- to three-leaf stage.
- Set growing conditions at 20/15 °C, 16-/8-h (day/night) photoperiod.
- Water plants three times a week with 0.35 g L^{-1} of 15-15-18 (15%N, 15% P, 18% K) fertilizer.
- After approximately 6 weeks (depends on the genotype), the first buds can be observed on the plants. Move plants to a lower temperature, 10/5 °C (day/night).
- Donor plants can remain productive for up to 6 months as long as they remain healthy and free from diseases and insects (Fig. 5.1a). Remove dead leaves and open flowers.

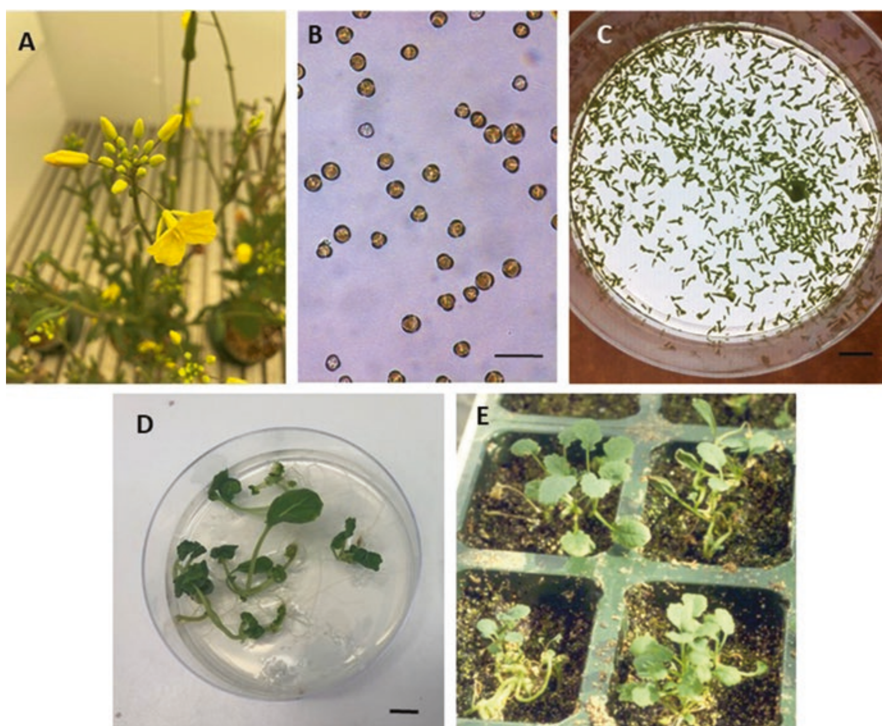

Fig. 5.1 Steps in the doubled haploidy process for *Brassica* species
(**a**) *Brassica* buds
(**b**) *Brassica* microspores at Day 0, scale bar = 50 μm
(**c**) Microspore-derived embryos at the cotyledonary stage, scale bar = 1 cm
(**d**) Regenerated plantlets on solid media, scale bar = 1 cm
(**e**) *Brassica* doubled haploid plants transferred to soil

5.3.4 Developmental Stage of the Pollen Grain

- Determine the developmental stage of the microspore. This can then be correlated with bud size for ease of bud selection (Fig. 5.1b). The developmental stage of the pollen grain and bud size can vary depending on the genotype and donor plant growing conditions; therefore, it is advisable to check the developmental stage when changing conditions or genotypes.
- On a microscope slide, crush an anther to release the microspores, and add a drop of 2 μg mL^{-1} DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) (Fan et al. 1988). Observe under a fluorescence microscope.

5.3.5 Microspore Culture

a. Selection and Sterilization

   - Remove buds (with microspores at the mid-late uninucleate stage) from the donor plants, and place on moist paper towels. Store buds in the refrigerator if bud selection is delayed or takes longer than 10 min.
   - Place approximately 50–75 buds in a Lipshaw basket, and surface sterilize in 6% sodium hypochlorite for 15 min on a shaker. Remove sodium hypochlorite solution and wash three times (5 minutes each) with sterile water.
   - We usually use six baskets per microspore isolation experiment.

b. Isolation of Microspores

   - It is important that the microspore isolation be done as quickly as possible after bud harvest as embryogenic frequency decreases with delays in the culture procedure.
   - Transfer buds to 50 mL beakers, and crush in 5 mL of half-strength B5-13 medium (B5 medium supplemented with 13% sucrose) (Gamborg et al. 1968) with a glass rod. Mini-blenders can also be used to crush buds. The objective is to break the anther wall to release the microspores but not damage the microspores.
   - Filter microspore suspension through a 44 μm nylon screen cloth into a 50 mL centrifuge tube.
   - Rinse beaker and filter three times with 5 mL of half-strength B5-13 and pour through the filter (final volume is 20 mL).
   - Centrifuge microspore suspension at 130–150 g for 3 min. Decant supernatant and add 5 mL half-strength B5-13 to the pellet.
   - Repeat previous step for a total of three washes. Prior to the third centrifugation step, place a drop of microspore suspension on a hemacytometer, and count the number of microspores.

c. Culture Media

- Add the required amount of modified Lichter medium (Lichter 1982) to achieve a density of 105 microspores mL−1. The medium used is NLN supplemented with 13% sucrose adjusted to pH 5.8.
- For B. rapa and B. juncea, NLN medium with 17% sucrose and 0.1 mg/l BA (benzyladenine) but without glutamine is used.
- Dispense 10 mL of microspore suspension into each 100 × 15 mm sterile Petri plate.
- Seal Petri plates with Parafilm, and label with the date, experiment number, genotype, and other pertinent information.

5.3.5.1 Culture Conditions

- Place Petri plates in a dark 32°C incubator.
- Remove plates after 72 h and place at 24 ºC, in the dark, for the remainder of 3 weeks.
- Embryos can usually be observed within 10 days of culture.
- For *B. rapa*, the microspores are incubated at 32 °C for 48 h. After 48 h, remove media from the Petri plates by pouring or pipetting into a centrifuge tube. Centrifuge for 3 min, at 130–150 g. Pour off the supernatant and add NLN with 10% sucrose, 0.8 g/l glutamine.
- For *B. juncea*, microspores are incubated at 35 °C for 48 h. After 48 h, the medium is changed, as in the process for *B. rapa*, to NLN medium with 13% sucrose and glutamine (0.8 g/l).

5.3.6 Embryo Culture

- Count embryos after 3 weeks. Take notes on embryo quality. At 3 weeks, embryos should be at the cotyledonary stage (Fig. 5.1c).
- Place Petri plates on a gyratory shaker (70 rpm) when embryos are at the cotyledonary stage. Culture conditions are 22 °C, 16-h photoperiod. Keep embryos on the shaker until green, usually about a week.
- Plate green cotyledonary embryos on solid B5 media (1% agar, 1% sucrose, pH 5.8) with ten embryos per Petri dish (100 mm × 15 mm).
- Petri plates are sealed with Parafilm and labeled with the necessary information (experiment number, date). This can be handwritten or using a barcode system.
- Culture conditions are 22 °C with a 14-h photoperiod and a light intensity of 150 μmol m^{-2} s^{-1}.
- To enhance regeneration, embryos can be placed in the cold (4 °C) for 2–3 weeks prior to plating on B5 media.

5.3.7 Plantlet Culture

- After 3 weeks, transfer the normal plantlets (i.e., shoot and root development similar to zygotic seedlings) to solid media (B5 with 0.8% agar, 2% sucrose, pH 5.8). Large Petri plates (25 × 100 mm), Magenta containers, or baby food jars can be used (Fig. 5.1d).
- Culture conditions are 12-h photoperiod, 22 °C.

5.3.8 Plantlet Transfer to Soil

- After an additional 3 weeks, remove plantlets from the Petri plates, and gently wash agar from the roots. Remove any dead leaves.
- Plant plantlets in flats containing a soil-less mix (Fig. 5.1e). Growth conditions are 20/15 °C, 16-h photoperiod. Keep flats covered to maintain high humidity.
- Slowly remove the lids over a period of a week to allow hardening of the young plantlets.
- Transplant into 15 cm pots and keep in the greenhouse. Keep plants well maintained to allow maximum seed set.

5.3.9 Chromosome Doubling

- Spontaneous chromosome doubling is very low in *Brassica* species; therefore, treating the microspores in vitro or treating the plantlets or plants with a chromosome doubling agent is required. We routinely use colchicine for in vitro chromosome doubling in our *B. napus* experiments. Other chromosome doubling agents can be used (i.e., trifluralin).
- Colchicine must be used with caution. Protective clothing, including gloves, mask, and eye protection, should be worn.
- Dissolve 3.4 g of colchicine in 1 L of water to make an aqueous solution of 0.34% colchicine which can be stored in the fridge in the dark.

a. In Vitro Chromosome Doubling

    - Culture microspores as outlined above.
    - Add NLN-13 media with colchicine (10^{-4} M) instead of regular NLN-13 culture initiation media.
    - After 72 h, change media to NLN-13 without colchicine. The media and microspores are removed from the Petri plate by pipette into a 50 mL centrifuge tube.
    - Centrifuge as before (130–150 g for 3 min).
    - Remove supernatant and discard appropriately.

- Add the same amount of NLN-13 media without colchicine and dispense into the same Petri plates. Reseal with Parafilm.
- For some *Brassica* species and for some genotypes, we have observed a slight decrease (<10%) in embryogenesis when colchicine is added to the media. Since the *Brassica* species tend to be embryogenic, we feel that this is a more efficient system than treating the plantlets.

b. Chromosome Doubling at the Plantlet Stage

- Prior to transferring plantlets to soil, remove plantlets from the agar, and submerge roots and crown in 0.34% solution of colchicine.
- After 1.5 h rinse roots and crown in water and transfer to a flat or pot with a soil-less mix. Grow as described above.

c. Chromosome Doubling at the Mature Plant Stage

- Early chromosome doubling techniques involved growing the plant to the flowering stage and determining if the plant had spontaneously doubled. If the plant was haploid, then the inflorescences would be cut back, and the soil would be washed from the roots. The roots and the crown would then be submerged in a colchicine solution (0.2% for 5–6 h) with aeration. This method is more time-consuming and requires more resources (chemical, personnel) than either the plantlet treatment or the in vitro approach.

The procedure from planting donor plant seed to harvesting seed from DH plants can take up to 9 months.

Doubled haploids can be used directly in a breeding program or as parents in crossing programs to bring new traits into the crop. *B. juncea* has a narrow genetic base, which may hinder the development of the crop. *B. nigra* is the B genome contributor to both *B. juncea* and *B. carinata* and could then be used to widen the genetic diversity of *B. juncea* (Ferrie and Caswell 2016). An efficient doubled haploidy protocol along with a microspore mutagenesis protocol would be one way to quickly generate variation in the B genome (Ferrie and Caswell 2016).

The development of microspore mutagenesis methodologies has been beneficial in generating DH lines with traits of interest in a number of different *Brassica* species. Combining mutagenesis with in vitro selection has increased the efficiency of identifying useful material and thereby accelerating the breeding of new cultivars with traits of interest. Chemical mutagenesis of microspores or resulting embryos or callus has been used to identify lines with alterations in the fatty acid profiles (Beaith et al. 2005; Ferrie et al. 2008; Daurova et al. 2020), glucosinolate content (Kott et al. 1996), disease resistance (e.g., *Sclerotinia sclerotiorum*, *Alternaria brassicicola*) (Ahmad et al. 1991; Liu et al. 2005), and herbicide resistance (Ahmad et al. 1991).

5.4 Speed Breeding

Speed breeding, also known as accelerated breeding, is a tool designed to shorten the life cycle of crop plants to hasten the breeding cycle in the long process of variety development. In addition to labs interested in hastening the breeding process, it has also been employed by NASA, along with Utah State University to assess how the constant light on space stations may influence food production. It has been tested on a number of crops including spring (*Triticum aestivum* L.), durum (*Triticum durum* Desf.) and winter wheat, barley (*Hordeum vulgare* L.), chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), and canola, evaluating extended daylength, light quality and intensity, and increased temperature (Ghosh et al. 2018; Watson et al. 2018). In addition, embryo rescue or immature seed germination has been used to bypass seed maturation for several species, including spring and winter wheat (Zheng et al. 2013; Ferrie and Polowick 2020) and legumes (Ribalta et al. 2017). There has also been a directed focus on hastening the life cycle of legumes, with an emphasis in early flowering and the germination of immature seed (Croser et al. 2016; Ribalta et al. 2017).

The systems developed resulted in a saving of 47–54 days per generation and the production of up to five generations of *Lupinus angustifolius* L. (lupin; Croser et al. 2016) or six generations of pea (Watson et al. 2018). Similarly, six (Watson et al. 2018) to eight (Zheng et al. 2013) generations of spring wheat or nine generations of barley (Zheng et al. 2013) could be cycled in 1 year, the latter study using embryo rescue to preclude seed maturation.

Conditions generally employed for hastening maturation include lighting (daylength/wavelength), temperature, pot size, watering, embryo rescue/immature seed germination, and higher density planting (Ghosh et al. 2018). Mobini et al. (2020) also tested the influence of cold temperatures and the addition of cytokinins, while Hickey et al. (2019) listed a number of potential improvements that could further shorten the generation time, including an elevated concentration of CO_2, hydroponics, and breaking of seed dormancy with plant growth regulators that promote germination. The combination of speed breeding treatments with rapid phenotyping has proven effective, for example, pod shattering resistance in canola (Watson et al. 2018) and disease resistance in wheat (Alahmad et al. 2018).

With canola and in the absence of DH technology, it normally takes five to six generations of backcrossing used to produce recombinant inbreeding lines (RILs) and near-isogenic lines (NILs). Using stress of a higher than normal temperature and long daylength (20 h) and with the added stress of restricted watering, Yao et al. (2016) found it was possible to complete five generations in 1 year, with the production of one pod per generation. With the addition of embryo culture with embryos as young as 10–12 days, depending upon the treatment, this was increased to seven generations. The limitation to a single pod restricts the ability to incorporate crossing, with too small a production of seed to accommodate segregation and selection.

Watson et al. (2018) were able to achieve four generations of canola, instead of the usual two to three per year achievable under greenhouse conditions. Using 22-h

days, high-pressure sodium lamps, and a temperature regime of 22 °C/17 °C, the average generation time under the speed breeding protocol was 98 days relative to normal greenhouse conditions that required 171 days on average. This meant the average generation time was reduced by 73 + 9 days, depending upon the genotype. The plants were sprayed with calcium nitrate to reduce calcium deficiency commonly associated with rapid growth. Normal pod development and seed yield was not significantly reduced; although the number was reduced somewhat in cv. Westar, it also appeared slightly increased in other cultivars (Watson et al. 2018 – supplemental data). While the number of generations achieved was not the same as with single-pod descent, this system could be used where segregation and selection for marker genes is required, including for multiple quantitative traits or for phenotyping.

5.5 Genetic Engineering

A further means of hastening breeding is through the use of genetic engineering. This can be used to directly incorporate desired genes/alleles from related species into elite lines without the requirements for interventions such as embryo rescue and to avoid the requirement for repeated backcrossing to remove the unwanted traits acquired through the wide cross. It can also be, and is more often, used to incorporate genes of interest that do not exist within the crop or its relatives (e.g., herbicide tolerance). In addition, genetic transformation is a valuable tool employed to validate gene function, through either over- or reduced expression prior to the work of traditional breeding for a desired trait.

Quest, the first transgenic *B. napus* variety registered for commercial purposes, was glyphosate (Roundup) herbicide tolerant and released by Monsanto in 1995 (History of Canola Seed Development | Canola Encyclopedia (canolacouncil.org)). Canola is one of the major crops represented by genetically modified varieties. According to the ISAAA database (GM Approval Database | GMO Database | GM Crop Approvals - ISAAA.org), as of January 2020, a total of 42 *B. napus* and 4 *B. rapa* canola varieties currently have approval for public use, commercialization, and importation worldwide. The main method of transformation of these varieties was through the use of *Agrobacterium tumefaciens*. Another common approach was through crossing with an existing transgenic line, with only a few generated through *A. rhizogenes* (1) and biolistics/particle bombardment (2). As might be expected, the majority (29) of the introduced traits involved herbicide tolerance and could also include pollination control (15). A total of nine lines produced a modified product, including four with modified oil/fatty acid composition (e.g., increased esterified lauric acid) or phytase for the breakdown of phytic acid; these lines were still largely developed in combination with herbicide tolerance. Most recently, there has been more of a focus on value-added modifications of the oils for health benefits to human consumers. For example, a canola line with a novel fatty acid, docosahexaenoic acid (DHA), was released (DHA Canola | GM Approval Database – ISAAA.

org), with levels of the healthy oils normally only found at similar levels in fish oil; this modification required the addition and expression of seven genes in the pathway (Petrie et al. 2020). Surprisingly, there are no lines registered with disease/insect/abiotic stress resistance.

In addition to material produced for commercialized lines of canola, transformation technology has been broadly used in canola (*Brassica napus* L.) as well as other members of the *Brassica* oilseed family, often for the validation of gene function. Some recent examples involve the testing from resistance to abiotic stresses (Lohani et al. 2020), frost-tolerant seed degreening (Perkins et al. 2019), and the production of antigens and adjuvants (Mohammadzadeh et al. 2020). There is continued interest in the production of novel healthy oils such as puccinic acid (Yang et al. 2020).

The more recent addition of gene-editing technology, using nucleases (e.g., zinc finger, TALENs, CRISPR/Cas9), permits minor changes in the endogenous DNA, through small deletions in a gene to alter or knock out function, substitutions to change gene function, or additions of short sequences (Chen et al. 2019a). Changes can be made in the promoter associated with a gene in order to manipulate the level of gene expression (Peng et al. 2017). It is also possible to make substitutions (Zhu et al. 2020) and small additions, but this is less common than deletions. In 2015, Cibus developed a transgene-free herbicide-tolerant canola using a proprietary genome-editing system to carry out an amino acid exchange using oligonucleotide-directed mutagenesis (Schinkel and Schillberg 2016), with cultivar now commercially available under the Clearfield banner. In some jurisdictions, gene editing can also bypass the burden of rigorous regulatory requirements surrounding GMO crops (Waltz 2018). For canola, some of the changes have included changes in plant architecture that result in more branches and higher yield (Stanic et al. 2020) or cause a reduction in the anti-nutritive phytase (Sashidhar et al. 2020).

Numerous protocols for the genetic transformation of canola have been published, dating back to the 1980s, some of which continue to be used, with local modifications (DeBlock et al. 1989; Moloney et al. 1989). Updated protocols deal with bottlenecks, especially as pertaining to the more recalcitrant genotypes, including elite commercial lines (Boszoradova et al. 2011; Chu et al. 2020).

The usual means of genetic transformation involve *Agrobacterium* and either hypocotyl sections or detached cotyledons (DeBlock et al. 1989; Moloney et al. 1989). Other tissues such as the epicotyl (Chu et al. 2020) or mesophyll protoplasts (Sahab et al. 2019) have been utilized; the latter can be useful for transient assays, including those to validate gene-editing components such as the guide RNA. The choice of explant can depend upon the selectable marker/selection chemical. Pre-co-cultivation culture and co-cultivation time are important (Cardoza and Stewart 2003), and there are often requirements for a series of five or more media during the course of preparation, co-cultivation, shoot initiation, shoot elongation, and regeneration. A range of cultivars can be used, but, as mentioned above, some genotypes are more amenable to transformation than others, and those, such as cv. Westar, are often utilized when the background of the material is not crucial. It should be noted that in the absence of efficient regeneration systems in cells that would allow direct uptake of the gene-editing components, the standard transformation protocols

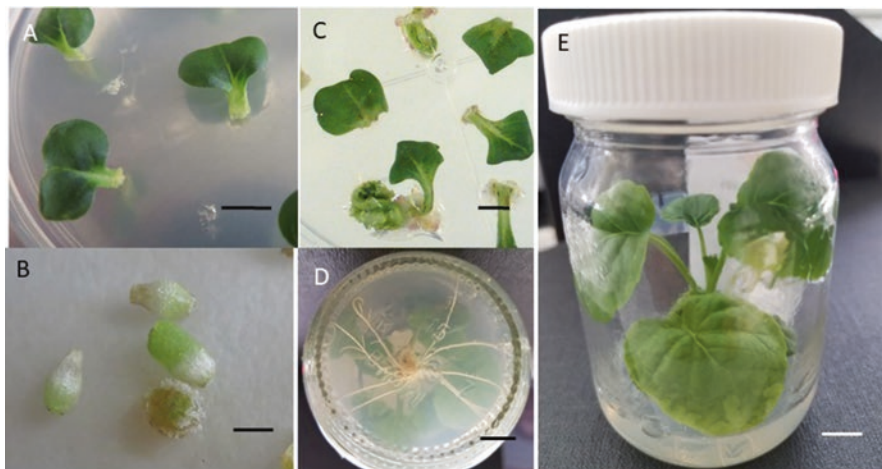

Fig. 5.2 Regeneration of genetically transformed *Brassica napus* plants
(**a**) Petiole explants with early stages of callus visible at base
(**b**) Hypocotyl segment explants in callus induction medium swollen in the center with early callus visible at ends
(**c**) Early shoot formation at the base of the petiole
(**d**) Roots formed at the bottom of shoot
(**e**) Rooted shoot in jar
Scale bar = 1 cm for A, C, D, E; 2 mm for B

remain in use for CRISPR technology (Sun et al. 2018; Stanic et al. 2020; Sashidhar et al. 2020).

The two protocols commonly used in our lab are detailed below. Regeneration through the bulk cotyledonary protocol is documented in Fig. 5.2.

5.5.1 Cotyledonary Petiole Transformation [Bulk Inoculation and Co-cultivation, from Lee (1996), as Modified from Moloney et al. (1989)]

5.5.1.1 Seed Sterilization and Germination

- Place approximately 15 mL of seed into a 50 mL screwcap (Falcon) tube. Add sufficient 70% ethanol to wet the seeds and leave for 15 s, drain, and fill the tube with full-strength commercial bleach (6.0% sodium hypochlorite) with a drop of wetting agent such as Tween 20.
- Keep for 15 min with occasional agitation. Pour off the bleach solution and add 0.025% mercuric chloride, also with a drop of Tween 20. Keep for 10 min, drain, and rinse well three times with sterile distilled water.

Note: For yellow-seeded *B. napus*, reduce the bleach concentration to 2.0% sodium hypochlorite. Unused seed can be dried in the laminar flow hood and used for subsequent experiments.

- Germinate seeds on seed germination medium (SGM; Table 5.2) in 15 × 60 mm Petri dishes, with media poured to within 4–5 mm of the top. Place 40 to 45 seeds per plate, pressing lightly into the surface for good contact, but not to submerge.
- Place the seeded plates (lid removed) into the lid of a larger sterile vessel (e.g., Magenta GA7 jars), and place the base over top. This allows the germinating seeds to grow tall and straight which makes it easier to harvest the cotyledons for inoculation.
- Keep at 25 °C, with 16 h light/8 h dark, approx. 80–100 µE light intensity at the shelf level.

Table 5.2 Composition of media used in cotyledonary petiole transformation (modified from Moloney et al. 1989)

Media component	Seed germination medium (SGM)	IM	SIM	SEM	RIM
MS/B5[a]	2.22 g/L	4.44 g/L		4.44 g/L	2.22 g/L
Murashige Minimal Organics (MMO)[b]			4.4 g/L		
2-(N-Morpholino) ethanesulfonic acid (MES)			500 mg/L	500 mg/L	500 mg/L
Benzyladenine (BA)			4.5 mg/L	0.5 mg/L	
Kanamycin			25 mg/L	25 mg/L	25 mg/L
Indole butyric acid (IBA)					0.2 mg/L
Timentin™ (added after autoclaving)			300 mg/L	300 mg/L	300 mg/L
Sucrose	1%	3%	3%	2%	1%
Bacto agar	0.80%				0.80%
Phytoblend agar[c]		0.70%	0.70%	0.90%	
pH	5.8	5.8	5.8	5.8	5.8
Plate size	15 × 60 mm	15 × 60 mm	25 × 100 mm	25 × 100 mm	4 oz. glass jars

[a]Murashige and Skoog (1962) macro- and micro-salts with Gamborg's B5 vitamins; Sigma Cat. No. M0404

[b]Murashige Minimal Organics consists of Murashige and Skoog inorganic salts with 100 mg/l i-inositol and 0.4 mg/L thiamine HCl; Sigma Cat. No. M6899

[c]Phytoblend agar – Caisson Laboratories Cat. No. PTP01

5.5.1.2 *Agrobacterium* Preparation

- Grow *Agrobacterium* containing the construct of interest overnight in 5 mL of LB medium in a 15 mL Falcon.
- Spin down (2000 G, 10 min.), discard the supernatant, and resuspend the pellet in the same volume of inoculation medium (IM; Table 5.2).

5.5.1.3 Explant Preparation

- Use 5-day-old seedlings. Inspect the plate for any obvious contamination.
- Cotyledons can be conveniently collected by holding the plate in the fingertips of one hand with the seedlings extending horizontally. Use fine scissors in the other hand to snip off the pairs of cotyledons. Let them drop as they are cut into a 15 × 60 mm Petri dish containing 4.5 mL of liquid IM. Take care not to cut too close to the cotyledonary node to avoid getting a piece of the shoot apical meristem. It is essential to have at least 3 mm of the petiole on the cotyledon, but longer is convenient for easier handling.
- Use a fresh plate for each jar of seedlings. Cotyledons are best inoculated fresh but if necessary can be stored overnight at 4 °C. If this is the case, collect them into a plate without medium and seal.

5.5.1.4 Inoculation with *Agrobacterium*

- Pipet a volume of *Agrobacterium* equal to 1/9 the volume of the inoculation medium bathing the cotyledons to provide a tenfold dilution (e.g., for 4.5 mL inoculation medium, add 0.5 mL of *Agrobacterium* suspension).
- Ensure the bacterium is thoroughly mixed with the inoculation medium and that all the explants have been well exposed and inoculated.
- Pipet off as much of the free liquid as possible.
- Seal plates and put into darkness (wrap with foil, place in box) in the tissue culture room at 25 °C. Keep for 2 days.
- Transfer to 4 °C, again in dark, and keep for 3 days.

5.5.1.5 Selection and Regeneration

- Transfer the explants to shoot induction medium (SIM; Table 5.2) with selection in 25 X 100 mm Petri plates, ten per plate. Explants must be standing with the petiole embedded in the medium and the lamina of the cotyledon standing clear of the surface. Cotyledons lying flat on the surface will quickly take up excessive moisture, swell, and vitrify.
- Keep in tissue culture room at 25 °C, 16-h light/8-h dark photoperiod, light intensity approx. 80–100 μE (Fig. 5.2a).

- Transfer to fresh selection every 2–3 weeks until shoots have initiated. Usually no more than one transfer to fresh medium is required as the first shoots will begin to appear after about 2 weeks (Fig. 5.2c).

5.5.1.6 Shoot Elongation

- Excise developing green shoots from the starting explant, and place onto shoot elongation medium (SEM; Table 5.2). Take only shoots that have a well-defined morphology. Some shoots will be thick and distorted, but will usually become more normal once removed from the high cytokinin medium.
- Remove as much callus as possible, but not so much that the shoot falls apart. Shoots sometimes have a glassy, waterlogged appearance. These vitrified shoots will often recover to normal-looking shoots after one or more subcultures on SEM.
- Subculture to fresh SEM every 2–3 weeks. There may be a number of escapes. When using kanamycin selection, escapes are usually, but not always, white or purple.

5.5.1.7 Rooting

- As shoots become normal and exhibit apical dominance, transfer them to rooting medium (RIM; Table 5.2). At this time, cut across the base of the stem to remove any remaining callus and to provide fresh exposure to the medium. Stand the shoot in the medium, but try not to bury the apex.
- Roots should begin to appear in 1–3 weeks (Fig. 5.2d). If a shoot fails to root, transfer to fresh rooting medium, again making sure all callus has been cut off and making a fresh, clean cut across the stem.
- Rooted shoots (Fig. 5.2e) can be transferred to soil. If you want to keep a backup copy of the shoot in vitro, you can propagate it clonally by cutting off the apical portion and placing it onto fresh rooting medium. The basal portion can be planted to soil to grow out.
- Prepare 8 in./20 cm pots with moistened wet Redi-Earth or other suitable growth medium, fortified with slow-release fertilizer 20-20-20.
- Gently pull the shoot from the jar and remove large chunks of agar by rinsing gently in tap water.
- Place the roots into a hole formed large enough to accommodate the roots without breaking, cover the roots with the growth medium, and pack gently.
- To harden the shoots and acclimate them to growing in soil, cover the shoot with a clear container such as a Magenta box, disposable drink glass, or glass jar.
- Place the shoots into a greenhouse or plant growth room under normal growth conditions.
- After being covered for 3–4 days, gradually expose the shoot to room air by tipping the cover back. Leave it for a couple of more days. If the plant is not wilting, the cover can be removed entirely.

5.5.2 Brassica napus *Hypocotyl Transformation*

5.5.2.1 Seed Sterilization and Germination

- Follow seed sterilization and germination steps from the protocol above for the bulk cotyledon method.

5.5.2.2 Explant Preparation

- Harvest hypocotyls from 4- to 5-day-old seedlings.
- Cut hypocotyls into 4–6 mm lengths, and place onto sterile filter paper (Whatman #1) on the surface of plates of preculture/co-cultivation medium (Table 5.3).
- Incubate 3 days in tissue culture chamber at 25 °C, 16 h/8 h light/dark.

5.5.2.3 Co-cultivation

- Grow *Agrobacterium* containing the construct of interest overnight in 5 mL of LB medium in a 15 mL Falcon tube with appropriate antibiotics, with incubation at 28 °C, on a rotary shaker (250 rpm).
- Spin down (2000 G, 10 min.), discard the supernatant, and resuspend the pellet in the same volume of inoculation medium.
- Collect hypocotyls by scraping them off the filter papers with a sterile spatula into a 70 mm Petri dish containing 2.7 mL of hormone-free MS medium. Add 0.3 mL of *Agrobacterium* suspension and mix well to ensure all tissue pieces are inoculated.
- Pipet off and discard excess fluid. Scoop the explants into a Petri dish containing two or three layers of dry, sterile filter paper. After excess fluid has been blotted away, transfer the explants to plates of preculture/co-cultivation medium covered with a sterile filter paper. About 80 to 100 explants can be accommodated per plate.
- Incubate 7 days in tissue culture chamber at 25 °C, 16 h/8 h light/dark.

5.5.2.4 Callus Induction

- Transfer explants to callus induction medium (CIM; Table 5.3) by picking up the edge of the filter paper with sterile forceps and turning it over onto the new plate. Tamp the back of the filter paper to lightly press the explants to the surface of the new medium, then peel of the filter paper, and discard.
- Use one plate CIM for each plate of inoculated explants.
- Keep in tissue culture chamber as before for 14 days (Fig. 5.2b).

5.5.2.5 Shoot Induction

- Transfer explants to shoot induction medium (SIM; Table 5.3). At this time the explants are transferred one by one, 20 explants per plate. Make sure the explants have good contact with the medium but do not bury them in the agar.
- Return to the tissue culture chamber.
- Subculture the explants after 2–3 weeks, at which time the first shoots should be present.

Table 5.3 Composition of media used for the *Brassica napus* hypocotyl transformation method (modified from DeBlock et al. 1989)

Media component	Seed germination medium (SGM)	Preculture/ co-cultivation medium	Callus induction medium (CIM)	Shoot induction medium (SIM)	Shoot elongation medium (SEM)	Root initiation medium (RIM)
MSB5[a]	1/2 strength	x	x	x	x	1/2 strength
2,4-D		1 mg/L	1 mg/L			
Kinetin		1 mg/L	1 mg/L			
MES[b]			500 mg/L	500 mg/L	500 mg/L	
Ag NO_3			5 mg/L	5 mg/L		
Benzyladenine (BA)				3 mg/L	0.5	
Kanamycin- or L-PPT – or hygromycin[c]				20 mg/L or 1–2 mg/L or 5 mg/L	20 mg/L or 5 mg/L or 5 mg/L	25 mg/L or 10 mg/L or 1 mg/L
Gibberellic acid (GA^3)					0.03 mg/L	
Phloroglucinol					150 mg/L	
Indole butyric acid (IBA)						0.5 mg/L
Timentin™ (added after autoclaving)			300 mg/L	300 mg/L	300 mg/L	300 mg/L
Zeatin (added after autoclaving)				1 mg/L		
Sucrose	1%	3%	3%	3%	2%	1%
Phytagar[c]		0.7%	0.7%	0.7%	0.9%	0.3% or 0.8% agar
pH	5.8	5.8	5.8	5.8	5.8	5.8
Petri plate size	15 × 70 mm	15 × 100 mm	15 × 100 mm	25 × 100 mm	26 × 100 mm	150 ml jar

[a]Murashige and Skoog (1962) macro- and micro-salts with Gamborg's B5 vitamins; Sigma M0404
[b]MES (2-[N-morpholino]ethanesulfonic acid)
[c]Phytagar – Gibco Laboratories, Cat. No. 670-0675, or Sigma P-8169

5.5.2.6 Shoot Elongation

- Excise developing green shoots to shoot elongation medium (SEM; Table 5.3). Take only shoots that have a defined morphology.
- Remove as much callus as possible, but not so much that the shoot falls apart. This can require some skill and judgment. Shoots sometimes have a glassy, waterlogged appearance. These vitrified shoots will often recover to normal-looking shoots after one or more subcultures on SEM.
- Maintain in the tissue culture chamber. Subculture to fresh SEM every 2–3 weeks. There may be a number of escapes. When using kanamycin selection, escapes are usually, but not always, white or purple. When using PPT selection, escapes will usually fail to root on 10 mg/L L-PPT.

5.5.2.7 Rooting and Planting

- As shoots become normal and exhibit apical dominance, transfer them to rooting medium (RIM; Table 5.3), first removing all callus by cutting across the stem. Stand the shoot in the medium, but try not to bury the apex.
- Roots should begin to appear in 1–3 weeks (Fig. 5.2d). If a shoot does not root, transfer to fresh rooting medium, again making sure all callus has been cut off and making a fresh, clean cut across the stem.
- Rooted shoots (Fig. 5.2e) can be transferred to soil. If you want to keep a backup copy of the shoot in vitro, you can propagate it clonally by cutting off the apical portion and placing it onto fresh rooting medium. The basal portion can be planted to soil to grow out.
- Prepare 20 cm pots with moistened wet Redi-Earth or other suitable growth medium, fortified with slow-release fertilizer 20-20-20.
- Gently pull the shoot from the jar and remove large chunks of agar by rinsing gently in tap water.
- Place the roots into a hole formed large enough to accommodate the roots without breaking, cover the roots with the growth medium, and pack gently.
- To harden the shoots and acclimate them to growing in soil, cover the shoot with a clear container such as a Magenta box, disposable drink glass, or glass jar.
- Place the shoots into a greenhouse or plant growth room under normal growth conditions.
- After being covered for 3–4 days, gradually expose the shoot to room air by tipping the cover back. Leave it for a couple of more days. If the plant is not wilting, the cover can be removed entirely.

5.6 Conclusion

There are numerous ways to shorten the time-consuming process of new cultivar development in crops, including the brassicas. In this chapter, we have described three of them, doubled haploidy (DH), accelerated/speed breeding, and genetic transformation/gene editing. These can further be combined with other technologies described in other chapters in this book, for example, the use of genomics and marker-assisted breeding. Each of these tools contributes to more rapidly enhancing genetic gain, increasing the ability to rapidly respond to new threats and, through the earlier release of adapted and superior cultivars, resulting in enhanced cost benefits. More importantly, the ability to do so and the adoption of all possible methods, as appropriate, is becoming increasingly crucial with population increases, and rapid changes in the environment result in more tenuous situations for crops in some of the poorest parts of the planet.

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Chapter 6
Achieving Genetic Gain for Yield, Quality and Stress Resistance in Oilseed Brassicas Through Accelerated Breeding

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Abstract Ever increasing global population enforces plant breeders to develop highly productive crop varieties, within a short period, to achieve food and nutritional security. This can be achieved through improving genetic gain at much faster rate, beyond the present level, in different agriculturally important crops largely consumed by the masses. Brassicas are mainly cultivated in different parts of the world for edible oil and vegetable purposes. Directed efforts in *Brassica* spp. have led to improvement in yield, quality and tolerance/resistance to various biotic and abiotic stresses, but at a slow pace. Various modern accelerated breeding approaches such as shuttle breeding, speed breeding, doubled haploid technology, marker-assisted selection, genomic selection, etc. have potential to improve the genetic gain to a significant level in different oilseed Brassica. In the recent past, these approaches have been used to improve different traits in *Brassica* spp. such as increase in oil content, reducing erucic acid, improving the genetic diversity, introgression of biotic and abiotic stress resistance and others. Of course, individual strength of these approaches has been well demonstrated; however, their integration with each other shall have potential to further improve efficiency and cost-effectiveness of the varietal development processes. In the present chapter, we will discuss different approaches deployed for accelerating breeding process and their implications in improving oilseed crops of genus *Brassica*.

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6.1 Introduction

Plant breeding has played a key role in improving genetic gain in almost all the agricultural crops and helped in achieving global food security. 'Green revolution' ushered during the 1960s, through the deployment of genetically improved crop varieties, application of mineral fertilizers and control of weeds, diseases and insect pests through chemicals, has led to a tremendous increase in crop productivity and saved billions of lives from hunger and malnutrition. Non-lodging semi-dwarf cultivars have reduced biomass, better response to the nutrient uptake and higher efficiency in the allocation of assimilates to the seed (Hedden 2003). The harmonic adjustment of biomass and harvest index in these modern-day varieties has helped in improving productivity. Dedicated efforts towards plant breeding and refining management practices led to the annual genetic gain of 0.8–1.2% in crop productivity (Li et al. 2018). However, this rate of genetic gain is not sufficient in meeting food demand for projected global population in 2050.

Genetic gain ($\Delta G = \sigma_A\, h\, i/L$) from a plant breeding programme can be determined by available genetic variance in a population (σ_A), selection intensity (i), heritability of the trait for which selection is practiced (h) and number of years per selection cycle (L). It is evident from the breeding equation that higher genetic gain can be achieved by increasing genetic variance, selection intensity or heritability and decreasing time to complete a cycle of selection. Increasing selection intensity by keeping very large population size and improving heritability by increasing the locations for phenotypic evaluation is resource consuming and largely inefficient in improving genetic gain. Therefore, decreasing time to complete a cycle of selection by raising more than one crop per year seems to be the most effective way in achieving higher genetic gain. Various conventional approaches along with advanced tools such as shuttle breeding, rapid generation advancement, speed breeding, doubled haploidy and molecular/genomic selection are helpful in accelerating the breeding process for crop improvement.

Rapeseed-mustard group of crop comprises of six cultivated species, namely, *Brassica rapa* (2n = 20, AA), *B. nigra* (2n = 16, BB) and *B. oleracea* (2n = 18, CC) are diploids; *B. juncea* (2n = 36, AABB), *B. napus* (2n = 38, AACC) and *B. carinata* (2n = 34, BBCC) are digenomic tetraploids. These tetraploid species evolved in nature following hybridization between the constituent diploid species. Five of these species, except *B. oleracea*, are being cultivated for edible oil. In general, tetraploid species are higher yielders and possess better stress tolerance capacities when compared to diploid progenitors. Due to this fact, these species are very popular among farmers and largely cultivated. Tetraploid *Brassica* species are

biologically self-pollinated and are being cultivated in diverse agro-ecologies. Therefore, regional efforts rely largely on the use of classical approaches such as hybridization followed by pedigree selection for the development of improved cultivars in these naturally inbreeding species. Such efforts were highly successful in development of biotic and abiotic stress-resistant/stress-tolerant varieties with improved oil and seed meal quality in rapeseed-mustard group of crops; however, this process takes more than 10 years to develop a new cultivar. To maintain the pace with the growing population and changing climate, plant breeders have to refine methods that can accelerate the breeding process for achieving the defined goals. Accelerating breeding will, thus, offer us an opportunity to tackle the problems and comes out with the product in a short span of time.

Limited genetic diversity in the working germplasm is one of the major challenges being faced by the *Brassica* breeders. The knowledge of management and evaluation of genetic diversity and relatedness in germplasm is needed for effective crop improvement programmes (Demeke et al. 1992). The genome of cultivated species need to be enriched with genes/alleles from cultivated, wild or weedy relatives. Both crossable and non-crossable types of relatives are known to harbour many useful traits. Tissue culture techniques such as embryo rescue and protoplast fusion provide us opportunity to exploit this variability, which otherwise is absent in cultivated types. Depending on genetic distance and load carried by an alien donor species/genus, more number of breeding cycles are needed to get rid of linkage drag and transfer novel trait(s). Involvement of tissue culture approach, poor stability of genotypes in the generated populations and pollen as well as seed sterility in the progenies obtained from wide crosses further delay the breeding process. Therefore, deployment of rapid generation advancement approaches shall help in speeding up breeding process and achieving a higher genetic gain.

6.2 Accelerated Plant Breeding

Successful plant breeding programmes involve multiple phases of hybridization among the genotypes, selection in segregating progenies and testing of synthesized lines. This process generally takes more than one decade to develop a cultivar. Long duration of the parents and progenies is the major impediment in achieving higher genetic gain per unit time. Efforts towards development of short-duration varieties using genetic options are helpful in improving cropping intensity but at the same time compromising the individual crop productivity. Accelerated plant breeding seems to be the most viable method for developing new varieties in short time through reconciliation of numerous cutting-edge advances that encourages a speedier and more productive breeding cycle (Kapiel 2018).Various approaches involving natural or artificial environments such as speed breeding/rapid generation advancement (RGA), shuttle breeding, doubled haploidy, marker-assisted selection (MAS) and genomic selection shall be helpful in this endeavour (Lenaerts et al. 2019).

6.2.1 Rapid Generation Advancement (RGA)

In nature, photoperiod sensitivity is selected for providing resilience to the populations during the course of evolution. Depending on the requirements of natural habitat, varied level of photoperiod sensitivity has been created in different plant species. Both short-day and long-day plants are amenable to initiate flowering under reduced and extended light period, respectively. This genetic trait can be manipulated by creating artificial environments to reduce the duration of crops and, thus, faster generation turnover. The speed of this process is highly dependent on the level of photoperiod and temperature sensitivity of a species. In a regular glasshouse, by changing photoperiod through extending or limiting light hours, adjusted temperature regime and application of growth regulators two to three generations per year can be taken. Recent research in development of high-throughput phenotyping facilities and procedures is opening new vistas in rapidly revealing intrinsic correlations among complicated physiological traits by reducing genotype x environment interactions and enhancing selection efficiency.

Goulden (1941) advocated rapid generation turnover using single-seed descent (SSD) method and delaying the selection until the homozygosity is achieved. This method enables retention of greater genetic variability up to F_5/F_6 generation for practising selection. Later, modifications of this method such as modified SSD, multiple-seed descent and single-pod descent methods were also advocated (Fehr 1991).

Researchers at the University of Queensland, in the year 2003, coined the term 'speed breeding' for the changed combination of artificial environmental conditions to accelerate the speed of breeding cycle in wheat (Watson et al. 2018). In speed breeding light, temperature and growth conditions are regulated in such a way that more generations of crop plants can be taken in a short span of time. This approach ensured six generations for spring both wheat species *Triticum aestivum* and *T. durum*, barley (*Hordeum vulgare*) and chickpea (*Cicer arietinum*) and four generations for canola (*B. napus*) instead of a single generation in the normal field conditions and two to three under glasshouse conditions (Watson et al. 2018). Since different species respond differently to changing photoperiod and temperature conditions, therefore, it is desirable to develop crop-specific speed breeding protocols. More concerted efforts are needed to improve the generation turnover efficiency for genus *Brassica*. Four generations can be taken in a year in *B. napus* through speed breeding; however, with short-duration species of this genus, such as *B. juncea* and *B. rapa*, possibility of better efficiency is there.

6.2.2 Shuttle Breeding

The concept of shuttle breeding was developed by the International Maize and Wheat Improvement Centre (CIMMYT). It was used by Dr. N. E. Borlaug for reducing the time for development of wheat varieties by taking winter crop at Obregon and summer crop at Toluca (Oritz et al. 2007; Lenaerts et al. 2019) and ultimately helped in ushering 'Green Revolution' during the 1950s. This approach uses diverse ecological environments to take two crops in a year and develop improved varieties with higher adaptability at a faster speed. Apart from generation advancement, selected lines and segregating generations can be screened for diverse diseases, pests and pathogens. Shuttling the breeding nurseries between two different locations enables advancement of breeding material by an extra generation per year; screening for diseases, insect pests and adaptation traits; and seed multiplication of promising genotypes. In china, through this approach a super high oil content cultivar Qinzayou-4 was developed which have more than 50% oil content (Guan et al. 2013). In India, off-season nurseries for oilseed brassicas are being taken in mountainous or sub-mountainous areas of the Himalayas in the north or Nilgiri Hills in the south, where temperature remains low during summers. These off-season sites also provide an opportunity to screen the breeding material against white rust, powdery mildew and *Alternaria* blight diseases and acidic soil conditions, hence helping in development of varieties with better stability of performance across crop-growing environments.

6.2.3 Doubled Haploidy

Production of the homozygous line is considered as one of the key steps in any breeding programme. Conventionally homozygous lines can be produced through six to seven generations of selfing, which takes an equal number of years if one crop is taken every year. Alternatively, if two crops are taken in a year, even then it will take 3–4 years for production of a pure line. Therefore, developing improved varieties generally takes more than a decade to reach the farmers' fields. Production of pure line through generating haploids and subsequently converting them to doubled haploid is a faster alternative to synthesize homozygous lines. Haploids contain one of the parental genomes only; it can be either maternal or paternal genome based on which it is called maternal or paternal haploid. These haploids, either spontaneously or through application of certain alkaloids such as colchicine, oryzalin and many others, can convert into diploid/doubled haploid (Murovec and Bohanec 2012).

Apart from the production of pure line, haploids are very useful in basic genetic studies of *Brassica* spp. DH are very useful genetic material for genome sequencing. Due to cross-pollination and inherent heterozygosity, it is very difficult to assemble the genome sequence in some *Brassica* species such as *B. oleracea*. Pure line(s) synthesized through chromosome doubling of haploids can be easily

deployed for genome assembly. In the genome sequencing of *B. rapa* (AA), doubled haploid line Chiifu-401-42 was used (Wang et al. 2011), whereas doubled haploid line 'YZ12151' was used for genome sequence of *B. nigra* (BB) (Yang et al. 2016). DH lines are ideal genetic material for QTL mapping as these take very less time for development of mapping population as compared to recombinant inbred line (RIL) or near-isogenic line (NIL). Furthermore, deployment of such genetic material is highly desirable in reverse breeding approach, a type of breeding where parental lines are recovered from the hybrid parent; thus, a hybrid can be reconstituted from the new set of parents (Dirks et al. 2009). Breeding at lower ploidy level in polyploid crops, known as analytical breeding, is highly fruitful. Apart from these, haploids are also useful in mutagenesis and genetic transformation experiments (Murovec and Bohanec 2012). DH technology is extensively utilized in *B. napus* and *B. oleracea* for varietal development and other abovementioned purposes. Many *B. napus* varieties currently cultivated at farmers' fields are doubled haploids. Primarily, two different approaches can be used for synthesis of haploids and doubled haploids in oilseed Brassicas. For details of DH approaches and their applications in *Brassica* improvement, Gil-Humanes and Barro (2009), Ferrie and Mollers (2011) and Watts et al. (2020) can be referred.

6.2.3.1 In Vitro Haploid Production

Production of haploid and subsequently converting them to doubled haploid is a challenging task. The most common method for haploid production involves culturing of haploid tissues such as anther, microspore or ovule and regenerates it into a complete plant. Firstly, haploids have been produced using anther culture in *Datura innoxia* by two Indian scientists Guha and Maheshwari in 1964. Later, haploids have been produced in many agriculturally important crops. Regeneration of complete plants from the pollen of *B. campestris* was first reported by Keller et al. in 1975. In the same year, embryogenic tissue from microspores of *B. napus* was also obtained. Licher (1982) has developed a system for in vitro plant regeneration using microspores of *B. napus*. The complete protocol is now available for regeneration of complete haploid plants from the haploid tissue in *B. napus* (Zhou et al. 2002; Weber et al. 2005), *B. juncea* (Lionneton et al. 2001) and *B. rapa* (Gu et al. 2003). Various factors such as genotype of the species and growth condition of the donor plant, length of the flower bud and selection of embryo influence the efficiency of tissue culture approach.

6.2.3.2 In Vivo Haploid Production

Due to the various technical constraints in labour-intensive tissue culture-facilitated haploid production, a simple in vivo method shall be highly desirable for inducing haploidy. Efficient chromosome elimination method of haploid production involving various wide crosses has not been established yet. However, partial to complete

elimination of one of the parental chromosomes is reported in some of the crosses involving wild species of Brassicaceae. In the cross between various *Brassica* spp. and *Orychophragmus violaceus*, a wild relative of genus *Brassica*, *O. violaceus* chromosomes get eliminated (Li and Ge 2007). Similarly, in the cross between *B. rapa* and *Isatis indigotica*, elimination of *I. indigotica* chromosome has been observed, and haploids have been recovered (Tu et al. 2009). Recently in *B. napus*, a new in vivo method of doubled haploid production has been developed. In this approach, a synthetic *Brassica* octaploid line (AAAACCCC, 2n = 8X = 76) was developed. This synthetic *Brassica* octaploid line on hybridization with *B. napus* leads to chromosome elimination and, thus, synthesis of doubled haploid line of *B. napus*. The frequency of doubled haploid recovery varied from 40 to 90% depending on the maternal parent genotype (Fu et al. 2018). Since mechanism of genome elimination is not well understood, therefore, these systems have not been practically utilized in *Brassica* breeding programmes.

6.2.4 Marker-Assisted Selection (MAS)

Conventionally, plant breeders select the parents based on phenotypic traits such as yield to achieve higher genetic gains. Further, with the advancement of quantitative genetics and statistics, best linear unbiased prediction (BLUP) approach was proposed and used by plant breeders to estimate the breeding value of the parents, thus enabling their selection. With the discovery and applications of molecular markers, several markers have been used in the different crop breeding programmes. Marker-assisted selection (MAS) was proposed as a strategy in which molecular markers are being employed to accelerate plant breeding and introgress gene of interest in relatively less time.

Backcross breeding method is followed to incorporate favourable gene(s) from the donor parent into an elite cultivar through the process of repeated backcrossing. However, this approach involves continuous backcrossing and selection of desirable genotypes in each generation, thus involving high amount of time and money. With the discovery of molecular markers and its application in plant breeding, this process of backcrossing can be accelerated. Apart from accelerated breeding and early selection, marker-assisted backcross breeding (MABB) also minimizes linkage drag and effectively selects target gene or alleles as compared to conventional backcrossing. This approach is highly efficient in reducing the time to transfer the gene of interest in any background. Further, MABB is not affected by the environmental conditions; therefore, selection under manipulated environmental conditions such as light-supported greenhouse, net house or off-season location is effective in screening of the desired genotypes.

Furthermore, MAS enables selection of traits at the seedling stage; therefore, its integration with speed breeding shall help in accelerating the development of improved varieties. This approach is being used in improving oil and seed meal quality and biotic and abiotic stress-related traits in brassicas. Spasibionek et al.

(2020) have used allele-specific CAPS markers and SNaPshot assay to improve the seed yield and other agronomical traits of lines which have high oleic acid and low linolenic acid. Different molecular markers such as RFLP, AFLP, RAPD, SSR and ISSR have also been developed to assess the genetic diversity among different *B. napus* genotypes (Ecke 2016; Havlíčková et al. 2014; Moghaieb et al. 2014 and Raza et al. 2019) and their associations with valuable traits.

6.2.5 Genomic Selection

Marker-assisted selection enables selection for simple traits by tracking them using linked DNA markers. As an alternative to MAS, genomic selection was proposed as a strategy through which complex traits such as yield can be tracked using the DNA markers covering the whole genome. In genomic selection, the breeding value of any genotype is estimated based on the large number of molecular markers covering whole genome. Therefore, it accelerates the breeding cycle through rapid selection of superior genotypes in a short span of time. With the advancement in genomic research and decrease in cost of sequencing, genomic selection is being integrated with the conventional plant breeding for better precision and speed. Genomic selection is extensively used in the animal breeding industry, where population size is very-very small as compared to angiosperms. Similarly in plants, genomic selection can be applied for assessing the breeding values of the parents. It allows the rapid selection of contrasting parents enabling higher breeding value in the segregating generations and, thus, tends to improve genetic gain significantly in a short span of time. In some crops such as rice, wheat and maize, it has already been demonstrated that genomic selection can be used as a strategy for accelerated plant breeding. However, there are many practical challenges such as sequencing, genotyping method and cost-effectiveness in deploying genomic selection in accelerating the plant breeding process.

Whole genome-based SNPs are very helpful in complete genome-assisted breeding. In *B. napus* 60K Illumina Infinium™ array was developed which contains 52,157 SNPs (Clarke et al. 2016). These kinds of SNP chips were highly useful for mapping of various traits in *B. napus* (Mason et al. 2017). Further these kinds of SNP chips are also required in other oilseed *Brassica* spp.

6.3 Special Implications of Accelerated Breeding in *Brassica* Improvement

6.3.1 Development of Genetic Resources

In addition to the rapid development of crop cultivars, accelerated breeding protocols are being used for generation of inbred/pure lines such as recombinant inbred lines (RILs), near-isogenic lines (NILs), alien introgression lines (ILs) and doubled haploid lines (DHs) which are required for conducting basic and applied research. These genetic resources are highly useful in gene discovery, QTL mapping, genetic mapping and their deployment in precise transfer of valuable traits to elite genetic backgrounds.

6.3.2 Recombination and Mutation Breeding

Conventionally, contrasting genotypes are hybridized to create selectable genetic variability through genetic process called recombination. It allows segregation in the following generations and provides opportunity to select plants with desirable combination of traits. This genetic phenomenon is being used for development of elite genotypes and achieving higher genetic gain. This process generally takes more than 10 years to develop a new variety and is not very efficient in achieving required genetic gain. The mutation breeding, on the other hand, is generally used to create novel genetic variability or some new traits. It allows creation of biodiversity directly in the germplasm (Kharkwal 2012; Shu et al. 2012a, b; Vries and Gager 1910). In oilseed Brassicas, efforts have been made through application of mutagens for development of genetic resources possessing high oil content, low erucic acid and high oleic acid, seed coat colour and resistance against different biotic and abiotic stresses. When a genotype is subjected to mutagen, a lot of changes occur in genome; thus, process of chromosomal rearrangement, fixation of derived lines and their characterization takes lot of time. Development of new lines of amphidiploid species through mutation breeding takes about seven to ten generations. This time span can be reduced by fast-forwarding the generation turnover using accelerated plant breeding approaches such as RGA, speed breeding, shuttling the breeding material between different location and doubled haploidy. These approaches can further be integrated with MAS or genomic approaches for improving results of selection process.

6.3.3 Resynthesis of Amphidiploids

In Brassicas, six species including three diploids, viz. *B. rapa* (earlier known as *B. campestris* AA; 2n = 2x = 20), *B. nigra* (BB; 2n = 2x = 16) and *B. oleracea* (CC; 2n = 2x = 18), and three amphidiploids, viz. *B. juncea* (AABB; 2n = 4x = 36), *B. napus* (AACC; 2n = 4x = 38) and *B. carinata* (BBCC; 2n = 4x = 34), are cultivated. The amphidiploid species developed de novo in nature by chance crosses between diploid progenitors (Nagaharu et al. 1935). The genetic variability available in the amphidiploid species is very less as compared to the constituting diploid species since amphidiploids are developed naturally either as a result of crossing of diploid species with unreduced gametes or autodiplodization of chromosomes after the crossing among diploid species. Therefore, resynthesis of amphidiploid species of genus *Brassica* is considered a novel method for enhancement of genetic diversity. Shyamprakash (1973) resynthesized *B. juncea* using *B. rapa* and *B. nigra*, and significant genetic diversity in the amphidiploid species was recovered. This approach involves interspecific hybridization between progenitor species, in vitro rescue of interspecific haploid embryos and chromosome doubling of resultant haploids through colchicine treatment (Srivastava et al. 2004; Chatterjee et al. 2016). It takes about five to seven generations, from the year of crossing, to develop a fixed line following tissue culture methods (embryo rescue, ovule culture, clonal propagation, etc.), chromosome doubling and selfing for achieving genomic stability through this approach. This process involves tedious tissue culture work, which is time taking. Due to these difficulties, resynthesis of *B. juncea* could not be routinely used by the *Brassica* breeders.

Any method bypassing the cumbersome and time-consuming in vitro techniques will, thus, speed up the process of development of synthetic amphidiploid lines. At ICAR-National Institute for Plant Biotechnology, New Delhi, while attempting resynthesis of *B. juncea* using *B. rapa* and *B. nigra* species, a unique *B. rapa* var. yellow sarson line 'NRCPB rapa 8' was identified that enables exceptionally high in vivo seed development on *B. rapa* without in vitro embryo rescue (Fig. 6.1). Seeds from such crosses were sterile amphihaploids having 18 chromosomes of which 10 were from *B. rapa* and 8 from *B. nigra*. Haploid seedlings were treated with colchicine for chromosome doubling (Fig. 6.2). The diplodized branches produce normal fertile flowers and yield amphidiploid seeds or resynthesized *B. juncea*. This is a unique genotype that could facilitate efficient resynthesis of *B. juncea* by saving resources and efforts; therefore, it was registered with ICAR-NBPGR, Delhi (IC0623820; INGR17050), in 2017 (Rao et al. 2019).

6.3.4 Wide Hybridization

The gene(s) which are not available in the primary gene pool of Brassicas, but important in imparting better productivity and stability of performance, can be searched in wild or distantly related species. The transfer of gene(s) from the secondary and tertiary gene pool is always a challenge for breeders as it is very difficult and time-taking process, especially to the amphidiploid species. The inter-species crossing resulted with linkage drag along with the introgression of desired gene(s), and it is generally very difficult to handle and break up the linkage between gene of interest and the drag (Griffiths et al. 2006). Most of the wild species are diploid, and trait transfer from these species to the cultivated amphidiploid species could be possible via using a diploid bridge species. Development of synthetic amphidiploid using the identified source germplasm and a bridge species requires tissue culture procedures followed by doubling of chromosome. This approach expands the total time required in transferring the gene(s). Once the trait/gene of interest is incorporated in the synthetic amphidiploid, it needs to be further crossed and/or backcrossed with the other cultivated amphidiploid genotypes. Sometimes embryo rescue is necessary at this stage also. Overall, the whole process of transferring trait from a wild/related species to cultivated amphidiploids takes more than 14 seasons to develop the stable alien introgression lines (ILs) with the desired traits. Different accelerated breeding approaches can be integrated and used for reducing the time for

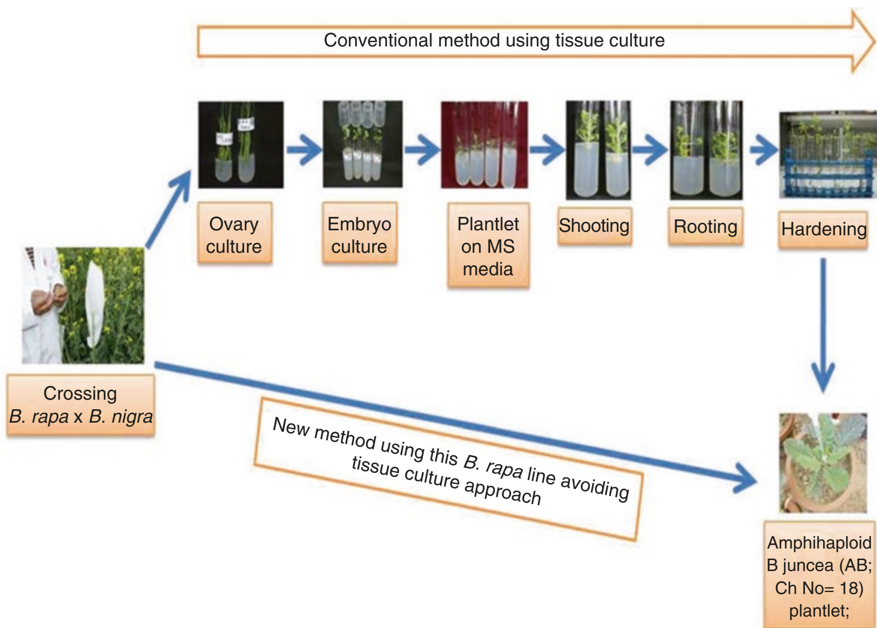

Fig. 6.1 Resynthesis of *B. juncea* eliminating the tissue culture interventions

Fig. 6.2 Large-scale in vivo resynthesis of *B. juncea*. (**a**) Haploid seedlings generated from hybridization of *B. rapa* (NRCPBrapa8) with *B. nigra* and (**b**) application of colchicine on the young emerging floral buds for chromosome doubling

development of ILs having valuable traits from wild and other related species/genera. Further, employing of different molecular markers shall help in accelerating the introgression process and discovery of new alleles. Vasupalli et al. (2017) have developed molecular markers based on the wild species *Diplotaxis erucoides* and used these markers to check the introgression in backcross progenies derived from *B. juncea* and *D. erucoides* crosses. Availability of genomic resources and rapid generation turnover methods shall help in efficient enrichment of cultivated species genomes with the novel traits embedded in the wild relatives, thus achieving higher genetic gain in different *Brassica* spp.

6.4 Conclusion

Accelerated breeding ensures development of homozygous and homogeneous lines by saving time and money. Rapid generation turnover under artificial environments, manipulated for photoperiod, temperature and growth regulators, along with other in vitro, doubled haploidy and genomic selection methods has potential to

drastically reduce the time required for development of new cultivars in many field crops including brassicas. Recent advancements in refining the accelerated breeding approaches are striving to contribute in varietal development process through fast-forwarding the genetic gain needed to achieve edible oil and nutritional security. Till now, the basic methodologies have been tested and refined for cost cutting and higher speed in *B. napus* among the oilseed brassicas. Individual strength of these approaches has been well demonstrated; however, their integration with each other is expected to further improve efficiency and results. It is also important to improve the selection efficiency through establishing better representation of G x E interaction, required for better stability of performance in the future varieties, which is generally ignored while focusing on speed of breeding process. Integrating field evaluation in this process may be desirable, but at the cost of speed of varietal development. Therefore, looking at the pros and cons, breeders have to select the most cost-effective and efficient approach for this purpose. Committed investment is desired at every level for further refinement of these techniques and procedures and integrates them with conventional plant breeding approaches directed towards achieving higher rate of genetic gain.

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Chapter 7
Genomic-Assisted Breeding for Enhanced Harvestable (Pod) and Consumable (Seed) Product, Yield Productivity in Groundnut (*Arachis hypogaea* L.)

J. Shanthala, S. Gazala Parveen, and Bharath Kumar P. Jambagi

Abstract Groundnut (*Arachis hypogaea* L.), an important oilseed crop of the world extensively cultivated in India, China, USA and other African countries, belongs to the genus *Arachis* family Leguminosae (2n = 4x = 40) originated in Brazil, Southern America. The complexity of the genome associated with the origin and domestication is the major bottleneck resulting in narrow genetic base of groundnut. The susceptibility to abiotic and biotic stresses, cultivation on marginal lands and limited response to high-input agriculture have further constrained its productivity throughout the world. Genetic resources and variability, the key factors for success of any crop improvement programmes, are reflected in large collection of groundnut germplasm, both cultivated and wild types, maintained at many national and international genebanks, globally. ICRISAT genebank has a core collection of 14,310 accessions, stratified by the botanical varieties within subspecies, and includes representative subsets, in the form of core and mini core collections or genotyping-based reference sets facilitating identification of several germplasm with specific traits, either resistance to abiotic and/or biotic stresses or superior agronomic and/or nutritional traits. Cutting-edge technologies such as genic markers developed from ESTs and genomic DNA libraries utilized for enriching genetic maps, in addition to DArT for diversity studies and for developing saturated linkage maps through introgression from wild species, have further intensified research progress in groundnut. All-encompassing contrasting populations, viz. RILs, NILs, NAM, AB-QTL, MAGIC, GWI, etc., have facilitated QTL mapping and association studies in

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groundnut. These emerging genomics technologies such as NGS and high-throughput marker genotyping using SNPs have enabled the development of en masse sequence data for groundnut.

Keywords Groundnut · *Arachis hypogaea* L. · Genebanks · Genic markers · Genomic-assisted breeding

7.1 Introduction

Groundnut is a multipurpose self-pollinated allotetraploid crop, globally established as food and oilseed crop. It is cultivated in more than hundred countries of tropics and subtropics of the world. It sustains and fulfils agriculture-dependent livelihood strategies and their nutritional necessity and thereby potentially mitigates malnutrition of masses in common. It is an invaluable source of proteins, calories, essential fatty acids, vitamins and minerals; its consumption bestows several health benefits in human nutrition (Kris-Etherton et al. 2008; Sabate et al. 2010; Guasch-Ferré et al. 2017; Willett et al. 2019; Ojiewo et al. 2020). Groundnut kernel is utilized as major ingredients in various commercial food products, with nutrient-dense bioavailability of active polyphenols, flavonoids and isoflavones for human nutrition. However, the plant in entirety is utilized for diverse purposes such as animal nutrition, livestock feed, fuel and fodder, and it also enriches soil fertility through nitrogen fixation and breaking disease and pest cycles (Varshney et al. 2018).

It belongs to the Fabaceae family (Stalker 1997; Valls and Simpson 2005) with a genome size of 2.54 Gb (Bertioli et al. 2019). It is believed to have originated in the southern Bolivia to northern Argentina region of South America. The genus *Arachis* contains 80 species, and most of them are diploid ($2n = 2x = 20$) with only two allotetraploids. The cultivated groundnut is an allotetraploid (AABB, $2n = 4x = 40$), which is believed to be the result of hybridization between two wild species, *Arachis duranensis* (AA genome, $2n = 2x = 20$) named as 'A genome ancestor' and *Arachis ipaensis* (BB genome, $2n = 2x = 20$) named as 'B genome ancestor', and subsequent chromosome doubling.

Groundnuts are now grown in most tropical, subtropical and temperate countries between 40°N and 40°S latitude, especially in Africa, Asia, North and South America. Groundnuts are a small erect or trailing herbaceous legume and grow to about 15 to 60 cm high. The fruit is a pod with one to five seeds that develop underground within a needle-like structure called peg. Currently, this crop is cultivated globally in over 28.5 million hectares which yielded 45.95 million tons of pods during 2018 (FAOSTAT 2019). The Asia (40.2%) and Africa (54.9%) regions hold together 95% of global groundnut cultivated area with the annual production contribution of 59.3% and 31.1%, respectively. All plant parts of groundnut are useful and are major sources of nutrition for both humans and livestock.

In India, groundnut accounts for about 22% of area (5.86 million ha) and 25% (8.26 million tons) of production of total oilseeds (DAC 2019). Most of the groundnut production in India is concentrated in six states, viz. Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra and Rajasthan. These six states account for about 90% of the total groundnut area. The remaining groundnut-producing areas are scattered in the states of Madhya Pradesh, Chhattisgarh, Uttar Pradesh, Punjab, Orissa and West Bengal. About 75% of cropped area of groundnut in India lies in low- to moderate-rainfall areas (parts of peninsular region and western and central regions), with a short period of distribution. Based on rainfall pattern, soil factors, diseases and pest situations, groundnut-growing areas in India have been divided into five agro-climatic zones (Table 7.1).

Table 7.1 Agro-climatic zones as suitable for production and productivity of groundnut cultivation of India

Zones	States in each zone	Rainfall (mm)	Soil type	Temp (°C)		Relative humidity (%)	Crop duration (days)	Pest and diseases
				Min.	Max.			
Zone I	Rajasthan, Haryana, Punjab, Uttar Pradesh	466–478	Sandy to sandy loam (saline alkaline soils in some cases)	12	38	57–88	120–150	White grub, termites, collar rot, stem rot and leaf-spots
Zone II	Gujarat, Southern Rajasthan	547–866	Medium black (calcareous; low soil depth)	19	35	76–94	115–130	Thrips, jassids, Spodoptera, collar rot, stem rot and leaf-spots
Zone III	Northern Maharashtra, Madhya Pradesh	610–939	Medium black to loamy (neutral soil with good depth)	16	37	65–90	100–110	Rust, leaf-spots, Spodoptera, Helicoverpa, thrips and jassids
Zone IV	Jharkhand, West Bengal, Odisha, North Eastern, Hill region	747–1268	Alluvium, sandy loam to clay-loam (mostly acidic)	I4	34	78–92	110–120	Aphids, thrips, rust, leaf spots and pod rot
Zone V	Southern Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu	455–900	Red-lateritic to sandy-loam (high alumina content forms hard crusts)	15	31	68–95	95–120	Rust, leaf-spots, dry root rot, stem rot, leaf miner, Spodoptera and termites

Table 7.2 The average area, production and productivity in different countries of the World –2018

Area (In Hectare)			**Production (In Tonne)**			**Productivity (In Hectogram/ Hectare)**		
Rank	Top 10 Countries	Harvested Area	Rank	Top 10 Countries	Production	Rank	Top 10 Countries	Yield
1	India	4,940,000	1	China	17,332,600	1	Uzbekistan	186,854
2	China	4,619,660	2	India	6,695,000	2	Israel	52,214
3	Sudan	3,065,000	3	Nigeria	2,886,987	3	Palestine	51,837
4	Nigeria	2,911,705	4	Sudan	2,884,000	4	Guatemala	50,037
5	Myanmar	1,028,960	5	United States of America	2,477,340	5	Iran (Islamic Republic of)	49,660
6	Senegal	962,905	6	Myanmar	1,599,149	6	Guyana	49,022
7	Tanzania	955,687	7	Tanzania	940,204	7	United States of America	44,732
8	Niger	919,834	8	Argentina	921,231	8	Malaysia	43,148
9	Chad	786,890	9	Chad	893,940	9	Nicaragua	42,152
10	Guinea	785,737	10	Senegal	846,021	10	Saudi Arabia	40,439
	Other Countries	7,539,007		Other Countries	8,474,428	48	India	13,553
	World	**2.9E+07**		**World**	**4.6E+07**		**World**	**16,114**

Source: Food and Agriculture Organization (FAO). (ON1407)

Major groundnut-growing countries of the world are India (19%), China (22%), Nigeria (11%) and the USA (2.0%). Globally, China (42%) and India (18%) are the largest producers followed by Nigeria (7.7%), the USA (4.3%) and Indonesia (1.8%). The average area, production and yield in different countries during 2018 are presented in Table 7.2. Area, production and productivity of groundnut in India for two decades (2000–2001 to 2018–2019) have been summarized in Fig. 7.1. State-wise area under groundnut in India and their percent contribution to total area under cultivation are presented in Table 7.3 and Fig. 7.2, respectively.

7.2 Nutritional Composition of Groundnut Kernels

Groundnut which is popularly known as poor man's almonds is a significant contributor to mitigate malnutrition due to its high nutritional content with fat and protein composition of 80% of seeds contents. Groundnut seeds are highly nutritious possessing fat (40–50%), protein (20–30%), carbohydrate (10–20%) and several other micronutrients and minerals (vitamin E, niacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium) (Pandey et al. 2012a). It serves as major reservoir of protein, oil and fatty acids the most beneficial nutrients required in human diet. The fatty acid composition of the oil has a great

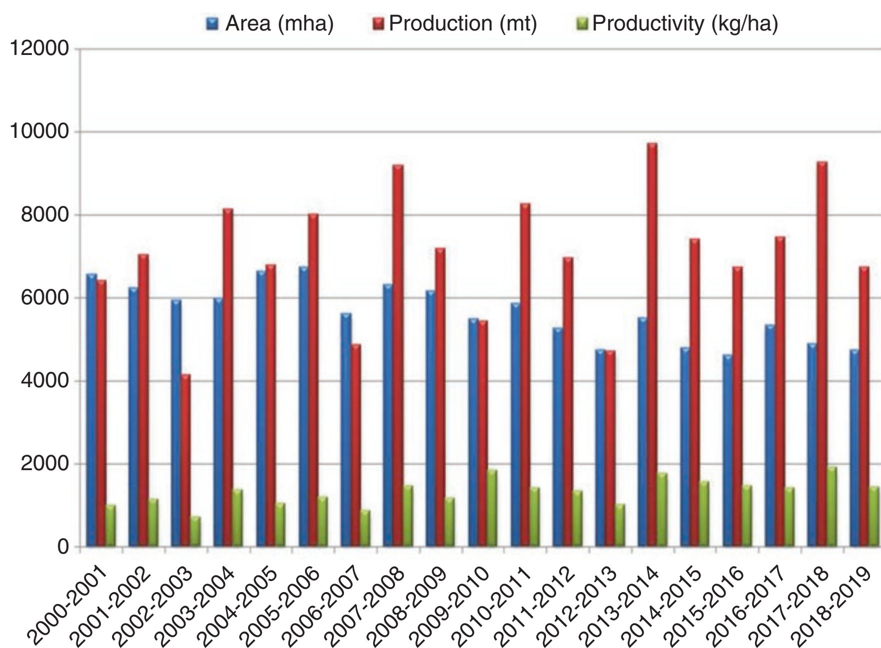

Fig. 7.1 Summary of area, production and productivity of groundnut in India for two decades: 2000–2001 to 2018–2019

consequence in determining shelf-life, nutrition and flavour of food products derived from groundnut (Gaydou et al. 1983). Groundnut oil is rich in monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) (Mercer et al. 1990). The percentage of MUFA and PUFA varies among genotypes derived from runner, Valencia, Spanish and Virginia types, and each habitat group differs for its kernel size, flavour and nutritional composition.

Virginia groundnuts owing to its large kernel size are the most preferable type for table purposes such as roasting, salting and other confectioneries as compared to the small kernels of Spanish groundnuts. Besides good-quality fatty acid profiles, the presence of various beneficial functional constituents like vitamin E, L-arginine, myo-inositol, soluble and insoluble fibre, phytosterols as well as water- and lipid-soluble phenolic antioxidants qualifies the kernels of groundnut as desirable and most nutritious plant food (Clements Jr and Darnell 1980; Isanga and Zhang 2007; Kris-Etherton et al. 2008). Groundnut kernels harbour a range of antioxidative phytochemicals including several phenolic acids, flavonoids and stilbenes, which have numerous beneficial effects on human health through apparent anti-inflammatory, antimicrobial and anticancer activities (Kris-Etherton et al. 1999; Griel et al. 2004). A compiled nutritional database of the composition of nutrients in different parts of the groundnut seed and the nutritional value are provided in Tables 7.4a and 7.4b.

Table 7.3 Selected state/season-wise area, production and productivity of groundnut in India (2018–19)

	Area (In ` 000 Hectare)			**Production (In ` 000 Tonne)**			**Productivity (In Kg./Hectare)**		
States/UT	**Kharif**	**Rabi**	**Total**	**Kharif**	**Rabi**	**Total**	**Kharif**	**Rabi**	**Total**
Andhra Pradesh	687.00	61.00	748.00	332.51	129.44	461.95	484	2122	618
Arunachal Pradesh	0.92	–	0.92	0.90	–	0.90	981	–	0.9
Bihar	0.86	–	0.86	0.87	–	0.87	1020	–	0.87
Chhattisgarh	30.12	–	30.12	40.30	–	40.30	1338	–	40.3
Goa	0.21	0.21	0.42	0.50	0.50	0.99	2370	2320	2345
Gujarat	1566.37	27.84	1594.21	2142.79	60.02	2202.82	1368	2156	1382
Haryana	3.70	–	3.70	3.33	–	3.33	900	–	900
Himachal Pradesh	0.04	–	0.04	0.04	–	0.04	1030	–	1030
Jharkhand	30.07	–	30.07	31.03	–	31.03	1032	–	1032
Karnataka	378.10	136.80	514.90	272.23	118.75	390.98	720	868	759
Kerala	0.11	0.08	0.19	0.14	0.10	0.24	1261	1227	1247
Madhya Pradesh	216.00	7.00	223.00	399.38	12.66	412.05	1849	1809	1848
Maharashtra	217.30	26.82	244.12	203.48	35.07	238.55	169	1308	977
Manipur	0.00	3.22	3.22	–	3.00	3.00	–	931	931
Nagaland	1.01	–	1.01	1.05	–	1.05	1040	–	1040
Odisha	12.95	14.92	27.87	13.91	21.68	35.59	1074	1453	1277
Puducherry	0.27	0.01	0.29	0.88	0.04	0.92	3197	3231	3199
Punjab	1.30	–	1.30	2.57	–	2.57	1980	–	1980
Rajasthan	668.89	4.48	673.37	1375.24	7.09	1382.32	2056	1583	2053
Tamil Nadu	196.54	138.95	335.31	426.30	485.07	911.37	2169	3491	2718
Telangana	13.00	113.00	126.00	32.55	281.26	313.81	2504	2489	2491
Tripura	1.26	0.99	2.25	1.67	1.43	3.10	1328	1438	1376
Uttar Pradesh	101.00	–	101.00	100.39	–	100.39	994	–	994
Uttarakhand	1.00	–	1.00	1.04	–	1.04	1038	–	1038
West Bengal	3.92	63.50	67.41	3.85	184.10	187.95	984	2899	2788
India	4131.94	598.82	4730.76	5386.97	1340.21	6727.18	1304	2238	1422

Source: Ministry of Agriculture and Farmers Welfare, Govt. of India. (ON2331).

7.3 Taxonomy and Evolution

Based on the patterns of reproductive and vegetative branching and on the pod morphology, the cultivated species is divided into two cultivated subspecies, that is, *A. hypogaea* subsp. *hypogaea* and *A. hypogaea* subsp. *fastigiata*. The subspecies are further divided into botanical varieties. The subsp. *hypogaea* is divided into *hypogaea* (Virginia) and *hirsuta*, while the subsp. *fastigiata* is divided into *fastigiata* (Valencia), *vulgaris* (Spanish), *peruviana* and *aequatoriana* (Krapovickas and Gregory 1994). Despite being a tetraploid, cultivated groundnut genetically behaves

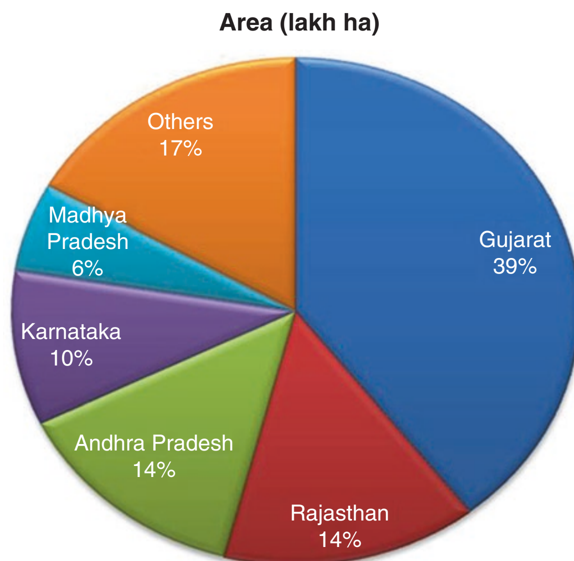

Fig. 7.2 State-wise area under groundnut in India (2018–2019)

Table 7.4a Chemical composition of various parts of groundnut seed (g/100 g)

Constituents	Testa	Embryo axis	Kernels
Moisture	9.0	–	3.9–13.2
Protein	11.0–13.4	26.5–27.8	21.4–36.4
Fat	0.5–1.9	39.4–43.0	35.8–54.2
Total carbohydrate	48.3–52.2	–	6.0–24.9
Reducing sugar	1.0–1.2	7.9	0.1–0.4
Sucrose	–	12.0	1.9–6.4
Starch	–	–	0.9–5.3
Crude fibre	21.4–34.9	1.6–1.8	1.6–1.9
Ash	1.9–4.6	1.9–3.2	1.8–3.1

(Satish and Shrivastava 2011

as diploid due to unusual pairing of AA and BB genome chromosomes during meiosis (Stalker 1991).

The cultivated groundnut *Arachis hypogaea* is an allotetraploid with an AABB genome constitution. However, the only wild allotetraploid progenitor *Arachis monticola*, which crosses freely to form fertile hybrids and hence belongs to gene pool-1, still remains unresolved. All the cytogenetic and molecular evidences support *Arachis duranensis* as the most probable progenitor and also the donor of A genome to the cultivated groundnut *Arachis hypogaea*. Likewise, *Arachis batizocoi* as the B genome donor however, RFLP banding pattern shows that it is more distantly related as compared to other species of the same section *Arachis*. Based on morphology, geographic distribution and cross compatibility of groundnut, which belongs to the genus *Arachis* is divided into nine intrageneric taxonomic sections (Krapovickas

Table 7.4b Nutritional values of groundnut seed (kernel)

Nutrients	Content in 100 g of seed	
	Raw	**Roasted**
Calories	564	582
Protein (g)	26	26
Fat (g)	47.5	48.7
Calcium (mg)	69	72
Iron (mg)	2.1	2.2
Thiamine (B1) (mg)	1.1	0.3
Riboflavin (B2) (mg)	0.13	0.13
Niacin (mg)	17.2	17.2

(Satish and Shrivastava 2011)

and Gregory 1994). The most diverse and the largest section *Arachis* consists of the most widely cultivated groundnut species *Arachis hypogaea* for its seeds and pods. Some of the ornamental as well as forage species are also found in this section, viz. *A. repens* and *A. pintoi*, *A. glabrata* and *A. sylvestris*, respectively. The species in other sections are mostly diploid and have very limited sexual compatibility with cultivable groundnut (Valls and Simpson 1994; Stalker and Simpson 1995). A unique cross between the wild diploid species *A. duranensis* (A genome) and *A. ipaënsis* (B genome) leading to hybridization followed by spontaneous chromosome doubling has led to the formation of the present-day cultivated groundnut species *Arachis hypogaea* (Kochert et al. 1996; Seijo et al. 2004). Single polyploidization outcome, followed by successive natural selection, has resulted in a highly conserved genome as a tetraploid, which genetically behaves as diploid. It is envisaged that the A and B genomes have nearly contributed equal amounts of DNA to the domesticated groundnut along with a single D genome species *A. glandulifera* (Stalker 1991; Singh et al. 1996).

7.4 Germplasm and Genetic Resources

Germplasm resources of any crop species are the building blocks which in turn forms the important sources of variability for different quantitative and qualitative traits that serve as reservoirs of umpteen number of potential genes for the existing stress-tolerant conditions that mitigate both biotic and abiotic stresses and emphasize breeding for future climate-resilient conditions. Hence, an array of groundnut accessions are globally conserved at national and international genebanks at different parts of the globe, viz. ICRISAT, the USA, Brazil, India and China (Ntare et al. 2006; Pandey et al. 2012a). Notable number of these accessions has been characterized for different morpho-agronomic and biochemical traits through the use of groundnut descriptors, and a large variation for both qualitative and quantitative traits along with the kernel quality traits and resistance to biotic and abiotic stress

has been observed (IBPGR and ICRISAT 1992; Jiang and Duan 1998; Barkley et al. 2016). Efficiency in exploitation of the available genetic diversity requires database information of the germplasm diversity existing for economically important traits in a given species through prebreeding activity, in turn enhancing its usage in crop improvement programmes; hence a list of major groundnut germplasm collections available throughout the world is enlisted in Table 7.5.

The narrow genetic base of the cultivated groundnut has been attributed to the lack of information of agronomic, morphological, biochemical and other economic traits and requires an extensive evaluation of the entire germplasm accessions of groundnut. Therefore, the development of a core collection could facilitate easier access to groundnut genetic resources, enhance their use in crop improvement programmes and simplify the genebank management. The development of a core collection from 14,310 accessions of groundnut was carried out at ICRISAT genebank. The ICRISAT groundnut collection was stratified first by the botanical varieties within subspecies, i.e. subsp. *hypogaea* var. *hypogaea* and var. *hirsula* Kohl and subsp. *fastigiata* var. *fastigiata*, var. *peruviana* Krapov et W. C. Gregory, var. *aequatoriana* Krapov et W. C. Gregory and var. *vulgaris* (Krapovickas and Gregory 1994; Hari 2003; Hari et al. 2003). The summary of the classification of the entire germplasm accession as per botanical variety and comparative differences of morphological descriptors among the entire and core collection of groundnut is presented in Table 7.6.

Conservation of co-adapted gene complexes that gives raise to new phenotypic associations should be prioritized while developing a core collection to have proper and adequate sampling (Ortiz et al. 1998). The Shannon-Weaver diversity index (H′) utilized for the development of core collection was found to be on par with that of the entire collection for all the descriptors, which indicates that the diversity of the entire collection is represented in the core collection. The average H′ in the core collection was 0.171 in *vulgaris* (0.157 in the entire collection), 0.283 in *aequatoriana* (0.294), 0.257 in *fastigiata* (0.228), 0.223 in *hirsuta* (0.216), 0.188 in *hypogaea* (0.167) and 0.264 in *peruviana* (0.257) suggesting that the diversity in each of the botanical variety was adequately sampled in this core collection (Table 7.7) (Hari 2003; Hari et al. 2003; Desmae et al. 2019).

7.5 Genetics of Quantitative Traits

Detailed reviews on groundnut genetics covering inheritance, cytogenetics, combining ability, genotypic and phenotypic coefficients of variation, heritability, genetic gain, genotype by environment interactions and trait correlations were published (Knauft and Wynne 1995). Most of the economically important traits such as yield, maturity and drought tolerance traits are quantitatively inherited (Knauft and Wynne 1995; Upadhyaya and Nigam 1998; Upadhyaya 2005; Ravi et al. 2011). The presence of genetic and nongenetic variances was reported for various traits (Upadhyaya et al. 1992; Pattanashetti and Gowda 2008; John et al. 2011; Janila et al. 2013). Low to high genotypic and phenotypic coefficients of variation, broad-sense heritability,

Table 7.5 Summary of Genetic resources maintained at different sources throughout the globe

Research centers	Cultivated genotypes	Wild genotypes	Total genotypes	Sources
Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India	14,968	477	15,445	Hari D. Upadhyaya, ICRISAT, India
National Bureau of Plant Genetic Resources (NBPGR), Indian Council of Agricultural Research (ICAR), India	–	–	14,585	http://www.nbpgr.ernet.in/monthly_progress/cons_july11.pdf
Plant Genetic Resource Conservation Unit (PGRCU), Griffin, U. S. Department of Agriculture (USDA), USA	9310	607	9917	Noelle Barkley, PGRCU, USA
Directorate of Groundnut Research (DGR), ICAR, India	8960	64	9024	T. Radhakrishan, DGR,
India Oil Crops Research Institute (OCRI), Chinese Academy of Agricultural Sciences (CAAS), China	7837	246	8083	Jiang Huifang, OCRI, China
Crops Research Institute (CRI), Guangdong Academy of Agricultural Sciences (GAAS), China	4210	–	4210	Xuanquiang Liang, CRI, China
Instituto Nacional de Technologia Agropecuaria (INTA), Argentina	3534	106	3640	Guillermo Seijo, IBONE, Argentina
EMBRAPA and CENARGEN, Brazil	1200	1220	2420	José F. M. Valls, EMBRAPA, Brazil
Instituto Agronomico de Campinas, Brazil	2140	–	2140	David J. Bertioli, University of Brasília, Brazil
Texas A&M University (TAMU), USA		1200	1200	Charles E. Simpson, TAMU, USA
North Carolina State University (NCSU), USA	740	406	1146	Tom H. Stalker, NCSU, USA
Instituto de Botánica del Nordeste (IBONE), Argentina	–	472	472	Guillermo Seijo, IBONE, Argentina

(Pandey et al. 2012a)

Table 7.6 Summary of classification of entire germplasm accession as per botanical variety and comparative differences of morphological descriptors among entire and core collection of groundnut

Descriptor	*Fastigata*	*Vulgaris*	*Aequatoriana*	*Peruviana*	*Hypogaea*	*Hirsuta*	Entire collection	Core collection	Differences
Stem color	2295	5089	15	249	6592	20	1.20 ± 0.003	1.22 ± 0.010	*
Stem hairs	2295	5092	15	249	6592	20	3.43 ± 0.008	3.49 ± 0.024	*
Branching pattern	2301	5098	15	249	6592	20	1.53 ± 0.004	1.53 ± 0.012	NS
Leaf Color	2300	5095	15	249	6605	20	2.50 ± 0.004	2.50 ± 0.013	NS
Leaf Shape	2296	5096	15	249	6605	20	2.99 ± 0.001	2.99 ± 0.002	NS
Leaf Hairs	2300	5094	15	249	6596	20	1.08 ± 0.003	1.10 ± 0.0012	*
Flower Color	2293	5086	15	249	6596	20	4.95 ± 0.002	4.95 ± 0.008	NS
Streak Color	2293	5086	15	249	6596	20	5.95 ± 0.002	5.98 ± 0.006	NS
Peg Color	2292	5081	15	249	6592	20	1.98 ± 0.001	1.97 ± 0.004	*
Pod Beak	2297	5091	15	249	6612	20	3.98 ± 0.011	4.00 ± 0.033	NS
Pod Constriction	2298	5091	15	249	6612	20	4.46 ± 0.010	4.45 ± 0.031	NS
Pod Reticulation	2298	5091	15	249	6612	20	4.42 ± 0.012	4.45 ± 0.038	NS
Seeds per Pod	2298	5090	15	249	6612	20	1.81 ± 0.034	1.89 ± 0.034 *	
Seed Color Pattern	2249	5038	15	249	6540	20	1.03 ± 0.001	1.03 ± 0.004	NS

(Adopted from Upadhyaya 2005; Gowda et al. 2011)

Table 7.7 Shannon-Weaver diversity index for 14 morphological descriptors in the entire and core collections of groundnut

Descriptor	Entire collection	Core collection
Stem color	0.217	0.231
Stem hair	0.246	0.264
Branching pattern	0.302	0.300
Leaf color	0.325	0.327
Leaf shape	0.009	0.022
Leaf hairs	0.111	0.128
Flower color	0.111	0.143
Streak color	0.061	0.090
Peg color	0.044	0.060
Pod beak	0.447	0.455
Pod constriction	0.396	0.419
Pod reticulation	0.468	0.493
Seeds per pod	0.489	0.512
Seed color pattern	0.054	0.063

(Adopted from Upadhyaya 2005; Gowda et al. 2011)

genetic advance and genetic advance as percentage of mean were reported for various traits including grain and pod yield, days to 50% flowering and plant height, shelling percentage, specific leaf area (SLA) and SPAD chlorophyll meter readings (SCMR), number of pods per plant and 100-seed weight (Upadhyaya 2005; Songsri et al. 2009; John et al. 2011; John et al. 2013; Padmaja et al. 2013; Patil et al. 2014; Rao et al. 2014; Padmaja et al. 2015). But quantitative inheritances were also reported for some of the traits such as oil content and quality (Dwivedi et al. 2002; Aruna and Nigam 2009; Khedikar et al. 2010; Sarvamangala et al. 2011; Sujay et al. 2012; Pandey et al. 2014; Shasidhar et al. 2017; Wilson et al. 2017).

In the case of trait correlations, grain and pod yield were reported to be positively correlated among themselves and with traits such as shelling percentage, biomass production, 100-seed weight, number of pods per plant and dry haulm yield (Padmaja et al. 2013; Padmaja et al. 2015; Rao et al. 2014) and also with drought-related traits such as harvest index (HI), SCMR and SLA (Songsri et al. 2009). On the other hand, negative correlations were reported for grain and pod yield with early leaf spot (ELS) resistance parameters, days to first flowering and days to 50% flowering (Padmaja et al. 2013; Gaikpa et al. 2015).

Most of morphological (e.g. growth and branching, leaf, pod and seed traits), quality (e.g. protein and oil) and disease resistance (leaf spots, rust) traits were reported to have predominantly qualitative inheritance (Upadhyaya and Nigam 1994, 1998, 1999; Pattanashetti and Gowda 2008; Jakkeral et al. 2013; Gangadhara and Nadaf 2016).

For quality traits negative correlations between protein content and oil content and between oleic acid and linoleic acid were reported (Sarvamangala et al. 2011).

Increasing the pod yield and oil content in addition to improving resistance/tolerance to various biotic and abiotic stresses has been the core objective of all the groundnut breeding programmes across the globe. Modern genomics hold great promise in accelerating the process of trait mapping, candidate gene discovery, functional gene identification, marker development and molecular breeding (Varshney et al. 2013; Pandey et al. 2016).

7.6 Varietal Development

The cultivated groundnut (*Arachis hypogaea L.)* is being subdivided into three distinct botanical groups, viz. Spanish (subsp. *fastigiata* var. *vulgaris*), Valencia (subsp. *fastigiata* var. *fastigiata*) and Virginia (subsp. *hypogaea* var. *hypogaea*). The Spanish and Valencia types the 'bunch' types are erect with light green foliage and pods in cluster at the base of the plant. The kernels are non-dormant and roundish with light rose testa (deep rose or purple testa in Valencia) colour. The *Virginia* group includes both spreading type Virginia runner and semi-spreading type the Virginia bunch which has dark green foliage with the branches trailing either partially or completely on the surface of the soil. The main stem is devoid of fruit and pods are scattered all along the branches. The seeds are dormant, oblong in shape with brownish testa. In general, the spreading and semi-spreading varieties mature late as compared with the bunch varieties. Groundnut is cultivated commercially in both *Kharif* and rabi/summer seasons.

Approximately about more than 20 public institutions representing 14 states are actively engaged in research and have released several high-yielding stress-tolerant varieties for cultivation in various agro-ecological zones of the country. Groundnut being a high-volume low-value crop, private contribution is limited. A comprehensive list of varieties released by various institutions and their specific characteristic features is presented in Tables 7.8a and 7.8b.

7.7 Major Constraints

Groundnut being the most important oilseed crop both as oilseed crop and fodder for cattle industry in the world, India stands first in area and a leading producer after China. However, due to its geocarpic nature of growth of pods, there are several biotic and abiotic factors hindering the performance and subsequently reducing yield levels and productivity of groundnut. It requires multidimensional strategies to combat these factors by developing an integral mechanism of tolerance/resistance genotypes.

Table 7.8a Groundnut varieties released at different institute and their botanical types

Sl. No.	Institution	Number of varieties released				Total
		Spanish bunch	Valencia	Virginia runner	Virginia bunch	
1	Achraya N.G. Ranga Agricultural University, Hyderabad	K-134, Kadiri-4, Kadiri-5, Kadiri-6, Kadiri Harithandra, Kadiri-9, Tirupati-2, Tirupati-3, Tirupati-4, JCG-88, Prasana, Abhaya, Kalahasti, Narayani, Greeshma,	–	Kadiri-71-1	Kadiri-2, Kadiri-3, Kadiri-7, Kadiri-8	23
2	Bhabha Atomic Research Centre, Mumbai	TG-3, TG-17, TG-22, TAG-24, TG-26, TG-37A, TG-38B, TG-51, TPG-41	–	Somnath	TG-1	11
3	Birsa Agricultural University, Kanke	–	–	–	BAU-13, BG-1, BG-2, BG-3	4
4	Chaudhary Charan Singh Haryana Agricultural University, Hissar	MH-1	MH-2, MH-4	–	–	3
5	Azad University of Agriculture & Technology, Kanpur	–	–	CSMG-84–1, CSMG-9510, Chandra, Chitra, T-28, Faizpur-1-5, Divya (CSMG-2003-19)	CSMG-884, T-64, Kaushal MA-16	11
6	Directorate of Oilseeds Research, Hyderabad	Mankya (DRG-12)	–	–	DRG-17	2
7	Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli	TKG-19A (TG-19A)	–	–	Konkan Gaurav	2

(continued)

Table 7.8a (continued)

Sl. No.	Institution	Number of varieties released				Total
		Spanish bunch	Valencia	Virginia runner	Virginia bunch	
8	Junagadh Agricultural University, Junagadh	J-11, GAUG-1, GG-2, GG-3, GG-5, GG-6, GG-7, GG-8, GJG-31 (J-71), GJG-9 (J-69)	–	GAUG-10, GG-11, GG-12, GG-14, GG-16, GJG-HPS-1 (JSP-HPS-44), GJG-17 (JSP-48)	GG-20, GG-21, GJG-22 (JSSP 36)	20
9	Indian Agricultural Research Institute Regional Station, Hyderabad	RSHY-1	–	–	–	1
10	International Research Institute for Semi-Arid Tropics, Patancheru	ICGS-1, ICGS-11, ICGS-37, ICGS-44, ICGV-86590, ICG (FDRS)-10, ICGV-91114	–	–	ICGS-5, ICGS-76, ICGS-86325	10
11	Jawaharlal Nehru Krishi Viswavidyalaya, Jabalpur	Jyoti, JGN-2, JGN-3, JGN-23	Ganga puri	–	–	5
12	Kerala Agricultural University, Thrissur	–	–	–	Sneha, Snigdha	2
13	Marathwada Agricultural University, Parbhani	LGN-1, TLG-45	–	–	LGN-2	3
14	Maharana Pratap University of Agriculture & Technology, Udaipur	Pratap Mungphalli-1, Pratap Mungphalli-2, Pratap Raj Mungphali	–	–	–	3
15	Mahatma Phule Krishi Vidyapeeth, Rahuri	SB XI, JL-24, JL-220, JL-286, JL-501	Koperg-aon-3	Karad-4-1	Kopergoan-1, B-95	9
16	Directorate of Groundnut Research, Junagadh	Girnar-1, Girnar-3	–	–	Girnar-2	3

(continued)

Table 7.8a (continued)

Sl. No.	Institution	Number of varieties released				Total
		Spanish bunch	Valencia	Virginia runner	Virginia bunch	
17	Odisha University of Agriculture & Technology, Bhubaneswar	Kisan, Jawan, Smruti (OG-52-1)	–	–	–	3
18	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola	AK-12-24, AK-159	–	UF-70-103	AK-265, AK-303	5
19	Punjab Agricultural University, Ludhiana	SG-84, SG-99	–	Punjab-1, M-13, M-37, M-335, M-548	c-501, M-45, M-522, M-197	11
20	Rajsthan Agricultural University,Bikaner	RG-141	–	RS-1, RG-382, Raj Mungfali-1 (RG-510)	RSB-87, RSB-138, HNG-10, TG-39, HNG-69, HNG-123, ICHG-00440, Raj Durga (RG-425)	12
21	Tamil Nadu Agricultural University, Coimbatore	Pollachi-1, Pollachi-2,ALR-2, CO-1, CO-2, CO-3, Co (Gn)-4, TMV-2, TMV-5, TMV-7, TMV-9, TMV-12, TMV (Gn)-13, VRI-1, VRI-2, VRI-3, VRI-4, ALR-3, VRI (Gn)-5, VRI (Gn)-6, BSR-1	TMV-11	TMV-1, TMV-3, TMV-4	TMV-6, TMV-8, TMV-10, ALR-1, ICGV-00348, VRI(Gn)-7, Co (Gn)-5, Co-6	33
22	University of Agricultural Sciences, Dharwad	Spanish Improved, Dh-3-30, Dh-8, Dh-86, GPBD-4, Dh-101, Mutant-III, KRG-1, S-206, GPBD-5, R-9251, R-8808, R-2001-3, R-2001-2	–	DSG-1, S-230	TGLPS-3	16
23	Vivekananda Parvatiya Krishi Anusandhan Shala, Almora	VL-Moongphali-1	–	–	–	1
	Total	**107**	**5**	**31**	**50**	**193**

(Rathnakumar et al. 2013)

Table 7.8b Specific characteristic features of groundnut varieties cultivated in different states of India

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
TLG 45	2007	MAU, Latur	1506	51	Maharashtra	Large-seeded (HSM = 59 g); medium maturity (114 days); recommended for kharif season
Narayani (TCGS 29)	2007	ANGRAU, Tirupati	3764	48	Andhra Pradesh	Tolerant to mid-season moisture stress conditions; recommended for both kharif and rabi-summer seasons
Phule Unap (JL 286)	2007	MPKV, Jalgaon	2231	49	Maharashtra	Tolerant to LLS, rust and stem rot; also tolerant to thrips, leaf miner and Spodoptera
Ratneshwar (LGN 1)	2007	MAU, Latur	1487	51	Maharashtra	Moderately resistant to LLS, stem rot, rust and PBND; tolerant to sucking pests; recommended for kharif season
Vasundhara (Dh 101)	2007	UAS, Dharwad	2877	50	West Bengal, Orissa, Jharkhand and Assam	Tolerant to stem rot and PBND; tolerant to thrips and Spodoptera; suitable for rabi-summer season

(continued)

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
ICGV 91114	2007	ICRISAT, Hyderabad	2000	48	Andhra Pradesh	Tolerant to rust and LLS; early maturity (100 days); tolerant to drought; recommended for kharif season
AK 265	2007	PDKV, Akola	1903	47	Southern Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu	Resistant to foliar diseases; drought tolerant; recommended for kharif season
M 548	2007	PAU, Ludiana	2185	51	Punjab	Tolerant to leaf spots and collar rot; recommended for kharif season
AK 303	2007	PDKV, Akola	2100	49	Maharashtra	Bold seeded (HSM = 80 g); recommended for kharif season
TG-39	2008	Raj AU, Bikaner	2054–3154		Arid and semi arid region of Rajasthan	Medium duration
TG 51	2008	BARC, Mumbai	2675	49	West Bengal, Orissa, Jharkhand and Assam	Tolerant to stem rot and root rot; suitable for rabi-summer season.
Ajeya (R 2001–3)	2008	UAS, Raichur	2440	46–48	Southern Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu	Resistant to PBND; drought tolerant; recommended for kharif season

(continued)

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
Girnar 2 (PBS-24030)	2008	NRCG, Junagadh	2907	51	Uttar Pradesh, Punjab, northern Rajasthan	Virginia bunch type with 'stay green' leaves and bold seeded (HSM =62 g); tolerant to rust, LLS PSND; recommended for kharif season
ICGV 00348	2008	TNAU, Vridhachalam	2013	47	Southern Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu	Tolerant to late leaf spot and rust; recommended for kharif season
VRI (Gn) 7	2008	TNAU, Vridhachalam	1865	48	Tamil Nadu	Moderately resistant to leaf miner, LLS and rust; recommended for kharif season
VL-Moongphali-1	2008	VPKAS, Almora	1943	–	Uttarakhand	Resistant to late leaf spot and root rot; recommended for kharif season
Utkarsh (CSMG 9510)	2009	CSAUAT, Mainpuri	21.92	49	Uttar Pradesh, Punjab, Northern Rajasthan	Resistant to rust, possess fresh seed dormancy up to 40–45 days; recommended for kharif season
VRI (Gn) 6 (VG 9816)	2009	TNAU, Vridhachalam	2259	47	Southern Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu	Tolerant to LLS, rust, PBND; recommended for kharif and rabi-summer seasons

(continued)

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
Jawahar Groundnut 23 (JGN 23)	2009	JNKVV, Khargone	1631	49	Madhya Pradesh	Tolerant to ELS and LLS; drought tolerant; recommended for kharif season
Kadiri 9	2009	ANGRAU, Kadiri	2500–3000	52	Andhra Pradesh	Tolerant of thrips, jassids, and nematodes. Tolerant to late leaf spot, rust, dry root rot and collar rot. Recommended for kharif season
Greeshma	2009	ANGRAU, Tirupati	2000–2500	49	Andhra Pradesh	Tolerant to LLS, drought, high temperature and aflatoxin; recommended for kharif and rabi-summer season
Kadiri 7	2009	ANGRAU, Kadiri	1643	47	Andhra Pradesh	Tolerant to sucking pests and leaf spots; bold seeded (HSM =65–75 g); recommended for kharif season
Kadiri 8	2009	ANGRAU, Kadiri	1523	47	Andhra Pradesh	Tolerant to sucking pests and leaf spots; bold seeded (HSM = 65–75 g)

(continued)

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
Mallika (ICHG00440)	2009	RAU, Hanumangarh	2579	48	All India	Resistant to collar rot and PBND; bold seeded (HSM = 73 g), recommended for kharif season
TGLPS 3 (TDG-39)	2009	UAS, Dharwad	2500–3000	–	Karnataka	–
GG 21 (JSSP 15)	2009	JAU, Junagadh	1843	53	Uttar Pradesh, Punjab, northern Rajasthan	Recommended for kharif season
JSP-39	2009	UAS, Dharwad	3000	49	AP, Karnataka, TN, Maharashtra	Tolerant to foliar diseases and root rot.
JL 501	2010	MPKV,	1661	48	Gujarat and southern Rajasthan	Suitable for early as well as late sown rainfed condition
Vijetha (R 2001–2)	2010	UAS,Raichur	1600	47	West Bengal, Orissa and Jharkhand Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu	Resistant to PBND; recommended for rabi-summer season
HNG 69	2010	RAU, Hanumangarh	2800	50	Uttar Pradesh, Punjab, northern Rajasthan	Tolerant to collar rot, stem rot and ELS; recommended for kharif season
Girnar 3 (PBS 12160)	2010	DGR, Junagadh	1520	45	West Bengal, Orissa, Manipur	Tolerant to leaf miner and thrips; recommended for kharif season

(continued)

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
Kadiri Haritandhra (K 1319)	2010	ANGRAU, Kadiri	3728	48	Karnataka and Maharashtra	Multiple diseases and insect pests resistant, possess fresh seed dormancy upto 20 days; recommended for rabi-summer season
VL-Moongphali-1	2010	VPKAS, Almora	1940	42.2	Uttarakhand (Kharif)	Resistant to LLS and root rot diseases. (State release)
GPBD-5	2010	UAS, Dharwad	1500	46	Jharkhanad and Manipur (K)	Resistant to LLS and rust.
GJG-HPS-1 (JSP-HPS-44)	2010	JAU, Junagadh	2120	49	Gujarat (Kharif)	Rose colour seed.
Phule vyas (JL-220)	2010	MPKV, Jalgaon	2000	52	Maharashtra	Early maturing, High oil content.
Bheema	2010	RARS, Tirupati	3500–5000	45	Andhra Pradesh	Suited to Kharif and rabi regions
Rohini	2010	RARS, Tirupati	3700–4000	50	Andhra Pradesh	Suited to Kharif and rabi areas. Tolerant to mid and end season.
Pratap Raj Mungphalli	2011	MAUT, Udaipur	1600–2200	48	Rajasthan	Moderately tolerant to ELS, LLS and PBND, Suited for Kharif and Summer
ALG-06-320	2011	TNAU, Aliayarnagar	3500	49	Vidharbha & Southern M.P.	Suitable for rabi/summer
RG-510	2011	RAU, Durgapur	2600	49	Rajasthan & Punjab	Resistant to collar rot, stem rot, early leaf spot, rust and stem necrosis.

(continued)

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
RG 425	2011	RAU, Durgapur	1800–3600	48	Rajasthan	Resistant to collar rot and tolerant to drought. Suitable for Kharif.
RHRG-6021	2011	MPKV, Rahuri	3800	51	Western Maharashtra	Resistant to rust, LIS and stem rot and spodoptera
Divya (CSMG-2003-19)	2011	CAUAST, Mainpuri	3000	49	Uttar Pradesh	Resistant to leaf spots and tolerant to BND.
HNG-123	2012	RAU, Hanumangadh	3000	49	Rajasthan, UP & Punjab	Virginia bunch variety, Tolerant collar rot, stem rot, LLS, Spodoptera
RARS-T-1	2011	ANGRAU, Tirupati	2500(K) 4000®	44	Andhra Pradesh	Kharif, Rabi-summer, Bold seeded pods
RARS-T-2	2011	ANGRAU, Tirupati	3734(K) 4200®	48	Andhra Pradesh	Kharif, Rabi-summer, Early maturity
ICGV-00350	2012	RARS, Tirupati	3000–4400	48	Tamil Nadu & Andhra Pradesh	Tolerant to LLS, rust, stem rot, High fodder value.
CO-6	2012	TNAU, Coimbatore	1914	50–51	Tamil Nadu	Kharif, Resistant to LLS & Rust
GJG-31 (J-71)	2012	JAU, Junagadh	1632	49	Gujarat	Tolerant to stem rot, Free from PBND, Suitable for Summer.
GJG-9 (J69)	2012	JAU, Junagadh	3483	49	Gujarat	Suitable for Summer, tolerant to stem rot.
GJG-22 (JSSP 36)	2013	JAU, Junagadh	1770	52	Gujarat	Suited to Kharif, semi spreading groundnut area. Tolerant to collar rot.

Table 7.8b (continued)

Variety	Year of release	Releasing centre	Yield Potential (Kg/ha)	Oil content (%)	Recommended for (state/ region)	Specific features
GJG-17 (JSP-48)	2013	JAU, Junagadh	1798	48	Gujarat	Suitable for Kharif, spreading groundnut area. Tolerant to stem rot
Dharani (TCGS-1043)	2013	RARS, Tirupati	3000		Andhra Pradesh	Recommended for all the three situations Kharif (rainfed): June–July Kharif (irrigated): May Rabi (irrigated): second fortnight of November-first fortnight of December. Timily sown Sandy Clay loams

7.7.1 Yield and Yield-Related Traits

Pod yield in groundnut is the most important economical part of the plant that fetches both kernels and oil and is a function of crop growth rate, duration of reproductive growth and the fraction of crop growth rate partitioned towards pod yield (Janila et al. 2013). The complexity nature of pod yield and the G X E interaction effects limiting the genetic gains form the major barrier for improvement of groundnut productivity (Nigam and Bock 1990). Majority of the efforts towards increasing yield in India came from improvement in seed size, seed weight and number of pods per plant. It was reported that improved varieties alone contributed to 30% yield increase in India since 1967 (Reddy and Basu 1989). JL 24, a high-yielding variety with wide adaptability, has been released in several countries. It was released as Phule Pragati in 1979 in India (Patil et al. 1980); subsequently, it was introduced to Africa and released as JL 24 in Congo (1990), Sera Leone (1993) and South Africa (2002), as Luena in Zambia (1999), as Kakoma in Malawi (2000), as Saméké in Mali (2000) and as ICG 7827 in Mozambique (2011) and is commercially cultivated in several other countries (Chiyembekeza et al. 2001). It was also released in 1985 as Sinpadetha 2 in Myanmar and in 1992 as UPL Pn 10 in the Philippines.

7.7.2 Quality Traits

Oil and oleic acid content and confectionery traits are among the important quality traits. Various physical sensory, chemical and nutritional factors determine the quality of groundnut for which substantial genetic variability exists (Dwivedi and Nigam 2005). Near-infrared reflectance spectroscopy (NIRS), a robust and nondestructive method, is gaining popularity for the estimation of oil, protein, carbohydrate and fatty acid contents (Pasupuleti and Nigam 2013). It is also cost-effective compared with wet chemistry. At ICRISAT, a large number of accessions screened had 34%–55% oil content (Dwivedi and Nigam 2005). Several advanced lines for high oil content have also been recently developed (Pasupuleti et al. 2016). In the case of oleic acid content, very few lines are officially released, specifically for high O/L ratio (e.g. SunOleic 95R and SunOleic 97R in the USA; PC 223 K8 and PC 223 K9). With regard to confectionery types, a large number of varieties have been identified (Mayeux et al. 2003; Monyo and Varshney 2016).

7.7.3 Biotic Stresses

In India, about 80 per cent of the crop is grown under marginal lands as rainfed situation which causes low productivity, coupled with several biotic and abiotic stresses, viz. diseases and insect pests and drought, salinity heat and cold stress, is compounded to limit the reproductive ability and productivity of groundnut (Nigam 2000). Although several nongenetic measures are available for the control of various foliar diseases, they prove to be very expensive, uneconomical and non-affordable for the small and marginal farmers besides polluting the environment. Hence, the most appropriate and viable option for minimizing economic losses to the farmers and maintaining good quality of the product is the development and cultivation of resistant cultivars (Tiwari et al. 2018). Although diversified resistant sources for various biotic stresses are existing, only limited use of resistant germplasm is made in crop improvement through hybridization. These could be utilized in resistance breeding programmes to produce stable and high-yielding resistant lines (Naidu et al. 2016). A concise list of diseases and their causal organisms and other insect pest damaging groundnut is presented in Tables 7.9a and 7.9b (Fig. 7.3).

Among foliar diseases, three major diseases, viz. rust (*Puccinia arachidis* Speg.), early leaf spot (ELS, *Cercospora arachidicola*) and late leaf spot (*Phaeoisariopsis personata* Ber.), are global constraints against groundnut production. These are considered as economically important foliar diseases as they are the most widely distributed and economically damaging diseases of groundnut. These diseases in addition to causing more than 70% yield loss are known to adversely affect the quality of the produce (pods, seeds and haulms). Late leaf spot is a major and widely distributed disease that is known to cause defoliation and reduce pod and fodder yields about 50% and adversely affect quality of its produce.

Table 7.9a List of different diseases along with their causal organisms affecting of Groundnut

Disease	Name	Causal organisms
Foliar diseases	Early Leaf spot (ELS)	*Cercospora arachidicola*
	Late Leaf spot (LLS)	*Phaeoisariopsis personata Ber.*
	Rust	*Puccinia arachidis Speg*
	Alternaria leaf spot and Veinal	*Alternaria arachidis*
	Necrosis	*Sclerotium rolfsii*
	Stem rot	*Sclerotium rolfsii*
	Sclerotium leaf spot	*Alternaria arachidis and A. tenuissima*
	Altenaria leaf blight	*Alternaria alternata (Fr.) Keissler*
	Pepper spot and leaf scorch	*Diplodia gossypina*
Seed and seedling	Collar rot	*Aspergillus niger, A. flavus*
	Crown rot	*Aspergillus pulverulentum*
	Stem rot	*Sclerotium rolfsii*
	Fusarium wilt	*Fusarium oxysporum*
	Dry root rot	*Macrophomina phaseolina (Tassi)*
	Pod rot	*Sclerotium rolfsii*
	Aflaroot	*Aspergillus flavus*
Bacterial diseases	Bacterial wilt	*Ralstonia solanacearum*
	Bacterial leaf spot	*Peanut Bud Necrosis Virus of Poty group*
Virus diseases	Peanut bud necrosis	*Tomato spotted wilt virus*
	Peanut yellow spot	*Aspergillus flavus*
	Peanut stripe virus	*Peanut stripe virus (PStv)*
	Goundnut rosette virus	*Groundnut Rosette Virus (GRV)*
Nematode diseases	Root knot Nematode	*Meloidogyne arenaria (Neal) Chitwood, M.hapla Chitwood*
	Kalahasti Malady	*Tylenchorhynchus brevilineatus Williams*

Table 7.9b List of insects along with their scientific names affecting groundnut

Category	Names	Scientific name
Sucking pests leaf miner	Leaf Minor	*Aproaerema modicella*
	Aphids	*Aphis crassivora*
	Jassids	*Empoasca kerri pruthi*
	Thrips	*Calliothrips spps*
Defoliators	Red Hairy Caterpillar	*Amsacta albistriga*
	Tobacco Caterpillar	*Spodoptera litura*
	Gram pod borer	*Helicoverpa armigera*
	White Grub	*Holotrichia Spp.*
Soil pests	White Grub	*Apogonia spps*
	Termites	*Microtermes spps*
Storage Pests	Bruchid Beetle	*Caryedonn seratus*

(Annual Report 2020)

Fig. 7.3 Evaluation of RIL population to explore their pod yield potential, biotic and abiotic stresses

7.7.3.1 Leaf Spots

Early leaf spot (ELS) and late leaf spot (LLS) which are caused by *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk & Curt.) Deighton, respectively, are the most common and serious diseases of groundnut, which can cause pod yield losses of over 50% (McDonald et al. 1985; Mayeux and Ntare 2001). Field and laboratory screening methods involve sowing genotypes in replicated plots with rows of a highly susceptible cultivar arranged systematically throughout the trial with good disease development ensured through the provision of inoculum (McDonald et al. 1985). A 9-point disease scale is used for measuring reactions separately for the two leaf spots. Earlier germplasm screenings resulted in the identification of promising lines for resistance sources (Subramanyam et al. 1985), and since then, many additional lines have become available as good sources of resistance (Izge et al. 2007; Kanyika et al. 2015; Monyo and Varshney 2016) (Figs 7.4 and 7.5).

Fig. 7.4 Screening of recombinant inbred lines for late leaf spot and rust disease under natural epiphytotic conditions during *Kharif season 2019*

7.7.3.2 Rust

Groundnut rust, caused by *Puccinia arachidis* Speg., is an economically important disease that significantly reduces the pod and fodder yield and oil quality. Rust is also an economic important disease causing yield losses ranging from 10 to 52%, in addition to a decline in seed quality. Protocols for screening genotypes at field condition involve the use of infector row technique (Subramanyam et al. 1985). Reviews on groundnut breeding for rust resistance are available (Subrahmanyam et al. 1997; Mondal and Badigannavar 2015). Earlier rust screening efforts identified some advanced rust-resistant lines such as ICG (FDRS) series (Reddy et al. 1987). Later, more accessions and advanced lines were identified (Subrahmanyam et al. 1998;

Fig. 7.5 Disease reaction of RILs against late leaf spot under natural epiphytotic condition

Reddy et al. 2001; Varshney et al. 2014; Monyo and Varshney 2016). Some of these lines combine rust and leaf spot resistance.

7.7.3.3 The Stem/Pod Rot and Peanut Bud Necrosis

The stem and pod rot caused by *Sclerotium rolfsii* Sacc. commonly occurs, and yield losses usually range from 10 to 25 per cent, but may reach up to 80 *per cent* in severely infected fields. Peanut bud necrosis virus (PBNV) caused by tomato spotted wilt virus is a severe problem in dry regions and may result in yield reduction up

to 80 per cent. The yield losses due to defoliating insect, tobacco cutworm (*Spodoptera litura* F.), range from 13 to 71 per cent. The different diseases and insect pests affecting groundnut cultivation are presented in Tables 7.7 and 8 (Chohan and Singh 1974; Ami 1983; Subrahmanyam et al. 1984; Nigam et al. 2012).

7.7.3.4 Rosette

Groundnut rosette disease (GRD) caused by the groundnut rosette virus (GRV), groundnut rosette assistor virus (GRAV) and satellite RNA (Pasupuleti and Nigam 2013) is a devastating disease. A method for simultaneous detection of the three causal agents has been published (Anitha et al. 2014). Sources of resistance were first discovered in cultivars from Burkina Faso and Cote d'Ivoire in 1952 (Subrahmanyam et al. 1998; Ntare et al. 2002). Resistance among these cultivars was effective against both chlorotic and green rosette forms of the disease and was governed by two independent recessive genes (Nigam and Bock 1990; Olorunju et al. 1992). Breeding through utilizing the cultivars resulted in the development of long-duration Virginia cultivars and early and medium maturing Spanish types (Ntare et al. 2002; Mayeux et al. 2003; Monyo and Varshney 2016).

7.7.3.5 Aflatoxin

Aflatoxin contamination induced by *Aspergillus flavus* and *A. parasiticus* is a major constraint to the global trade of groundnut. Low-altitude warmer ecologies with low precipitation support high occurrence and distribution of *aflatoxigenic aspergilli* in soil and high aflatoxin B1 contamination in groundnut. Three resistance mechanisms have been focuses of aflatoxin resistance breeding: (a) preharvest natural seed infection, (b) aflatoxin production and (c) in vitro seed colonization (IVSC). Nigam et al. (2009) described a large number of groundnut lines that showed IVSC resistance (15% or fewer seeds colonized) and seed infection resistance (<2% seed infection) including five elite lines recommended for cultivation in SA. In WCA, three varieties were reported for resistance to aflatoxin (Mayeux et al. 2003). More recently, seven accessions with consistent very low aflatoxin accumulation were identified (Waliyar et al. 2016). However, G × E interaction remains a major issue in screening for aflatoxin resistance (Nigam et al. 2009), and generally, little progress has been made in using conventional breeding for enhancing host-plant resistance to aflatoxin contamination (Waliyar et al. 2016). Even if some elite lines were recommended for cultivation in India (Nigam et al. 2009), so far no prominent variety has been officially released with aflatoxin resistance. Two varieties (J 11 and 55-437) released for yield and agronomic performance are known to have a good level of resistance and serve as standard checks. Recent efforts using biotechnology options have reported a high level of resistance in groundnut by overexpressing antifungal plant defensins

MsDef1 and MtDef4.2 and through host-induced gene silencing of aflM and aflP genes from the aflatoxin biosynthetic pathway (Sharma et al. 2018).

7.7.4 Abiotic Stress

Among the abiotic stresses, drought, salinity and high or low temperature are the most prominent stresses observed worldwide and widespread in countries cultivating groundnut (Karim et al. 1990; SRDI 2003). Based on the time of occurrence, drought could be characterized as early-season, mid-season and end-of-season drought, of which mid- and end-of-season droughts prove to be very crucial and critical as they affect both the pod yield and quality. Besides, end-of-season drought predisposes preharvest *Aspergillus* infection in the field that further affects the quality of the produce. Linked closely with drought is high temperature stress. Two key stages for heat stress in groundnut are flowering including microsporogenesis (3–6 days before flowering) and fruit set (Craufurd et al. 2003). Understanding the underlying mechanism of drought tolerance significantly helps in achieving progress in groundnut over the years, which could be made possible through the development of efficient physiological trait-based and empirical selection approaches (Nigam et al. 2005) to breed for drought tolerance in groundnut.

The interrelation between crop yield and water use can be expressed as yield = transpiration × WUE × harvest index, where WUE is the amount of biomass produced per unit of water transpired. It is associated with drought avoidance mechanisms adopted by plants under water stress conditions (Anyia and Herzog 2004). WUE is positively associated with SCMR (SPAD chlorophyll meter readings), but it is negatively related with specific leaf area (SLA) (Songsri et al. 2009). As the scope to achieve a high level of transpiration (T) under drought-prone environment, increasing WUE will offer an avenue for maintaining high yield in water stress (Table 7.10, Fig. 7.6).

The surrogate trait-based approaches measuring WUE which employs SPAD (soil plant analysis development) and SLA (specific leaf area) for drought tolerance, and they are often used in combination with empirical approach. Drought-adaptive traits such as root traits are identified as and are used as selection criteria for drought resistance. However, it is limited because elaborate phenotyping protocols are required. So far, studies on heat tolerance in groundnut were limited to few screening studies reporting tolerant lines for heat stress (Craufurd et al. 2003; Hamidou et al. 2013).

In addition to conventional breeding methods, marker-assisted selection establishes to be an important tool to enhance tolerance or resistance to these stresses and genetic improvement of popular varieties for targeted traits. It is efficiently introgressing and transferring targeted traits into the desirable cultivar within 2–3 years through marker-assisted backcrossing (MABC) as against 6–8 years required by the conventional methods (Janila et al. 2013; Kanyika et al. 2015; Varshney 2016).

Table 7.10 List of publicly available SSR markers linked to WUE related traits and other yield related traits

Character	QTL name	Nearest marker	Position (cM)	Highest LOD	R^2	Reference
Specific leaf area	SLApreTest04-VII	PM427	0.1	2.97(3.1)	3.5	Varshney et al. (2009)
	SLAHar0.5-XVII	IPAHM 105	0.1	3.45(3.3)	4.2	Varshney et al. (2009)
SPAD at vegetative stage	SPAD pre Trt04-XVI	pPGPSeq2B09	2.3	6.02(2.9)	10.6	Varshney et al. (2009)
	SPAD005-XVII	IPAHM 105	0.1	4.47(2.5)	6	Varshney et al. (2009)
SPAD at stage of harvest	SPAD stress start 04-XVI	pPGPSeq2B09	2.1	4.58(3.0)	8.2	Varshney et al. (2009)
	SPAD07 understress04-Xia	Ah-193	2.1	3.21(2.9)	5.1	Varshney et al. (2009)
	SPADD1005-X	IPAHM 165	0.1	4.23(2.5)	5.7	Varshney et al. (2009)
	SPADD1005-XVII	IPAHM 165	0.1	3.03(2.8)	4.5	Varshney et al. (2009)
	SPADD1505-X	IPAHM 165	0.1	3.64(5.5)	5.1	Varshney et al. (2009)
	SPADD1505-XVII	IPAHM 165	0.1	2.08(1.5)	2.9	Varshney et al. (2009)
100 kernal weight	–	PM 137	0.8	3.21(2.5)	6.9	
	–	PGS19D09	1.2	3.01(2.3)	5.1	
	–	PM384	1.5	2.5(2.0)	3	
100 pod weight	–	PM375	1.7	3.21(2.3)	9.1	Varshney et al. (2009)

(Varshney et al. (2009))

7.8 Genomic Resources

Although in recent years a range of marker systems including hybridization-based Diversity Array Technology (DArT) and sequence-based markers such as single nucleotide polymorphisms (SNPs) have become available, simple sequence repeat (SSR) or microsatellite marker is still the preferred marker system especially for genetics and breeding applications. SSRs exhibit polymorphism in terms of variation in the number of repeat units as revealed by amplification of unique sequences flanking these repeat units. They show co-dominant inheritance and therefore are suitable for genotyping segregating populations (including F_2). Multi-allelic nature of the markers enables them to detect a large number of allelic variants in the germplasm collection. Availability of genomic resources in groundnut is presented in Table 7.11.

Until recently, development of SSR markers was largely based on screening of SSR-enriched or size-selected DNA libraries; however mining of ESTs (expressed

Fig. 7.6 Indicating stress imposed on RIL population and its recovery after releasing stress

sequence tags) or BAC-end sequences (BESs) have become popular approaches for development of SSR markers. SSR markers developed from ESTs or cDNA sequences are referred to as 'genic SSR' or 'genic markers' (Varshney et al. 2010). By using a range of different approaches mentioned above, 3000–6000 SSR markers have become available in the target SAT legume crops. For instance, in the case of chickpea, ca. 2000 SSR markers have been developed from genomic DNA libraries (Varshney et al. 2007), ESTs (Varshney et al. 2009), 454/FLX transcript reads (Hiremath et al. 2011) and BESs. Similarly, another set of 487 novel functional markers including 125 EST-SSRs, 151 intron targeted primers (ITPs), 109 expressed sequence tag polymorphisms (ESTPs) and 102 SNP markers has been developed at the National Institute of Plant Genome Research (NIPGR). In the case of pigeon pea, a large number of SSR markers have been developed from BESs and 454/FLX sequences. After mining 88,860 BESs, a set of 3072 SSR markers was developed. In addition, 3583 SSRs were identified from ESTs and 454/FLX sequences (Dutta et al. 2011). Furthermore, by scanning the draft genome sequence of pigeon pea,

Table 7.11 Availability of genomic resources in groundnut

Specification	Information	Reference
BAC libraries	ca. 5.3 × –Diploid (BB); ca. 7.4 × –diploid (AA)	
BAC-end sequences	182,784 and 36,435	Wang et al. (2012)
EST	253,274	Pandey et al. (2012a)
SSR	>6000	Pandey et al. (2012a)
TILLING population	3400 mutant M2 lines	
DArT clones	ca. 15,000	Varshney et al. (2010)
454/FLX reads	1000,000	
SNPs	>2000 SNPs, 768-SNP	Pandey et al. (2012a)
Mapping populations	Diploid (AA) – 5, Diploid (BB) – 1, Tetraploid –39 Diploid (AA)-3, Diploid (BB)-2, Tetraploid-13 maps and one reference consensus map	Pandey et al. (2012a)
Complete genome sequence	In progress	

(Adopted from Varshney et al. 2013)

309,052 SSRs have been identified (Varshney et al. 2012), and they can be used to enrich genetic maps with more number of molecular markers and also to tag QTL/genes for important traits. In the case of groundnut, >6000 SSRs have become available by the international groundnut community (Pandey et al. 2012a); Wang et al. 2012). After screening 4500 SSR markers on parental lines of several mapping populations, 199 highly informative SSR markers with polymorphism information content (PIC) value of >0.50 were identified (Pandey et al. 2012c). Similarly, more recently a set of 66 highly informative SSRs (>0.5 PIC) with long TC repeats has been reported (Macedo et al. 2012).

DArT marker system is another marker resource mainly used for diversity studies, for saturating linkage maps and also for identifying introgressions from other species. ICRISAT in collaboration with DArT Pty Ltd., Australia, has developed DArT arrays with 15,360 features for chickpea, groundnut and pigeon pea crops (Varshney et al. 2010). Screening of elite germplasm of the SAT legume crops with these DArT arrays, however, showed very little polymorphism. Interestingly, DArT markers have been found very useful for monitoring the genome introgression in the cultivated species of pigeon pea from the wild species (Mallikarjuna et al. 2011). Because of higher abundance and amenability to high throughput, SNP markers are becoming popular marker system in several crop species.

Once SNPs are identified, development of an appropriate SNP genotyping platform is very critical to make the SNP genotyping cost-effective. In the SAT legume crops, a range of SNP genotyping platforms have become available. For instance, the University of California-Davis, USA, in collaboration with some partner

institutes has developed Illumina GoldenGate assays for genotyping 768 SNPs in chickpea, pigeon pea and diploid *Arachis* species. Similarly, the University of Georgia, USA, has also developed an Illumina GoldenGate SNP array comprising of 1536 SNPs with high confidence for *Arachis* species. These assays are most suitable when a relatively large number of SNPs (>500) need to be genotyped with a large number of samples. However, in the case of certain molecular breeding applications which generally require less number of markers (b400), GoldenGate-based SNP arrays are not very cost-effective (Hiremath et al. 2011).

7.9 Mapping Populations and Marker-Trait Associations in Groundnut

Molecular markers have enabled the development of different kinds of genetic maps utilizing various mapping populations. Development of different types of genetic populations is the quintessential need of the hour for effective usage of marker-trait association with agronomically important traits. An array of genetic population would be developed and utilized in molecular approaches, viz. recombinant inbred lines (RILs), F_2 population, near-isogenic lines (NILs), backcross introgression lines (BILs), natural populations such as groundnut reference set or mini core collection, nested association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) populations (Pandey et al. 2012b; Varshney et al. 2013). The first SSR-based genetic map was developed with 135 loci using a recombinant inbred line (RIL) population (Varshney et al. 2009). A schematic representation of integrated breeding approach for trait improvement in groundnut has been presented in Varshney et al. (2013)).

7.10 Genomic-Assisted Breeding for Trait Improvement

Genomic-assisted breeding refers to integration and use of genomic tools in breeding practices for developing superior groundnut cultivars with enhanced biotic or abiotic stress tolerance and improved yield levels. GAB includes a range of approaches including genomics, transcriptomics and proteomics to identify the molecular markers associated with traits of interest to the breeders that help prediction of phenotype from the genotype to assist breeding. With the advent of next-generation sequencing (NGS) technologies (Varshney et al. 2009) and high-throughput genotyping technologies, it has been possible to use the genome-wide marker profile/allele data for prediction of phenotype of progenies for selection to the new cycle in breeding programmes. There are various GAB strategies that can be utilized in plant breeding activities, viz. marker-assisted backcross breeding (MABC), marker-assisted recurrent selection (MARS), genomic selection, genome-wide association studies and advanced-backcross QTL analysis.

Genomic-assisted breeding (GAB) could be practised either through simple marker-assisted selection (MAS) approach or marker-assisted backcrossing (MABC). The selection of breeding lines in GAB requires three categories of markers: (a) foreground selection, which involves using molecular markers for selecting the target gene or QTL; (b) recombinant selection, which involves selecting of backcross progenies containing the target gene, and recombination events between the target locus and linked flanking markers; and (c) background selection, wherein the plants/progenies are selected based on recovery of the highest proportion of recurrent parent genome.

7.11 Advanced-Backcross QTL Analysis-Based Breeding (AB-Breeding)

The molecular breeding approaches MABC, MARS and GS are reclaimable only if superior alleles for the trait of interest are available in the breeding in primary gene pool which is not assured always. Hence, novel approach of advanced-backcross QTL-based breeding (AB-breeding) is appropriate for introduction of novel alleles from wild relatives to the cultivated species. In AB-breeding approach, a selected wild species is backcrossed to a cultivar or a variety, and then, selection is imposed in segregating BC_2F_2 or in BC_2F_3 population to identify and preserve individuals with desirable traits in the population. Both genotyping and phenotypic data are generated with this segregating BC_2F_2 or in BC_2F_3, and these data sets will be subjected to QTL analysis to identify QTL and QTL-associated markers and also to check whether any of these QTLs are involved in trait improvement in the progenies that are preserved (Tables 7.12a and 7.12b). Therefore, AB-QTL strategy involves the parallel discovery and transfer of desired QTL from an unadapted germplasm into selected breeding lines (Tanksley and Nelson 1996). In addition, AB-QTL strategy postpones the QTL mapping up to BC_2 or BC_3 generations to avoid problems associated with incompatibility and pollen fertility in the initial backcross populations as well as to ensure maximum genome recovery from the recurrent parent. AB-breeding can help in tracking alien genomic regions, and hence, the linkage drag can easily be taken care of. Two major studies by Simpson et al. (1993) and Fa'vero et al. (2006) reported development of three amphiploids using a range of wild AA and BB genome species like *A. cardenasii*, *A. diogoi* and *A. batizocoi*, *A. ipaensis*, *A. duranensis*, *A. gregoryi* and *A. linearifolium*. In order to diversify the primary gene pool and conduct AB-QTL analysis, ICRISAT has developed a set of 17 amphiploid and autotetraploid groundnuts (Mallikarjuna et al. 2011). Furthermore, two AB-QTL mapping populations, namely, ICGV 91114 (cultivated) × ISATGR 1212 (*A. duranensis* ICG 8123 × *A. ipaensis* ICG 8206, synthetic amphidiploid) and ICGV 87846 (cultivated) × ISATGR 265-5A (*A. kempff-mercadoi* ICG 8164 × *A. hoehnei* ICG 8190, synthetic amphidiploid), have been developed (Mallikarjuna et al. 2011) (Table 7.13). Off-late, the advanced mapping populations

Table 7.12a Reported QTLs for important traits of breeding interest in groundnut

Trait	Total[a]	PVE	Major	Population	Reference
Number of QTLs identified for agronomic and yield component traits					
GH, plant spread, MSH, PH, total biomass, DF, PoM, LNB, haulm weight, shell weight, shelling %, HI, pod number pod weight, seed number 100-SW, pod beak, pod constriction, pod length, pod width,seed width, seedlength, FSD	7	9.19–17.69	5	Tamrun OL01 × BSS 56	Selvaraj Gomez et al. (2009)
	106	8.50–26.70	29	[Fleur 11 × (*A. ipaënsis* × *A. duranensis*)[4×]]	Fonceka et al. (2012)
	23	4.80–28.20	17	Satonoka×Kintoki	Shirasawa et al. (2012)
	25	6.20–30.40	9	*A. ipaënsis* (K30076) × *A. magna*(K30097)	Leal-Bertioli et al. (2015)
	31	8.30–26.00	263	*A. ipaënsis* (K30076) × *A. magna(K30097)*	Leal-Bertioli et al. (2015)
	24	1.69–18.70	11	Zhonghua 10 × ICG12625	Huang et al. (2015)
	18	4.85–20.52	8	Zhonghua 10 × ICG 12625	Huang et al. (2016)
	22	2.55–7.95	0	Zhonghua 5 × ICGV 86699	Zhou et al. (2016)
	39	1.25–26.11	13	Fuchuan Dahuasheng × ICG 6375; Xuhua 13 × Zhonghua 6	Chen et al. (2016)
	2	22.14–71.21	2	ICGV 00350 × ICGV 97045	Vishwakarma et al. (2016)
	7	6.12–22.53	2	79,266 × D893	Li et al. (2017)
	25	4.46–17.01	5	Yuanza 9102 × Xuzhou 68–4	Luo et al. (2017a)
	42	3.68–27.84	11	Yuanza 9102 × Xuzhou 68–4	Luo et al. (2017b)
	86	3.84–15.06	6	TAG 24 × GPBD 4	Khedikar et al. (2018)
	–	12.00–32.30	6	TMV 2 × TMV 2-NLM	Hake et al. (2017)
Number of QTLs identified for quality traits					

(continued)

Table 7.12a (continued)

Trait	Total[a]	PVE	Major	Population	Reference
Linoleic acid, oleic acid, O/L ratio and other fatty acids	3	5.10–9.70	0	TG 26 × GPBD 4	Sarvamangala et al. (2011)
	27	1.04–42.33	17	SunOleic 97R × NC94022, Tifrunner × GT-C20	Pandey et al. (2014) and Wang et al. (2013)
	191	0.16–40.56	34	SunOleic 97R × NC94022, Tifrunner × GT-C20	Wang et al. (2015)
	11	1.72–20.20	7	Zhonghua 10 × ICG 12625	Huang et al. (2015)
	48	2.00–17.00	5	Florunner × TxAG-6	Wilson et al. (2017)
	21	8.40–78.60	20	ICGV 06420 × SunOleic 95R	Shasidhar et al. (2017)
	–	15.10	1	TMV 2 × TMV 2-NLM	Hake et al. (2017)
Oil content	1	11.03	1	Tamrun OL01 × BSS 56	Selvaraj Gomez et al. (2009)
	4	1.50–9.10	0	TG 26 × GPBD 4	Sarvamangala et al. (2011)
	15	2.53–10.23	5	SunOleic 97R × NC94022; Tifrunner × GT-C20	Pandey et al. (2014) and Wang et al. (2013)
	1	14.36	1	Zhonghua 10 × ICG 12625	Huang et al. (2015)
	13	2.00–18.00	2	Florunner × TxAG-6	Wilson et al. (2017)
	8	5.60–22.10	2	ICGV 07368 × ICGV 06420	Shasidhar et al. (2017)
Protein content	6	1.50–10.70	2	TG 26 × GPBD 4	Sarvamangala et al. (2011)
	–	26.40	1	TMV 2 × TMV 2-NLM	Hake et al. (2017)
Number of QTLs identified for resistance to abiotic stress					

(continued)

Table 7.12a (continued)

Trait	Total[a]	PVE	Major	Population	Reference
T, TE, SLA, LA, SCMR, CI, CC, yield components measured under drought stress	38	2.90–17.60	6	TAG 24 × ICGV 86031	Varshney et al. (2009)
	105	3.28–33.36	–	TAG 24 × ICGV 86031	Ravi et al. (2011)
	178	1.70–40.10	0	ICGS 76 × CSMG 84–1; ICGS 44 × ICGS 76	Gautami et al. (2012b)
	12	8.50–31.20		*A. ipaënsis* (K30076) × *A. magna* (K30097)	Leal-Bertioli et al. (2016)
	13	10.40–20.10		[Fleur 11 × (*A. ipaënsis* × *A. duranensis*) $^{4\times}$]	Fonceka et al. (2012)
Number of QTLs identified for resistance to biotic stress					
Rust resistance	12	1.70–55.20	1	TAG 24 × GPBD 4	Khedikar et al. (2010)
	15	2.54–82.96	7	TAG 24 × GPBD 4; TG 26 × GPBD 4	Sujay et al. (2012)
	13	5.80–59.30	2	*A. ipaënsis* (K30076) × *A. magna* (K30097)	Leal-Bertioli et al. (2015)
	6	10.2–70.4	6	TAG 24 × GPBD 4	Kolekar et al. (2016)
	8	42.7–83.6	8	TAG 24 × GPBD 4	Pandey et al. (2017a)
Leaf spot resistance	5	4.6–53.00	3	*A. duranensis* (K7988) × *A. stenosperma* (V10309)	Leal-Bertioli et al. (2009)
	28	–	13	TAG 24 × GPBD 4; TG 26 × GPBD 4	Sujay et al. (2012)
	11	1.70–6.50	0	TAG 24 × GPBD 4	Khedikar et al. (2010)
	50	5.95–27.35	10	Tifrunner × GT-C20	Wang et al. (2013)
	20	3.41–19.12	7	Zhonghua 5 × ICGV 86699	Zhou et al. (2016)
	4	14.1–44.5	4	TAG 24 × GPBD 4	Kolekar et al. (2016)
	42	3.88–16.88	12	SunOleic 97R × NC94022	Khera et al. (2016)
	31	6.26–15.55	11	Tifrunner × GT-C20	Pandey et al. (2017b)
	3	9.00–63.10	2	TAG 24 × GPBD 4	Pandey et al. (2017a)

(continued)

Table 7.12a (continued)

Trait	Total[a]	PVE	Major	Population	Reference
RKN resistance	10	–	7	Florunner × TxAG-6	Burow et al. (2014)
	8	5.70–43.70	6	*A. duranensis* × *A. stenosperma*	Leal-Bertioli et al. (2016)
TSWV resistance	2	12.90–35.80	2	SunOleic 97R × NC94022;	Qin et al. (2012)
				Tifrunner × GT-C20	
	24	4.40–34.92	6	Tifrunner × GT-C20	Wang et al. (2013)
	2	10.02–22.70	1	Florida- EPTM "113" × Georgia Valencia	Tseng et al. (2016)
	6	4.36–29.14	4	SunOleic 97R × NC94022	Khera et al. (2016)
	11	6.74–14.41	1	Tifrunner × GT-C20	Pandey et al. (2017b)
Thrips resistance	3	5.86–19.43	2	Tifrunner × GT-C20	Wang et al. (2013)
Bruchid resistance	44	11.00–82.00	13	VG 9514 × TAG 24	Mondal et al. (2014)

such as RILs and reasonably large number of polymorphic molecular markers and linkage-mapping-based marker analysis are gaining importance, so as to locate the QTLs for drought tolerance-related traits (Varshney et al. 2009; Ravi et al. 2011), resistance to foliar disease (Khedikar et al. 2010; Sujay et al. 2012) and nutritional quality traits (Sarvamangala et al. 2011) in groundnut.

7.12 Rapid Generation Advancement/Speed Breeding

Classical breeding approaches render breeding programme inefficient in meeting the ever-increasing population consumer demand of groundnut and replacement in farmers' field. Hence, the new technology 'rapid generation advancement' or 'speed breeding' has come to the rescue by shortening the life cycle of a crop species and accelerating the development by enabling the plant breeders to increase the number of breeding generations per calendar year which can considerably improve the efficiency of breeding programmes (Sysoeva et al. 2010). Although this concept is not new for groundnut (O'Connor 2012; O'Connor et al. 2013), the recent emphasis on it has brought more awareness and realization for this technology (Watson et al. 2018).

Speed breeding technologies involve the growth of plants in controlled environmental conditions (CEnvC), lamps with 24-h high-intensity photosynthetic active radiation (PAR) and optimal temperatures (28–32 C) in a greenhouse environment.

Table 7.12b List of markers associated with major QTL/genes for different traits in groundnut

Traits studied	QTL/genes	Markers linked	PVE (%)	References
Agronomic & yield				
Flowering date	qFD02.1	AHGS2736-AHGS1251	19.5	Shirasawa et al. (2012)
Angle of branch	qAB05.1	AHGS2534-AHGS2622	11.9	Shirasawa et al. (2012)
Length of main stem	qLMS04.2	AHGS2155-AHGS3725	19.2	Shirasawa et al. (2012)
	qLMS05.2	AHGS2020-AHGS2450	15.7	Shirasawa et al. (2012)
Length of the longest branch	qLLB06.2	AhTE0697-Ah1TC3H7	21.1	Shirasawa et al. (2012)
	qLLB01.2	AHGS1813b-AhTE1016	14.2	Shirasawa et al. (2012)
Number of branches	qNB06.2	AhTE0967-AhTE0074	15.6	Shirasawa et al. (2012)
Weight of plant	qWP06.2	AhTE0697-Ah1TC3H7	11.8	Shirasawa et al. (2012)
Mature pod wt/plant	qWMP09.2	AHGS0422-AHGS2635	28.1	Shirasawa et al. (2012)
Length of pod	qPL05.1	AhTE0601-AHGS1413	28.2	Shirasawa et al. (2012)
	qPL06.2	AhTE0745-AhTE0826	20.5	Shirasawa et al. (2012)
Pod thickness	qPT07.1	AHGS1803a-AhTE0025	21.7	Shirasawa et al. (2012)
Pod width	qPW07.1	AhTE0025-pPGPSeq2E6b	15.2	Shirasawa et al. (2012)
	qPW08.2	AHGS1286-AHGS2249	25.5	Shirasawa et al. (2012)
Pod constriction	qCP09.2	AHGS0362-AhTE0726	18.1	Shirasawa et al. (2012)
Seed weight	qWS08.2	AhTE0846-AhTE0974	19.1	Shirasawa et al. (2012)
Stem diameter	SD02	pPGPseq2G3–TC7A02	24.1	Liang et al. (2009)
Total dry weight (TDW)	Total DWWW09_AhIX	TC7E04–GM1949	22.39	Gautami et al. (2012a)
Harvest index (HI)	HI Control 08_AhIX	GM1922–GM2050	40.1	Gautami et al. (2012a)
Shoot dry weight (SDW)	ShDWWS08_AhVII	GM1979–GM1919	22.09	Gautami et al. (2012a)
Haulm weight	HaulmWtWW08_IV	TC1D02–TC3E05	33.36	Ravi et al. (2011)
Biomass	ShootBiomass04_XI	GM1971b–Ah193	20.32	Ravi et al. (2011)

(continued)

Table 7.12b (continued)

Traits studied	QTL/genes	Markers linked	PVE (%)	References
Canopy conductance	ISC04_Iva	19H03–PM418	22.24	Ravi et al. (2011)
Biotic stress				
Leaf rust	$QTL_{rust}01$	IPAHM103	55.2	Khedikar et al. (2010)
	QTLR4-rust01/ QTLR5-rust01	GM2009–GM1536	82.27	Sujay et al. (2012)
	QTLR4-rust02	GM1536–M2301/ GM207	62.35	Sujay et al. (2012)
	QTLR4-rust03/ QTLR5-rust02	IPAHM103–GM1954	82.96	Sujay et al. 2012
	QTLR5-rust03	RN16F05–GM1988	29.02	Sujay et al. (2012)
Late leaf spot (LLS)	QTL- R4-LLS01 QTL- R4-LLS02/QTL-R5-LLS01 QTL-R4-LLS02/ QTL- R5-LLS01	GM1573–pPGPSeq2D09 GM2009–GM1536	62.34 67.98	Sujay et al. (2012) and Sujay et al. (2012)
	QTL- R4-LLS04/QTL-R5-LLS03	IPAHM103–GM1954	–	Sujay et al. (2012)
	QTL- R4-LLS04/QTL-R5-LLS03	IPAHM103–GM1954	42.66	Sujay et al. (2012)
	QTL- R5-LLS02	GM2504–GM2746	22.46	Sujay et al. (2012)
Aspergillus flavus invasion	*Af01*	TC11H06–TC4H07	22.7	Liang et al. (2009)
Tomato spotted wilt virus (TSWV)	*qTSWV1*	IPAHM287	12.9	Qin et al. (2012)
	qTSWV2	Seq12F07	35.8	Qin et al. (2012)
Aphid vector of groundnut	QTL	M1-TTG/M-GAA1	76.16	Herselman et al. (2004)
Nematode resistance	*Rma*	S197, GM565	–	Chu et al. (2007)
				Nagy et al. (2010)
Oil and protein				
Protein content	QTL 1	TC2E05-TC3E02	10.2	Sarvamangala et al. (2011)
Oil content	QTL 1	TC6H03-TC11A04	10.7	Sarvamangala et al. (2011)
		IPAHM103-PM36	10.2	Sarvamangala et al. (2011)
High oleate trait	*FAD2A*, *FAD2B*	aF19/1056R, bF19/ R1FAD	89.7	Chu et al. (2007)
				Shirasawa et al. (2012)

Table 7.13 Genetic maps for diploid and tetraploid *Arachis* species

Population	Population size	Marker loci mapped	Marker type	LG	Total map distance (cM)	References
AA Genome						
A. stenosperma × *A. cardenasii*	87 F_2	132	RFLP	11	1063.00	Halward et al. (1993)
A. stenosperma × *A. cardenasii*	44 BC_1F_1	206	RAPD, RFLP	11	800	Garcia et al. (2005)
A. duranensis (K7988) × *A. stenosperma* (V10309)	93 F_2	204	SSR	11	1230.89	Moretzsohn et al. (2005)
	93 F_2	369	SSR, anchor, AFLP, NBS profiling, SNP, RGA-RFLP SCAR	10	–	Leal-Bertioli et al. (2009)
	89 F_5	597	SSR, TE	10	544.00	Shirasawa et al. (2013)
	90 F_5	384	SNP, SSR	10	705.10	Bertioli et al. (2014)
	93 F_6	502	SNP, SSR, RGA, anchor, morphological	10	1004.10	Leal-Bertioli et al. (2016)
A. duranensis (PI 475887) × *A. duranensis* (Grif 15,036)	94 F_2	1724	SNP, SSR, SSCP, RGC	10	1081.30	Nagy et al. (2012)
BB Genome						
A. ipaënsis (K30076) × *A. magna* (K30097)	93 F_2	149	SSR	10	1294.00	Moretzsohn et al. (2009)
	94 RILs	798	SSR, TE	10	461.00	Shirasawa et al. (2013)
	94 RILs	399	SSR, TE	10	678.00	Leal-Bertioli et al. (2015)
K 9484 (PI 298639) × GKBSPSc 30,081 (PI 468327) in *A. batizocoi*	94 F_2	449	SSR	16	1278.60	Guo et al. (2012)
AABB Genome						
Florunner × TxAG-6 {[*A. batizocoi* K9484 × (*A. cardenasii* GKP10017 × *A. diogoi* GKP10602)]$^{4×}$}	78 BC_1F_1	370	RFLP	23	2210.00	Burow et al. (2001)
	78 BC_1F_1	91	SSR	22	1321.90	Wilson et al. (2017)

(continued)

Table 7.13 (continued)

Population	Population size	Marker loci mapped	Marker type	LG	Total map distance (cM)	References
ICG 12991 × ICGV-SM 93541	60 F_2	12	AFLP	5	139.4	Herselman et al. (2004)
[Fleur 11 × (*A. ipaënsis* × *A. duranensis*)$^{4\times}$]	88 BC_1F_1	298	SSR	21	1843.70	Foncéka et al. (2009)
Yueyou 13 × RILs Zhenzhuhei	142	131	SSR	20	679.00	Hong et al. (2008)
TAG 24 × ICGV 86031	318 RILs	135	SSR	22	1270.50	Varshney et al. (2009)
	318 RILs	191	SSR	22	1785.40	Ravi et al. (2011)
Yueyou 13 × Zhenzhuhei	142 $F_{4:6}$	132	SSR	19	684.90	Hong et al. (2010)
Yueyou 13 × Fu 95–5	84 $F_{4:6}$	109	SSR	21	540.69	Hong et al. (2010)
Yueyou 13 × J11	136 $F_{4:6}$	46	SSR	13	401.70	Hong et al. (2010)
TAG 24 × GPBD 4	268 RILs	56	SSR	14	462.24	Khedikar et al. (2010)
	266 RILs	188	SSR	20	1922.40	Sujay et al. (2012)
	266 RILs	289	SSR, TE	20	1730.80	Kolekar et al. (2016)
TG 26 × GPBD 4	146 RILs	45	SSR	8	657.90	Sarvamangala et al. (2011)
	146 RILs	181	SSR	21	1963.00	Sujay et al. (2012)
ICGS 44 × ICGS 76	188 RILs	82	SSR	15	831.40	Gautami et al. (2012b)
ICGS 76 × CSMG 84–1	177 RILs	119	SSR	20	2208.20	Gautami et al. (2012b)
SunOleic 97R × NC94022	352 RILs	172	SSR, CAPs	22	920.70	Qin et al. (2012)
	352 RILs	206	SSR, CAPs	20	1780.60	Pandey et al. (2014)
	352 RILs	248	SSR	21	1425.90	Khera et al. (2016)

(continued)

Table 7.13 (continued)

Population	Population size	Marker loci mapped	Marker type	LG	Total map distance (cM)	References
Tifrunner × GT-C20	94 F_2	318	SSR	21	1674.40	Wang et al. (2012)
	248 RILs	239	SSR, CAPs	26	1213.40	Qin et al. (2012)
	248 RILs	378	SSR, CAPs	20	2487.40	Pandey et al. (2014)
	248 RILs	418	SSR	20	1935.40	Pandey et al. (2017b)
YI-0311 × Nakateyutaka	186 F_2	326	SSR, TE	19	1332.90	Shirasawa et al. (2012)
Satonoka × Kintoki	94 F_2	1114	SSR, TE	21	2166.40	Shirasawa et al. (2012)
A. hypogaea "Runner IAC 886" × (*A. ipaensis* × *A. duranensis*)^{4x}	91 RILs	1469	SSR, TE	20	1442.00	Shirasawa et al. (2013)
	89 F_6	772	SNP, SSR	20	1487.30	Bertioli et al. (2014)
Zhonghua 5 × ICGV 86699	166 RILs	1685	SNP, SSR	20	1446.70	Zhou et al. (2014)
VG 9514 × TAG 24	164 RILS	95	SSR	24	882.90	Mondal and Badigannavar (2015)
	164 RILs	190	SSR, ISSR, TE, RGC	21	1796.70	Mondal et al. (2014)
Zhonghua 10 × ICG12625	232 F_2	470	SSR	20	1877.30	Huang et al. (2015)
	140 RILs	1219	SSR, TE	20	2038.75	Huang et al. (2016)
Fuchuan Dahuasheng × ICG6375	218 $F_{2:3}$	347	SSR	22	1675.60	Chen et al. (2016)
Xuhua 13 × Zhonghua 6	282 $F_{2:3}$	228	SSR	22	1337.70	Chen et al. (2016)
Florida- EP™ "113" × Georgia Valencia	163 F_2	30	SSR, SNP	1	157.80	Tseng et al. (2016)
ICGV 00350 × ICGV 97045	268 F_2	1152	DArT, DArTseq	20	2423.12	Vishwakarma et al. (2016)
79,266 × D893	151 RILs	231	SSR	23	905.18	Li et al. (2017)
Yuanza 9102 × Xuzhou 68–4	195 RILs	743	SSR	22	1232.57	Luo et al. (2017a)
	195 RILS	830	SSR	20	1386.19	Luo et al. (2017b)
ICGV 07368 × ICGV 06420	184 F_2	854	DArT, SSR	20	3526.00	Shasidhar et al. (2017)

(continued)

Table 7.13 (continued)

Population	Population size	Marker loci mapped	Marker type	LG	Total map distance (cM)	References
ICGV 06420 × SunOleic 95R	179 F_2	1435	DArT, DArTseq	20	1869.00	Shasidhar et al. (2017)
Tamrun OL07 × Tx964117	90 RILs	1211	SNP	20	–	Liang et al. (2017)
TMV 2 × TMV 2-NLM	432 RILs	91	TE	20	1205.66	Hake et al. (2017)
3 populations	–	175	SSR	22	885.40	Hong et al. (2010)
2 populations	–	225	SSR	20	1152.90	Sujay et al. (2012)
3 populations	–	293	SSR	20	2840.80	Gautami et al. (2012b)
2 populations	–	324	SSR	21	1352.10	Qin et al. (2012)
11 populations	–	897	SSR	20	3863.60	Gautami et al. (2012a)
16 populations	–	3693	SSR, TE	20	2651.00	Shirasawa et al. (2013)

The only report on the use of 24-h light-growing systems has been that published by the National Aeronautics and Space Administration's (NASA) Advanced Life Support (ALS) programme (Rowell et al. 1999). This study concluded that a 24-h photo-period resulted in significantly greater vegetative biomass, but lower pod and mature kernel yields compared to the control treatment with a 12-h photo-period.

The speed breeding is ideally suited to a backcrossing breeding strategy, where the major objective is to incorporate a relatively simple inherited trait, e.g. one controlled by one or two genes, into a new variety. For example, the high oleic acid trait in groundnut, which is controlled by two recessive genes (ol1 and ol2), in runner-type populations, would be an ideal candidate for this strategy (O'Connor 2012; O'Connor et al. 2013). Among major applications of speed breeding, the major possible applications in groundnut include (a) faster development of genetic populations such RILs, NAM, MAGIC and NILs for trait mapping, (b) accelerated domestication and faster generation advancements for synthetic groundnuts, (c) integration with MABC/MAS/pyramiding for faster development of molecular breeding products and (d) fast-forwarding genomic selection breeding through rapid generation advancement. In summary, the speed breeding has great potential in speeding up the process of genetic population development, accelerated domestication, trait mapping, MAS/MABC and genomic selection breeding in groundnut.

7.13 Conclusion

Groundnut is important for food and nutritional security as well as for improving soil fertility. The complexity of the genome associated with the origin and domestication is the major bottleneck for narrow genetic base of groundnut. The susceptibility to abiotic and biotic stresses, cultivation on marginal lands and limited response to high-input agriculture have further constrained its productivity, particularly in developing countries. Genetic resources and variability are the key to the success of any crop improvement programmes. Large collection of groundnut germplasm, both cultivated and wild types, is maintained in many national and international genebanks globally. The strategic research on development of representative subsets, in the form of core and mini core collections or genotyping-based reference sets and subsequent to their extensive evaluation, have resulted in identification of several germplasm with specific traits, that is, resistance to abiotic and/or biotic stresses or superior agronomic and/or nutritional traits. Groundnut is no more orphan crop but genomic resource-rich crop, which has enabling effects towards identifying and tracking allelic variants associated with beneficial traits and identifying segregants with specific attributes, thus accelerating molecular breeding in pod groundnut improvement. Genomic resources and associated genotyping platforms have also enabled researchers to monitor introgression of wild segments carrying useful genes in cultivated groundnut.

Use of genomic tools in breeding programme results in enhanced rate of genetic gain for target traits and also enables to combine multiple traits. Besides, molecular markers also enable tapping of desirable alleles from wild species without the burden of linkage disequilibrium. The development of molecular markers linked to target traits is a key step in integrating genomics with groundnut breeding. Construction of molecular marker linkage maps in groundnut and identification of markers associated with gene/QTL(s) for important target traits paved the way for deployment of genomic tools in breeding programme. With the identification of markers linked to gene/QTL(s), MAS is now common and moving towards gene pyramiding for combining multiple traits. For example, markers linked to LLS and rust resistance and markers for high oleic acid content are being used to introgress these traits into short-duration, high oil-containing drought-tolerant cultivars. Different types of populations such as GWI, AB-QTL, MAGIC, NAM, RILs, NILs, etc. are now available to map QTLs and carry out association studies in groundnut. Emerging genomics technologies such as NGS and high-throughput marker genotyping using SNPs have enabled the generation of a lot of sequence data for groundnut. The draft genome sequences for the two diploid progenitor species are now available in groundnut. But the accessibility and utilization of integrated breeding (e.g. use of MAB) are expected to expand with improved affordability of using genomic tools with advances in molecular techniques.

The last decade has witnessed the rapid development of genomic tools helping to better understand the groundnut genome. MAS and MABC have proved useful for

selected traits. Emerging trait mapping approaches are expected to help the search for linked markers for other traits and develop diagnostic markers for breeding applications. The availability of the diploid and tetraploid genome sequences will provide more opportunities to identify the useful genetic variation for breeding at a genome scale, discover the genes of breeding interest and identify additional molecular markers amenable for high-throughput genotyping. High-throughput genotyping technologies are advancing fast with genotyping costs getting cheaper. It will not be far for such technologies to be routinely utilized by many breeding programmes, if not all, for screening segregating populations, purity testing, genetic mapping, targeted resequencing of specific genomic regions and other studies. In summary, groundnut improvement tools are available to exploit and build on past achievements for new discoveries to enhance and accelerate the genetic gain of breeding programmes such that processes for the development and release of improved varieties are speedy, technically efficient and cost-effective (Desmae et al. 2019).

(Chuni Lal et al. 2015; Subrahmanyam et al. 1981)

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Chapter 8
Genomics-Assisted Breeding for Resistance to Leaf Spots and Rust Diseases in Peanut

R. S. Bhat, Venkatesh, M. P. Jadhav, P. V. Patil, and K. Shirasawa

Abstract Peanut, an important oilseed, food and fodder legume crop, suffers heavily from the foliar diseases like early leaf spot (ELS), late leaf spot (LLS) and rust diseases, worldwide. The symptoms and the factors causing yield loss have been identified. Though use of chemicals is a good measure of control, breeding for resistant genotypes has been a preferred approach. The components of resistance for ELS, LLS and rust have been worked out, and the genotypes within the cultivated types and its wild relatives possessing resistance have been identified and employed in breeding-resistant varieties. However, the current approach of genomics-assisted breeding has seen a considerable progress with the developments of genomic resources in terms of genome sequencing, marker development, trait mapping, gene discovery, marker/QTL validation and translational genomics to develop foliar disease-resistant genotypes with enhanced precision and efficient selection. The advancements made so far in genomics-assisted breeding for resistance to leaf spots and rust diseases in peanut have been reviewed in this chapter.

Keywords Peanut · Early leaf spot · Late leaf spot and rust disease · Components and genetics of resistance · Breeding efforts · Accelerated breeding · Development of resistant genotypes with genomic tools

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8.1 Introduction

Peanut is an important legume oilseed and food crop apart from being a source of fodder. Globally, groundnut is cultivated on an area of 29.6 million hectares with a production of 48.8 million tons (http://www.fao.org/faostat/en/#data/QC/visualize) and productivity of 1647 kg/hectare (in 2019). Over half of this groundnut produce goes for oil extraction, while the remaining is consumed as raw and processed food. Because of its rich nutrient contents in terms of oil, proteins, fibres, polyphenols, antioxidants, vitamins and minerals, groundnut is popularly called as 'poor man's almond'.

Breeding work on groundnut started in 1976 with the efforts of Gibbons (1976). The primary objective of groundnut breeding is to develop cultivars with high yield potential, earliness, adaptation to specific environments and production systems, resistance or tolerance to environmental stresses and resistance to diseases and insects and better nutritional quality. Because groundnuts are grown under many different cropping systems across a wide array of agroecological conditions, the specific objectives of breeding programmes vary considerably. However, improving its resistance to foliar diseases like leaf spots and rust is an important component in any groundnut breeding programme.

Early leaf spot (ELS) [*Cercospora arachidicola* Hori. (teleomorph: *Mycosphaerella arachidicola*)], late leaf spot (LLS) [*Phaeoisariopsis personata* (Berk. & Curt) V. Arx. (teleomorph: *Mycosphaerella arachidis*)] and rust [*Puccinia arachidis* Speg.] are the widespread and destructive fungal foliar diseases of groundnut. The leaf spots are probably the most important diseases of groundnuts on a worldwide scale depending on the genotype and the environment. In India, late leaf spot occurs more regularly and reaches high levels in the rainy season. But the occurrence of early leaf spot is less common and rarely reaches levels high enough to permit field resistance screening. Rust is now of economic importance in almost all groundnut-growing areas of the world (Hammons 1977; Subrahmanyam et al. 1979) though previously it was unimportant outside the Americas (Bromfield 1971).

Although the diseases can be controlled by fungicides, adoption of resistant varieties and cultivars by the farmers is the best option to minimize losses at farm level and maintain good product quality (Dwivedi et al. 1993). Though the progress in groundnut breeding was at a slow phase initially due to late initiation of breeding programmes and regional importance of the crop (Wynne et al. 1991), considerable efforts were made from 1976 onwards with the identification of numerous germplasm accessions as sources of disease resistance (Hammons 1977). Identification of the component traits and mechanism of resistance also contributed for the success of disease resistance breeding programmes. The breeding methods could also overcome the challenge due to the positive relationship between both low pod yield and late maturity with resistance to leaf spots observed in breeding material and germplasm (see Miller et al. 1990). In this regard, the breeding efforts (Wynne et al.

1991; Isleib et al. 1994; Janila et al. 2013a; Liao 2014; Mondal and Badigannavar 2015; Desmae et al. 2019) made significant progress with the development of the first late leaf spot-resistant and high-yielding variety Southern Runner (Gorbet et al. 1987). Subsequent developments and the application of genomics and the genomic tools have triggered better understanding of resistance to leaf spots and rust. Also, the molecular breeding (Pandey et al. 2020; Daudi et al. 2021) has been contributing immensely for the development of disease-resistant genotypes precisely and efficiently.

8.2 Loss of Pod Yield Due to Leaf Spots and Rust

Since leaf spots and rust diseases develop mainly on the photosynthetically active leaves which are the main site of carbohydrate synthesis in plants, they affect crop growth and pod yield. However, LLS is also known to reduce the yield indirectly through defoliation, which enhances peg deterioration leading to pod drop (Bourgeois et al. 1991). Since the late leaf spot produces lesions on the petioles, and lateral and main stems, apart from forming the lesions on the leaves during severe epidemics, these lesions interrupt translocation along laterals, thereby accelerating maturity (Hemingway 1954).

Leaf spots and rust diseases develop more rapidly during the rainy season than in the irrigated post-rainy season (Subrahamanyam et al. 1980). Rust and leaf spots normally occur together, but the incidence and severity of each disease vary with environment, location and genotype (Mehan et al. 1996). These factors make it difficult to estimate the yield due to them separately. During the rainy season of 1979, Subrahamanyam et al. (1980) attempted to estimate yield losses by applying fungicides: Daconil to control leaf spots and rust, Bavistin to control only leaf spots and Calixin to control only rust to susceptible and disease-resistant genotypes. Yield losses were less in the resistant than in the susceptible genotypes. The yield loss in the susceptible genotype (Robut 33-1) could reach up to 59% by leaf spots, 52% by rust and 70% by both leaf spots and rust diseases.

Recently, meta-analyses were conducted over 140 datasets to quantify relationships between end-of-season defoliation and yield loss. Proportion of yield loss with increasing defoliation was estimated separately for Virginia and runner market-type cultivars. Yield loss for Virginia types was described by an exponential function over the range of defoliation levels, with a loss increase of 1.2–2.2% per additional percent defoliation, while the runner market-type cultivars showed linear increase in yield loss at the rate of 2.2–2.8% per 10% increase in defoliation for levels up to 95% defoliation, after which the rate of yield loss was exponential. Defoliation thresholds to prevent economic yield loss for Virginia and runner types were estimated at 40 and 50%, respectively (Anco et al. 2020).

These diseases damage plant by reducing the green leaf area available for photosynthesis and by stimulating leaflet abscission leading to extensive defoliation (McDonald et al. 1985) which results in lower seed quality and reduced seed size and seed weight and oil content besides affecting the haulm production and quality (see Sudini et al. 2015). With the heavy disease pressure, the resistant genotypes do not show significant decline in yield, instead the yield continues to increase with maturity (Knauft et al. 1988), while the susceptible lines show more rapid reduction in the yield probably due to the loss of harvestable yield through deterioration of pegs and other plant tissue.

Yield and economic analyses show that groundnut is able to compensate for various levels of defoliation early in the growing season. However, defoliation around peak pod fill (80 DAE) leads to significant yield and economic loss, indicating that minimizing defoliation during reproductive growth is important (Santos and Sutton 1982; Abbott 2020). In a Virginia bunch groundnut, it was observed that only a small proportion of the flowers which formed early in the flowering phase contributed to pod yield at maturity with various levels of defoliation. Defoliation after pod formation caused a reduction in pod yield by inhibiting fruit formation and degenerating the pods already formed (Santos and Sutton 1983).

Progress of LLS in terms of disease-induced defoliation and necrotic leaf area was evaluated by Bourgeois et al. (1991) to check its effect on accumulation of dry matter, leaf area index (LAI) and pod production in groundnut. The leaf dry weight, LAI and the dry weight of the total biomass were significantly different between fungicide-treated and non-treated plots. LLS reduced the potential yield (harvested and dropped pods) by 37–46%. The predictions of pod yield with the measures of healthy leaf area duration (HAD) and healthy area absorption (HAA) were adequate for fungicide-treated plots where pod losses were minimal. However, HAD and HAA were inadequate for predicting pod yield of a groundnut crop severely infected by LLS, primarily because this predictive approach does not account for losses of dropped pods (Bourgeois et al. 1991).

Assessing the defoliation of groundnut caused by late leaf spot and reflected radiation (800 nm wavelength) of sunlight from groundnut canopies weekly beginning 60 days after planting until 1 week prior to digging using the critical-point yield-loss models where either percentage defoliation or percentage reflectance values were used as the independent variable and pod yield (kg/ha) was used as the dependent variable indicated that the best critical-point models for both the assessment methods were observed at 2–3 weeks prior to digging. The models with percent reflectance as the independent variable explained more of the variation in peanut pod yield than those models using percent defoliation values as the independent variable. This study indicated that the healthy green leaf area (as estimated by percent reflectance) can be a good indicator for assessing the pod yield (Nutter Jr and Littrell 1996).

Adomou et al. (2005) used the CROPGRO-Peanut model to predict and simulate the crop and pod dry matter over time by providing percent diseased leaf area and percent defoliation. Percent main-stem defoliation above the fourth node and

percent diseased leaf area estimated from visual leafspot score were found to be the most useful disease assessments.

In an attempt to determine canopy photosynthesis in the three foliage layers in response to leafspot, defoliation and combinations of disease and defoliation, the canopy C exchange rate (CER), photosynthetic uptake of $^{14}CO_2$, leaf area and light interception by leaves were measured in three canopy layers. The upper 42% of the canopy leaf area intercepted 74% of the light and fixed 63% of the total $^{14}CO_2$. Removal of 25% of the total leaf area, primarily from the upper half of the canopy, reduced $^{14}CO_2$ uptake by 30% and canopy CER by 35%. Photosynthesis of diseased canopies was reduced not only by loss of leaves which abscised as a result of infection but also because diseased leaves which remained on the plants were less efficient in fixing CO_2 (Boote et al. 1980).

In order to study the response of groundnut canopy carbon dioxide exchange rate (CER) to degrees of foliage loss at different dates throughout the season, the plants were manually defoliated by 25%, 50% and 75% on different dates during the season in comparison with the control (0% defoliation). Weekly CER measurement on these plants indicated the initial reduction in canopy CER by 45–70% with 75% defoliation [leaf area index (LAI) to about 1.0]. However, a considerable CER recovery was observed later probably due to two mechanisms: leaf area production and re-adaptation of previously shaded leaves to full sun. Subsequently, the efficiency of utilizing photosynthetically active radiation improved with probably increasing specific leaf weight but not with an increase in LAI. New leaf production diminished as the plants matured and progressed into pod setting and pod filling stage (Jones et al. 1982).

Ability to partition the assimilates to fruits is a key factor in enhancing the pod yield. Therefore, Miller et al. (1990) and Aquino et al. (1992) suggested a more appropriate method of selecting true resistance and reducing the influence of inefficient partitioning while selecting for the specific components of resistance (Green and Wynne 1986; Chiteka et al. 1988). However, Duncan et al. (1978) could observe a significant improvement in the partitioning of assimilate to fruits from 41 to 98% due to breeding activity. But the crop growth rate did not differ significantly among peanut cultivars (Duncan et al. 1978).

8.3 Symptoms of Leaf Spots and Rust Diseases

Understanding the characteristic field symptoms of ELS, LLS and rust (Subrahmanyam et al. 1992) (Fig. 8.1) is important to diagnose the diseases based on their pattern of occurrence and macroscopic symptoms in order to manage these diseases effectively. Apart from diagnosing the field symptoms with naked eye or handheld lens (~10x), microscopic examination of the diseased tissues and fruiting structures of the pathogen may be required in certain cases as the disease symptoms are influenced by genotype and environment.

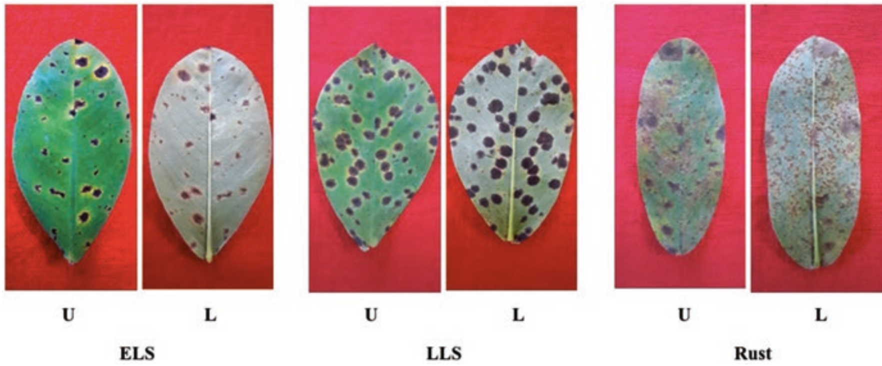

Fig. 8.1 Symptoms of early leaf spot (ELS), late leaf spot (LLS) and rust diseases in peanut (*U* upper surface, and *L*, lower surface)

8.3.1 Early Leaf Spot

Early leaf spot in peanut is caused by *Cercospora arachidicola* Hori. The symptoms appear within 8–10 days after emergence, depending on the prevailing temperature, relative humidity (≥95%), planting time, previous cropping history and preparatory tillage practices (Smith 1980b). The initial macroscopic symptoms appear on the adaxial side of the lower leaves. Subcircular, dark brown necrotic lesions with a chlorotic bright yellow halo are found on the upper leaflet surface where most sporulation occurs, and a lighter shade of brown on the lower leaflet surface. Appearance of grey-coloured tufts of mould, which can be seen with a 10X hand lens, during sporulation on the upper leaf surface for early leaf spot and on the lower leaf surface for late leaf spot is a reliable method of distinguishing these two leaf spots (Damicone 2017). Sporulation occurs in the necrotic tissues of the lesions on the upper surface. Irregular or elliptical dark brown to black lesions are also produced on petioles, stems, stipules and pegs. But these lesions are oval to elongate and have more distinct margins than leaflet lesions. When the disease attack is severe, affected leaflets become chlorotic and then necrotic, and lesions often coalesce, resulting in premature senescence and shedding of leaflets. The defoliation progresses from lower to upper leaves.

8.3.2 Late Leaf Spot

Late leaf spot in peanut is caused by *Phaeoisariopsis personata* (Berk. & M.A. Curtis) Van Arx (previously known as *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton) (see McDonald et al. 1985), and peanut is the only known natural host for *P. personata*. Temperature of 16–20 °C and relative humidity of more than 93% for 12 h a day are favourable for germination of *C. personatum*

conidia (Sommartya and Beute 1986; Shew et al. 1988). Germination declines gradually after 28 or 30 °C. The lesions are nearly circular and darker (than early leaf spots) on the lower surface of the leaflets. The sporulation occurs in the black and slightly rough lesions in the lower surface. Circular rings of fruiting structures are seen on the lesions of the lower surface with the aid of a hand lens. Under severe disease conditions, the affected leaflets become chlorotic and then necrotic, and lesions often coalesce, resulting in premature senescence and shedding of the leaflets. Oval to elongate lesions similar to those of early leaf spot are also formed on petioles, stems, stipules and pegs. Late leaf spot attack is usually coincident with that of rust.

8.3.3 Rust

Rust disease in peanut is caused by *Puccinia arachidis* Spegazzini. Temperature ranging between 20 and 28 °C, free water on the leaf surface and high relative humidity are the favourable environmental conditions for disease development (Mallaiah and Rao 1979). Orange-coloured rust pustules appear on all aerial parts except on flowers. Pustules appear on the lower surfaces of leaflets and rupture to release masses of reddish-brown spores. The primary pustules may be surrounded by secondary pustules. Then they spread to adaxial surfaces. Pustules on the stem are elongate. The leaves infected with rust become necrotic and dry up, but tend to remain attached to the plant in contrast to the rapid defoliation associated with leaf spots.

8.4 Components of Resistance to Leaf Spots and Rust

Identification of components and mechanism of resistance is the prerequisite for the success of disease resistance breeding programmes. An understanding of how the components of resistance operate is required to estimate their relative importance in evaluating the resistance and to explore the means of enhancing it. Assessing the correlation between the component traits in the controlled condition and the field condition and checking the correlation between these two conditions are important in breeding for resistance. The component trait(s) reflecting the actual resistance level in the field would be of great utility in the breeding programmes.

8.4.1 Early Leaf Spot

A wide range of traits have been used by different researchers to evaluate leaf spot resistance; they are degree of sporulation, lesion number, necrotic area, latent period, defoliation and disease-index parameters. Disease assessment method based on the defoliation ratio and the visual estimation of percentage of leaves with leaf spots was found to be efficient and reliable (Hassan and Beute 1977).

The component traits contributing for resistance to ELS include number of lesions per leaflet, lesion diameter, latent period, time to leaflet loss, percentage defoliation and degree of sporulation (see Waliyar et al. 1989). However, it is important to determine which components of the resistant genotypes differ quantitatively from those of susceptible genotypes and whether components are the same for all resistant genotypes (Ricker et al. 1985).

Peanut genotypes with larger areas under disease progress curves (AUDPC) had faster rates of disease increase (Johnson et al. 1986). Disease level at 103–110 days after planting was highly correlated with AUDPC, which in turn were highly correlated with latent period, percent lesions sporulating, spore production and time to defoliation than with infection and rate of defoliation. Percent lesions sporulating was the component of resistance which showed the highest correlation with disease progress in the field. Identifying the trait(s) reflecting ELS resistance both in the greenhouse and field is also significant. Creen and Wynne (1986) observed that the necrotic area measured in the field was significantly correlated with that measured in the greenhouse. Similarly, sporulation measured in the greenhouse was significantly correlated with increase in lesion in the field. This study showed a possibility of evaluating and selecting the genotypes for components of partial resistance in the greenhouse to develop resistant lines for the field.

8.4.2 Late Leaf Spot

Components of resistance to LLS include longer latent period, fewer lesions per leaf, smaller lesion diameter, reduced sporulation, lower sporulation index, less leaf area damage and marginal defoliation (Nevill 1981). Among them, sporulation, lesion size and latent period are predominant and are highly correlated with each other and with percent leaf necrotic area (Chiteka et al. 1988). Small sample size, low repeatability of evaluation techniques and genotype × environment interaction (Chiyembekeza et al. 1993) resulted in the poor correlation between the resistance observed in the greenhouse and field conditions. However, Subrahmanyam et al. (1982) found correlation for lesion diameter, defoliation and sporulation between greenhouse and field environments. Thus, resistance to LLS is partially due to longer incubation and latent periods, and resistant genotypes show reduced infection than the susceptible genotypes (Nevill 1981; Green and

Wynne 1986; Anderson et al. 1993; Waliyar et al. 1993b; Dwivedi et al. 2002). The progenies of the genotypes selected for ELS were tested for LLS in the greenhouse for latent period, lesion area and amount of sporulation. These traits showed strong correlation in the field, indicating that these variables could be used as measurements of resistance to predict the performance for LLS in the field (Walls et al. 1985).

The components of resistance to LLS were studied among the interspecific derivatives of *A. duranensis*, *A. batizocoi* and *A. cardenasii* in greenhouse and field experiments (Pande et al. 2002). Interspecific derivatives showed significantly longer incubation and latent periods; lower lesion number and frequency; smaller lesion diameter; and less sporulation indices, leaf area damage and defoliation under both the environments compared to the susceptible cultivars. Among them, latent period, lesion diameter, sporulation index and defoliation were the major contributing components to the resistance among the interspecific derivatives.

A scoring scale of 1–10 or 1–5 was proposed based on the leaf area affected by disease and/or defoliation for evaluating a large number of entries (Smith 1980a). Later, a visual 9-point scale was developed at ICRISAT for preliminary screening of germplasm for leaf spot resistance.

Resistance has been attributed to various morphological and anatomical characteristics of the host plant and to chemical constituents of leaves (Stalker 1984). A positive correlation between the size of the stomatal aperture and the susceptibility of groundnuts to *C. arachidicola* was observed by Hemmingway (1957) and confirmed by D'Cruz and Upadhyaya (1961). However, a contrasting observation was reported by Hassan and Beute (1977) when they found that the decreased stomatal aperture failed to increase the resistance, and the stomatal size changed due to changes in growth environments.

8.4.3 Rust

The rust-resistant genotypes exhibit reduced rate of disease development due to longer incubation and latent periods, fewer pustules per leaf, smaller pustule diameter and lower sporulation index. In general, infection frequency, pustule diameter, percent ruptured pustules and leaf area damage are correlated with each other and with the mean field rust score. The incubation period is negatively correlated with other components. Rust resistance components appear to work additively (Subrahmanyam et al. 1983; Reddy and Khare 1988; Mehan et al. 1994; Dwivedi et al. 2002). The wild *Arachis* species and their interspecific derivatives show small and slightly depressed uredosori containing very few uredospores which are not readily released. These mechanisms could be different from those in *A. hypogaea* which hints at combining rust resistance of wild and cultivated species to give more effective and stable resistance in the cultivated peanut (Subrahmanyam et al. 1983).

8.5 Genetics of Resistance

Better understanding of the genetics of disease resistance will enable breeders to design an efficient breeding strategy. Considerable efforts have been made to understand the genetic control of resistance to leaf spots and rust. Various views have been reported depending on the genotypes used for the investigation.

LLS resistance was reported to be governed by multiple recessive genes (Kornegay et al. 1980; Nevill 1982; Vasanthi and Raja Reddy 1997; Motagi 2001) and two genes (Tiwari et al. 1984). Partial resistance (not complete, as several components influence the resistance) similar to 'slow rusting' was also reported for LLS (Singh et al. 1997). A five-locus recessive gene model was also reported by Sharief et al. (1978) in the crosses involving cultivated peanut and wild *Arachis* species. Inheritance study involving interspecific derivatives showed that the resistance to LLS is controlled by a combination of both nuclear and maternal gene effects (Janila et al. 2013b).

A study using 6 × 6 F_2 full diallel populations from six parents (African genotypes) revealed that additive and non-additive gene actions were involved in the inheritance of the ELS resistance (Zongo et al. 2019), with a predominant additive gene action. Cytoplasmic effect was also observed for ELS resistance.

Resistance to rust in peanut is conferred either by a few recessive genes (Bromfield and Bailey 1972; Kalekar et al. 1984; Tiwari et al. 1984; Knauft 1987; Paramasivam et al. 1990; Motagi et al. 2013) or two to three genes acting in duplicate complementary interactions in rust resistance (Vasanthi and Raja Reddy 1997). The resistance is predominantly controlled by additive, dominance and additive × additive and additive × dominance genetic effects (Reddy et al. 1987; Varman 1991).

Singh et al. (1984) concluded that rust resistance in diploid species is partially dominant as compared to the recessive resistance in *A. hypogaea*. R-genes have been studied in groundnut. Nucleotide-binding-leucine-rich repeat (NB-LRR)-encoding genes are of particular interest because they confer resistance against pests and diseases. Bertioli et al. (2003) identified 78 resistance gene analogs (RGAs) based on the nucleotide-binding site (NBS) regions from *A. hypogaea* 'Tatu' and four wild relatives (*A. duranensis*, *A. cardenasii*, *A. stenosperma* and *A. simpsonii*). Yuksel et al. (2005) identified 234 RGAs from *A. hypogaea* L. cv. Florunner UF-439-16-1003-2 based on the primer sequence information from NBS-leucine-rich repeats (NBS-LRR) and LRR-Toll-like motif (LRR-TM). Proite et al. (2007) identified 35 putative non-redundant RGAs and 26 pathogenesis-related expressed sequence tags (ESTs) from *A. stenosperma* which is resistant to rust and other foliar diseases. Genome sequencing has identified 345 and 397 NB-LRR genes in *A. duranensis* and *A. ipaensis*, respectively (Bertioli et al. 2016). The largest clusters were on distal regions of chromosomal pseudomolecule 02, the lower arms of chromosomal pseudomolecule 04 and the upper arms of chromosomal pseudomolecule 09. The genome assemblies could associate QTLs with candidate genes.

8.6 Sources of Resistance

Though most commonly cultivated Spanish bunch types of groundnut are highly susceptible to LLS, several sources of resistance to leaf spots and rust have been reported in *A. hypogaea* (Anderson et al. 1993; Waliyar et al. 1993b; Mehan et al. 1996; Singh et al. 1997). A majority of the resistant germplasm belong to subsp. *fastigiata* and are landraces from South America (Subrahmanyam et al. 1989). Screening of 500 peanut plant introductions from the USA could identify 33 genotypes with partial resistance to LLS (Adomou et al. 2005). Use of the diploid species, *A. cardenasii*, has resulted in several breeding lines with levels of resistance to late leaf spot more than that is found in cultivated peanuts.

Wild *Arachis* species resistant to LLS in sections Erectoides, Triseminalae, Extranervosae, Rhizomatosae and Caulorhize have small and nonsporulating lesions, whereas species in section *Arachis* have accessions either with nonsporulating lesions or with variably sporulating lesions. Frequency of infection (number of lesions per square centimetre of leaf area) and defoliation vary greatly within each section and species (Subrahmanyam et al. 1985). Most of the wild *Arachis* species in sections Erectoides, Triseminalae, Extranervosae and Rhizomatosae show immunity to rust with no recognizable symptoms of the disease appearing even after an incubation period of 40 days (Rao 1987). Several diploid wild species of the genus *Arachis*, viz. *A. diogoi*, *A. stenosperma*, *A. cardenasii*, *A. duranensis*, etc., show very high levels of resistance to fungal and rust pathogens (Pande and Rao 2001). Alien introgressions from *A. cardenasii* Krapov. & W.C. Gregory in IAC 322 resulted in LLS resistance (Lamon et al. 2020). Efforts to utilize the diploid *Arachis* species to transfer LLS resistance to cultivated peanut the development of synthetic allotetraploids was demonstrated at ICRISAT. These tetraploids were resistant to LLS (Mallikarjuna et al. 2012).

Valencia-type germplasm lines originating from the region of Tarapoto (Peru) possess a high degree of resistance to rust and moderate levels to LLS (Singh et al. 1997). Majority of resistant sources belong to subspecies *fastigiata* var. *fastigiata* and are landraces from South America (Subrahmanyam et al. 1989). But they are limited for utilization in groundnut breeding because of many undesirable attributes. They possess thick shell, low productivity, poor adaptation, late maturity and highly reticulated and constricted pods which are commercially unacceptable (Subrahmanyam and McDonald 1983; Wynne et al. 1991; Anderson et al. 1993; Hegde et al. 1995). However, later, screening of the germplasm originating from secondary centres of diversity resulted in identification of some resistant sources with good agronomic backgrounds. But even then, low productivity is the major constraint (Singh et al. 1997).

With the development of minicore in peanut (Upadhyaya et al. 2002), 184 accessions were screened for LLS and rust under artificial epiphytotic conditions (Sudini et al. 2015). Accessions showed significant variations for disease resistance; 53 accessions were moderately resistant, 86 accessions were susceptible and 45 accessions were highly susceptible for LLS, while 10 accessions were resistant, 115

accessions were moderately resistant and 59 accessions were susceptible for rust. ICGs 4389, 6993, 11426, 4746, 6022 and 11088 were selected as superior accessions in terms of disease resistance and yield.

8.7 Breeding for Foliar Disease Resistance

Genetic improvement of groundnut faces challenges like narrow genetic base of the cultivated gene pool and the tetraploid and complex nature of genome. Only limited genetic diversification has been achieved in the past through interspecific hybridization between cultivated groundnut and other species of section *Arachis* due to differences in ploidy levels and the linkage drag. Eliminating the linkage drag involves a lengthy process that also results in dilution of the level of resistance present in wild relatives of *Arachis*.

Standard breeding methods for self-pollinated crops like pedigree and bulk selection methods have been used to develop groundnut cultivars. Backcross breeding was not extensively used in groundnut improvement because economically important traits are quantitatively inherited (Janila et al. 2016c). Mutation breeding was also used successfully to release the improved varieties in groundnut. Considerable efforts have been made to screen the diploids and tetraploids with resistance to LLS and rust. The gene pools were categorized into immune, highly resistant, resistant, moderately resistant and susceptible for LLS and rust diseases (Stalker 1992). They were used as the genetic resource for foliar disease resistance in groundnut improvement (Singh and Nigam 2016).

Wild species are the valuable source of genes for resistance/tolerance to different diseases/insect pest including yield-related traits and oil content. Wild species were used to broaden the genetic base of groundnut (Simpson 1991; Mallikarjuna et al. 2011). There are hurdles in the use of wild species as a source of resistance due to ploidy differences, cross incompatibility, linkage drag with undesirable traits and unavailability of tools to track introgression of chromosomes. Therefore, genes from these wild species are generally incorporated into cultivated groundnut through triploids, autotetraploids and amphiploids and directly from tetraploid wild species (Simpson 2001; Bertioli et al. 2011). Improved breeding lines with resistance to foliar fungal diseases were developed (Singh et al. 2003), and 'Southern Runner' was the first moderate LLS-resistant cultivar to be released in the USA (Gorbet et al. 1987). ICG 7878, an ELS- and LLS-resistant variety, was released in Mali (Waliyar et al. 1989, 1993a).

Interspecific derivatives were developed first by crossing diploid *Arachis* species with the cultivated groundnut to get a triploid, which was then backcrossed to cultivated groundnut to get a stable tetraploid. Garcia et al. (1995) obtained interspecific hybrids from *A. hypogaea* × *A. cardenasii*, and several LLS-resistant germplasm lines have been released from progenies of this cross (Moss et al. 1997; Stalker et al. 2002). Rust-resistant interspecific hybrids have been selected from progenies of *A. hypogaea* × amphiploid (*A. batizocoi* × *A. duranensis*) and (*A. correntina* ×

A. batizocoi) (Singh 1986). Using backcross method of triploid, hexaploid and autotetraploid route, interspecific hybrids ICGV 86699 and ICGV 87165 were developed at ICRISAT. ICGV 86699 is an interspecific derivative from the cross (*A. batizocoi* × *A. duranensis*) × *A. hypogaea*. It is a high-yielding germplasm with resistance/tolerance to early and late leaf spots, rust, groundnut bud necrosis, groundnut mottle virus and stem and pod rots. ICGV 87165 is a high-yielding foliar disease-resistant interspecific derivative obtained from *A. hypogaea* × *A. cardenasii*. An interspecific derivative, ICGV-SM 86715, was released as cultivar Veronica in Mauritius. ICGV 87853 was released with high-level resistance to rust and moderate resistance to late leaf spot (Reddy et al. 2000).

Several interspecific derivatives were developed with resistance to foliar diseases, but they could not be released as commercial cultivars because of linkage drag of undesirable traits like late maturity and poor pod and seed characteristics. Therefore, the foliar fungal disease-resistant varieties developed in the 1980s and 1990s had poor pod and kernel features due to linkage disequilibrium; consequently, despite high pod yield and resistance, they did not find acceptance among farmers (Nigam 2000). Combining foliar fungal disease resistance and early maturity has remained a challenge despite availability of several donors.

Some interspecific derivatives, ICGVs 99005, 99003, 99012 and 99015 with rust resistance and ICGVs 99006, 99013, 99004, 99003 and 99001 with late leaf spot resistance, could be desirable parents for use in resistance breeding programmes (Dwivedi et al. 2002). ICGV 86855 is an interspecific derivative between *A. hypogaea* × *A. cardenasii*, and it is resistant to rust and late leaf spot. ICGV 86855 was used in the development of GPBD 4, a Spanish bunch groundnut genotype resistant to rust and late leaf spot (Gowda et al. 2002).

Several sources of resistance to LLS and rust have been reported in tetraploid *A. hypogaea* (Anderson et al. 1993; Waliyar et al. 1993a; Singh et al. 1997). Mainly, these resistant sources belong to subspecies *fastigiata* var. *fastigiata* and are landraces from South America (Subrahmanyam et al. 1989). Valencia germplasm line PI 259747 is a landrace which is resistant to LLS and rust, and this line was used in the development of ICGV 86590. ICGV 86590 is a Spanish bunch groundnut genotype, resistant to rust and late leaf spot (Reddy et al. 1993).

Induced mutagenesis offers an alternative approach to improve disease resistance while retaining desirable agronomic traits. Qiu and Feng (1998) reported some mutants with improved resistance to leaf spot. Several cultivars were developed with mutants as crossing parents (Qiu 1992). Rust-resistant mutant, VL 1 (Valencia mutant), was derived from DER (Dharwad Early Runner) by EMS treatment (Gowda and Nadaf 1992). Genotypes like 28-2, 45 and 110 were selected for foliar disease resistance after EMS-induced mutation from the VL 1 (Motagi et al. 1996). GG13, a rust-resistant mutant variety, was released in Gujarat (Basu 2002). Few groundnut varieties ALR 1, ALR 2, ALR 3, ICG (FDRS) 10, ICGV 86590 and Girnar 1 were released in India with resistance to LLS and rust diseases (Rathnakumar et al. 2013). With an objective of developing the induced mutants for superior productivity and resistance to foliar diseases in peanut, two introgression lines derived from ABK genomes of peanut were subjected to gamma and sodium azide

mutagenesis. Mutants with high level of resistance to ELS, LLS and rust were selected (Joshi et al. 2019).

8.8 Genomics-Assisted Breeding

Traits like resistance to leaf spots and rust are difficult to manage through conventional phenotypic selection because they co-occur and lead to defoliation. However, such traits can be handled with genomics-assisted tools like markers. Advantages of genomics-assisted breeding (GAB) have been well demonstrated through translational genomics for breeding LLS- and rust-resistant genotypes in peanut (see Pandey et al. 2020). Integration of genomic tools with conventional breeding has accelerated peanut improvement with precision in breeding for traits with complex genetic control. Considerable progress has been made on GAB with the development of several thousands of markers, several genetic maps, dense consensus genetic maps, QTL mapping and molecular breeding. Developments in peanut genomics have greatly contributed for GAB. Peanut genomics was not much explored till the 1980s due to its large size (~2.7 Gb), high fraction of receptive DNA and allotetraploidy with two closely related genomes. However, with the sequencing of expressed sequence tags (ESTs) (Wang et al. 2006; Proite et al. 2007; Guo et al. 2008; Bi et al. 2010), peanut genomics research was initiated during the late 1980s to characterize species relationships and investigate more efficient methods to introgress genes from wild species to *A. hypogaea.* Relatively low-density genetic maps were developed initially from inter- and intra-specific crosses to map disease resistance genes. With the development of more markers, construction of high-density maps was reported later. These developments marked the start of peanut genomics (Paterson et al. 2004; Stalker et al. 2009; Pandey et al. 2012b; Stalker et al. 2013; Ozias-Akins et al. 2017) and picked the pace in post-genome sequencing era.

As a first step towards characterizing the genome of cultivated peanut, the genomes of the two diploid ancestors (*A. duranensis* V14167 and *A. ipaensis* K30076) of cultivated peanut were sequenced and analysed (Bertioli et al. 2016) to overcome the challenge in assembling of chromosomal pseudomolecules. Both these accessions were collected from the most likely geographic region of origin for the cultivated peanut. In the same year, Chen et al. (2016) sequenced *A. duranensis* (accession PI475845 from Bolivia) as well as four synthetic tetraploids and their six diploid parents [two A genomes and four B genomes, including the suspected B genome progenitor, *Arachis ipaensis*] to gain insight into peanut evolution. Based on the draft genome of *A. duranensis*, the gene models with 50,324 protein-coding genes were proposed. Also, Lu et al. (2018) sequenced *A. ipaensis* and recorded ~1.39 Gb genome with 39,704 predicted protein-encoding genes.

The first reference quality assembly of the *A. monticola* (PI263393) genome was developed with a genome size of ~2.62 Gb (Yin et al. 2018). The efficiency of the current state of the strategy for de novo assembly of the highly complex

allotetraploid species based on whole-genome shotgun sequencing, single-molecule real-time sequencing, high-throughput chromosome conformation capture technology and BioNano optical genome maps was demonstrated. Subsequently, Yin et al. (2020) re-sequenced 17 wild diploids from AA, BB, EE, KK and CC groups and 30 tetraploids and compared the previously sequenced genome of *A. monticola* (Yin et al. 2018).

During 2019, two reference genomes, one for the subsp. *fastigiata* and the other for subsp. *hypogaea* of the cultivated tetraploid, were reported. The IPGI-led initiative (Bertioli et al. 2019) completed the sequencing of Tifrunner (PI644011 with registration number CV-93) (Holbrook and Culbreath 2007), a runner type belonging to *Arachis hypogaea* subsp. *hypogaea* by deploying several modern sequencing and assembly technologies such as PacBio and Hi-C data/technology. A genome of ~2.56 Gb with 20 pseudomolecules and 66,469 predicted genes was reported. Similar advanced technologies were deployed by two independent efforts in China leading to the development of high-quality reference genome assemblies for 'Shitouqi' (Zhuang et al. 2019) and 'Fuhuasheng' (Chen et al. 2019) both belonging to *A. hypogaea* subsp. *fastigiata.* The variety 'Shitouqi' (zh.h0235) is a well-known Chinese landrace and breeding parent belonging to subspecies *fastigiata* and botanical-type *vulgaris* (agronomic-type Spanish), while 'Fuhuasheng' is a landrace from North China. For Shitouqi, a genome of ~2.54 Gb with 83,709 predicted genes across 20 pseudomolecules was reported, and the heterozygosity was very low (1/6537 nucleotides), while a genome of ~2.55 Gb with 83,087 predicted genes across 20 pseudomolecules was reported for Fuhuasheng.

8.8.1 Marker Development

Availability of genome-wide genetic markers is essential for trait mapping and marker-assisted breeding. In peanut, the initial efforts on isozyme and seed protein analyses identified only limited variability among the cultivated peanuts (see Lu and Pickersgill 1993; Stalker et al. 1994) though substantial diversity exists within the cultivated peanut genotypes for various morphological, physiological and agronomic traits (Stalker 1992). Random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) approaches also failed to detect any DNA variation in the cultivated peanut (Halward et al. 1991, 1992; Kochert et al. 1991; Paik-Ro et al. 1992). However, these approaches could identify genetic variability among the wild types (Halward et al. 1991). Later, He and Prakash (1997) reported polymorphic DAF and AFLP markers in cultivated peanut. SSR markers were developed in the cultivated peanut using DNA library (Hopkins et al. 1999), and the polymorphism was detected. SSRs were also developed from *Arachis pintoi* to identify the variation in *Arachis pintoi* (Palmieri et al. 2002) and the accessions belonging to the section *Caulorrhizae* (*Arachis*, Fabaceae) (Palmieri et al. 2005). Fifty-six SSRs were developed from the cultivated peanut from SSR-enriched library (He et al. 2003), of which 19 showed polymorphism. STMS markers were

developed in cultivated peanut to detect variation (Ferguson et al. 2004). Subsequently, several efforts were made to develop genic and non-genic SSRs (Moretzsohn et al. 2004, 2005; Hong et al. 2008; Zhang et al. 2012; Huang et al. 2016; Peng et al. 2016). Single-locus marker offers an advantage over multi-locus marker in genetic and breeding studies since its alleles can be assigned to specific genomic locus. Zhou et al. (2016a) developed 1790 single-locus SSR markers from the de novo assembly of peanut sequence reads. Using the reference genome sequences of *A. duranensis* and *A. ipaensis*, Luo et al. (2017) identified 264,135 and 392,107 SSRs from which 84,383 and 120,056 SSR markers were developed. Highly informative genic and genomic SSR markers facilitating molecular breeding in cultivated groundnut have been reviewed (Pandey et al. 2012a). CAPS markers were developed for detecting the mutations at *AhFAD2A* and *AhFAD2B* (Chu et al. 2009). Diversity Array Technology (DArT) (Kilian 2008) and Diversity Array Technology Sequencing (DArTSeq) (Shaibu et al. 2020) marker platforms have also been developed for peanut.

When compared to aforementioned marker systems with low polymorphic rate (5–6%), the transposable element-based marker system with higher polymorphic rate (up to 22%) was developed (see Bhat et al. 2019b). This was named as *Arachis hypogaea* transposable element (AhTE) marker system, which detects the polymorphism for the insertion of 205 bp long *Arachis hypogaea* miniature inverted-repeat transposable element (*AhMITE1*). AhTE marker system was proposed by developing just one marker (Bhat et al. 2008; Gowda et al. 2010, 2011); and subsequently a large number of such markers were developed in peanut. Shirasawa et al. (2012a) developed 504 AhTE markers using *AhMITE1*-enriched libraries. The representative *AhMITE1* exhibited a mean length of 205.5 bp and a GC content of 30.1%, with AT-rich, 9 bp target site duplications and 25 bp terminal inverted repeats. Later, Shirasawa et al. (2012b) developed additional 535 AhTE markers using transposon-enriched libraries of other cultivars. Since these AhTE markers were highly polymorphic and user-friendly (Kolekar et al. 2016b), they were successfully used to construct linkage maps (Shirasawa et al. 2013; Kolekar et al. 2016b) and to identify QTL for resistance to LLS and rust (Kolekar et al. 2016b). Later, AhTE markers were also used for marker-assisted backcross breeding in peanut (Yeri and Bhat 2016).

With the availability of the genome sequences of the diploid progenitors of peanut (Bertioli et al. 2016), efforts were made to identify the genome-wide distribution of *AhMITE1* (Gayathri et al. 2018). For this, a set of diverse genotypes (33), including the genetically unstable peanut mutants which show hyperactivity of *AhMITE1* (Hake et al. 2018), were used to discover the *AhMITE1* insertion polymorphic sites. WGRS reads from these diverse genotypes were analysed using the computational method polymorphic TEs and their movement detection (PTEMD) (Kang et al. 2016) for the de novo discovery polymorphic sites and to develop 2957 *AhMITE1* markers (Gayathri et al. 2018).

Currently, the advent of next-generation sequencing and genotyping technologies has enabled the detection of SNPs, which have emerged as the marker of choice in mapping (Bertioli et al. 2014), and several studies (see the section on

trait mapping) have reported identifying the SNP markers for mapping and population genomics. Hale et al. (2020) reviewed the methods to reduce per-sample costs in high-throughput targeted sequencing projects, minimal equipment and consumable requirements for targeted sequencing while comparing several alternatives to reduce bulk costs in DNA extraction, library preparation, target enrichment and sequencing. A cost calculator was developed for researchers considering targeted sequencing.

We attempted to analyse the WGRS data of 231 genotypes available in the public domain (NCBI SRA BioProject accession numbers: PRJDB4621, PRJDB5785, PRJDB5787, PRJDB0473, PRJNA340877, PRJNA490832, PRJNA490835, PRJNA511348 and PRJNA525866) for the SNPs as an effort towards population genomics and peanut pan-genomics (unpublished data). In comparison to the reference genome of Tifrunner, as high as 4,309,724 SNPs were detected (unpublished data) with a range of 113,363 (chromosome 18) to 433,957 (chromosome 03). On an average, a greater number of SNPs were noticed for A subgenome than that of B subgenome.

8.8.2 Mapping of Resistance to Leaf Spots and Rust

Due to the greater simplicity of diploids as genetic models, molecular genetic studies initially progressed using diploids rather than tetraploid cultivated types in peanut. Halward et al. (1993) mapped 117 RFLP markers on a 1063 cM map using a F_2 population derived from *A. stenosperma* × *A. cardenasii*. Genetic maps for diploid peanut have also been reported by Moretzsohn et al. (2005) (1230.89 cM, AA genome) and Moretzsohn et al. (2009) (1294.0 cM, BB genome). Considering the importance of foliar diseases, the development of several mapping populations segregating for LLS and rust resistance using GPBD 4 as one of the parents at the University of Agricultural Sciences, Dharwad, was reported by Bhat et al. (2012). Two hundred and sixty-eight RILs of TAG 24 × GPBD 4 were used for map (462.24 cM; 56 loci mapped on 14 LGs) construction using 59 SSR markers (out of total 67) (Khedikar et al. 2010). Similarly, a map (657.90 cM; 45 loci mapped on 8 LGs) was constructed using the RILs of TG 26 × GPBD 4 (Sarvamangala et al. 2011). Improving these maps with the additional markers led to TAG 24 × GPBD 4 (1922.4 cM; 188 loci mapped on 20 LGs) and TG 26 × GPBD 4 (1963; 181 loci mapped on 21 LGs) (Sujay et al. 2012). Further, using 143 markers common to the 2 maps, a consensus map with 225 SSR loci and total map distance of 1152.9 cM was developed (Sujay et al. 2012).

LLS and rust resistance-linked QTLs have been identified using the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4 (Sujay et al. 2012). The genomic region on linkage group AhXV carried three QTLs, GM2009-GM1536, GM1536-GM2301/GM2009 and IPAHM103-GM1954, contributing for both LLS and rust resistance. The highest phenotypic variance explained (PVE) across the seasons ranged from 62.35 to 82.96% for rust resistance and 17.37–67.98% for LLS resistance among

the three QTLs. Another region on AhXII flanked by GM1573-GM1009-pPGPseq8D09D exhibited 62.34% PVE for LLS resistance.

The linkage map for the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4 was further improved by adding new SSR and transposable element (TE) markers (Kolekar et al. 2016a, b). A linkage map of 1742.44 cM with inter-marker distance of 6.13 cM was constructed from the RILs of TAG 24 × GPBD 4 using 326 (SSR and AhTE) marker data. Similarly, a linkage map of 1230.77 cM with inter-marker distance of 5.56 cM was constructed from the RILs of TG 26 × GPBD 4 using 242 (SSR and AhTE) marker data. Also, a consensus map of 1727.39 cM with average inter-marker distance of 4.96 cM was constructed using 348 markers from the individual maps of TAG 24 × GPBD 4 and TG 26 × GPBD 4. QTL analysis based on genotypic and comprehensive phenotypic data for LLS and rust from 11 seasons could identify a region on linkage group AhXV (B03 linkage group of B genome) which contributed significantly towards LLS and rust resistance. QTL analysis detected five major QTL regions for LLS resistance and eight major QTL regions for rust resistance in TAG 24 × GPBD 4. $QTL_{MPI\text{-}LLS1}$ (GM1839-GM1009) had the highest R^2 of 5.16–92.34% for LLS resistance, and $QTL_{MPI\text{-}Rust1}$ (AhTE0498-GM2009) had the highest R^2 of 61.33–84.37% for rust resistance. QTL analysis detected five major QTL regions for LLS resistance and eight major QTL regions for rust resistance in TAG 24 × GPBD 4. $QTL_{MPI\text{-}LLS1}$ (GM1839-GM1009) had the highest R^2 of 5.16–92.34% for LLS resistance, and $QTL_{MPI\text{-}Rust1}$ (AhTE0498-GM2009) had the highest R^2 of 61.33–84.37% for rust resistance. $QTL_{MPI\text{-}LLS1}$ was identified in a maximum of four traits over four seasons followed by $QTL_{MPI\text{-}LLS5}$ appearing in three traits across three seasons. $QTL_{MPI\text{-}Rust2}$ appeared in as many as nine traits over four seasons. One major QTL region (GM2009-IPAHM103) was common for both LLS and rust resistance in TAG 24 × GPBD 4. Four major QTL regions flanked by AhTE0498-GM2009, AhTE0621-AhTE0360, AhTE0360-AhTE0498 and GM2009-GM2079 were common for LLS and rust resistance in TG 26 × GPBD 4. The RILs of VG 95149 × TAG 24 showed strong linkage of SSR marker GO340445 with rust resistance (Mondal et al. 2012a). The marker was closely linked (11.9 cM) to IPAHM103. Also, several main-effect and epistatic QTLs for the morphological and yield-related traits from TAG 24 × GPBD 4 RIL population were identified.

With an objective of identifying the candidate resistance genes for LLS and rust diseases in peanut, double-digest restriction site-associated DNA sequencing (ddRAD-Seq) was used based on next-generation sequencing (NGS) for genotyping the RILs of TAG 24 × GPBD 4 (Shirasawa et al. 2018). A total of 171 SNPs from the ddRAD-Seq were used along with the previously mapped markers to construct a map of 1510.1 cM. QTL analysis revealed major genetic loci for LLS and rust resistance on chromosomes A02 and A03, respectively. Heterogeneous inbred family-derived near-isogenic lines and the pedigree of the resistant gene donor, *A. cardenasii*, including the resistant derivatives of ICGV 86855 and VG 9514 as well as GPBD 4, were employed for whole-genome resequencing analysis. The results indicated the QTL candidates for LLS and rust resistance were located in 1.4 and 2.7 Mb genome regions on A02 and A03, respectively. In these regions, four

and six resistance-related genes with deleterious mutations were selected as candidates for LLS and rust resistance, respectively. All these studies showed a major region on A02 and A03 contributing for LLS and rust resistance. A few other studies with different mapping populations also pointed the same regions for LLS and rust resistance (Mondal et al. 2008, 2012a, b, 2013; Mondal and Badigannavar 2009, 2010). In addition, Mondal and Badigannavar (2018) identified SSR markers linked to rust resistance based on fine mapping using the RILs of VG 9514 × TAG 24. Also, they identified a R-gene (*Aradu.Z87JB*) of TIR–NB–LRR category and four glucan endo-1,3-β-glucosidase genes (*Aradu.RKA6 M*, *Aradu.T44NR*, *Aradu.IWV86* and *Aradu.VG51Q*) in the map interval of 1.25 cM corresponding to 331.7 kb region on the physical map of *A. duranensis*. Regions on A02/B02 and/or A03/B03 were also identified to be the candidate regions for LLS other populations (Khera et al. 2016; Pandey et al. 2017b; Han et al. 2018; Chu et al. 2019). However, Zhou et al. (2016b) identified QTL for LLS resistance on other chromosomes using the RIL population of Zhonghua 5 × ICGV 86699.

Mapping of ELS resistance was also attempted in various populations. Using the RIL population of SunOleic 97R × NC94022 mapped with 248 marker loci, Khera et al. (2016) identified 48 QTLs with PVE ranging from 3.88 to 29.14% for ELS on various chromosomes including B04. Subsequent studies identified QTL for ELS resistance on B04 and its homeologous chromosome (A04) apart from other chromosomes (Liang et al. 2017; Pandey et al. 2017b; Han et al. 2018). An $F_{2:6}$ RIL population derived from Tamrun OL07 and Tx964117 was used to get a map with 1211 SNP markers derived from ddRAD-Seq. Six QTLs with LOD score values of 3.2–5.0 and PVE ranging from 11 to 24% were identified for ELS resistance (Liang et al. 2017). Mapping with the RILs of Tifrunner × GT-C20 could detect nine QTLs for resistance to ELS (Pandey et al. 2017b). A RIL population consisting of 192 individual lines derived from Florida-07 × GP-NC WS 16 was subjected for GBS to construct a high-resolution map with 2753 SNP markers (Han et al. 2018). Two major QTLs located on chromosomes A03 and B04 were associated with resistance genes for ELS resistance.

A considerable progress has been made to involve wild germplasm in the recent genomics approaches, which not only expedite QTL mapping, fine mapping and gene discovery but also help variety development since they involve the simultaneous transfer of QTLs into popular breeding lines. In general, when wild relatives are used, inbreeding after crossing results in sterility, thus making it difficult to generate a large, random array of segregants for mapping. Advanced backcross QTL (AB-QTL) populations help overcome this problem. Wild species (donor) are crossed to a popular variety (recurrent parent), and the F_1s are backcrossed. An array of BC_2 or BC_3 lines, each containing a small number of random introgressions from the donor wild species in a popular varietal background, is used as the AB-QTL population (Tanksley and Nelson 1996). Recently, such a mapping population was developed from ICGS 76 (LLS susceptible) and an LLS-resistant synthetic allotetraploid, ISATGR 278-8 (*A. duranensis* × *A. batizocoi*). QTL analysis in this population identified (Varshakumari 2013) the genomic regions previously mapped using the RILs of TAG 24 × GPBD 4 and TG 26 × GPBD 4.

QTL-seq was employed to map LLS and rust resistance in peanut (Pandey et al. 2017a) and only LLS resistance (Clevenger et al. 2018). The candidate region co-localized with an alien introgression from the diploid A genome species, *A. cardenasii* (Clevenger et al. 2017).

Whole-genome resequencing (WGRS) of mapping populations has facilitated development of high-density genetic maps essential for fine mapping and candidate gene discovery for traits of interest in crop species. Agarwal et al. (2018) generated WGRS data for Tifrunner × GT-C20 RIL population, developed a SNP-based high-density genetic map of 3120 cM with an average distance of 1.45 cM and conducted fine mapping, candidate gene discovery and marker validation for ELS, LLS and TSWV. Also, 35 main-effect QTLs exhibiting PVE of 6.32% to 47.63%, with 2 major-effect QTLs for ELS on B05 (47.42% PVE) and B03 (47.38% PVE), 2 QTLs for LLS on A05 (47.63% PVE) and B03 (34.03% PVE) and 1 QTL for TSWV on B09 with 40.71% PVE were identified.

8.8.3 Association Mapping

Association mapping based on linkage disequilibrium is another method of identifying marker-trait association. A comprehensive analysis of marker-trait association (MTA) on LLS and rust resistance was done using a multi-location and multi-season data collected on a 'reference set' of groundnut genotypes. MTAs were identified for early leaf spot, late leaf spot and rust resistance (Pandey et al. 2014).

Recently, transposable element markers associated with LLS- and rust-resistant traits from an association panel of independent mutants were identified in peanut. Marker-trait association analysis for 110 markers resulted in 23 highly significant MTAs for foliar disease resistance. Seventeen MTAs with phenotypic variance explained (PVE) above 50% were observed for resistance to late leaf spot (LLS) and rust. The genic and non-genic AhTE markers associated with LLS- and rust-resistant traits were analysed for their genomic location and functional annotation (Hake et al. 2017).

GWAS was attempted using Affymetrix version 2.0 SNP array with 120 genotypes mainly coming from the US peanut minicore collection. A total of 46 quantitative trait loci (QTLs) were identified with phenotypic variation explained (PVE) from 10.19 to 24.11%, in which 18 QTLs are for resistance to ELS and 28 QTLs for LLS. A total of 74 non-redundant genes were identified as resistance genes, among which 12 candidate genes were in significant genomic regions including 2 candidate genes for both ELS and LLS and other 10 candidate genes for ELS (Zhang et al. 2020).

8.8.4 QTL Validation

Some 'significant' QTL may be false positives, and QTL responsible for significant variation within and between populations can be missed if the tested genotypes are fixed by chance for alleles with similar effects. Therefore, QTLs should be confirmed by repeated experiments using the same and different strains or genotypes before they are considered for breeding programmes. In general, the QTL/markers are validated by testing them in different genetic background. In general, stabilized populations, cultivated genotypes, popular cultivars and near-isogenic lines (NILs) are used as genetic material for validating the QTLs/markers.

Considering the importance of marker utility in molecular breeding to improve LLS and rust resistance in peanut, LLS and rust resistance-linked QTL and markers were validated (Khedikar et al. 2010; Sujay et al. 2012) using a set of 46 resistant and susceptible germplasm lines with different genetic background including released varieties, hybrid derivatives from North Carolina Accessions, interspecific derivatives, mutant lines, cultivars from South American landraces and advanced breeding lines. The makers linked to LLS and rust resistance were also validated using the RILs of a new cross, TG 19 × GPBD 4, and three introgression line populations from ICGS 76 × ISATGR 278-18, DH 86 × ISATGR 278-18 and DH 86 × ISATGR 5. The type of allele at three LLS resistance-linked markers (GM1009, GM1573, pPGPseq8D09), six LLS and rust resistance-linked markers (GM1536, GM1954, GM2009, GM2301, GM2079 and IPAHM103) and one rust resistance-linked marker (GO340445) loci was checked for the co-segregation with the phenotype. The resistant genotypes had the resistant allele at all marker loci. They were validated statistically by single-marker analysis, Kruskal-Wallis test and locus-by-locus AMOVA (Sukruth et al. 2015). Markers were also validated using the NILs derived from TAG 24 × GPBD 4 and TG 26 × GPBD 4 population (Yeri et al. 2014). Many of this (IPAHM103, GM2301, GM1536, GM2079 and pPGPseq8D09) were also validated using 95 diverse genotypes; majority of these markers were on LG AhXV followed by LG AhXII on consensus genetic map (Gajjar et al. 2014). LLS and rust resistance-linked markers were also validated by Divyadharsini et al. (2017) among the derivatives of CO 7 × COG 0437 using single-marker analysis.

8.9 Transcriptomics

Numerous efforts have been made in peanut to collect and study the transcriptome (see Chen and Liang 2014) using the initial efforts with microarrays (Chen et al. 2012; Zhu et al. 2014), real-time polymerase chain reaction (PCR) and the current studies with RNA sequencing. Luo et al. (2005) attempted to identify resistance genes in response to leaf spot disease using microarray and real-time PCR. Gene expression profiles of the resistant and susceptible genotypes were studied for 384 unigenes selected from 2 expressed sequence tag (EST) cDNA libraries. A total of

112 spots representing 56 genes in several functional categories were up-regulated. Seventeen of the top 20 genes with known function were validated. The resistant genotype showed higher expression for these genes (Luo et al. 2005). They showed similarity with genes encoding lipid transfer protein precursors, pathogenesis-related protein, glucosyl hydrolase family protein and LRR protein family.

In another study, transcriptomic and proteomic analysis identified several genes for proteins involved in cell wall strengthening, hypersensitive cell death, resistance-related proteins, metabolism and signal transduction in resistant wild groundnut species *A. diogoi* for late leaf spot pathogen (Kumar and Kirti 2015b). Zhou et al. (2016a) confirmed that nucleotide-binding-leucine-rich repeat (NB-LRR)-encoding genes were involved in the LLS resistance mechanism by comparing QTL locations for LLS resistance from genetic linkage map with the physical map.

A total of 214 expressed R-genes were identified from cultivated peanuts that are naturally infected by early and late spot pathogens (Dang et al. 2018). Further, efforts were made to identify the association of specific R-genes to leaf spot resistance for providing molecular targets for marker-assisted breeding strategies (Dang et al. 2021). Advanced breeding lines from different pedigrees were evaluated for leaf spot resistance, and 76 candidate R-genes were analysed for their expression among the susceptible and resistant lines. Thirty-six R-genes were differentially expressed and significantly correlated with resistant lines, of which a majority were receptor-like kinases (RLKs) and receptor-like proteins (RLPs) that sense the presence of pathogen at the cell surface and initiate protection response. The largest group was receptor-like cytoplasmic kinases (RLCKs) VII that were involved in pattern-triggered kinase signalling resulting in the production of reactive oxygen species (ROS). Four R-genes were homologous to TMV-resistant protein N which is known to confer resistance against tobacco mosaic virus (TMV). When mapped to peanut genomes, 36 R-genes were found to be distributed in all the chromosomes except a pair of homeologous chromosomes (A09 and B09). Low levels of gene expression in resistant lines suggested that the expression is tightly controlled to balance the cost of R-gene expression to plant productively (Dang et al. 2021).

Gong et al. (2020) used RNA-Seq to identify 133 differentially expressed genes (DEGs) between 904 (resistant to ELS) and 1006 (susceptible to ELS) RILs of Florida-07 × GP-NC WS 16. Coiled-coil nucleotide-binding leucine-rich repeat (NLR)-type resistance genes and phytoalexin deficient 4 (PAD4) regulator of effector-triggered immunity mediated by NLR resistance proteins and polyphenol oxidase (PPO) genes were among the DEGs.

RNA-Seq was used to identify the DEGs between Yuanza 9102 (resistant to LLS) and its EMS-derived mutant M14 (susceptible to LLS) (Han et al. 2017). In total, 2219 genes showed differential expression in the leaf tissue, of which 1317 were up-regulated and 902 were down-regulated. Pathogenesis-related (PR) protein-coding genes were significantly up-regulated, while those related to photosynthesis were down-regulated in the susceptible M14 in comparison to Yuanza 9102. The study suggested that the susceptibility in M14 could be associated with the down-regulation of chloroplast genes and plant hormones related to plant growth and up-regulation of WRKY transcription factors.

DEGs were also identified for resistance to rust in peanut using RNA-Seq (Rathod et al. 2020). DEGs uniquely up-regulated in resistant genotype (GPBD 4) when compared to JL 24 (susceptible) included pathogenesis-related (PR) proteins, MLO such as protein, ethylene-responsive factor, thaumatin and F-box. Down-regulated genes in the susceptible genotype were caffeate O-methyltransferase, beta-glucosidase and transcription factors (WRKY, bZIP, MYB). Selected DEGs were also validated using RT-qPCR.

8.10 Proteomics

Regulations during transcription, translation and posttranslational modifications are widespread; therefore the mRNA content not necessarily corresponds with the protein content (Dhingra et al. 2005). Proteomics is constantly advancing to bridge the gap between DNA sequence, transcriptome and phenotype under the diverse and dynamic stages of growth and development. Since proteins influence important phenotypes and are the products of genes and epigenetic or posttranslational mechanisms, population proteomics has the potential to provide key insights into functional and metapopulation ecology, adaptation and acclimation processes under various climate and environmental conditions. Proteomics approaches also help identify genetic loci underlying disease resistance and for the development of biomarkers. In peanut, the low DNA polymorphism coupled with high morphological variation might involve differences in the proteomics. Katam et al. (2014) reviewed the progress made on the proteomics in peanut especially on peanut allergens and adaptive responses to various stresses. Proteomics analyses have been reported for response to high-oleic acid content in seed (Liu et al. 2020), cadmium detoxification and translocation (Yu et al. 2019), allergen production (Mamone et al. 2019), response to water stress (Kottapalli et al. 2009, 2013; Katam et al. 2016), gynophore development (Sun et al. 2013; Zhao et al. 2015), development of aerial and subterranean pods (Zhu et al. 2013), response to salinity (Jain et al. 2006) and polyphenol content (Muralidharan et al. 2020).

Kumar and Kirti (2015b) employed 2D proteomics to confirm a few selected DEGs identified (233) through cDNA-AFLP when *Arachis diogoi* was challenged with LLS pathogen. From this study, three candidate genes, *AdLEA*, *AdSGT1* and *AdTLP*, were further characterized and confirmed for their function in imparting disease resistance. *Arachis diogoi* late embryogenesis abundant (AdLEA) protein-coding gene (identified from the above study) was characterized and shown to impart resistance to multiple stresses in tobacco (Sharma et al. 2016). *AdSGT1* (suppressor of G2 allele of SKP1), an essential signalling component in R-gene-mediated resistance response against various plant pathogens (Kumar and Kirti 2015a), and *AdTLP*, a pathogen-induced thaumatin-like protein gene (Singh et al. 2013), were also characterized. These genes were found to be candidate genes for enhancing stress resistance in crop plants.

8.11 Epigenomics

Low DNA sequence polymorphism despite enormous phenotypic variations in peanut indicates the possible role of epigenetic variations. Detection of the epigenetic marks (along with associated expression) provides high power to identify genomic regions associated with traits or evolutionary processes such as fitness, phenotypes and selection. In peanut, Bertioli et al. (2019) observed lower methylation in the transcribed regions and characteristic decline in methylation at transcription start and end sites like in most plant genomes. Genome-wide methylation per cytosine content was higher in pericentromeric regions than the chromosome arms. Methylation was lower in the A subgenome than the B subgenome, with 76.0% and 80.5% methylation at CG sites, 61.7% and 65.1% methylation at CHG sites (where H is an A, T or C) and 5.14% and 5.51% methylation at CHH sites, respectively.

A genome-wide DNA methylation pattern and its influence on gene expression were reported across 11 peanut genotypes differing for foliar disease resistance (Bhat et al. 2019a). Bisulphite sequencing and RNA-Seq of 11 genotypes after 21 days of sowing (DAS) differentially DNA-methylated sites between the foliar disease-resistant (GPBD 4, VG 9514, ICGV 86855, ICGV 99005 and ICGV 86699) and disease-susceptible (TAG 24, TMV 2 and JL 24) genotypes. Foliar disease-resistant genotypes showed significant differential DNA methylation at 766 sites corresponding to 25 genes. Of them, two genes (*Arahy.1XYC2X* on chromosome 01 and *Arahy.00Z2SH* on chromosome 17) coding for senescence-associated protein showed differential expression where the resistant genotypes recorded higher FPKM at their epialleles.

8.12 Marker-Assisted Backcrossing (MABC) for Foliar Disease Resistance

Genomic tools can enhance the efficiency of breeding programmes through their use in marker-assisted selection (MAS) where selection of target traits can be achieved indirectly using molecular markers that are closely linked to genes or gene itself. Marker-assisted backcrossing (MABC) is a precise and effective method to introgress a gene or genomic region of interest while retaining the essential characteristics of the recurrent parent (Hospital and Charcosset 1997; Hospital 2001; Collard and Mackill 2008). Marker-assisted foreground, background and recombinant selection would be done in the marker-assisted backcrossing (MABC) unlike time-consuming selection based on only phenotype in the conventional backcross breeding. The first successful example of marker-assisted selection (MAS) was the development of a nematode-resistant cultivar, NemaTAM (Simpson et al. 2003). Chu et al. (2011) developed the high-oleic Tifguard cultivar, by pyramiding the nematode resistance and the high-oleic trait in less than 3 years by using molecular markers associated with these traits.

The genomic resources developed for LLS and rust resistance have been employed for developing backcross lines in peanut with enhanced precision and efficiency (see Pandey et al. 2020) with the first effort of transferring the genomic region on linkage group AhXV governing rust resistance from GPBD 4 to three rust-susceptible varieties, ICGV 91114, JL 24 and TAG 24, through MABC using IPAHM103, GM2079, GM1536 and GM2301 (Varshney et al. 2014). Two to three backcrosses and selfing could yield 200 backcross lines from all the 3 crosses. Field evaluation of 81 lines confirmed their improved resistance to rust. These lines had significantly increased pod yields (56–96%) in infested environments compared to the susceptible parents (Janila et al. 2016a). Based on the genotype × environment interactions for these MABC lines, location-specific genotypes were suggested (Rathnakumar et al. 2020). Through other efforts, LLS- and rust-resistant backcross lines were developed in JL 24 (Yeri and Bhat 2016) and TMV 2 (Kolekar et al. 2017; Ramakrishnan et al. 2020) using GPBD 4 as the donor. Majority of these genotypes are under multi-location testing or large-scale farm testing (Fig. 8.2) for variety development and commercialization.

Molecular breeding for improving oleic acid content was reported by transferring fatty acid desaturase mutant alleles (*ahFAD2A* and *ahFAD2B*) from the donor SunOleic 95R to ICGV 06110, ICGV 06142 and ICGV 06420 (Janila et al. 2016b). Through MABC, high-oleate lines were developed in ICGV 05141 (Bera et al. 2018), GPBD 4 (Nawade et al. 2019) and ICGV06100 (Bera et al. 2019) using SunOleic 95R as the donor. Huang et al. (2019) developed high-oleate MABC lines in Yuhua 15, Yuanza 9102, Yuhua 9326 and Yuhua 9327 using the donor parents (KN176, DF12 and KX016). Efforts to combine foliar disease resistance with high oleate have also been successful where GJG 9, GG 20 and GJGHPS 1 were used as the recurrent parents and the GPBD 4 and SunOleic 95R were used as the donor parents (Shasidhar et al. 2020). Similarly, Deshmukh et al. (2020) improved an elite variety, K 6, for foliar disease resistance (ICGV 13193 as the donor) and oleate content (ICGV 15033 as the donor). Combining foliar disease resistance with high

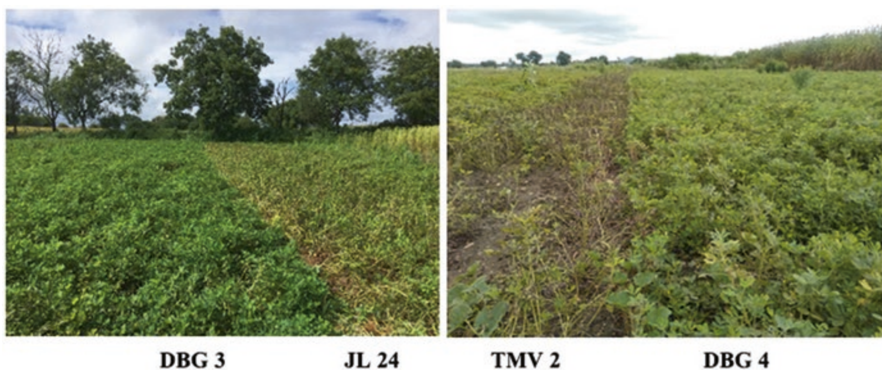

Fig. 8.2 Field performance of foliar disease-resistant backcross lines DBG 3 (in JL 24 background) and DBG 4 (in TMV 2 background)

oleic acid is also being attempted in GPBD 4, G 2-52, TMV 2 and JL 24 apart from a bold seeded variety ICGV 06189 (our unpublished data). AB-QTL method of transferring foliar disease resistance from the synthetic amphidiploids to cultivated peanut varieties (ICGS 76 and Dh 86) could identify superior genotypes (Paratwagh and Bhat 2015).

8.13 Transgenic Approach

With the identification of the candidate genes in peanut and other sources, transgenics have been developed in peanut for improving various traits including resistance to foliar diseases. Pathogenesis-related (PR) proteins, SniOLP (*Solanum nigrum* osmotin-like protein) and Rs-AFP2 (*Raphanus sativus* antifungal protein-2), were overexpressed in peanut. Transgenic peanut plants showed enhanced disease resistance to late leaf spot based on a reduction in number and size of lesions on leaves and delay in the onset of disease (Vasavirama and Kirti 2012).

Peanut cv ICG 13942 was transformed with *Tcchitinase-I* gene (Marka and Nanna 2021). T_1 transgenic plants when evaluated for ELS, LLS and rust showed longer incubation, latent period and lower infection frequencies in comparison to non-transformed plants. A significantly negative correlation was recorded between chitinase activity and the infection by ELS, LLS and rust pathogens.

A chitinase gene from rice (*Rchit*) was introduced into three varieties of peanut through *Agrobacterium*-mediated genetic transformation (Prasad et al. 2013). Evaluation of the transgenic plants for LLS and rust using detached leaf assays showed longer incubation, latent period and lower infection frequencies when compared to their non-transformed counterparts. A significant negative correlation was observed between the chitinase activity and the frequency of infection to the three tested pathogens. Overexpression of a fusion defensin gene from radish and fenugreek also improved the resistance against leaf spot diseases in peanut (Bala et al. 2016).

8.14 Conclusions and Future Perspectives

A considerable progress has been made so far in understanding the symptoms and the components of resistance to the foliar diseases like ELS, LLS and rust in peanut. Initial efforts have identified the sources of resistance both in the cultivated peanut and its wild relatives. Through different routes of utilizing the wild diploids, the genetic variability is being expanded, and the novel alleles governing disease resistance in the wild relatives have contributed for the success of breeding for improved varieties. These efforts have also marked an opportunity to overcome the undesirable linkage drag associated with disease resistance. But recent extensive progress in genomics and molecular breeding has successfully demonstrated the translational

genomics in peanut. Availability of genome sequences of cultivated peanut, its primitive allotetraploid progenitor (*Arachis monticola*) and the two diploid parents could enhance the understanding on ELS, LLS and rust resistance. Genome-wide marker development, identifying and dissecting QTL to identify the candidate genes and a great effort to validate the QTL and markers coupled with the transcriptome data have facilitated marker-assisted backcross breeding to develop foliar disease-resistant lines in the elite varieties such as JL 24, TMV 2, ICGS 76, Dh 86, TAG 24, ICGV 91114, GJG 9, GG 20, GJGHPS 1 and Kadiri 6. These lines are in the advanced stage of testing and release for commercial cultivation.

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Chapter 9
Safflower Improvement: Conventional Breeding and Biotechnological Approach

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Abstract Safflower (*Carthamus tinctorius* L.) is one of humanity's oldest oilseed crops, although it is a minor crop with limited distribution due to environmental factors and the crop's spiny nature. In India, the crop has traditionally been produced in combinations with other "rabi" crops such as wheat and sorghum during the "rabi" or winter dry season. It is a self-pollinated crop with 5–40% outcrossing due to the action of numerous insects, primarily honeybees. It's mostly utilized in the production of vegetable oil, animal feed, biofuel, plant-based pharmaceuticals, and industrial oil. The crop was traditionally grown for its flowers, which were used to color and flavor dishes, as well as make dyes (particularly before cheaper aniline dyes became accessible) and medicines. Lower oil content and seed yield, insect pest susceptibility, and disease resistance are all characteristics that reduce safflower production and quality, contributing to its underutilized status. The limited genetic diversity of local and traditional varieties necessitates collecting accessions from all over the world to explore the genetic diversity of the available germplasm.

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These collections will provide information that will improve future safflower conservation and utilization. Genetic diversity in breeding lines and cultivars among global germplasm and main origins must be characterized in order to develop effective breeding strategies. Cultivar improvement ought to have played a role in the enhanced yield levels. Nonetheless, oil content remained fairly consistent, ranging between 28% and 30%, with only a few cultivars attaining an average of 35% oil. In recent years, several countries' research efforts have mostly concentrated on increasing seed or oil yield. Pure line selection is the most often employed breeding approach for cultivar growth in India when it comes to safflower improvement. This is shown by the fact that local selection has resulted in the development of more than 17 varieties for commercial production in the country. Through both genetic and cytoplasmic male sterility systems, hybrid vigor has been commercially exploited for the production of hybrids in safflower. Now there is shift in objectives in development of non-spiny cultivars which can address the problem of operational costs. In recent years, biotechnological methods have played a supporting role in safflower breeding. However, because safflower is an "orphan" of the genomics revolution, breeding efforts have been impeded by a lack of molecular tools that might otherwise allow for faster development. However, in recent years, this scenario has begun to shift. Safflower research is dispersed, and there is an urgent need to concentrate on the crop's untapped potential. The diverse floral and physiological features, flower yield, pigment content (carthamin, carthamidin, and luteolin), leaf and medicinal components, and antioxidant activity of safflower have not been studied genetically. There have been no studies on proteomics of safflower. Biotechnology can be used to further investigate the medicinal application of safflower for pharmaceutical objectives. For safflower breeding, advances in molecular farming and transcriptome research to identify key genes (e.g., gene incorporation in enzymatic and nonenzymatic antioxidant biosynthesis) are recommended. Hence, there is urgency of biotechnological interventions to make cutting-edge breakthrough in case of safflower.

Keywords Safflower · *Carthamus tinctorius* L. · Underutilized crop · Genetic diversity · Breeding strategies · Hybrid vigour · Biotechnology

9.1 Introduction

Safflower (*Carthamus tinctorius* L.) is one of the most ancient oilseed crops in the world. Worldwide, safflower is a minor crop compared with other oilseeds. It is believed to have originated in Southern Asia and has been cultivated in China, India, Persia, and Egypt since prehistoric times. It is a Compositae or Asteraceae family annual oilseed crop grown commercially in Australia, Ethiopia, India, Mexico, the USA, and other countries (Hashemi et al. 1994). The countries with the longest traditions of growing safflower as an oil plant are India and Ethiopia (Weiss 2000).

Ethiopia, Afghanistan, and India were suggested as centers of origin for cultivated Safflower by Vavilov (1951). Safflower is known in India and Pakistan as "kusum," derived from the Sanskrit "kusumbha" (Chavan 1961), and in China as "honghua" or red flower. Other names for safflowers include "agnisikha," "asfiore," "assfore," "asfrole," "brarta," "carthami flos," "flase," "ghurtom," "golzardu," "hebu," "kahil," "kajena-goli," "kamal lotarra," "kar," and "karar" (Smith 1996).

Safflower is a thistlelike annual or winter annual with a lot of branches. The leaves typically have a lot of long, sharp spines. Plants range in height from 30 to 150 cm tall with globular flower heads (capitula), and bright yellow, orange, or red flowers are common. Achenes are flat and four-sided and lack pappus in most cases (Dajue and Mundel 1996). Over 60 countries grow safflower, but over half is produced in India mainly for the domestic vegetable oil market (Popov and Kang 2011).

Safflower has high adaptability to low moisture conditions. Therefore, its production all over the world is mainly confined to areas with scanty rainfall (Singh and Nimbkar 2006). Safflower is an important industrial crop moderately tolerant to abiotic stresses (Hussain et al. 2016). Researchers, Madaan et al. (2011), discovered phytoremediative properties of safflower while researching the biochemistry of heavy metal accumulation in plants. They discovered that the absorption of these metals through roots is translocated by the plant, accumulating in the seed, and that the plant can act as a phytoremediative plant in areas polluted with Hg (mercury) and Se (selenium). When safflower plants are used for this purpose, however, seeds should only be used for biodiesel production and should never be consumed by humans or animals (Menegaes and Nunes 2020).

Safflower, a multipurpose crop, has been grown for the orange-red dye (carthamin) extracted from its brilliantly colored flowers (used as a fabric dye and for food coloring) and for its quality oil rich in polyunsaturated fatty acids (linoleic acid, 78%), and it also includes a mixture of oleic and other acids, as well as serotonin and its conjugates, polyphenols, lignans, and other compounds.

There is growing demand for high polyunsaturated food products in developed countries. Furthermore, there are signs of recent growth in the safflower industry, as well as a premium price for safflower oil and promising prospects, which have prompted a number of countries to adopt safflower as an oilseed crop (Johnson and Marter 1993). Oleic-rich safflower oil can be used in the production of varnishes, alcohols, paints, lubricants, cosmetics, detergents, and bio-based plastics (Velasco and Martínez 2001).

Apart from this, the scavenging activities of safflower petals have been shown to contain a variety of colors from orange to white with varying intensities (Imami et al. 2010). Therefore, this plant is used for numerous culinary and textile purposes (Delshad et al. 2018). Carpet-weaving industries in Eastern Europe, the Middle East, and the Indian subcontinent relied heavily on safflower dyes (Dajue and Mundel 1996). It is vastly utilized in traditional medicine for various medical conditions, namely, dysmenorrhea, amenorrhea, postpartum abdominal pain and mass, trauma, and joint pain (Delshad et al. 2018). Other biological activities associated with its water extract include anticoagulant, vasodilator, antihypertensive, antioxidative, neuroprotective, immunosuppressive, and anticancer effects, as well as

inhibitory effects on melanin synthesis (Zhao et al. 2009). Safflower forage is palatable, and its feed value and yields are similar to or better than those for oats or alfalfa. Thus, each part of safflower has a value attached to it resulting in a high-quality edible and industrial oil and bird feed (Bergman et al. 2007).

Safflower, a diploid with 12 chromosome pairs (Ashri and Knowles 1960), is primarily self-pollinating, but pollen transfer by a variety of insects may result in significant outcrossing (Rudolphi et al. 2008). Furthermore, the degree of outcrossing is determined by genotype and environmental factors. Ashri and Knowles (1960) divided about 25 species of wild safflower into different sections based on chromosome number. Many of these are weedy, such as *C. oxyacanthus*, which is a noxious weed in the USA, making its regeneration at the USDA Western Regional Plant Introduction Station more difficult (WRPIS). Species with 12 chromosome pairs are more likely to cross. These involve *C. tinctorius*, *C. persicus* Desf. Ex Willd, *C. oxyacanthus*, and *C. palaestinus*. With its many and various uses, safflower (*C. tinctorius* L.) has benefited from the richness of genetic resources conserved and distributed by gene banks.

Zhang and Johnson (1999) compiled a safflower germplasm directory that listed 18 different collections from 14 different countries. The US safflower collection, which dates back to the late 1940s, is housed at the WRPIS in Pullman, Washington. Germplasm from more than 50 countries is embodied in the US collection, and accessions are open to scientists all over the world (Kisha and Johnson 2012). *Carthamus palaestinus* Eig, a self-compatible wild species restricted to the deserts of southern Israel and western Iraq (Zeven and Zhukovsky 1975), with white and yellow flowered forms, is the progenitor from which the weedy species *C. oxyacanthus* Bieb., a mixture of self-compatible and self-incompatible species, is descended. Ashri and Knowles (1960) considered these to be the parental species of the cultivated species *C. tinctorius* L.

The level of improvement made in both yield and oil content largely determines the success of safflower as a commercial oilseed crop in traditional areas and its expansion into new areas. After being cultivated for a long time across vast and diverse regions in the Old World, safflower established significant diversity, and there is evidence of incipient genetic differentiation (Knowles 1989). The simplest method for crop improvement is introduction, which has been used successfully to grow many oilseed crops in new areas (Knowles 1983). The genetic diversity of global safflower germplasm has been assessed in a number of studies. Prior to the 1990s, the majority of this research focused on morphological and agronomic traits. Understanding the extent and distribution of genetic variation among species will aid in the development of potential safflower breeding programs (Padulosi et al. 1999). Via recombination, the abundance of genetic diversity preserved in world collections, as well as regional divergence within them, can be exploited to produce even more variability. Molecular markers may be used to recognize duplicate accessions, to establish and evaluate specific groups within collections (such as core collections), to estimate and compare diversity across countries or regions, to identify acquisition needs, and to perform genetic mapping. In case of safflower, molecular markers have been widely used for genetic diversity analysis, phylogenetic studies,

and marker-assisted breeding. Various molecular markers including RAPD (Mahasi et al. 2009), AFLP (Zhang et al. 2006), ISSR (Golkar et al. 2011a, b), and EST-SSR (Barati and Arzani 2012) were used to assess the germplasm diversity. Chapman et al. (2009) developed a polymorphic EST-SSR marker which made comparative map-based analysis easier in the case of safflower. To improve germplasm management and utilization, further characterization of safflower with molecular markers from various world sources is needed, regardless of the type of molecular marker used (Kisha and Johnson 2012).

For safflower breeding, advances in molecular farming and transcriptomic studies (such as the identification of essential genes in enzymatic and nonenzymatic antioxidant biosynthesis) are recommended. Little is known about the form of gene action that improves plant stress resistance by increasing tocopherol content (as a nutritional factor) and inheritance of physiologic-related traits (chlorophyll a, b, carotenoids). For (bacterial and fungal) disease resistance, there is a significant gap in gene action. In case of safflower, little has been done to establish mapping populations and molecular markers. There appears to be a lack of knowledge on safflower trait mapping, and further genetic studies in this area will aid breeders in locating the gene position of important agronomic and oil quality-related traits so that cultivars with improved productivity and resistance to biotic and abiotic stresses could be developed. One of the major reasons for low productivity of safflower in India is infestation of aphids with estimates of yield loss as high as 74% (Bhardwaj et al. 1990). No resistance to this pest has been identified so far. There appears to be no research on the proteomics of safflower. As a result, it is suggested that this significant gap in safflower research be filled by conducting the required studies. To overcome the cytological and genetic barriers to introgression between cultivated safflower and wild related species, embryo rescue techniques must be established. Finally, to accelerate safflower breeding programs, close collaboration between research institutes involved in modern plant breeding, germplasm conservation, biotechnology, and bioengineering is needed (Golkar 2014).

Numerous authors have reported that genotype-environment interactions have a significant impact on the composition of safflower oil (Amini et al. 2014). The manipulation and combination of the major genes that regulate fatty acid levels in the safflower oil has been widely investigated in recent years by the fields of genetic breeding and engineering (Gecgel et al. 2007).

9.2 Description About the Crop

9.2.1 Germplasm Resources

There is a wide array of germplasm depicting different species and wild species, with a good amount of diversity in terms of various traits. Due to the sterility of hybrids, however, there is a small exchange of genetic information between species

(Ashri and Knowles 1960). Natural crosses between *C. tinctorius* and its wild relative *C. oxyacantha* with 2n = 24 chromosomes have been reported near Isfahan, Iran, and in the experimental field at Abu Ghraib station near Baghdad, Iraq. In India, however, it is still limited because the flowering times of both species do not coincide (Ashri and Knowles 1960).

The Indian Institute of Oilseeds Research in Hyderabad, India (Anonymous 2002), has 7318 germplasm accessions, including exotic and Indian genotypes. At the international level, the Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington, which is part of the USA; the National Plant Germplasm System (NPGS); and the US Department of Agriculture's (USDA) Agriculture Department (Bradley and Johnson 2001) maintain safflower accessions obtained from more than 50 countries. The germplasm that has been identified in this way provides a rich resource for various agronomic traits as well as biotic and abiotic stresses (Li et al. 1993; Cervantes-Martinez et al. 2001). The UAS Dharwad has very rich collection of germplasm accessions (Fig. 9.1) promising for various traits, number of capitula per plant (GMU-2757, GMU 2365), size of the capitula (GMU 3625 and GMU 3621), and sources for high density planting (Fig. 9.2).

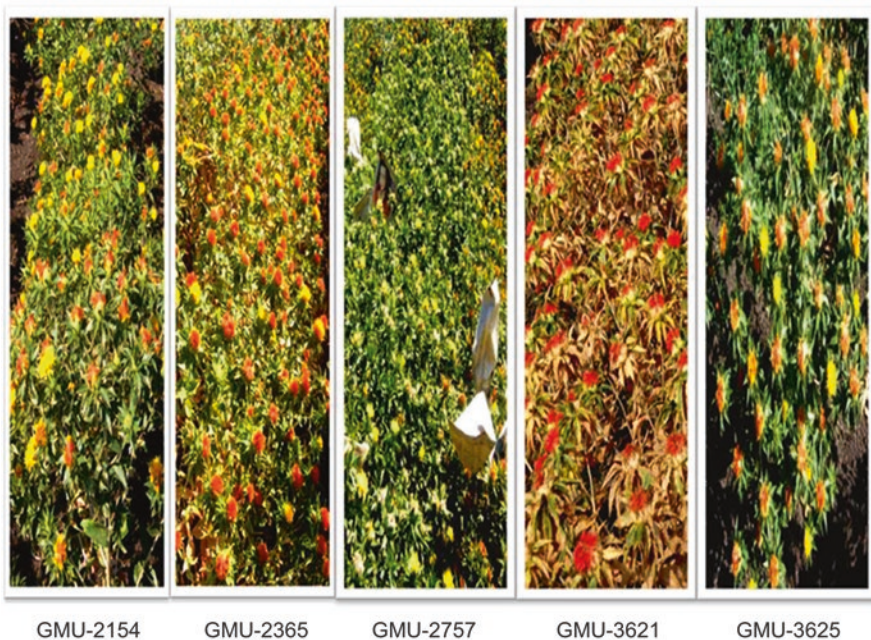

Fig. 9.1 Promising germplasm accessions of safflower

Fig. 9.2 Appressed types of safflower accessions

9.2.2 Conventional Breeding

The importance of gene action in the selection of any breeding methodology cannot be overstated. As a result, understanding these different aspects is critical, as discussed in the following paragraphs. Plant height as a yield component trait, for example, has been shown to be affected by additive gene action (Kotecha 1979; Shahbazi and Saeidi 2007; Golkar et al. 2012b). Extranuclear genes are said to have no impact on this morphological trait (Mandal and Banerjee 1997). As a result, cyclic selection can be used to improve target trait. Two other traits, stem diameter and leaf length, have been confirmed to be influenced by additive and nonadditive gene action (Kotecha 1979). Gupta and Singh (1988b) discovered that additive gene effects play a significant role in the genetic regulation of the number of branches per plant. However, Narkhede and Patil (1987) identified an epistasis effect in controlling the number of branches per plant. Contrary to popular belief, Golkar et al. (2012b) found that epistasis has no impact in this regard. Given these contradictory results, it appears that the functional application of epistasis knowledge in breeding is a challenging task that will need more biometric research (Golkar et al. 2012b). One more important trait in safflower is the number of branches per plant, which indirectly leads to higher yield. This trait is regulated both digenically and environmentally in safflower (Deokar and Patil 1975). Apprised branching is regulated both digenically and monogenically and is recessive to separating types (Deokar and

Patil 1975). Head diameter is genetically regulated by dominance gene effects (Golkar et al. 2012b). Camas and Esendal (2006) found it to have low broad-sense heritability. This result highlights the significance of external influences on head diameter, which is a good indicator for safflower ornamental applications. With GMA, the additive-dominance model has also been proposed for genetic regulation of node number on the main stem (Abel 1976). Internode distances have also been linked to epistatic effects (Abel 1976).

9.2.3 Seed Related Traits

Safflower seeds appear in a range of colors, from white to creamy. It is made up of 55–65% kernel and the rest is hull (Singh 2007). In typical hull types, the entire seed contains 27–32% oil, 5–8% moisture, 14–15% protein, 2–7% ash, and 32–40% crude fiber (Weiss 2000). Oil content varies depending on hull content, varying from 27% to 67%. Oil content and grain yield have a clear negative association. The oil percentage in the plant's whole seeds ranges from 25% to 37%, but in very thin hull types, it rises to 46–47%. The pericarp (hull) of a safflower seed is high in fiber, while the embryo component is high in oil and protein (Urie 1986). As a result, reducing the hull portion of the seed increases the product value. According to the same researcher, the partial hull is recessive to white hull. Striped seed and reduced pericarp are dominated by the recessive genes th and stp, according to Ebert and Knowles (1966). They also reported that the stripped hull inheritance was under the monogenic influence.

9.2.4 Nutritional Parameters

One of the most important aspects of nutritional quality is protein content. There is currently little knowledge on the genetics of safflower protein content. The additive-dominance model regulates the protein content (Pahlavani et al. 2007; Golkar et al. 2012a). As a result, the pedigree method with later generation selection may be used to enhance it. Oil content is an essential seed nutrient that is influenced by many factors including genotype, environment, and genotype X environment interaction. In safflower breeding, the focus should be on improving both the quality and quantity of oil (Hamdan et al. 2008). In the genetic regulation of seed oil yield, both additive (Golkar et al. 2011a) and dominance (Gupta and Singh 1988a, b) gene effects are observed, according to the literature. Epistatic effects had a major influence on the genetic control of safflower oil, as per Pahlavani et al. (2007). The dominance alleles involved in the genetic regulation of safflower oil content outnumbered the recessive ones, that according to Ramachandram and Goud (1981). Camas and Esendal (2006) recorded a low value for heritability affecting oil yield, which may be affected by the high effect of environmental conditions on oil content.

9.2.5 Non-spiny Type

The safflower crop's spiny nature is one of the main factors limiting its popularity and cultivation. In most genotypes, the crop has a lot of sharp spines on the leaves and bracts (Bradley et al. 1999). As a result, the production of high-yielding *non-spiny* varieties with high oil yield is the main focus in safflower breeding (Golkar et al. 2010). The inheritance of spininess has been recorded in many reports (Classen 1952; Narkhede and Deokar 1990; Golkar et al. 2010). Spininess was dominant over spinelessness with four genes (Sa, Sb, Sc, and Sd), according to Narkhede and Deokar (1990); however, Golkar et al. (2010) documented monogenic inheritance and that the spiny trait was fully or partially dominant. Spininess is influenced by an unknown number of modifier genes, according to Classen (1952). Spiny varieties, in general, have a higher yield potential than *non-spiny* types (Dajue and Mundel 1996).

9.2.6 Nutritional Properties

For its healthy vegetable oil, safflower is an important crop. In its oil composition, the oil is a rich source of the greatest variability of fatty acids (Camas and Esendal 2006). Palmitic acid (6–8%), stearic acid (2–3%), oleic acid (16–20%), and linoleic acid (16–20%) make up the fatty acid content of conventional safflower seed oil (71–75%). Because of this, determining the genetic control of safflower oilseed and its fatty acid composition is crucial in breeding programs aimed at increasing oil yield. Although quantitative inheritance for safflower oil content has been reported, nonadditive gene effects for the genetic regulation of oil content have also been reported (Golkar et al. 2011a). Epistatic effects played a significant role in the genetic regulation of gasoline, according to Yermanos et al. (1967). The different fatty acids and oil content of safflower have been stated to have both broad- and narrow-sense heritabilities (Golkar et al. 2011a). Additive gene effects are thought to play a role in the genetic regulation of linoleic acid (Hamdan et al. 2008), oleic acid (Hamdan et al. 2009b), palmitic acid, and stearic acid (Hamdan et al. 2009a). Golkar et al. (2011a) investigated the maternal effects on safflower linoleic acid and stearic acid content. Fernandez-Martinez et al. (1993) showed that recessive alleles are responsible for the high oleic acid content. Stearic acid inheritance is monogenic, according to Ladd and Knowles (1971).

9.2.7 Yield and Yield Components

Knowledge of agronomic trait inheritance aids in the development of an effective strategy for increasing yield potential. Golkar et al. (2012b) suggested that additive gene effects were important in the genetic control of seed yield, but their findings

contradict with Ragab and Fried (1992), Mandal and Banerjee (1997), and Singh et al. (2008), who found that dominance controlled seed yield. The number of capsules per plant is a significant component of yield that has been linked to genetic control dominance gene effects (Pahlavani et al. 2007). Deshmukh et al. (1991) used a line tester to determine high heterosis for capsules/plant. According to Shahbazi and Saeidi (2007), additive × dominance and dominance × dominance epistasis play important roles in the genetic control of capsules/plants. The importance of the additive-dominance model for genetic control was highlighted by Sahu and Tewari (1993). This means that the selection breeding approach could be used to enhance seed/capsule value. In the genetic regulation of seed weight, additive gene effects have also been discovered to play a significant role (Golkar et al. 2012b). Seed weight has also been found to be affected by the digenic model (additive-dominance) (Shahbazi and Saeidi 2007).

9.2.8 Inheritance to Biotic and Abiotic Stresses

Different pathogens, such as fungi, bacteria, and nematodes, cause different responses in safflower. A review of the literature shows that little is understood about safflower disease resistance. Resistance to *Pythium ultimum* was genetically regulated, according to Ghaderi et al. (2011), with both simple and digenic interaction effects. Identification and resistance screening may provide useful sources as the first step toward future cultivar development. Targeting tolerance to environmental stresses is critical for increasing safflower yield with large adoption in different ecogeographical climates. Since safflower is primarily grown in drought-prone areas, tolerance-related traits like rootlet length and fresh and dry plantlet weight are important, while dominance gene action appears to affect shoot length and leaflet number. Under drought conditions, the additive model [d] was fitted for branches/plant, seeds/capsule, and seed yield/plant and the simple additive-dominance model [d, h] for number of seeds/plant. To suit the model as [d, h, l] for capsule/plant and dry weight/plant, the dominance x dominance epistasis [l] has been applied. Finally, the genetic models [d, h,i] and [d, i] for genetic regulation of plant height and seed weight, respectively, have been fitted. These results can be used to improve safflower salt tolerance genotypes.

9.3 Safflower Improvement: Conventional Breeding

9.3.1 Breeding Methods

The main thrust of safflower research in general and India, in particular, is breeding for high grain and oil yield. As a result, a major emphasis was placed on increasing safflower yield and oil content, which are both negatively correlated. To

demonstrate safflower's genetic improvement, this condition necessitates the dismantling of undesirable associations. While safflower is an often cross-pollinated crop, breeding methods to breed self-pollinated crops have been used to improve it. Details regarding safflower varieties /hybrids developed in India are given in Table 9.1. The methods used to increase the yield and oil content of safflower cultivars are outlined below.

9.3.1.1 Introduction and Pure Line Selection

Introduction is the simplest form of crop improvement, and it has been used widely all over the world. Introduced varieties, in general, need few cycles of adaptation, selection, and evaluation before being formally released for commercial production, since the plants of introduced cultivars respond differently to changes in the environment. As a result, before the population is subjected to selection for identifying promising selections and subsequent evaluation for release as a variety, the introduced cultivars must be acclimatized.

In India, for safflower improvement, selection is the most popular method for the development of cultivar. This is reflected in the number of varieties developed, which account for more than 17 of the 41 varieties of India. A-300, N-630, Nagpur-7, JSF-1, N-62-8, CO-1, Manjira, S-144, K-1, Bhima, Type-65, APRR-3, HUS-305, Sharda, JSI-7, A-2, PBNS-12, and others are among the varieties produced using this process. Pure line selection from local safflower cultivars led to the development of many germplasm lines with several desirable safflower traits. As previously mentioned, safflower has enormous diversity for various economic traits; however, since it is a rainfed crop of minor economic importance, proper utilization of this diversity is inadequate. Because of the abundance of untapped variability for various traits in safflower, many of the cultivars grown in India have been established through pure line selection, which is still considered the most successful method for varietal development in safflower.

9.3.1.2 Hybridization

Hybridization is a technique for combining favorable alleles for multiple traits in a single background and generating genetic variability. Apart from the generation of variability, it has long been the most common method for deciphering the genetic makeup of various traits, which paves the way for the development of the most suitable methodology for crop improvement.

The first step in hybridization is the selection of parents, which decides the success of the crop improvement. The following are important considerations in hybridization: (1) selection of male and female parents based on per se performance, (2) consideration of the parents' genetic diversity to bring desired genes of diverse origins together, (3) consideration of the degree of expression in yield components,

Table 9.1 List of safflower varieties/hybrids in India

Variety	Year of release	Pedigree	Developed by	Salient features	Recommended state/region situations
N-630	1942	Local germplasm	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola		Maharashtra
NAGPUR-7 (N-7)	1953	Local germplasm	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola		Maharashtra
A-300	1957	Selection from local bulk	AICRP (Safflower) Centre, Agricultural Research Station, Annigeri, University of Agricultural Sciences, Dharwad	Resistant to wilt, moderately tolerant to *Alternaria* leaf blight and aphid	Karnataka
N-62-8	1959	Local germplasm	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola		Maharashtra
CGKUSUM-1	1969	Selection from GMU-7368	AICRP (Safflower) Centre, Indira Gandhi Krishi Vishwavidyalaya, Raipur	Moderately tolerant to wilt, aphid	Chhattisgarh plains
ANNIGERI-1 (A-1)	1969	A-482-1 x A-300	AICRP (Safflower) Centre, Agricultural Research Station, Annigeri, University of Agricultural Sciences, Dharwad	Moderately tolerant to wilt, aphid	Karnataka, Bihar, Orissa, Rajasthan
SSF-13-71	1969	Bhima x NARI-44	AICRP (Safflower) Centre, Solapur, Mahatma Phule Krishi Vidyapeeth, Rahuri	Moderately tolerant to aphid and wilt, tolerant to *Alternaria* leaf spot	Zone I (Maharashtra, Andhra Pradesh, Telangana, and Karnataka)
TSF-1	1969	Selection from NASH-92-1	AICRP (Safflower) Centre, Tandur, Professor Jayashankar Telangana, State Agricultural University, Hyderabad	Resistant to *Fusarium* wilt, tolerant to *Alternaria* leaf spot, and moderately tolerant to aphid	Safflower-growing areas of Telangana

(continued)

Table 9.1 (continued)

K1	1969	Pure line selection from American spiny variety	Tamil Nadu Agricultural University, Coimbatore	Oil content 32% spiny florets	Suitable for southern districts of Tamil Nadu
MANJIRA	1976	Direct selection from C-438	AICRP (Safflower) Centre, Tandur, Professor Jayashankar Telangana State Agricultural University, Hyderabad		Andhra Pradesh, Telangana
S-144	1976	Direct selection from GMU 372	Regional Agricultural Research Station, Raichur, University of Agricultural Sciences, Raichur	Tolerant to aphid	Karnataka, Bihar
TARA	1976	N-62-8 × *C. palaestinus*	AICRP (Safflower) Centre, Jalgaon, Mahatma Phule Krishi Vidyapeeth, Rahuri	Erect growth	Western Maharashtra
TYPE-6503	1977	Local selection	Uttar Pradesh	Moderately tolerant to aphid	Uttar Pradesh
CO 1	1979	Pure line selection from CTS 7403 (non-spiny)	Tamil Nadu Agricultural University, Coimbatore	Non-spiny, tolerant to *Alternaria*, moderately resistant to wilt, oil 32.7%.	Tamil Nadu
BHIMA (S-4)	1982	Selection from A-300	Dry Farming Research Station, Solapur, Mahatma Phule Krishi Vidyapeeth, Rahuri		Western Maharashtra
JAWAHAR SAFFLOWER-1 (JSF-1)	1984	Sel. IC 11839	AICRP (Safflower) Centre, Indore, Rajmata Vijayaraje Scindia, Krishi Vishwavidyalaya, Gwalior		Madhya Pradesh and Chhattisgarh

(continued)

Table 9.1 (continued)

Sagarmutyalu (APRR-3)	1985	Direct selection from EC-27250 (SF429)	AICRP (Safflower) Centre, Tandur, Professor Jayashankar Telengana State, Agricultural University, Hyderabad	Resistant to rust	Andhra Pradesh, Telangana
MALVIYA KUSUM	1986	Germplasm (identity number unknown)	AICRP (Safflower) Centre, Banaras Hindu University, Varanasi	High oil type, tolerant to salinity, moderately tolerant to *Alternaria* leaf blight, wilt and root rot	Uttar Pradesh and West Bengal area adoption: Indo-Gangetic plains and salt affected areas especially for Sunderban areas of 24- Paraganas district of West Bengal
NIRA (NRS-209)	1987	NS1572 × EC32012	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan		Irrigated areas of Maharashtra
GIRNA (JLSF-88)	1990	A1 × G1254	AICRP (Safflower) Centre, Oilseeds Research Station, Jalgaon, Mahatma Phule Krishi Vidyapeeth, Rahuri	Moderately resistant to wilt	Khandesh region of Maharashtra
SHARDA (BSF-168-4)	1990	Sel. No. 168	AICRP (Safflower) Centre, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani	Moderately tolerant to aphid and wilt	Marathwada region of Maharashtra
JAWAHAR SPINELESS SAFFLOWER-7 (JSI-7)	1990	Sel. JSF1909	AICRP (Safflower) Centre, Indore, Rajmata Vijayaraje Scindia, Krishi Vishwavidyalaya, Gwalior	Non-spiny	Madhya Pradesh and Chhattisgarh
ANNIGERI-2 (A-2)	1997	(A1 × 166–6) × 328	AICRP (Safflower) Centre, Agricultural Research Station, Annigeri, University of Agricultural Sciences, Dharwad	Reduced hull, tolerant to aphid	Rainfed regions in Karnataka

(continued)

Table 9.1 (continued)

JAWAHAR SPINELESS SAFFLOWER-73 (JSI-73)	1997	JSI-42 × JSI-7	AICRP (Safflower) Centre, Indore, Rajmata Vijayaraje Scindia, Krishi Vishwavidyalaya, Gwalior	Non-spiny, moderately resistant to rust, powdery mildew, wilt, and aphid	Madhya Pradesh and Chhattisgarh
NARI-6	2000	Co-1 × JL6	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan	Non-spiny, moderately tolerant to wilt	All India
PARBHANI KUSUM (PBNS-12)	2001	PBNS-9-97 × PI 248567	AICRP (Safflower) Centre, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani	Moderately tolerant to aphid, *Alternaria* leaf blight	All India
PHULE KUSUMA (JLSF-414)	2003	JLSF-103 × GMU 216 (a)	AICRP (Safflower) Centre, Oilseeds Research Station, Jalgaon, Mahatma Phule Krishi Vidyapeeth, Rahuri		All India
JAWAHAR SAFFLOWER-97 (JSF-97)	2004	NS133-1 × JSI-62	AICRP (Safflower) Centre, Indore, Rajmata Vijayaraje Scindia, Krishi Vishwavidyalaya, Gwalior	Non-spiny	Madhya Pradesh and Chhattisgarh
JAWAHAR SAFFLOWER-99 (JSF-99)	2004	Mexican dwarf × BH-5	AICRP (Safflower) Centre, Indore, Rajmata Vijayaraje Scindia, Krishi Vishwavidyalaya, Gwalior	Extra-early, semi-spiny	Madhya Pradesh and Chhattisgarh
AKS-207	2006	[(Bhima × Tara) × N7] × [(AKS 15 × A1) × AKS 68]	AICRP (Safflower) Centre, Oilseeds Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola		Vidarbha region (Akola, Buldana, Washim, Amaravati) of Maharashtra
PARBHANI KARDI-40 (PBNS-40)	2006	C-40-Pro.3 (mutation breeding)	AICRP (Safflower) Centre, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani	Non-spiny, moderately tolerant to wilt, *Alternaria* leaf blight, and aphid	All India

(continued)

Table 9.1 (continued)

NARI-38	2007		AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan	Spiny, resistant to wilt	All India
SSF-658	2009		Mahatma Phule Krishi Vidyapeeth, Solapur	Tolerant to wilt and aphids	All India
SSF-708	2010	NARI-2 × JSI-99	AICRP (Safflower) Centre, Solapur, Mahatma Phule Krishi Vidyapeeth, Rahuri	Moderately tolerant to aphid	Safflower-growing areas of Maharashtra
PKV- PINK (AKS-311)	2012	NARI 6 × JLSF 344	AICRP (Safflower) Centre, Oilseeds Research UnitDr. Panjabrao Deshmukh Krishi, Vidyapeeth, Akola	Distinct petal color, pale yellow turning to pink after fading, tolerant to wilt	Vidarbha region of Maharashtra
NARI-57	2015	Carmax × C-2829-5-2	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan	Resistant to wilt	Irrigated areas of all safflower-growing states in India (Maharashtra, Karnataka, WestBengal, Rajasthan, Uttar Pradesh, Punjab, Jharkhand)
NARI-96	2016	DMST-10-1-16 × D-151-4-3	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan		Irrigated areas of all safflower-growing states in India (Maharashtra, Telangana, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Rajasthan)

(continued)

Table 9.1 (continued)

ISF-1	2018	(A1x 9-5-7)7-50-5-1	ICAR-Indian Institute of Oilseeds Research Hyderabad	High oleic acid content (76%)	All India (Maharashtra, Karnataka, Telangana, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Bihar, Uttar Pradesh, Rajasthan)
ISF-764	2018	SFS-2042 × EC523360	ICAR-Indian Institute of Oilseeds Research, Hyderabad	High oleic acid content (76%)	All India (Maharashtra, Karnataka, Telangana, Andhra Pradesh, Madhya Pradesh, Chhattisgarh, Bihar, Uttar Pradesh, Rajasthan)
SSF-12-40	2019	Bhima x A1	AICRP (Safflower), Solapur, Mahatma Phule Krishi Vidyapeeth, Rahuri	Moderately tolerant to aphid	Zone-I (Maharashtra, Karnataka, Andhra Pradesh, and Telangana)
RVSAF 14-1	2019	JSI-120 × JSF-1	AICRP (Safflower) Centre, Indore, Rajmata Vijayaraje Scindia, Krishi Vishwavidyalaya, Gwalior		Entire Madhya Pradesh
Annigeri 2020 (A-2020) Figure 9.3	2020	ANN-2-04 * APS-09-8	AICRP (Safflower) Centre, Annigeri, UAS, Dharwad	High yielding, drought tolerant	Karnataka, Maharashtra, Andhra Pradesh, and Telangana

Hybrids					
Hybrids	Year of release	Pedigree	Developed by	Salient features	Recommended state/ region situations
DSH-129	1980	MS 9(O) × A1	AICRP (Sunflower) Centre, University of Agricultural Sciences, Bengaluru	Resistant to wilt, moderately tolerant to *Alternaria* leaf blight and aphid	All India
MKH-11	1997	MS-5008 × NMK-3480	MAHYCO, Jalna	Moderately tolerant to wilt, *Alternaria* leaf blight, and aphid	All India

(continued)

Table 9.1 (continued)

NARI-NH-1 (PH6)	2001	MMS × C2829-5-3a-6	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan	First non-spiny safflower hybrid, moderately tolerant to *Alternaria* leaf blight and aphid	All India
NARI-H-15	2005	MSV-10-1-5 × GMU 2369	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan	Moderately tolerant to aphid	Assured irrigated areas in India
MRSA-521	2006	MS-1308 × MK-1018	MAHYCO, Jalna	CMS-based hybrid, resistant to wilt	All India (area adoption: Maharashtra and Karnataka)
NARI-H-23	2013	TMS-3-1-9-1 × D-152-12	AICRP (Safflower) Centre, Nimbkar Agricultural Research Institute, Phaltan	Tolerant to wilt	Assured irrigated zones of Maharashtra, Karnataka, Madhya Pradesh, Chhattisgarh, Rajasthan, West Bengal
DSH-185	2013	CMS-A-133 × 1705-p22	ICAR-Indian Institute of Oilseeds Research, Hyderabad	Resistant to *Fusarium* wilt	All India (Maharashtra, Karnataka, Andhra Pradesh, Telangana, Madhya Pradesh, Chhattisgarh)

and (4) identification of the best general combiners and cross combinations using the diallel cross approach.

Pedigree

Depending on the trait to be improved, one of the following strategies is used to handle segregating populations in F_2 and subsequent generations. This method is most commonly used to improve important traits like grain yield, oil yield, and other desired traits (Knowles 1969; Ranga Rao et al. 1977). In safflower, the standard pedigree method is used, which is briefly listed below.

Around 5–10% of plants with desired traits are selected in F_2 populations, harvested, and threshed separately to increase plant-to-progeny rows in the F_3 generation using this method. In a replicated trial, F_3 progenies can be evaluated along with standard checks for various abiotic and biotic stresses for seed yield and desired traits. In subsequent years, selected progenies are advanced to the F_4, F_5, and F_6 generations. Inter- and intraprogeny selection of promising types occurs in each

Fig. 9.3 A high-yielding and drought-tolerant safflower variety, Annigeri 2020 (A-2020) developed by AICRP (Safflower) Centre, Annigeri, UAS, Dharwad, India

generation. At every stage of the selection process, the selected plants must be selfed, as this ensures homozygous progenies by the time they reach the F_6 generation. At this stage, uniform and homozygous progenies may be considered for yield trials, with the most promising ones subjected to individual plant selections. Individual plant progenies are then evaluated in replicated trials to determine which lines are the most promising for multilocation testing. Until releasing the most adaptable line, a multilocation evaluation is needed to determine their adaptability to various agroclimatic conditions. The following are safflower cultivars grown in India and other countries using the pedigree process, as well as the years they were released for commercial production: A-1, Tara, Nira, Girna, JSI-73, NARI-6, Phule Kusuma, Leed, Sidwill, Hartman, Rehbein, Oker, Girard, Finch Sahuaripa 88, Ouiriego 88, San Jose 89, AC Stirling, and AC Sunset (Hegde et al. 2002).

Bulk Population Method

Individual plants with desired traits will be bulked and advanced to the next generation using this method. The main goal was to allow for natural selection pressure and not to test individual progeny. As a result, natural selection plays an important role in the development of variety, and another advantage is that breeders can handle

multiple populations at the same time due to the simplicity of the method. During the F_7 and F_8 generations, individual plants will be selected, and their progenies will be evaluated in a replicated trial with checks. Multilocation testing will be conducted on the most promising ones. The important point to remember here is that individual population bulk should be selfed; otherwise, there would be a large number of heterozygous plants in the F_7 generation.

Single-Seed Descent Method

In this process, randomly selected F_2 plants will be advanced to the next generation until they reach F_5 or F_6. From F_7 onward, a large number of plants are chosen, and the progenies are evaluated in replication for abiotic and biotic stresses, as well as end-use quality resting. This approach helps the breeder to use his expertise in plant selection.

Recurrent Selection (Backcrossing)

Backcrossing is a recurrent selection method that has been used across the globe to incorporate a trait of interest into a widely adopted genetic background (referred to as the recurrent parent). In general, this method works well for traits that are influenced by oligogenes. Numerous backcrosses will be attempted until the target trait is genetically improved, after which they will be selfed to grow homozygous plants for the trait of interest. This approach is used in safflower breeding to incorporate dominant genes to control diseases like *Phytophthora drechsleri* root rot (Thomas et al. 1960; Rubis 2001) and the development of high oleic acid safflower (Knowles 1968 and Hamdan et al. 2009a, b).

9.3.2 Hybrid Breeding

Safflower is a strong choice for the exploitation of hybrid vigor in the crop because of its often cross-pollinated nature, high heterosis for seed and flower yield, presence of several commercially important traits, and presence of genetic male sterility (GMS) and cytoplasmic male sterility (CMS) systems. Since the 1970s, reports of high heterosis for seed yield and other desirable traits in safflower have encouraged many researchers to look for simple and easy-to-use commercial-scale hybrid seed production methods (Urie and Zimmer 1970a and Karve et al. 1979). In India, the identification of genetic male sterility sources in safflower (Heaton and Knowles 1980; Joshi et al. 1983; Ramachandram and Sujatha 1991; Singh 1996, 1997) and the development of agronomically superior genetic male-sterile lines resulted in the development and release of spiny safflower hybrids DSH-129 and MKH-11 in 1997, the first non-spiny hybrid safflower NARI-NH-1 IN 2001(Singh et al. 2003a), and

the spiny hybrid NARI-H-15 in 2005. In general, these hybrids have a 20–25% higher seed and oil yield than the national check A-1. India is the only country in the world that grows hybrid safflower.

In the development of hybrid cultivars in safflower, both genetic and cytoplasmic male sterility systems are used. The GMS system, on the other hand, is the male sterility system used in India for the production of safflower hybrids. The GMS systems found in safflower are monogenic recessive as well as dominant.

9.3.2.1 Single Recessive Genetic Male Sterility

In safflower, the GMS sources controlled by single recessive genes are as follows:

1. UC-148 and UC-149 GMS lines developed by Heaton and Knowles (1980)
2. GMS lines developed by Ramachandram and Sujatha (1991)
3. MSN and MSV male-sterile lines developed by Singh (1996)
4. DMS male-sterile lines associated with dwarfness developed by Singh (1997)

Male sterility sources are segregated into 1 male-sterile to 1 male-fertile plant ratio. The presence of a pinched capitulum opening in male-sterile plants and a normal opening in male-fertile plants distinguishes male-sterile and male-fertile plants at flowering.

9.3.2.2 Dominant Genetic Male Sterility

Joshi et al. (1983) found that male sterility in safflower is regulated by a dominant gene. At the flowering stage of the crop, as in single recessive genetic male sterility, identification of sterile and fertile plants is possible. The hybrids and male-sterile lines in this system segregate in a ratio of 1 male-sterile to 1 male-fertile plant due to the dominant nature of the gene conferring male sterility. The presence of 50% MS plants in the hybrid population hampered the success of hybrids based on this source, which hindered the hybrid's yielding ability if honeybee activity is insufficient to give 100% seed setting in the male-sterile plants.

9.3.2.3 Cytoplasmic-Genetic Male Sterility

In safflower, cytoplasmic-genetic male sterility (CGMS) has been noted to be used for hybrid development (Hill 1989). CMS hybrids were compared to GMS-based hybrids in India, and the seed yield of CMS hybrids was only half that of the corresponding GMS hybrids. Furthermore, all CMS-based hybrids separated into sterile and fertile plants, implying that the sterile cytoplasm was not restored to fertility (Singh et al. 2000). The commercialization of CMS-based hybrids is still awaited.

At the Nimbkar Agricultural Research Institute (NARI), Phaltan (Singh et al. 2001a), and the Directorate of Oilseeds Research, Hyderabad, efforts are also

underway to establish a CGMS system for safflower. Interspecific crossing and streptomycin mutagenesis are used to develop the CGMS systems at NARI. Cytoplasmic male sterility (CMS) has been established in safflower as a result of both programs. In both cases, genotypes causing a complete restoration of fertility to the sterile cytoplasm have been identified (Singh 2005). In the sterile cytoplasm, efforts are being made to develop appropriate maintainer genotypes that can maintain 100% male sterility.

9.4 Safflower Improvement: Biotechnology

9.4.1 Molecular Markers

9.4.1.1 Genetic Diversity

Safflower genomic research has progressed a lot more than other related fields including transcriptomics and proteomics. Molecular markers have mainly been used to assess local cultivars, landraces, and germplasm accessions, as well as to partition genetic variation geographically (Khan et al. 2008). For assessing germplasm diversity, RAPD (Sehgal and Raina 2005; Mahasi et al. 2009), AFLP (Zhang et al. 2006; Johnson et al. 2007), ISSR (Yang et al. 2007; Golkar et al. 2011b), and EST-SSR (Barati and Arzani 2012) have been used. These are the preferred markers for crops with limited genomic resources because they do not need prior sequence knowledge and scan the entire genome, including repeated sequences. To estimate genetic variation in safflower, researchers used a combination of molecular polymorphism and phenotypic variation (Johnson et al. 2007; Yang et al. 2007; Amini et al. 2008; Khan et al. 2008). In safflower, Chapman et al. (2009) developed a collection of polymorphic EST-SSR markers as a useful resource for comparative map-based analysis. EST-SSR markers were found to be useful in determining the genetic purity and heterozygosity of safflower hybrids by Naresh et al. (2009). Safflower, on the other hand, has a lot of variability and many characteristics that can be genotyped using available molecular marker systems (Sujatha 2008). According to Zhang and Johnson's IPGRI germplasm directory, a total of 25,179 safflower accessions, including wild species, are stored in 22 gene banks across 15 countries (safflower.wsu.edu/saff-dir.pdf).

9.4.1.2 Phylogenetic Analysis

Dysploidy is present in safflower (x = 12, 11, and 10) and dysploid evolution may be descending from x = 12, ascending from x = 10, or both ascending and descending from x = 11. The primary determinant of karyological evolution in the genus *Carthamus* is descending dysploidy, according to an analysis focused on ITS sequences and karyology (Vilatersana et al. 2000). *Carthamus* sectional, species,

and subspecies classification have all derived from the use of RAPD markers. Although the genus *Carthamus* was originally divided into five sections based on chromosome numbers, correlation of molecular analysis data with morphological and karyological characters resulted in the number of sections being reduced from five to two, namely, *Carthamus* and *Atractylis*. Previously, species with 2n = 20 were divided into two sections – Odontagnathius (*C. dentatus* spp. *dentatus*) and Lepidopappus (*C. glaucus*, *C. boissieri*, *C. tenuis*, and *C. leucocaulos*) – but molecular analysis supported grouping all species into one section (Vilatersana et al. 2005). Chapman et al. (2007) used universal markers unique to the Asteraceae to characterize *Carthamus* species with 2n = 24 and found *C. palaestinus* to be the progenitor species of cultivated safflower. Cultivated safflower proved to be distantly related to *C. oxyacanthus* and *C. persicus*. Among the *Carthamus* types, cultivated safflower had the least nucleotide diversity, *C. oxyacanthus* had the most, and *C. palaestinus* was in the center. Per 95 bp of sequence, one single nucleotide polymorphism (SNP) was found on average.

9.4.1.3 Genomics and Marker-Assisted Selection

Ravikumar et al. (2008) used RAPD primers to develop the first linkage map of safflower with three linkage groups (LG), and Mayerhofer et al. (2009) used a set of SSR and RFLP markers to produce the complete linkage map with 12 LG groups in safflower Each linkage group (LG) included 6 to 40 markers and ranged in size from 30.7 to 105.3 (cM). In safflower, more molecular markers will provide a foundation for fine map growth. A physical map of the safflower chloroplast genome has been developed (Ma and Smith 1985). According to Lulin et al. (2012), *Carthamus tinctorius* L. has 567 nucleotide sequences, 41,588 expressed sequence tags (ESTs), 162 proteins, and 0 genes which have been deposited until October 2011 in the NCBI's gene bank database. Thippeswamy et al. (2013) found 146 distinct and novel ESTs in safflower that were linked to drought-responsive genes. For MAS in safflower, SCAR and RAPD molecular markers linked to Li (the regulating gene for very high linoleic acid) and Ms (nuclear male sterility) (Hamdan et al. 2008) as well as Tph2 (high gamma-tocopherol) genes (Garcia-Moreno et al. 2011) were found. The ol (high oleic acid content) gene was linked to the SSR marker ct365, which was mapped to the T3 linkage group in another study conducted by Hamdan et al. 2012). Kammili (2013) explored a correlation between male sterility and a non-spiny marker that could be used to produce pure F_1 hybrid seeds. Pearl et al. (2014) identified 61 QTL (quantitative trait loci) at different safflower linkage groups that were linked to several traits including the number of heads, flower color, and fatty acid content.

9.4.1.4 Transcriptomics and Proteomics

Safflower transcriptomics seems to have received less attention than that of other oilseeds. Li et al. (2011) discovered that safflower contains at least 236 recognized microRNAs (miRNA). By sequencing and assembling the safflower flower transcriptome, Lulin et al. (2012) reported four genes and new pathways that could regulate flavonoid and secondary metabolite synthesis in safflower and indicated that these genes encoded other anthocyanidin-related products that have not yet been detected in the flower. Knutzon et al. (1992) described and partially sequenced two protein species with molecular masses of 34 and 40 KD correlated with thioesterase activity. From high oleat genotypes of safflower, Mizukami et al. (2000) isolated a cDNA clone (CTOS1) that probably encoded a novel protein. The sequencing of the functional and vital proteins in the safflower genome appears to require the completion of cDNA libraries.

9.4.2 Tissue Culture

Sujatha (2007) reviewed very well regarding seedling tissues, including roots and mature embryo explants, which are used in tissue culture protocols for both American and Indian safflower cultivars. Organogenic and embryogenic pathways, as well as direct and callus-mediated approaches, may be used to regenerate safflower (Sujatha 2007). The past studies were aimed to improve plant regeneration protocols to achieve a high frequency of shoot regeneration. Vijaya Kumar et al. (2008) have recently expanded the tissue culture technique for the development of *Alternaria carthami*-resistant plants. Embryogenic and organogenic calli were selected for shoots on medium supplemented with 40% *A. carthami* fungal culture filtrate (5 × 105 conidia/ml) in this protocol. In the R0, R1, and R2 generations, resistance was increased to 100, 97.6, and 84%, respectively, over the power.

9.4.3 Genetic Engineering

Sujatha (2008) presented keynote on biotechnological interventions for genetic improvement of safflower during the 7th International Safflower Conference held in Australia from November 3 to 9, 2008, and explained beautifully about the genetic engineering work done at DOR, Hyderabad. Both Indian and American cultivars of safflower have been transformed using *Agrobacterium*-mediated transformation protocols (Orlikowska et al. 1995; Rohini and Rao 2000). Tissue culture regeneration with cotyledons and primary leaves was used in the genetic transformation of American cultivars. In in planta transformation with embryo, explants have been developed for Indian cultivars. Only constructs with a widely used reporter (uidA) and selectable marker genes were used in the transformation studies (nptII).

Cocultivation conditions and the *Agrobacterium tumefaciens* strain used were found to have an impact on the studies. The confirmation of transgenes was accomplished using transient assays based on GUS expression and molecular study of primary transformants using PCR and Southern hybridization assays. Despite a high frequency of shoot regeneration from transformed tissues (15–34.3%), rooting of transformed shoots has proven difficult. The protocols have not been used to create transgenics with agronomically desirable characters (Sujatha 2008).

9.5 Breeding for End Use

Oil and bird feed are the most popular end uses of safflower. It is grown in rainfed environments all over the world. As a result, the severity of disease and pest infestation is stated to be low. However, under favorable conditions, they can cause significant crop damage, as what happened in India in 1997–1998, when an outbreak of *Alternaria* wiped out the entire safflower crop in the major safflower-growing states of Maharashtra and Karnataka (Anonymous 1997–1998). In light of the foregoing, the primary focus of safflower improvement has been on seed yield; however, to meet the needs of local agroclimatic conditions, cropping patterns, and market demands, safflower improvement has also focused on developing disease- and pest-resistant cultivars, as well as improved oil content and quality.

9.5.1 Disease Resistance

Many diseases affect safflower, including those caused by fungi, bacteria, viruses, or physiological disorders caused by abiotic stresses. According to Patil et al. (1993), safflower is infested by 57 pathogens worldwide, including 40 fungi, 2 bacteria, 14 viruses, and 1 mycoplasma. *Alternaria* leaf stain, caused by *Alternaria carthami*, and *Fusarium oxysporum* wilt, caused by *Fusarium oxysporum*, are the most damaging of these, causing 13–49% losses and wiping out the entire crop in the area under favorable conditions, as in India.

Breeding safflower for disease resistance is the most cost-effective and practical way to combat major safflower diseases. Mundel and Huang (2003) detailed how to control major safflower diseases through breeding and cultural practices. For most diseases, the genetics and mode of inheritance of disease resistance and tolerance in safflower have not been studied (Li and Mundel 1996). Though several germplasm lines or cultivars have been identified as having partial or complete resistance to some of the major diseases, the genetics of only a few have been defined. Resistance to *Alternaria carthami* Chowdhari, *Cercospora carthami* Sund and Ramak, *Ramularia carthami* Zaprom, *Fusarium oxysporum* Sehl. ex. Fries, *Rhizoctonia bataticola* Bult, and *Rhizoctonia Solani* Kuhn is conferred by single dominant genes, as per Karve et al. (1981). The regulation of inhibitory gene action in the

expression of wilt resistance in safflower was found in a study of wilt (*Fusarium oxysporum*) resistance in safflower (Singh et al. 2001b). The source of wilt resistance has been detected in local germplasm lines (Sastry and Ramachandram 1992). The development of wilt-resistant genotypes in safflower following backcrossing resulted in a 31% increase in seed yield over the national check A-1 (Singh et al. 2003b). The germplasm line VFR-1 was developed through the breeding of safflower varieties for resistance to multiple diseases. This line was developed from the Nebraska 4051 breeding line and showed resistance to *Verticillium* wilt, *Fusarium* wilt and root rot, and *Rhizoctonia* root rot (Thomas 1971). Backcrosses have resulted in the development of the Australian safflower cultivar Sironaria, which is resistant to *Alternaria* blight and moderately resistant to *Phytophthora* root rot (Harrigan 1987, 1989). In the USA, safflower cultivars resistant to *Alternaria* blight have been produced, including Sidwill, Hartman, Oker, Girard, and Finch (Bergman and Riveland 1983; Bergman et al. 1985, 1987, 1989a, b). In a disease nursery that began in the early 1960s, these cultivars were developed by crossing existing cultivar AC-1 with mass-selected *Alternaria*-resistant line 87-42-3. Dart cultivar has *Phytophthora drechsleri* resistance to all of the most common races of root rot (Abel and Lorance 1975). *Sclerotinia* head rot (caused by *Sclerotinia sclerotiorum* (Lib.) de Bary) resistance was incorporated into the first Canadian safflower cultivar Saffire by mass selection (Mundel et al. 1985).

9.5.2 Oil Content and Quality

Except for HUS-305, NARI-6, and non-spiny hybrid NARI-NH-1, which each contains 35% oil, safflower varieties released for commercial production in India have low oil content of 28–32%. The development of high-oil-content varieties and hybrids with disease and pest resistance has recently been emphasized in India's national safflower improvement program. Many studies have found a negative correlation between safflower hull content and oil content (Ranga Rao et al. 1977; Sangale et al. 1982; Mandal 1990). As a result, lowering the hull content directly raises the oil content. In safflower, several genes for different hull types have been identified, including partial hull (par par), which is recessive to the normal hull and is inherited independently of the thin hull (th th) and striped hull (stp stp) (Urie 1981); gray-striped hull (stp2) (Abel and Lorance 1975); and reduced hull (rh rh), which has small dark blotches on the seed. Reduced hull is recessive to partial hull (Urie 1986). However, depending on the normal hull genotype used in the crossing program, normal hull is dominant or partially dominant over reduced hull (Urie and Zimmer 1970). Safflower seed cultivars grown in the USA have significantly increased the oil content of the seed (Bergman et al. 1985; Rubis 2001). Oker is a safflower cultivar with 45% oil content (Bergman et al. 1985). Rubis (2001) has registered a safflower line with up to 55% oil content.

The fatty acid composition of any oil determines its consistency, and oils rich in poly- or monounsaturated fatty acids are considered good because they help lower

blood cholesterol levels. Given the above, safflower oil is the best, since it contains high levels of polyunsaturated (linoleic acid, 70–75%) and monounsaturated (oleic acid, 70–75%) fatty acids. The best example of a crop with variable fatty acid composition in seed oil is said to be safflower (Knowles 1989). Around 6–8% palmitic acid, 2–3% stearic acid, 16–20% oleic acid, and 71–75% linoleic acid make up standard safflower oil (Velasco and Fernandez-Martinez 2001). In the released materials, variants with higher stearic acid content (4–11% of total fatty acids), intermediate oleic acid content (41–53%), high oleic acid content (75–80%), and very high linoleic acid content (87–89%) have been found (Fernandez-Martinez et al. 1993; Johnson et al. 1999). Velasco and Fernandez-Martinez (2001) described the development of lines with a modified fatty acid composition that included high palmitic acid content (10.3% of total fatty acids), medium or high stearic acid content (3.9 and 6.2%), high or very high oleic acid content (>78 and 86%), and low levels of the saturated fatty acids palmitic and stearic acid (5%). They also found sources of high total tocopherol content (up to 400 mg kg^{-1} seed) and increased gamma-tocopherol content (up to 9.9% of total tocopherols). Futehally investigated the genetics of oleic, linoleic, stearic, and palmitic acids in seed (reported by Knowles 1989). The genetics of fatty acids in safflower showed that three independent recessive genes, ol ol, li li, and st st, regulate the development of oleic, linoleic, and stearic acids, respectively. Knowles and his colleagues released the first high oleic (oleic acid = 78.3%) safflower variety, “UC-1,” in 1966 in the USA, followed by the release of “Oleic leed” in 1976 (Urie et al. 1979). Other high oleic acid-containing cultivars released for commercial production include “Alameda” and “Rinconada,” produced by Fernandez-Martinez and Dominguez in Spain in 1986, and “Montola 2000” and “Montola 2001,” developed by Bergman in the USA, all with >80% oleic acid (Li and Mundel 1996). All other safflower varieties released for commercial production in various countries are of the high linoleic form (linoleic acid = 70–75%). The fatty acid profile, genetic variability for fatty acids, and genetic control of fatty acids all indicate that the fatty acid composition in safflower can be changed as needed.

9.5.3 Insect Resistance

The most common pest of safflower is the aphid, which can cause up to 50% damage. In safflower, germplasm lines with a stable tolerance to aphids have been reported. Two wild species, *C. flavescens* and *C. lanatus*, have been found to bear safflower fly resistance genes (Kumar 1993). The genetics of aphid resistance in safflower has been stated to be additive as well as nonadditive. However, nonadditive gene action was found to be the most important factor (Singh and Nimbkar 1993).

9.5.4 *Spineless Safflower*

Safflower is a spiny crop in general. In China, however, spineless cultivars account for the majority of safflower production. Except for China, all safflower production is done with spiny cultivars. Because of its spiny nature, safflower production has been severely hampered, especially in nontraditional areas and in areas where mechanized cultivation has yet to be implemented. Spiny cultivars also dominate safflower production in India. While spineless cultivars CO-1 and JSI-7 were available, they were unable to command a large safflower area due to their poor yielding capacity when compared to spiny cultivars. In 2001 and 2002, the non-spiny variety NARI-6 and the non-spiny hybrid NARI-NH-1 (Singh et al. 2003a) were released for all-India production. The two cultivars produce comparable yields to their spiny counterparts, and they are said to be more resistant to foliar and wilt diseases than spiny cultivars. As a result, these cultivars are gaining popularity among farmers in India's safflower-producing states.

9.6 Future Direction

Safflower crop is the most neglected oilseed crop. Hence, the scientific literature available and information on genetic and linkage maps are meager. To evolve new varieties with outstanding yield along with other improved economic traits, this information is very much essential and it needs immediate attention. In this crop, introgression with wild species is not favorable. To overcome such barriers, modern techniques like embryo rescue and other biotechnological tools will come for rescue. Heterosis breeding through hybrid development should be explored to break yield barrier. Flower yield and pigment content of the flowers are the other traits that have gained economic importance recently, due to an increasing demand for safflower flowers as a source of natural food color in European and other western countries and their use in medicines for curing several chronic diseases. The improvement in yield of flowers and pigments in flowers would certainly help in increasing total remuneration from the crop to the farmer. Genetic transformation of safflower to impart resistance to biotic and abiotic factors, in addition to development of seeds with altered fatty acid and protein profiles, is another area that has received very little attention. Conventional breeding techniques, though used for these purposes, have not been very successful. Therefore, genetic modification of safflower would be of enormous importance in improving productivity, production, and remuneration per unit area from the crop, which in turn would certainly help in increasing safflower area in the world.

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Chapter 10
Enhancing Genetic Gain in Coconut: Conventional, Molecular, and Genomics-Based Breeding Approaches

S. V. Ramesh, R. Sudha, V. Niral, and M. K. Rajesh

Abstract Coconut (*Cocos nucifera* L.) is a monotypic genus and an important plantation crop with an enormous application value as a food, fuel, and fiber. The crop has also been recently attaining great importance in the field of nutraceuticals worldwide. Development of high yielding cultivars, genotypes having high copra content, genetic sources with characteristic features such as disease resistance [root (wilt) disease and lethal yellowing] and pest resistance (rhinoceros beetle, red palm weevil, mites, and rugose spiraling whitefly), and speciality cultivars (such as makupuno, sweet kernel, and aromatic nut water) are the main objectives of coconut breeders. Various breeding approaches such as conventional breeding techniques of selection and hybridization, molecular breeding approaches namely marker-assisted selection, QTL identification, marker-trait linkage analysis, instances of association analysis, etc. have been resorted to achieve the above-cited breeding objectives. Advances in high-throughput techniques such as genomics, proteomics, transcriptomics, and metabolomics have resulted in the generation of voluminous data and buildup of genomic resources that have a greater role to play in the future molecular and omics-based breeding approaches in coconut. However, it is pertinent to recognize that applying high-throughput techniques in coconut is largely hampered due to its perennial nature, long juvenile phase, outcrossing behavior, and consequently high heterozygous nature. This chapter comprehensively summarizes the advancements made in the field of coconut breeding, including the varietal development programs in India and elsewhere in the world, followed by advancements in molecular breeding techniques. It also provides glimpses of achievements in multi-omics approaches in coconut and discusses the prospects and applications of various high-throughput techniques in the improvement of coconut.

Keywords Coconut varieties · QTL mapping · Molecular breeding in coconut · Palms · Omics

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10.1 Introduction

Coconut (*Cocos nucifera* L.), a member of the palm family Arecaceae in the subfamily Cocoideae, is an economically important, multipurpose palm widely grown in the humid tropics. It is the only species under the genus *Cocos*, and it is a diploid with 32 chromosomes (2n = 2x = 32). Coconut offers nutritious food, reviving drink, oil for edible and non-edible purposes, fiber of commercial value, shells for industrial and fuel uses, timber, and a range of miscellaneous products for both household and industrial uses. In the past few years, coconut is increasingly being considered as a health food, with tender coconut water, virgin coconut oil, and coconut inflorescence sap being encouraged for consumption. The palm is grown in more than 90 countries, comprising primarily of coastal areas and island ecosystems. Currently, the coconut production in the world is estimated at 68833 million nuts from 12.08 million ha and productivity of 5777 nuts ha^{-1} (ICC 2019). Mainly the world production is concentrated in tropical Asia, with Indonesia, the Philippines, and India jointly accounting for more than 70% of the total area and production.

Worldwide, coconut populations have been classified into two main groups: the Pacific group with five subgroups (Southeast Asia, Micronesia, Polynesia, Melanesia, and the Pacific coastline of South and Central America) and the Indo-Atlantic group (Perera et al. 2009). The genetic resources in coconut, a crucial component of coconut breeding programs, have been widely exploited through breeding methods such as selection and hybridization for several desirable traits, which have resulted in the development of numerous coconut varieties. Breeding approaches are mostly confined to conventional breeding methods such as mass selection and hybridization, besides using individual palm selection for novel traits. The perennial nature of the palm, heterozygosity, extended juvenile phase, and lack of mass propagation technologies for palms having desirable traits are the major challenges in coconut breeding efforts.

The advent of DNA-based molecular markers has offered novel opportunities such as marker-assisted selection (MAS) to identify target traits of economic and agronomic importance irrespective of the crop's phenological stage and prevailing environmental conditions. These advancements have greatly aided the plant breeders to achieve the crop improvement goals in a relatively easy and effective manner. The accelerated developments and improvements in the field of next-generation sequencing (NGS) techniques have generated voluminous data at the DNA, RNA, protein, and metabolite levels leading to an era of "big data"-enabled molecular breeding. Nevertheless, consolidation of large-scale information and integrating it with particular plant phenotype would not only help in comprehending the molecular and genetic basis of a trait expression but also would aid in their introgression into a desired genotype by adopting multi-omics-based crop breeding. This integration of multi-omics approaches in molecular breeding has greatly assisted the improvement of many crop plants which could not be emulated in coconut. The main reasons for lag in application of multi-omics technologies in coconut are its inherent heterozygosity, stemming from its breeding behavior and lack of genomic resources. This chapter discusses the

achievements made in the conventional breeding of coconut leading to robust varietal development programs, followed by the use of molecular markers for genetic diversity assessment and mapping of quantitative trait loci (QTLs). Further, the glimpses of the recent advancements in the field of genomics, proteomics, transcriptomics, and metabolomics in coconut are enumerated discussing the future prospects to enhance the breeding efforts in this economically important palm crop.

10.2 Coconut Genetic Resources

The varietal development programs and the current status and future strategies for coconut breeding worldwide are discussed herein. Despite being a monotypic genus, *Cocos nucifera* L. has substantial genetic diversity in its populations (Arunachalam and Rajesh 2017; Niral and Jerard 2018). The varieties of coconut could be distinguished based on their qualitative traits such as size, shape, nut color, and pest/disease resistance. In contrast, quantitative traits such as precocity of flowering, bunch, nut numbers, and inflorescence/fruit characteristic features are also used to investigate the diversity. Nevertheless, the genetic basis of these phenotypic variations is poorly understood. A global network of coconut growing countries called the International Coconut Genetic Resources Network (COGENT) was set up by the International Plant Genetic Resources Institute (IPGRI) in 1992 (Batugal et al. 2005). The COGENT coordinates the collection of important coconut varieties and their conservation at respective national gene banks and appropriate duplicates at multisite International Coconut Genebank (ICG). The regional-level ICG gene banks are hosted at Indonesia for Southeast and East Asia, India for South Asia and the Middle East, Côte d'Ivoire for Africa and the Indian Ocean, Papua New Guinea for the South Pacific, and Brazil for Latin America and the Caribbean (Ramanatha Rao and Batugal 1998). Though COGENT's International Coconut Genetic Resources Database (CGRD) reveals that over 1416 coconut accessions are being conserved, national breeding programs utilize less than 5% of that germplasm (Batugal 2004, 2005a). However, development of catalogs of conserved germplasm (Ratnambal et al. 1995; Ratnambal et al. 2000; Bourdeix and Batugal 2005; Bourdeix et al. 2010), compiling of descriptors of salient traits of coconut accessions via CGRD (Hamelin et al. 2005), and the improved accessibility of coconut germplasm in ICG and national genebanks have greatly ensured more accessions are being integrated into the crop improvement programs worldwide.

10.3 Coconut Breeding: Current Status

In coconut, inadequate adaptability to wide environmental conditions, lack of high and stable yielding genotypes, and consequent low farm-level productivity are the major limitations (Batugal 1999). Presently, 1837 accessions are conserved in the

24 gene banks and are potential sources for development of high yielding varieties taking into consideration the national requirements and the needs of the local coconut communities (Nampoothiri and Parthasarathy 2018). Hence, the development of coconut varieties possessing disease resistance is of utmost importance in South American and African countries because of the prevalence of the lethal yellowing disease in these regions. In contrast, Vanuatu focuses on varieties that are resistant to coconut foliar decay. Since coconut is largely grown in marginal, rainfed areas in Sri Lanka, India, and Tanzania, the main breeding objective is to develop drought-tolerant genotypes in these countries. While China is involved in developing cold-tolerant lines, the Pacific and Caribbean countries are breeding for cyclone-tolerant varieties.

10.4 Breeding Programs

The first International Coconut Breeders Meet was held in Côte d'Ivoire to standardize the research on coconut breeding techniques during 1996 (Batugal and Ramanatha Rao 1998). Various national breeding programs, in general, aim to evaluate local cultivars against the introduced varieties obtained from the more advanced breeding centers. A follow-up survey conducted by COGENT during 2001–2003 (Batugal 2004) indicated that locally produced hybrids were predominant in national varietal performance trials. Along with the progeny test, most of the coconut growing countries have conducted phenotypic and genotypic characterization of coconut genotypes, evaluation of collected coconut genotypes for general and specific combining abilities, mother palm selection, pollination, and hybrid seed nut production.

10.4.1 Coconut Breeding Program in India

10.4.1.1 Selection

India has been one of the earliest countries to initiate work on coconut breeding, with focus on collection and conservation of germplasm, following the establishment of the Central Coconut Research Station (presently, ICAR-Central Plantation Crops Research Institute) at Kasaragod in the year 1916. The indigenous germplasm collection was strengthened with the introduction of coconut cultivars from the major coconut growing countries, viz., the Philippines, Malaysia, Indonesia, Sri Lanka, Vietnam, and Fiji, way back in 1924. ICAR-CPCRI hosts the National Active Germplasm Site (NAGS) for coconut and maintains the largest collection of 455 accessions, representing coconut germplasm from around 28 countries, encompassing South and Southeast Asia, Caribbean Islands, Indian Ocean Islands, Pacific Ocean Islands, and African countries. In addition, India also hosts the International Coconut Genebank for South Asia (now referred to as International Coconut

Genebank for South Asia and Middle East) with 91 accessions, comprising designated Indian germplasm, germplasm from regional member countries, viz., Sri Lanka and Bangladesh, and accessions collected through prospection from the Indian Ocean Islands of Madagascar, Mauritius, Seychelles, Maldives, Comoros, and Reunion (Niral et al. 2019). India was also the first country to develop a catalog of coconut germplasm, following standardized coconut descriptors (IPGRI 1995), describing 48 conserved germplasm of diverse origin with photographs of the different plant parts and textual information, for the benefit of coconut researchers (Ratnambal et al. 1995). Subsequently, a second volume of the descriptors, describing another 26 accessions, was compiled and distributed in CD-ROM (Ratnambal et al. 2000). Comprehensive characterization of genetic resources has resulted in identification of trait-specific germplasm and registration of seven trait-specific germplasm with the ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR). In India, evaluation and selection of promising coconut accessions maintained at the ICAR-CPCRI (Indian Council of Agricultural Research-Central Plantation Crops Research Institute, Kasaragod, Kerala) as well as the several coordinating research centers under the All India Coordinated Research Project on Palms (AICRP on Palms) and State Agricultural Universities (SAUs) have led to the development and release of 30 improved coconut varieties. In the initial years, the focus was on evaluation for higher nut/copra/oil yield (Niral et al. 2009). Subsequently development of varieties for tender nut purpose and inflorescence (neera) sap production was also given greater emphasis (Samsudeen et al. 2013; Niral et al. 2014; Sudha et al. 2019). In tune with changing user needs, Chowghat Orange Dwarf (COD) was the first to be released as an exclusive tender nut variety (Fig. 10.1), and more recently, Kalpa Ratna was released as a multipurpose variety suitable for copra, tender nut, and inflorescence sap production (Fig. 10.2). Screening

Fig. 10.1 COD, a popular dwarf tender coconut variety

Fig. 10.2 Kalpa Ratna, a high yielding multipurpose coconut variety

of germplasm for biotic/abiotic tolerance, with special emphasis on root (wilt) disease tolerance, drought tolerance, and high/low temperature tolerance/climate resilience, is also in progress, and stress-tolerant accessions have been identified (Rajagopal et al. 1990; Nair et al. 2004; Kasturi Bai et al. 2006; Hebbar et al. 2013; Hebbar et al. 2018).

The varieties released so far for cultivation in the country are highlighted in Table 10.1.

10.4.1.2 Exploitation of Hybrid Vigor

The first hybridization attempt in India was made in 1930, involving tall and dwarf types [West Coast Tall (WCT) × Chowghat Green Dwarf (COD)] (Patel 1937). Since then, many hybrids have been tested involving many tall × dwarf (T × D), dwarf × tall (D × T), and tall × tall (T × T) crosses. Also, the advantages of hybrids compared to the local tall cultivars were well recognized. Even though hybrids from both the cross combinations are high yielding, hybrids derived from D × T cross have a discrete advantage over hybrids from T × D cross as large-scale production of these hybrids is possible by emasculating dwarf mother palms and allowing natural pollination with the pollen of tall palms in the vicinity. Hence, in the recent past, the production of D × T hybrids has been promoted rather because of their relative ease in production (Nair et al. 2016). Also, hybrid coconuts are characterized with early bearing and high yielding traits (nut, oil, and copra yield palm^{-1}).

Further, offsprings of the crossing of tall and dwarf coconut accessions were evaluated at ICAR-CPCRI and other AICRP on palm (AICRPP) centers resulting in development of many hybrid varieties. Consequently, the crossing of unrelated

Table 10.1 Improved coconut varieties developed for cultivation in India through selection

Variety	Salient features	Institution responsible for the release
Chandra Kalpa	Drought-tolerant, high oil (72%)	ICAR-CPCRI
Kera Chandra	High yield, dual purpose for tender nut and copra	ICAR-CPCRI
Kalpa Pratibha	High yield, dual purpose for tender nut and copra	ICAR-CPCRI
Kalpa Mitra	High nut and oil yield, drought-tolerant	ICAR-CPCRI
Kalpa Dhenu	High nut and oil yield, drought-tolerant	ICAR-CPCRI
Kalpatharu	Drought-tolerant, high yield, ball copra	AICRP on Palms (AICRPP), ICAR-CPCRI. Kasaragod
Pratap	High yield	Dr. BalasahebSawant Konkan Krishi Vidyapeeth (Dr. BSKKV), Maharashtra
Kamarupa	High yield	Assam Agricultural University (AAU), Assam
Aliyarnagar Tall 1-ALR (CN) 1	High yield	Tamil Nadu Agricultural University (TNAU), Tamil Nadu
KeraBastar	High yield	AICRPP, ICAR-CPCRI
KeraKeralam	High yield	AICRPP, ICAR-CPCRI
Aliyarnagar Tall 2-ALR (CN) 2	High yield	TNAU, Tamil Nadu
VPM-3	High yield, drought-tolerant	TNAU, Tamil Nadu
Kera Sagara	High yield	Kerala Agricultural University (KAU), Kerala
Double century	High yield	Acharya N. G. Ranga Agricultural University (ANGRAU), Andhra Pradesh
Kalpa Haritha	Green color fruits, less eriophyid mite damage, dual purpose for tender nut and copra	ICAR-CPCRI
Kalyani Coconut	High yield	Bidhan Chandra KrishiViswavidyalaya (BCKV), West Bengal
Kalpa Shatabdi	Large fruit, dual purpose for copra and tender nut	ICAR-CPCRI
Kalpa Ratna	Multipurpose for tender nut, copra/oil, inflorescence sap (neera) production, tolerant to moisture stress	ICAR-CPCRI
Kalparaksha	Semi-tall, green color fruits, high nut/copra yield in root (wilt) disease (RWD) prevalent areas, tender nut purpose	ICAR-CPCRI
Kera Madhura	Semi-tall, dual purpose for tender nut and copra	KAU, Kerala

(continued)

Table 10.1 (continued)

Variety	Salient features	Institution responsible for the release
Gautami Ganga	Dwarf, green fruits	Dr. YSR Horticultural University (Dr. YSRHU), Andhra Pradesh
CARI-C1 (Annapurna)	High copra content and tender nut purpose, dwarf, green color fruit	ICAR-Central Island Agricultural Research Institute (ICAR-CIARI), Port Blair, Andaman and Nicobar Islands
Chowghat Orange Dwarf (COD)	Dwarf, orange color fruit, tender nut purpose	ICAR-CPCRI
Kalpa Jyothi	Dwarf, yellow color fruit, tender nut purpose	ICAR-CPCRI
Kalpa Surya	Dwarf, orange color fruit, tender nut purpose	ICAR-CPCRI
Kalpasree	Dwarf, green fruits, superior oil, high yield in RWD areas	ICAR-CPCRI
CARI-C2 (Surya)	Ornamental purpose, orange color fruit	ICAR-CIARI, Andaman and Nicobar Islands
CARI-C3 (Omkar)	Ornamental purpose, yellow color fruit	ICAR-CIARI, Andaman and Nicobar Islands
CARI-C4 (Chandan)	Ornamental purpose, orange color fruit	ICAR-CIARI, Andaman and Nicobar Islands

genotypes of T × T was also performed to produce varieties with high yield and superior-quality copra possessing a certain degree of stress tolerance. Production of T × T hybrids was carried out exploiting palms with high breeding value. Diallel analysis of 16 diverse coconut parental genotypes showed that Gangabondam Green Dwarf (GBGD) is a good general combiner. The combination LCT × GBGD is most suited for an appreciable increase in nut and copra yield based on specific combining ability analysis (Nampoothiri et al. 1999). The crosses WCT × COD, COD × WCT, LCT × COD, MYD (Malayan Yellow Dwarf) × TPT (Tiptur Tall), MYD × WCT, ECT (East Coast Tall) × MOD (Malayan Orange Dwarf), ECT × MGD (Malayan Green Dwarf), GBGD × ECT, ECT × MYD, and CGD × WCT are instances of successful coconut hybrids in India (Fig. 10.3). In order to exploit the possibility of developing high yielding dwarf hybrids combining desirable traits of early flowering and higher rate of bunch observed in dwarfs, work on development of D × D hybrids was initiated at ICAR-CPCRI during 1999, and 21 dwarf × dwarf hybrid combinations were planted for evaluation during 2003 (ICAR-CPCRI 2004). The D × D hybrids recorded positive heterosis for growth as well as yield traits and were also observed to show earliness in flowering. Earliest flowering, 15 months after planting, with regular bunch production was recorded in MYD × CGD. The MYD × NLAD (Niu Leka Dwarf) hybrid (Fig. 10.4) recorded compact crown, large inflorescence with more number of medium-sized fruits, and high tender nut water content and sturdy trunk, while COD × GBGD showed early flowering with high nut yield, highlighting the prospects of D × D hybrids for commercial exploitation (ICAR-CPCRI 2014).

Fig. 10.3 Chandra Sankara, a popular Dwarf × Tall coconut hybrid

In India, 21 coconut hybrids (11 T × D; 8 D × T; and 2 T × T) have been developed by ICAR-CPCRI and SAUs under the AICRPP for commercial cultivation in different regions of the country (Niral et al. 2019). The hybrid MYD × WCT (released as Kalpa Samrudhi) also recorded higher fruit, copra, and oil yield as compared to the MAWA (MYD × WAT) hybrid (Jerard et al. 2015). Table 10.2 lists the coconut hybrids released for commercial cultivation in different regions of India.

10.4.2 Coconut Breeding Program in Sri Lanka

The coconut breeding program has been in vogue in Sri Lanka since the setting up of the Coconut Research Institute (CRI) of Sri Lanka in 1928. The requirements of the local coconut industry has led to the identification and selection of coconut accessions with high nut yield and copra content (Peries 1994; Liyanage et al. 1988).

Fig. 10.4 MYD × NLGD, a promising Dwarf × Dwarf coconut hybrid

In Sri Lanka, coconut production depends on the annual rainfall pattern since it is widely grown under rainfed conditions, and tall coconut cultivars are predominantly grown in Sri Lanka. In the early 1940s, coconut improvement programs had commenced with crossing selected Sri Lanka Tall cultivars to develop the improved T × T hybrids (CRIC 60). During 1965, breeding for D × T hybrid (CRIC 61) was also initiated. In 1955, the first isolated seed garden was established for the mass production of improved cultivar CRIC 60. In the early 1970s, the coconut biotechnology program was initiated at CRI.

A coconut germplasm conservation program initiated in Sri Lanka during 1984 has led to the preservation of over 90 distinct accessions and diverse ecotypes in CRI field genebanks (Samarajeewal et al. 2005). In Sri Lanka, coconut germplasm conservation has primarily focused on ex situ conservation of phenotypically diverse coconut collections and randomly identified collections from different parts of Sri Lanka. Later, in situ conservation of farmers' collections was given due importance for sustainable production. In 1992, a new and uncommon dwarf form, Sri Lankan

Table 10.2 Coconut hybrids released for commercial cultivation in India

Hybrid	Parents	Important traits	Institution responsible for release
Chandra Sankara	COD × WCT	High yield	ICAR-CPCRI
Kera Sankara	WCT × COD	High yield, drought-tolerant	ICAR-CPCRI
Chandra Laksha	LCT × COD	High yield, drought-tolerant	ICAR-CPCRI
Kalpa Samrudhi	MYD × WCT	Dual-purpose variety, drought-tolerant, good nutrient use efficiency	ICAR-CPCRI
Kalpa Sankara	CGD × WCT	Tolerant to root (wilt) disease, high yield	ICAR-CPCRI
Kalpa Sreshta	MYD × TPT	Dual-purpose variety, high yield	ICAR-CPCRI
Laksha Ganga	LCT × GBGD	High yield	KAU, Kerala
Ananda Ganga	ADOT × GBGD	High yield	KAU, Kerala
Kera Ganga	WCT × GBGD	High yield	KAU, Kerala
Kera Sree	WCT × MYD	High yield	KAU, Kerala
Kera Sowbhagya	WCT × SSAT	High yield	KAU, Kerala
VHC-1	ECT × MGD	High yield	TNAU, Tamil Nadu
VHC-2	ECT × MYD	High yield	TNAU, Tamil Nadu
VHC-3	ECT × MOD	High yield	TNAU, Tamil Nadu
Godavari Ganga	ECT × GBGD	High yield	ANGRAU, Andhra Pradesh
Konkan Bhatye Coconut Hybrid 1	GBGD × ECT	High yield	Dr. BSKKV, Maharashtra
Kalpa Ganga	GBGD × FJT	High yield, suitable for ball copra production	UHS, Bagalkot, Karnataka
Vasista Ganga	GBGD × PHOT	High yield	Dr. YSR Horticultural University (Dr. YSRHU), Andhra Pradesh
Abhaya Ganga	GBGD × LCT	High yield	Dr. YSRHU, Andhra Pradesh
VHC-4	LCT × CCNT	High yield	TNAU, Tamil Nadu
Vynateya Ganga	PHOT × GBGD	High yield	Dr. YSRHU, Andhra Pradesh

Brown Dwarf, with a high number of female flowers and yield, was identified and used in hybrid production. Sri Lanka Brown Dwarf was used to produce two new coconut hybrids by crossing it with Sri Lankan Tall (hybrid CRISL2012 or Kapsuwaya) and San Ramon Tall (hybrid CRISL2013 or Kapsetha). CRI has developed and released six improved coconut cultivars for coconut growers, and among them, four were D × T hybrids (Dissanayaka et al. 2012).

10.4.3 Coconut Breeding Program in Indonesia

In Indonesia, the breeding program under the aegis of the Research Institute for Coconut and Palme (RICP) have led to the industrialization of the coconut industry. The aims of the breeding program are the development of coconut hybrids possessing early bearing, high copra yield, suitable for marshy or drought regions, resistant to diseases, requiring low input, and suitable for food industry purposes (Hengky et al. 1998).

Collection of coconut ecotypes from adjoining areas of Java was the initial research activity followed by surveys in 11 provinces of Indonesia (Liyanage 1974) to identify coconuts for seed gardens and select useful genotypes for the coconut improvement program. The best performing populations, viz., Tenga Tall (DTA) (North Sulawesi), Nias Yellow Dwarf (GKN) (North Sumatra), Bali Tall (DBI) (Bali Island), and Palu Tall (DPU) (Central Sulawesi), were planted at the Mapanget Experimental Garden. The research priority was accorded for the production of D × T hybrid seeds by setting up appropriate seed gardens. Later collections were planted at the research farm at Pakuwon, West Java, and coconut accessions from different parts of Indonesia were planted at the Bone-Bone Experimental Garden, South Sulawesi (Novarianto et al. 1998).

Initially, the main objective of systematic coconut breeding in Indonesia was to identify the diverse coconut populations in Moluccas Provinces and East Nusa Tenggara and initiate efforts to characterize the accessions and conserve them at ICG for Southeast and East Asia (ICG-SEEA) at Sikijang, Riau, Indonesia. These exploratory surveys have identified seven ecotypes (six Talls and one Dwarf) (Novarianto et al. 1998). Later the exploration surveys in different provinces of Indonesia collected 107 accessions and are being conserved in experimental gardens of the Indonesian Coconut and Palmae Research Institute (ICOPRI) and the ICG-SEEA. Notable among them is Mamuaya Tall from North Sulawesi, which is currently being used as genetic material for breeding and distribution in seed gardens.

Around 15 coconut ecotypes were used in the national breeding program. The addition of dwarfs and talls sourced from local and exotic lands has further increased the genetic variability. For instance, Igo Daku, Mapanget, Bali, Riau, Sawarna, Tenga, Palu, and other local talls were found to yield high copra.

The hybrid PB 121 was introduced from Port Bouet, Côte d'Ivoire, in 1975 to develop coconut hybrids having resistance to nut fall and bud rot as these two disorders were a serious menace. Exploratory surveys jointly conducted by the Directorate General of Estate Crops and Coconut Division Director of IRHO (Institut de Recherches pour les Huiles of Oléagineux) to identify sources resistant to bud rot disease caused by *Phytophthora* sp. revealed that most of the standing hybrids were susceptible to the disease. However, some genotypes such as RLT, DJP, PYT, and DBI (Bali Tall) were relatively resistant to bud rot, while hybrid PB 121 and WAT were susceptible. Hence, the hybrid of MYD × PYT was found to be highly resistant to the disease. Three D × T hybrids, namely, KHINA-1 (Nias Yellow Dwarf ×

Tenga Tall), KHINA-2 (Nias Yellow Dwarf × Bali Tall), and KHINA-3 (Nias Yellow Dwarf × Palu Tall), which yield 4–5 t copra ha^{-1} year^{-1} and flower within 3 years after planting were released during 1984 by the Ministry of Agriculture. Later four T × T hybrids, developed by hybridization between selected Mapanget Talls (MPT), viz., KB-1, KB-2, KB-3, and KB-4, with potential to yield 4–4.5 t copra ha^{-1} year^{-1} (Balitka 1989), were also released. The other T × T hybrids produced were Tenga Tall (TGT) × Bali Tall (BAT), TGT × TGT, BAT × TGT, TGT × Palu Tall (PUT), BAT × PUT, and BAT × BAT (Novarianto et al. 1998).

Further, to develop new hybrids with high yield, early bearing and requiring medium inputs, the following crosses were made: Raja Brown Dwarf (RBD) × Mapanget Tall (MPT), NYD × Takome Tall (TKT), Bali Yellow Dwarf (BYD) × MPT, and BYD × TKT. To develop coconut hybrids with high yield, early bearing and suited for swampy area conditions, the following crosses were made: NYD × Riau Tall (RUT), TebingTinggi Dwarf (TTD) × RUT, and Salak Dwarf (SKD).

More than 90 coconut germplasm have been selected and collected in the International Coconut Genebank (ICG) at Indonesian Palm Crops Research Institute (IPCRI) and the Assessment Institute for Agricultural Technologies, North Sulawesi. Approximately 40 coconut accessions were officially released as superior national varieties and superior local varieties (Novarianto et al. 1998).

In situ exploration and characterization of coconut germplasm in early 2016 led to the discovery of Bido coconut in Morotai Island, North Maluku Province (Novarianto et al. 2016). The Bido coconut begins flowering at the age of 3 years and produces many fruits of large fruit size with the fresh meat weight of 534 g nut^{-1}, with short stems; the rate of growth in the height of the trunk is slower than the local tall coconut. Bido coconut pollen has been used as a male parent to pollinate the three superior Dwarf coconut varieties (Nias Yellow Dwarf (NYD), Yellow Dwarf Bali (BYD), and Raja Brown Dwarf (RAD)). The offsprings of these crosses were expected to be superior coconut hybrids. Table 10.3 lists the important coconut varieties released in Indonesia.

10.4.4 Coconut Breeding Program in the Philippines

Traditionally, coconut stands in the Philippines are dominated by talls (97%), as MYD × WAT hybrids and Philippine Coconut Authority (PCA) local hybrids (PCA 15-1, PCA 15-2, and PCA 15-3) occupied relatively less area (Magat 1993). The prominent tall populations grown are Baybay (BAY), Bago-Oshiro (BAO), Macapuno (MAC), Laguna (LAG), San Ramon (SNR), Hijo Tall (HJT), and Tagnanan (TAG). The dwarf varieties include Tacunan (TAC), Catigan (CAT), Aromatic (ARO), and Kinabalan (KIN).

The Bureau of Plant Industry (BPI), Tiaong, Quezon; Visayas State College of Agriculture (ViSCA), Baybay, Leyte; and College of Agriculture, University of the Philippines (UPLB), Los Baños, Laguna, were involved in germplasm collection (Santos et al. 1984). The collected germplasm was planted in the PCA Research

Table 10.3 Coconut varieties of Indonesia released by the Ministry of Agriculture

No.	Variety	Important traits	Origin
1.	Mapanget Tall	High yield	North Sulawesi
2.	Tenga Tall	High yield	North Sulawesi
3.	Bali Tall	High yield	Bali
4.	Palu Tall	High yield	Central Sulawesi
5.	Sawarna Tall	High yield	West Java
6.	KimaAtas Tall	High yield	North Sulawesi
7.	Banyuwangi Tall	High yield	East Java
8.	Jepara Tall	High yield	Central Java
9.	LubukPakam Tall	High yield	North Sumatera
10.	Rennel Tall	High yield	Rennell Island, Pacific
11.	Takome Tall	High yield	North Maluku
12.	Sikka Tall	High yield	Nusa Tenggara Timur
13.	BojongBulat Tall	High yield	Jogyakarta
14.	Kramat Tall	High yield	Gorontalo
15.	Molowahu Tall	High yield	Gorontalo
16.	Adonara Tall	High yield	Nusa Tenggara Timur
17.	Panua Tall	High yield	Gorontalo
18.	Mastutin Tall	High yield	Nusa Tenggara Barat
19.	Sri Gemilang Tall	Swampy tolerant	Indragiri Hilir
20.	Kopyor PuanKalianda Tall	Soft endosperm	South Lampung
21.	Buol St-1	Semi-tall	Central Sulawesi
22.	Nias Yellow Dwarf	dwarf	Nias, North Sumatera
23.	Bali Yellow Dwarf	dwarf	Bali
24.	Salak Dwarf	Many nuts per bunch, dwarf	South Kalimantan
25.	Raja Dwarf	dwarf	North Maluku
26.	Kopyor Green Dwarf	Soft endosperm	Pati, Central Java
27.	Kopyor Brown Dwarf	Soft endosperm	Pati, Central Java
28.	Kopyor Yellow Dwarf	Soft endosperm	Pati, Central Java
29.	Kopyor PuanKalianda	Soft endosperm	Lampung
30.	KB-1 (MT #32 × MT # 32)	High yield	IPCRI
31.	KB-2 (MT #32× MT #2)	High yield	IPCRI
32.	KB-3 (MT #32 × MT #83)	High yield	IPCRI
33.	KB-4 (MT #32 × MT #99)	High yield	IPCRI
34.	KHINA-1 Hybrid	High yield	IPCRI
35.	KHINA-2 Hybrid	High yield	IPCRI
36.	KHINA-3 Hybrid	High yield	IPCRI
37.	KHINA-4 Hybrid	High yield	IPCRI
38.	KHINA-5 Hybrid	High yield	IPCRI
39.	Red Cungap	High antioxidant	Banten
40.	Bido Tall	High yield, early bearing Morotay	North Maluku Source

Source: Elsje Tenda (2004), Novarianto et al. (1994), and Tampake et al. (2002)
KB-Kelapabaru = new hybrid; KHINA = Kelapa Indonesia (Indonesian coconut)

Centers at Davao and Zamboanga (both in Mindanao) and Albay (Luzon) (Santos and Rivera 1998). Currently, the Philippines have 224 coconut accessions in the International Coconut Genetic Resources Database (CGRD) of the COGENT. Sixteen coconut varieties were registered with the Philippine National Seed Industry Council (NSIC), whereas the report of the Research, Development, and Extension Branch of the Philippine Coconut Authority (RDEB-PCA) states that 15 coconut hybrids are registered.

The use of nine tall and seven dwarf promising populations has led to the development of 97 hybrids since the early 1970s. Screening of 31 cultivars for cadang-cadang disease (either by artificial inoculation or natural screening) resistance led to the development of 6 hybrids and 3 selfed lines at Albay Research Center.

Three PCA-recommended hybrids, Malayan Red Dwarf or MRD × TAG (PCA 15-2), MRD × BAY (PCA 15-3), and CAT × LAG (PCA 15-1), are being produced by assisted pollination technique. BAY, a local tall cultivar, is also recommended as planting material. Promising varieties like CAT, TAC, MRD, ARO, BAY, and RIT are used for the multiplication and purification of seed nuts for seed gardens. Eleven accessions, viz., Rennel Island Tall (RIT), West African Tall (WAT), Gazelle Peninsula Tall (GPT), Vanuatu Tall (VTT), Markham Valley Tall (MVT), Malayan Red Dwarf (MRD), Malayan Yellow Dwarf (MYD), Sri Lanka Green Dwarf (SGD), Karkar Tall (KKT), Equatorial Guinea Green Dwarf (EGD), and Aromatic Green Dwarf (AROD), are of foreign origin as a part of global coconut breeding program of COGENT. Apart from the 11 introduced accessions, 22 genotypes are hybrid/line collections. The first three locally produced hybrids, namely, PCA 15-1 (CATD × LAGT), PCA 15-2 (MRD × TAGT), and PCA 15-3 (MRD × BAYT), were mass-produced using the assisted pollination breeding technique for the planting/replanting program. Other hybrids that produced PCA 15-4 (CATD × TAGT) and PCA 15-5 (CATD × BAOT), among others, were also registered with the National Seed Industry Council (NSIC).

Santos et al. (2000) reported that these hybrids were selected based on their stable yield performance and economic profitability. Registered local Tall and Dwarf varieties are TACD, CATD, TAGT, BAOT, and BAYT. The PCA has introduced the SynVar 001, known as Genetically Multi-Ancestored Farmers Coconut Variety (nicknamed "GMA Coconut Variety"), which is considered the hybrid of hybrids. The F1 hybrids derived from six Tall populations having reasonably a good general combining ability formed the base populations of the GMA. GMA is thus an open or cross-pollinating population of highly heterozygous individual palms. Farmers can use the subsequent seed generation for successive planting and making them more self-reliant. Two Dwarf varieties of the Philippines, Tacunan Green Dwarf (TACD) and Galas Green Dwarf (GALD), which were superior to the famous Thai aromatic varieties Nam Hom (HOM) and Nam Wan (WAN), were developed for young tender coconut.

The introduced hybrid MYD × WAT (MAWA) produced inflorescence earlier than the local talls and consequently produced fruits a couple of years earlier than the local genotypes. MAWA produced small-sized nuts compared to local talls and

yielded an average of 229 g of kernel per nut, whereas local tall like BAY yielded 476–534 g of kernel per nut.

The PCA recommended nine hybrids derived from the local cultivars, viz., Tagnanan Tall (TAGT), Catigan Green Dwarf (CAT), Laguna Tall (LAGT), Baybay Tall (BAYT), and Bago-Oshiro Tall (BAOT), and the introduced varieties Malayan Red Dwarf (MRD) and Polynesian Tall (PYT). These hybrids started flowering from the third to fourth year onward. The average number of nuts per palm ranged from 117 to 155, and copra yield per hectare ranged from 4 to 6 tons. The local Tall BAYT was comparatively good, producing 114 nuts per palm with a copra yield of 5 t ha^{-1}. Among the nine hybrids, MRD × TAGT (PCA 15-2) and MRD × BAYT (PCA15-3) were outstanding, giving the highest number of nuts (144–155 $palm^{-1}$) and copra yield (6 t ha^{-1}).

10.4.5 Coconut Breeding Program in Thailand

Coconut germplasm collection was established in Thailand in 1965 when a few cultivars from local and foreign countries were collected and exploited in the Chumphon Horticulture Research Centre (CHRC). A coconut germplasm genebank (COGENT/ADB project), with 20 local coconut accessions, was later established at Kanthuli, Surat Thani Province, in 1997 (Petchpiroon and Thirakul 1998).

The talls were traditionally preferred. Initially, the coconut palm was confined to the west and east coasts and off-shore islands; however, coconut has expanded to inland areas. Phenotypic differences were observed between the coconut varieties grown on the two coasts of peninsular Thailand. Pak Chok (PCK) and Thalai Roi (TLR) were the two populations grown on West Coast. Owing to their small to medium-sized nuts with more husk and less meat than the predominant talls, these varieties are not grown on a large scale. The commercial coconuts are Maphrao Yai or Thai Tall (THT), which has large, green, or reddish-brown round-shaped fruit. In the country's central region, the Toddy variety is another tall population grown because of its relatively high sugar content in the inflorescence sap. Besides, dwarfs are also being grown for a tender nut purpose (Petchpiroon and Thirakul 1998).

In the national coconut genebank at Kanthuli, Thailand, 34 coconut ecotypes were collected and conserved. Characterization was done for the 20 existing accessions maintained in Chumphon Horticulture Research Centre (CHRC). The tall forms include Hua Ling, Thalai Roi, Pak Chok, Pulak Wan, Klang, Maphraeo, So, Yai, and YaiPhiset. The dwarf form includes Mu Si, Nok Khum, Nam Hom, Mu Si Khieo, Thung Khlet, Mu Si Luang, Nalike, Mu SI Som, Fai, and Pathiu. Besides, a miscellaneous group consists of rare coconut varieties whose affinities are not clearly understood; this includes Phuang, TuenDok, Thale Ba, Nim, Lao Tan, and KonChuk.

Suricha, Thalaeba, and Saiboa were the promising tall coconut varieties for sap and sugar production, yielding about 4–6 L of fresh sap $palm^{-1}$ day^{-1}, whereas Kheekai and Krati varieties produced 3–4 L sap $palm^{-1}$ day^{-1}. Because of their high

sap yield per spathe and their strong leaf petiole to support tappers (sap collectors), these tall varieties were preferred by farmers. Sawi Hybrid No. 1 was also identified as a suitable variety for sugar production because of more spathe production and sap yield stability. Aromatic Dwarf and Green Dwarfs were also being grown for sap production. Among the Dwarfs, Green Dwarfs such as aromatic coconut (Nam Hom) and sweet water coconut (Nam Wan) were extensively grown on a commercial scale.

In contrast, other Dwarfs, such as Yellow, Red, and Brown Dwarfs, were found growing in home gardens for tender nuts. These dwarf varieties are considered rare and endangered. A pink mesocarp-type palm from Nam Wan variety was also found to be a rare variety. Recommended hybrids of Thailand include Sawi Hybrid No. 1, Chumphon Hybrid 60 (THT × WAT), and Chumphon Hybrid No 2 (MYD × THT). Studies have shown that the MYD × WAT hybrid was the most precocious with the highest yield, followed by the THT × WAT hybrid, whereas the THT yielded the least. The results of the local hybrid varieties trial had shown the MYD × THT hybrid was also precocious as that of MYD × WAT and had bigger nuts. Higher yields and drought resistance are the objectives of the current coconut improvement program.

10.4.6 Coconut Breeding Program in Vietnam

The Institute for Research on Oils and Oil Plants, also known as the Oil Plants Institute of Vietnam (OPI), established in 1980, undertakes research activities pertaining to coconut. The objectives of the Vietnam coconut breeding program are to produce elite planting materials that can adapt to a wide range of ecological conditions in the country. In the field genebank of Dong Go Experiment Station, 45 coconut accessions (11 exotic and 34 local accessions) have been conserved. These accessions possess traits for oil, copra content and tolerance to the acid sulfate soil of the Mekong Delta and adapted to alluvial soils of the Mekong Delta, sandy soil of Central Vietnam's coastline, for the industrial zones, highlands and mountain areas of Central Vietnam, island area, for tender nut purpose for the Mekong Delta, and rare and precious traits (Long 1998).

Superior performance was observed with some populations, e.g., Sri Lanka Green Dwarf, Catigan, West African Tall, Malayan Yellow Dwarf, Hijo Tall, and San Ramon. The coconut hybrids have been produced locally using the available genetic materials PB121, PB141, JVA1, JVA2, MYD × Ta Tall, Tam Quan × Hijo Tall, MYD × Rennel Tall, and MYD × Palu Tall, and the Rennell Tall and Palu Tall pollens were collected from Indonesia.

Ta is the most extensively grown traditional variety in the country. It has large-sized fruits with 260–280 g of copra nut^{-1}. Dau is the second most promising variety under cultivation, with a high number of medium size nuts per bunch and high copra content of 180–220 g of copra nut^{-1}. Giay is another popular variety in the central region, particularly along with the coastal areas, and it has big-sized nuts and a high

number of nuts per bunch. However, Bi or Bung coconut variety has the largest nut size (2.7 kg) but with a low number of nuts bunch^{-1}. Some genotypes with special characters are Ngot (sweet), Sap (Macapuno), Soc (stripe), and Dua (aromatic).

Eo, Xiem, and Tam Quan were the three distinct dwarfs mainly preferred for nut water because of their aroma and high water sugar content (9.8%). Eo variety produces brown color small-sized nuts (20–40 nuts per bunch). Xiem variety has green color nuts with big size (15–20 nuts bunch^{-1}). Tam Quan coconut variety has yellow color nuts with good fruit component parameters. Among the dwarf types, Tam Quan is considered the most promising material.

PB 111, PB 121, PB 132, and PB 141 hybrid seed nuts were introduced into the country in 1984, followed by introducing JVA1, JVA2, and CRIC 65 in 1986. The seedlings of indigenous hybrids, i.e., Tam Quan × Ta, Eo × Ta, and Tam Quan × BAOT, were evaluated in Dong Go Station, and MYD × Palu Tall, MYD × Rennel Tall, and MYD × Ta were being evaluated at Binh Thanh Experimental Station. Trang Bang coconut seed garden is producing the hybrid PB 121, and it is the only coconut seed garden in the country operating under the assistance of IRHO.

10.4.7 Coconut Breeding Program in Papua New Guinea

Nationwide coconut prospection surveys were conducted by the Coconut Breeding Section of Papua New Guinea (PNG) Cocoa and Coconut Research Institute (CCRI), leading to the planting of 42 talls (Rennell Tall and 41 local) and 11 dwarfs (5 among them are exotic). A hybridization program was initiated involving the crossing of selected local talls with three dwarfs, viz., Malayan Red Dwarf (MRD), Malayan Yellow Dwarf (MYD), and PNG Brown Dwarf (PBD). The progenies from these crosses were planned to undergo both general combining ability tests to identify suitable hybrid combinations. The trials include both population and single plant improvement to select the best parents for future hybridization programs. The IPGRI and the Government of PNG signed a Memorandum of Agreement through the Department of Agriculture and Livestock to establish the International Coconut Genebank for the South Pacific (ICG-SP) with PNG-CCRI as host (Faure and Moxon 1998).

The high yield and early bearing of the MAREN hybrid (Malayan Yellow Dwarf × Rennell Tall) than the local cultivars have been demonstrated in PNG at Bubia and Kerevat (Brook 1985). However, MAREN is susceptible to beetle attack. Compared with MRD × RT, the low yield was observed with MAWA (MYD × WAT), which is also susceptible to beetle attack. Rennell Tall outyielded the local tall varieties; however, it is also susceptible to beetle attack. The common insect pests include two beetles, one weevil, and a tree hopper. Most of the exotic accessions and cultivars are susceptible to beetle attack causing palm death. The 78 series of D × T hybrids developed are being field-tested. Besides, four Dwarf and four Tall accessions have been used to develop new progenies for GCA trials.

10.4.8 Coconut Breeding Program in Fiji

In Fiji, the Taveuni Coconut Centre (TCC) has been maintaining four dwarf varieties (MD, MYD, MRD, and NLD) along with three tall varieties (FJT, RLT, and ROT). Progenies of Fiji Tall palms, which were selected from two populations of Taveuni, were maintained and monitored by TCC. It is the source for germplasm selection for breeding programs, mainly on pure Fiji Tall or on hybrids of Fiji Tall. The notable precocity of the hybrids is inherited from Malayan Dwarfs. The Niu Leka Dwarf confirmed its peculiar character of being a late bloomer (only 33% of palms flower after 50 months) (Kauvere 1998).

TCC has established a cooperation scheme involving Fiji, France, and the European Economic Council (EEC). Under bilateral cooperation between Fiji and France, regular breeding activities are carried out. In 1992, hybridization work commenced, and emphasis was given to breeding for total copra content since wide variation exists in copra/nut ratio between accessions. The trait has a high heritability value and makes sure its rapid improvement by selection.

The germplasm maintained at TCC is characterized according to CIRAD (Centre de Coopération Internationale en RechercheAgronomique pour le Développement) standards and is utilized in breeding programs. Susceptibility of the germplasm to the endemic disease, coconut foliar decay (CFD), is evaluated in the field and by artificial inoculation under controlled condition.

The D × T hybrid evaluation is one of the major breeding activities. Four D × T hybrids were developed; these hybrids had Malayan Red Dwarf as their female parent and Fiji Tall, Rennell Island Tall, Rotuman Tall, and Niu Leka as male parents. The performance of the hybrids was then compared with Fiji Tall.

CFD is a severe disease that restricts the exploitation of exotic materials for varietal improvement. Hence, the research priority is given to the less sensitive ecotypes to CFD, like Rennell Island Tall, to improve the local cultivars. Further selected ecotypes will be crossed with the local tolerant ones.

10.4.9 Coconut Breeding Program in Vanuatu

Vanuatu germplasm collection, which comprises local and imported ecotypes, is entirely maintained in a field genebank. Talls, T × T hybrids, and D × T hybrids are the three major coconut types grown in Vanuatu.

The Vanuatu Agricultural Research and Training Centre (VARTC), located at Saraoutou, Santo Island, is engaged in undertaking coconut breeding programs. The main goals of the breeding program are tolerance to CFD, high yield, nut size at least equivalent to that of Vanuatu tall (VTT), germination curve similar to that of the VTT, tolerance to low levels of fertilizer, and ability to adapt under the local cultural management conditions. Hence, tall and of T × T hybrid development activities remain important, although D × T would give a higher yield (Duhamel 1998).

The exploitation of CFD tolerance sources existing in the local ecotypes is important for developing planting materials in Vanuatu. Introgressions of genes from exotic ecotypes were carried out to rectify the productivity limitations of these ecotypes. This recombination enforces the application of selection pressure at each generation for tolerance to CFD.

Presently, the Vanuatu coconut germplasm resources consist of three types of cultivars, namely:

- A tall cultivar (VTT) – CFD tolerance, precocious, small nuts, and average productivity
- A local hybrid [VRD (Vanuatu Red Dwarf) × VTT] – CFD tolerance, very small nuts, and very slow germination
- Hybrids of introduced ecotypes – productive but CFD susceptible

The breeding program of VARTC has several lines of actions, aiming to produce different types of improved cultivars. Hybrids involving the local cultivars VTT and VRD and the introduced Rennell Island Tall (RIT) and Brazilian Green Dwarf (BGD) were developed. The MRD was also crossed with RIT to develop hybrids that performed better than the local VTT in copra yield but are highly susceptible to CFD. The BGD crossed with either VTT or RIT produced the best copra yields of 4.4–5.2 t ha^{-1}; however, the hybrids were highly susceptible to CFD. Relatively low copra yield (3.3–3.7 t ha^{-1}) was observed with VRD × VTT hybrids, but these were found to be more tolerant to CFD. The lowest copra yields of 2.6–2.8 t ha^{-1} were observed with both the traditional and improved VTT types, but the yield is comparable with the hybrid MRD × RIT.

10.4.10 Coconut Breeding Program in Côte d'Ivoire

The Centre National de Recherche Agronomique (CNRA) is the authority for agronomic research nationwide in Côte d'Ivoire. The Marc Delorme Research Station in Abidjan located in the southern part of the Côte d'Ivoire is the headquarters for the Coconut Program of CNRA. From 1967 to 1986, a total of 53 coconut accessions were introduced. Coconut research activities were executed mostly in collaboration with the French Government. Conservation and evaluation of coconut accessions in the field genebank, utilization of the germplasm to detect the best combinations among the ecotypes, and improvement through the production of hybrids identified through progeny tests were the breeding strategies in Côte d'Ivoire (De Lamothe 1970; Gascon and De Lamothe 1976). This method has been restructured into two different axes (D × T and T × T) using the reciprocal recurrent selection (Bourdeix et al. 1990, 1991a, b).

The coconut yield is often limited by phytopathological problems. *Phytophthora katsurae* is the only disease with economic significance causing bud rot and premature nut fall in infected palms. In addition to chemical control methods, few disease-tolerant hybrids are being released by the Marc DELORME Research Station. The

Aceria mite is an important pest that damages fruits, causing a reduction in the copra content. At Marc Delorme Research Station, production performance and physiological characteristics under drought conditions of young and old coconut varieties and hybrids have also been studied (Konan 1997; Repellin et al. 1994a, b), with the objective of identifying drought-tolerant lines. Further, in collaboration with Ghana, 30 varieties and hybrids from CNRA have been tested for resistance to LYD (Konan et al. 2002).

Côte d'Ivoire germplasm collection became the centerpiece of the International Coconut Genebank for Africa and the Indian Ocean (ICG-AIO) from 1996 onward. Generally, an average Ivorian coconut grove consists of 52% West African Tall (WAT) and 48% hybrids. PB121 (Malayan Yellow Dwarf × WAT) is predominant among the hybrids. PB111 (Cameroon Red Dwarf × WAT) and PB141 (Guinean Green Dwarf × WAT) were the other cultivated hybrids. WAT is the only local ecotype that is phenotypically very homogeneous and represents more than 50% of coconut palms. The yield difference between the West African Tall and the hybrids was highly significant. Best F_1 hybrids yielded twice as much as the local WAT at the Marc DELORME Research Station. The copra yield was 3.1 t and 2.1 t for the hybrids and the WAT, respectively (De Taffin et al. 1991). Similarly, the hybrids outyielded the local cultivars in several cultivated areas of Côte d'Ivoire.

In 1953, the Institut de Recherches pour les Huileset Oléagineux (IRHO) introduced 53 ecotypes from different tropical areas at the Marc DELORME Research Station since local cultivars showed limited genetic variability. These ecotypes display broad geographic diversity within a large population size. The different ecotypes have their origin from Latin America, Africa, the Caribbean, the Pacific, the Indian Ocean, and Southeast Asia. This important field genebank consists of 36 talls and 17 dwarfs.

In addition to the 53 ecotypes, 160 selfed families of West African Tall, which are selected genitors, and 25 tall × tall hybrids were used for breeding and germplasm conservation.

A total of 121 inter-ecotype hybrids were tested in Côte d'Ivoire from 1965 to 1993. The first genetic trials compared 35 hybrid combinations from intercrossing 35 parent ecotypes wherein WAT was used as a control. Seven inter-ecotype hybrids were identified, which was significantly superior to the control WAT. None of them performed less than the control, and four of these yielded twice that of the WAT. The other early breeding trials used PB121 as a control (De Lamothe and Benard 1985). These trials resulted in new promising hybrids which were very productive during the adult stage (9–12 years) and highly tolerant to *Phytophthora* than the control PB121. PB213 (WAT × Rennell Tall) and improved PB121 are being used as a control for the breeding trials since 1993 (Bourdeix et al. 1992). Thirty years of continuous breeding research resulted in 121 inter-ecotype hybrids (26% of the possible combinations). In the tall × tall hybrids, the basic trials comprised crossing every new accession with two complementary tall testers with known characters. WAT and Rennell Tall are being used as testers in Côte d'Ivoire. In Dwarf × Tall hybrids, basic trials consisted of crossing every new accession with a single tester. The tall cultivars were tested with the Malayan Yellow Dwarf, while for the dwarfs one tall

ecotype was used as a tester (WAT and improved WAT × Rennell Tall hybrid). The complementary trials have more complicated crossing schemes. These trials are intended to provide genetic information on the type of gene action involved in coconut hybrid vigor.

Relative precocity is the main advantage of the Dwarf × Tall hybrids. Nevertheless, this advantage over the T × T hybrids is not sufficient for eliminating the latter. The yield of the WAT × RIT hybrid is equivalent to the widely grown hybrid PB121 in the ninth year. However, this T × T hybrid later out yielded the PB121 control (IDEFOR/DPO 1992).

In 1970, the second phase of the breeding program was initiated (Gascon and De Lamothe 1976) to improve the best detected hybrids individually. In this method, to improve PB122 (MYD × Polynesian Tall), 45 selected TAT (Tahitian Tall) palms were crossed individually with the same Malayan Yellow Dwarf (MYD) population. These half-sib progenies were tested in comparative hybrid trials.

The improvement of an inter-ecotype hybrid involved two complementary crossing designs: palms from each population were crossed individually onto a set of palms in the other population and reciprocally. When unequal levels of variability exists in two populations, then this approach can be simplified. The male parents were tested individually and were later selfed to obtain progenies for conservation and multiplication. Pollen obtained from these self-pollinated progenies will be used for hybrid seed production. The results showed that selecting 7–8% of the best families resulted in 15–30% genetic gain depending on the trials (Bourdeix et al. 1989). Based on the trials, the following guides were formulated for effective breeding strategies:

- Progeny test is important, especially for genitor selection, and it cannot be replaced with a phenotypic selection of parents.
- Genetic progress is mainly due to the improvement in the number of nuts per bunch. In some cases, the percentage of copra in the fruit without water is slightly improved.

Since 1976, the complex hybrids were developed in Côte d'Ivoire. Evaluation of the genetic variability of hybrid progenies and selection of outstanding individuals for multiplication were the objectives of this program. The following combinations of crosses were being tested:

GYD × (WAT × TAT)	(WAT × TAT) × RIT
GYD × (WAT × RIT)	LMT × (WAT × RIT)
(CRD × MYD) × (WAT × RIT)	(CRD × RIT) × (EGD × WAT)
(MYD × WAT) × (EGD × RIT)	(MRD × RIT) × (WAT × TAT)
(MRD × MYD) × (WAT × TAT)	(MRD × WAT) × (TAT × VNT)

Le Saint and de Lamothe (1987) reported that the hybrid between the Malayan Yellow and Red Dwarfs yielded 3.8 t of copra ha^{-1} during the adult phase, at a planting density of 170 palms ha^{-1} in Côte d'Ivoire. The control cultivar MYD produced one t of copra ha^{-1} in the same trial, which was less than the hybrid, and the yield is

comparable to the D × T materials (IRHO-CIRAD 1989). However, dwarfs are not broadly adapted, and they are not tolerant to drought (Ziller 1962). These undesirable traits could be transmitted to their hybrid progenies, and there is less genetic variability than talls. Thus, the long-term genetic potential of the dwarfs is limited. However, the precocity and the more number of bunch production are valuable traits of the dwarf ecotypes. For accumulating such desirable genes, D × D hybrids should be created and incorporated in a breeding scheme, although the primary goal may not be to release this type of material (Bourdeix et al. 1991a).

D × T and T × T were the two important breeding schemes. Production of three-way hybrids using D × D, D × T, or T× T female genitors was suggested by Harries (1991). Some accessions with composite characters could be exploited for fruit or hybrid seed nuts production based on the demand. Based on the outcome of the genetic trials (Bourdeix et al. 1990, 1991a, b), new directions of the coconut breeding program were proposed at the Marc DELORME Station. The proposed method was based on the reciprocal recurrent selection (RRS) method (Comstock et al. 1949). Improvement of T × T and D × T hybrids are the two main areas. The conception of the D × T axes was relatively simple. The dwarfs and the talls are two different types with some complementary characters, and the combining ability between these two types is very good. The conception of the T × T was more difficult. The tall ecotypes represented the main component of the genetic variability in coconut. Some combinations expressed high heterosis. Morphological approaches (Harries 1978; N'Cho et al. 1993) provide some idea on the partitioning of the tall population. However, the current knowledge of coconut genetic diversity is not enough for efficient exploitation in breeding programs.

There is a lack of accurate technique for assessing genetic distances between and among ecotypes in the T × T hybrid improvement. Two artificial populations were created and improved in respect of each other by half-sib RRS based on two founder ecotypes. The choice of the founder ecotypes should take into account the different constraints of the specific breeding program. The availability of the material and phytopathological status have to be considered. One of the ecotypes should be a local variety. The analysis of genetic trials guided the choice of the WAT (Côte d'Ivoire and Benin) and the RIT (Pacific) as founders in Côte d'Ivoire. These two ecotypes showed good combining ability between themselves as well as with other dwarf and tall ecotypes. Some genitors with excellent general combining ability were identified from these two ecotypes (Bourdeix et al. 1989, 1992), and using these genitors as testers has several advantages.

Further, multilocation trials to identify suitable coconut hybrids and varieties for Africa, Latin America, and the Caribbean were also undertaken. Côte d'Ivoire, along with six other countries, viz., Tanzania, Benin, and Mozambique in Africa; Brazil and Mexico in Latin America; and Jamaica in the Caribbean, was involved in the implementation of the Common Fund for Commodities (CFC)-funded project, a collaborative activity between IPGRI-COGENT, CFC, and the Portuguese governments, which included testing six hybrids from Côte d'Ivoire and ten hybrids from the participating countries (Konan 2002).

CNRA experiments identified two varieties, Vanuatu Tall and Sri Lankan Green Dwarf, as highly tolerant of LYD in Ghana. Using these varieties as parents, the CNRA is now producing hybrids to check the spread and destruction of the disease. Initially, the CNRA MarcDelorme Coconut Station identified six outstanding hybrids, viz., PB111 (CRD or Cameroon Red Dwarf ×WAT), PB121 (MYD × WAT), PB123 (MYD × RIT), PB 132 (MRD × TAT or Tahitian Tall), PB 213 (WAT × RIT), and PB 214 (WAT × VTT). These hybrids were precocious (40–57 months after field planting) under Côte d'Ivoire conditions. The nut yield is 100–132 nuts palm^{-1} year^{-1} which is 34–138% higher than the control, WAT. Besides, their copra yields ranged from 3.15 to 4.8 t ha^{-1} or from 86 to 135% more compared with WAT.

10.4.11 Coconut Breeding Program in Ghana

The germplasm collection maintained in Ghana was brought from other countries, particularly Côte d'Ivoire. Eight accessions, viz., Tacunan Green Dwarf, Catigan Green Dwarf, Panama Tall, Tagnanan Tall, Laccadive Ordinary Tall, Andaman Ordinary Tall, Vanuatu Tall (VTT) and West African Tall (Benin), and three hybrids, viz., Sri Lanka Green Dwarf (SLGD) × VTTV, VTT × Panama Tall, and MYD × VTT, were collected from the Marc Delorme Station in Côte d'Ivoire to expand the lethal yellowing (LY) trials for resistant varieties in Ghana. All the cultivars in the country are considered to be at risk of Cape St. Paul Wilt Disease (CSPWD), a lethal yellowing-type disease because of the nature of CSPWD. Hence, the most pressing problem of the coconut industry in Ghana is the CSPWD. In Ghana, this lethal yellowing disease is causing widespread death of palms. Similar diseases are also noticed in Florida, the Caribbean, South America, Togo, Cameroon, and Nigeria (Romney 1972). Efforts are being made to devise suitable disease control measures. Some coconut hybrids, particularly MYD × Panama Tall, are considered resistant to the Caribbean strain of lethal yellows; they are, however, reported to be susceptible to the disease in East Africa. The occurrence of different strains of lethal yellowing phytoplasma in different parts of the world might be the reason for this. Researchers at Rothamsted Research Station in the UK proved that the East and West African strain of LY MLO is different (Tymon et al. 1998).

VTT, SLGD, MYD × VTT showed some degree of tolerance against LY disease. In Jamaica, crosses of MYD with Panama Tall exhibited high tolerance against LY disease. MRD, CRD, and MRD × Polynesian Tall (PYT) were the other cultivars or hybrids which have shown a lesser degree of tolerance. Dery and Philippe (1995) reported that VTT is relatively tolerant to LYD (also locally known as Cape St. Paul Wilt Disease or CSPWD). Bourdeix (2000) reported that the VTT cultivars in Ghana were introduced from Côte d'Ivoire, which is quite variable. Harries (1995) also confirmed the general variability of VTT. The coconut breeding program in Ghana is currently geared toward developing hybrids resistant or highly tolerant to CSPWD.

10.4.12 Coconut Breeding Programs in Other Countries

10.4.12.1 Bangladesh

The Agricultural Research Institute (BARI) has developed two high-yielding coconut hybrids: BARI Narikel-1 and BARI Narikel-2. These hybrids are broadly adapted and capable of producing 65–70 nuts palm^{-1} and suitable for cultivation throughout Bangladesh. In addition, Sri Lankan Tall and Malayan Yellow Dwarf are the two introduced varieties recommended by BARI to the country's coconut growing communities (Batugal 2005b).

10.4.12.2 China

Hybrid derived from Malayan Yellow Dwarf (MYD) and the local Hainan Tall (HAT) was recommended by Wenchang Coconut Research Institute. This hybrid (WY78F1) is early flowering (3–4 years) and has three- to fourfold increase in terms of nut yield (80 nuts palm^{-1} year^{-1}) and copra yield (4 t ha^{-1} year^{-1}) (Batugal 2005b).

10.4.12.3 Tanzania

Evaluation of six hybrids, with the local East African Tall (EAT) as the pollinator, was undertaken at the Mikocheni Agricultural Research Institute (MARI). The mother palms involved Malayan Green Dwarf (MGD), CRD, Pemba Red Dwarf (PRD), MYD, and MRD and improved EAT populations. Apart from the yield performance, the hybrids are also being tested for their resistance to lethal disease and tolerance to drought stress (Batugal 2005b).

10.4.12.4 Mexico

The development of hybrids resistant to lethal yellowing disease is the main objective of coconut research at the Instituto Nacional de Investigacion Agropecuaria Y Forestal. Hybrids were mostly derived from crosses between improved Pacific Tall populations and MYD. Intrapopulation crosses of selected Pacific Tall were also done, and these are being tested (Batugal 2005b).

10.5 Application of Molecular Markers in Coconut Improvement Programs

Molecular markers have been widely employed in investigating the evolutionary lineage, in the reconstruction of phylogenetic relationships, in the investigation of heterosis, in hybrid authenticity, in the assessment of genetic diversity of the germplasm holdings, in genetic mapping and QTL mapping studies, in marker-assisted breeding, including marker-assisted backcross breeding, and in association mapping studies, etc. (Nadeem et al. 2018). Among these, marker-assisted selection (MAS) is a concerted strategy of utilizing traditional breeding approaches in conjunction with DNA, RNA, or protein markers linked to agronomic or economic traits of importance. In the context of coconut, the development of an array of molecular markers and relatively dense genetic linkage maps has greatly aided in the crop improvement programs. DNA-based molecular markers such as inverse sequence-tagged repeat (ISTR) (Rohde et al. 1995), randomly amplified polymorphic DNA (RAPD) (Ashburner et al. 1997), restriction fragment length polymorphism (RFLP) (Lebrun et al. 1998), and amplified fragment length polymorphism (AFLP) (Perera et al. 1998) were effectively developed and employed generally for germplasm diversity analysis. Later the developments in the field of genome sequencing and high-throughput sequencing platforms have enabled the generation of simple sequence repeat (SSR) markers (Perera et al. 1998; Perera et al. 2000; Rivera et al. 1999; Teulat et al. 2000; Meerow et al. 2003; Rajesh et al. 2008; Ribeiro et al. 2010). The use of molecular markers in coconut has greatly facilitated the identification of genetic distinctness of a genotype and assessment of genetic diversity, markers linked to eriophyid mite resistance, lethal yellowing disease resistance, hybrid authenticity, etc. (Shalini et al. 2007; Rajesh et al. 2015; Jerard et al. 2017; Preethi et al. 2020). As stated above, great quantum of research work on molecular markers in coconut has been dedicated to germplasm diversity analysis; however, investigations pertaining to marker-trait analysis, association mapping studies, and use of novel genomics tools are not uncommon (Cardena et al. 2003; Shalini et al. 2007; Rajesh et al. 2013, 2014; Boonkaew et al. 2018; Saensuk et al. 2016). Application of molecular markers in coconut improvement programs is enlisted in Table 10.4.

Market-trait association analysis in coconut using bulked segregant analysis of DNA of contrasting coconut accessions (West African Tall, Malayan Yellow Dwarf, Atlantic Tall) identified 12 RAPD molecular markers putatively linked with the lethal yellowing disease resistance (Cardena et al. 2003). Similarly, five molecular markers linked to coconut mite resistance were identified by Shalini et al. (2007) based on SSR and RAPD analysis. Besides biotic resistance, marker-trait association studies have been performed for agronomic traits such as palm habit, which profoundly influence plant protection and harvest operations. Rajesh et al. (2013) identified RAPD markers, later developed into sequence-characterized amplified region (SCAR) markers, to differentiate tall and dwarf genotypes of coconut. Genotypic differentiation of "pandan-like" aromatic and non-aromatic coconut

Table 10.4 Applications of molecular markers in coconut improvement

Sl. no.	Applications	Molecular markers employed	References
1.	Genetic differentiation of coconut genotypes	Restriction fragment length polymorphism (RFLP)	Lebrun et al. (1998)
2.	Genetic diversity and evolutionary lineage analysis	Randomly amplified polymorphic DNA (RAPD)	Ashburner et al. (1997)
3.	Genetic diversity of talls and dwarfs	Amplified fragment length polymorphism (AFLP)	Perera et al. (1998)
4.	Genetic diversity	Inverse sequence-tagged repeat (ISTR)	Rohde et al. (1992)
5.	Genetic diversity of accessions from various geographic regions	Inter simple sequence repeats (ISSR)	Manimekalai and Nagarajan (2006)
6.	Genetic polymorphism	Simple sequence repeats (SSRs)	Rivera et al. (1999)
7.	Genetic distinctness analysis	SSRs	Meerow et al. (2003)
8.	Genetic markers linked with mite resistance	SSRs	Shalini et al. (2007)
9.	Genetic markers linked with LYD resistance	SSRs	Konan et al. (2007)
10.	Genetic diversity	SSRs	Rajesh et al. (2014)
11.	Genetic diversity and the population structure analysis	SSRs	Jerard et al. (2017)
12.	Genic SSRs for genetic diversity analysis	EST-SSRs	Preethi et al. (2020)
13.	DNA polymorphism studies	Start codon targeted polymorphism (SCoT) markers	Rajesh et al. (2015)
14.	Distinction of aromatic and nonaromatic coconuts	Gene-specific markers	Vongvanrungruang et al. (2016)
15.	Detection of biallelic SNPs linked to aroma trait	Gene-specific markers from transcriptome sequences	Saensuk et al. (2016)
16.	Differentiating tall and dwarf genotypes	RAPD converted to SCAR marker	Rajesh et al. (2013)
17.	Hybrid detection	RAPD	Rajesh et al. (2014)

accessions was performed by developing a type-specific DNA marker (Vongvanrungruang et al. 2016) and a functional marker that could distinguish SNP variations between these two genotypes (Saensuk et al. 2016).

Nevertheless, large-scale utilization of molecular markers in backcross breeding to incorporate a gene of agronomic or economic importance or association mapping analysis is severely lacking in coconut. The untapped potential of molecular breeding in coconut could be attributed to serious impediments such as a relatively long breeding cycle, difficulties in identifying and following appropriate selection protocols for yield and yield attributing traits, pest and disease resistance conferring genes, etc., specific to perennial crops. In this context, it is imperative to identify

molecular markers tightly linked to the trait of interest, and genetic linkage maps are very useful resources.

10.6 Genetic Linkage Maps in Coconut: QTL Mapping

A genetic linkage map refers to describing the relative positions of the molecular markers and distances among them along a chromosome or linkage group. Availability of a good-quality genetic linkage map plays a significant role in genetic analysis of a trait, accelerates molecular breeding programs, and aids in identifying genetic loci that govern agronomic traits of importance or loci that are linked to biotic or abiotic stress tolerance. Thus, a linkage map is an integral component of any marker-assisted breeding scheme. Even though the physical maps could provide the order of molecular markers, genetic maps are required for validating them and would greatly assist in improving de novo genome assemblies. Also, the characterization of genetic regions linked to quantitative traits and mapping them in linkage maps refer to QTL mapping. It would help analyze the segregation pattern of QTLs and assist the genomics-based breeding in coconut. In coconut, both the strategies of genetic mapping, a) linkage mapping and b) association mapping or linkage disequilibrium (LD) mapping, are followed though the latter is minimally explored (Table 10.5).

Generally, the biparental population is developed from F_2 backcrosses, recombinant inbred lines (RILs), double haploids (DHs), and near-isogenic lines (NILs) (Xu et al. 2017). Owing to its perennial nature, the development of these experimental populations, along with their pedigree information for QTL mapping in coconut, consumes a huge time. Despite the inherent limitations such as limited recombination between the parents causing less mapping resolution (QTLs getting localized to 10–20 cM intervals) and limited phenotypic diversity between the parents, biparental population-based linkage mapping is widely employed in perennials such as coconut. Furthermore, instead of following a conventional strategy of genotyping all the individuals of a population, bulked sample analysis is found to be very effective and cost-efficient for studying the major gene effect or QTLs with a large effect. Rajesh et al. (2013) have effectively utilized this strategy to investigate the palm habit in coconut and identified DNA marker linked to the trait. The strategies of multiparent mapping populations such as nested association mapping (NAM) and multiparent advanced generation intercrosses (MAGIC) in field crops to overcome the limitations of biparental mapping populations remain a challenging task in coconut. Hence, the concept of natural population-based genetic mapping is an invaluable tool to perform linkage mapping studies in crops like coconut due to its high resolution, allelic richness, do away with the tedious development of a mapping population. Thus, linkage disequilibrium or genome-wide association studies (GWAS) utilize the principle of linkage disequilibrium in a set of crop accessions to identify QTLs. This strategy thereby utilizes the phenomenon of historical recombination since the population diversion.

Table 10.5 Salient achievements of trait mapping in coconut through linkage mapping and association or linkage disequilibrium (LD) mapping strategies

Sl. no.	Mapping population	Map length features	QTLs	Molecular markers	References
A. Biparental populations					
1.	Malayan Yellow Dwarf (MYD) and Laguna Tall (LAG)	–	–	ISTR	Rohde et al. (1999)
2.	Laguna Tall × Malayan Yellow Dwarf	Laguna Tall: 2226 cM; MYD 1266 cM	Six QTLs governing precocious germination and yield	AFLP, ISSR, ISTR, and RAPD	Herran et al. (2000)
3.	Cameroon Red Dwarf (CRD) × Rennell Island Tall (RIT)	1971 cM	Nine QTLs linked to yield and yield attributing traits	AFLP and SSR	Lebrun et al. (2001)
4.	Cameroon Red Dwarf (CRD) × Rennell Island Tall (RIT)	1849.8 cM	48 QTLs linked to fruit traits	AFLP and SSR	Baudouin et al. (2006)
5.	African Tall (EAT) × Rennell Island Tall (RIT)	2739 cM	46 QTLs linked to epicuticular wax and other component traits	AFLP and SSR	Riedel et al. (2009)
6.	West African Tall (WAT)-Malayan Yellow Dwarf (MYD)-Atlantic Tall (AT)	–	Markers linked with lethal yellowing disease resistance	RAPD	Cardena et al. (2003)
B. Natural population-based mapping					
1.	79 genotypes across the world	–	SSR locus CnCir73 is linked to fruit component traits	SSRs	Geethanjali et al. (2018)
2.	80 accessions (6 populations, vis., Red Dwarf, Yellow Dwarf, Hainan Red, Hainan Tall, MAWA, and Aromatic Green Dwarf)	–	11 SSR loci linked to the fatty acid content Allele CnFAtB3-359 with a major positive effect	SSRs	Zhou et al. (2020)

In coconut, GWAS-based analysis is very rare. However, Geethanjali et al. (2018) and Zhou et al. (2020) employed this strategy to study the population architecture and the trait fatty acid content. Analysis of genetic diversity of 79 coconut accessions revealed 2–7 alleles and 2 major clades differentiating talls of Indo-Atlantic and South Asia from Indo-Pacific and SE Asia region accessions. Also, SSR locus CnCir73 has been linked to fruit component traits (Geethanjali et al. 2018). Recently, Zhou et al. (2020) performed linkage analysis in 80 accessions for fatty acid content resulting in a grouping of germplasm into subgroups comprising higher-fatty acid and a lower-fatty acid group. Further, Zhou et al. (2020) identified

SSR markers linked to fatty acid content in chromosome 11 and donor genotype (Aromatic Green Dwarf) carrying an allele CnFAtB3-359 with a major positive effect for use in coconut oil breeding. However, applying high-throughput sequencing technologies and the development of suitable bioinformatics and statistical tools are expected to open up the genotyping strategies for rapid genetic mapping of crop plants. Instances of such tools are genotyping-by-sequencing (GBS) (Elshire et al. 2011) and restriction site-associated DNA sequencing (RAD-seq) (Peterson et al. 2012), which have significantly supported the genome-wide rapid discovery of molecular markers, which in turn aids in QTL mapping of traits of importance (Torkamaneh et al. 2017). From a coconut perspective, it is anticipated that integration of GBS and the use of biparental mapping population could be a powerful tool to dissect complex traits. In this context, the availability of whole-genome sequence assemblies of coconut (Xiao et al. 2017; Lantican et al. 2018; Rajesh et al. 2020) has been further utilized to perform GBS to generate a high-density linkage map (Yang et al. 2021). Combining the utility of backcross-mapping population [MYD × (MYD × WAT)] and the high-throughput nature of GBS, the coconut genome sequence has been arranged on to 16 pseudomolecules ensuring over three-fourth of coconut genes in the 16 linkage groups (Yang et al. 2021). This chromosome-scale assembly of the coconut genome is an important step toward establishing a robust genomics-assisted breeding schema in coconut.

10.7 Whole-Genome Assemblies

The inherent complexities of plant genomes could be attributed to their polyploid nature and the presence of repetitive DNA elements interspersed throughout the genome. Among the repetitive sequences, transposable elements (TEs) comprise a major component. They pose a serious impediment to genome sequencing efforts in crops, especially in gene mapping and genome assembly construction (Jackson et al. 2011). However, developments in the field of sequencing technologies and rapid downfall in the cost of sequencing and resequencing have created a massive wealth of genome information of crops, including perennials like coconut. It has created a paradigm shift in crop improvement strategies, especially in marker-trait linkage analysis, QTL mapping, deciphering the expression profile of critical genes, etc. (Jackson et al. 2011). In coconut, the whole-genome sequencing efforts have been undertaken by three independent research groups belonging to China, the Philippines, and India resulting in the genome assemblies of cultivars Hainan Tall, Catigan Green Dwarf, and Chowghat Green Dwarf, respectively (Xiao et al. 2017; Lantican et al. 2019; Rajesh et al. 2020).

10.7.1 Genome Assembly of the Chinese Hainan Tall Cultivar

The Chinese Academy of Tropical Agricultural Sciences sequenced the tall cultivar Hainan Tall, and the draft genome sequence of which was published (Xiao et al. 2017). The cultivar was chosen since it occupies a major area under the crop cultivation in the province of Hainan. The cultivar is also known to exhibit abiotic stress (salinity and drought) tolerance. Genomic analysis revealed the expansion of gene families such as Na^+/H^+ antiporters and ion channels, suggesting their role in imparting abiotic stress tolerance. Further molecular evolutionary analysis of coconut with its relative oil palm suggested that coconut diverged from its relative around 46 mya (Xiao et al. 2017).

10.7.2 The Genome of the Philippine Cultivar Catigan Green Dwarf

A combination of multiple sequencing platforms, namely, Pacific Biosciences (PacBio), Illumina MiSeq, and Dovetail Chicago, and various computational pipelines were utilized to assemble and annotate the genome of dwarf cultivar Catigan Green Dwarf (CATD) (Lantican et al. 2019). The hybrid genome assembly was created using the long sequence reads of PacBio followed by correction using the short Illumina reads. The draft genome assembly was further analyzed with Dovetail Chicago, resulting in 97.6% of genome coverage. Comparative genomic analysis of the dwarf CATD and tall HAT genomes identified 58,503 SNPs for use in coconut molecular breeding. Further, over 7000 genomic and functional SSRs having an immense role in conferring biotic, drought tolerance and involved in oil biosynthetic pathways were also mined. Evolutionary analysis of palm genomes further suggested that palms could have undergone at least three rounds of whole genomic duplications (WGD) during the course of evolution (Lantican et al. 2019).

10.7.3 Genome of Disease-Resistant Cultivar Chowghat Green Dwarf

Rajesh et al. (2020) uncovered the whole nuclear and organellar genome sequences of indigenous cultivar Chowghat Green Dwarf (CGD), which possess root (wilt) disease-resistant trait. Furthermore, the predicted coding sequences in the genome assembly were validated using the multiple transcriptome sequence data available in the public domain. A diverse group of nucleotide-binding site and leucine-rich repeat (NBS-LRR) class resistance-conferring genes was identified from the genome. Though the number of NBS-LRR genes identified was comparable to that of other palms, it was way too less compared to other cereal crops. The candidate

R-genes identified in this investigation warrants a functional validation and further molecular characterization to utilize this genetic repertoire for breeding disease resistance in coconut (Rajesh et al. 2020).

A comparison of genome assemblies of three coconut cultivars is given in Table 10.6.

10.8 Multiple Omics Approaches in Coconut

Application of multiple omics technologies such as transcriptomics, small RNA sequencing, proteomics, and metabolomics has been on the rise in plant breeding to characterize the role of functional elements, RNA, proteins, and metabolites in the

Table 10.6 A comparison of genome assemblies of three coconut cultivars

Parameters	Hainan Tall (Xiao et al. 2017)	Catigan Green Dwarf (Lantican et al. 2019)	Chowghat Green Dwarf (Rajesh et al. 2020)
Sequencing platform(s)	Illumina HiSeq 2000	Pacific Biosciences (PacBio) SMRT, Illumina MiSeq, and Dovetail Chicago	Illumina HiSeq 4000, Pacific Biosciences (PacBio) RSII
Predicted protein coding genes	28,039	34,958	13,707
Repeat elements (% in genome)	72.75%	78.33%	77.29%
BUSCO assessment	74.1%	85.3%	84.6%
Total sequences	111,366	7998	26,885
Total bases	2,202,455,121	2,102,417,611	1,930,087,115
Average sequence length	19776.73	262867.92	71790.48
Median sequence length	1139	120,849	41,589
N50 length	1,217,559	570,487	128,735
Features	First draft genome sequence of coconut	First genome sequence of a dwarf cultivar	Nuclear and organellar genome sequences of a dwarf coconut cultivar
	Role of Na^+/H^+ antiporters in abiotic stress tolerance	Identified SNPs by comparing tall and dwarf cultivar for use in molecular breeding	Mined 112 NBS-LRR genes (40 NBS-LRR loci, 20 CC-NBS-LRR loci, 29 NBS loci, 20 CC-NBS loci, 2 RPW8-NBS-LRR loci, and a single TIR-NBS locus) involved in the disease resistance mechanism

cellular context. Along with genomic technologies, coconut has witnessed the use of other omics approaches in deciphering diverse biological questions ranging from biotic stresses, abiotic stress, fatty acid biosynthesis, and post-harvest management of coconuts (Table 10.7). Notable among them is a study deciphering the molecular basis of root (wilt) disease tolerance in indigenous dwarf cultivar Chowghat Green Dwarf (Rajesh et al. 2018). Besides identifying the differential gene expression pattern of healthy and diseased palms, a molecular model describing the host-pathogen interaction was presented (Rajesh et al. 2018). Earlier, Nejat et al. (2015) investigated the yellow decline disease and provided the molecular basis for coconut-phytoplasma interaction.

On the other hand, investigations pertaining to somatic and zygotic embryogenesis were performed utilizing RNA-seq experiments (Bandupriya et al. 2016; Rajesh et al. 2016), which have a great application potential for in vitro multiplication of elite coconut genotypes. The biochemical features and quality profile of coconuts in the transcriptomic studies by Fan et al. (2013) and Saensuk et al. (2016) help identified protein factors responsible for enhanced lauric acid content and genetic basis for the expression of "pandan-like" aroma, respectively. Analyzing the effect of water-deficit stress in coconut seedlings, Ramesh et al. (2020) characterized the differential response of coconut genotypes to drought conditions. Exploration of small RNAs in coconut, though very limited, has provided significant leads in understanding the phenomenon of embryogenesis (Sabana et al. 2020). Similarly, investigations pertaining to coconut proteins have been initially confined to fractionation of various protein components and studying their antioxidant properties (Li et al. 2018; Zheng et al. 2019); however, protein profiling has helped in the identification of functional markers involved in the process of embryogenesis and cold stress acclimatization (Lakshmi Jayaraj 2019; Yang et al. 2020). Application of metabolomics in coconut has been confined to investigating the changes in nut water composition at different maturing stages or during the postharvest period (Zhang et al. 2020; Kumar et al. 2021).

10.9 Conclusions and Recommendations

Precious coconut germplasm is threatened by genetic erosion due to serious biotic and abiotic stresses and anthropological activities which compete for land on which coconuts are grown. These activities hasten the loss of important coconut diversity needed to produce improved varieties. The capacity building in existing national and international genebanks should be continued for sustainable coconut conservation and breeding program. COGENT and its associate institutions like CIRAD, the International Coconut Community [the erstwhile Asian and Pacific Coconut Community (APCC)], and others play an important role in capacity building activities. COGENT is presently working through capacity building and promoting research collaboration among its 38 coconut producing countries and advanced laboratories worldwide. Other than the production of hybrids, emphasis is provided

Table 10.7 Application of multi-omics approaches to enhance the genetic gain in coconut

Sl. no.	Biological phenomena	Genotype(s)	Inference(s)	References
A. Transcriptome sequencing				
1.	RNA-seq analysis of leaf and fruit tissue	Hainan Tall	Expression of genes encoding fatty acyl-ACP thioesterases is involved in the accumulation of medium-chain fatty acids (i.e., lauric acid)	Fan et al. (2013)
2.	Maturing gelatinous endosperm, mature embryo and young leaf	Fragrant dwarf coconut	RNA-directed DNA methylation is an important factor. Small RNA-mediated epigenetic regulation during seed development	Huang et al. (2014)
3.	Coconut yellow decline disease	Diseased and healthy Malayan Red Dwarf	Reprogramming of defense-related gene(s), upregulation of GA-*2ox* (gibberellin-2-oxidase) reduced gibberellins leading to stunted growth, necrosis of inflorescence and premature nut fall. Upregulation of ABC transporter genes was linked to sugar import to maintain the energy source of phytoplasma	Nejat et al. (2015)
4.	Somatic embryogenesis	West Coast Tall	Transcripts involved in somatic embryogenesis [receptor-like kinases (SERK and CLV1), mitogen-activated protein kinase (MAPK), transcription factors (WUS, AP2/ERF, PKL, ANT, and WRKY)], extracellular proteins (AGP, GLP, ECP, and LEA) were studied	Rajesh et al. (2016)
5.	Embryogenesis	Immature embryo, mature embryo, microspore-derived embryo, and mature leaves	Transcripts with putative roles in embryogenesis, viz., chitinase, β-1,3-glucanase, ATP synthase CF0 subunit, thaumatin-like protein, and metallothionein-like protein, were identified	Bandupriya et al. (2016)
6.	“Pandan-like” aroma	Aromatic Green Dwarf coconut of Thailand	Differences in length of transcripts encoding 2AP in aromatic (2371 bp) and nonaromatic (1921 bp) palms	Saensuk et al. (2016)
7.	Host-pathogen interaction during root (wilt) disease	Healthy and diseased CGD palms	A molecular model for coconut-pathogen interaction was put forth	Rajesh et al. (2018)

(continued)

Table 10.7 (continued)

Sl. no.	Biological phenomena	Genotype(s)	Inference(s)	References
8.	Water-deficit stress	Kalpasree (dwarf) and Kalpatharu (tall) having contrasting water use efficiency trait	Genotypic differences in molecular response to water-deficit stress Genic SSRs and the role of long ncRNAs deciphered	Ramesh et al. (2020)
B. Small RNA transcriptomics				
1.	Mature and immature endosperm	–	Mature endosperm-specific miRNA expression pattern identified	Li et al. (2009)
2.	Leaf transcriptome	–	16 miRNAs (of 11 miRNA families) identified	Naganeeswaran et al. (2015)
3.	Embryogenesis		27 novel miRNAs of 15 diverse miRNA families	Sabana et al. (2018)
4.	Cross-kingdom miRNA interaction	Mature and immature nut water	Diverse miRNAs in mature nut water than immature coconut water	Zhao et al. (2018)
5.	Embryogenesis	Embryogenic and non-embryogenic calli	Identified 110 conserved miRNAs and 48 miRNAs specific for embryogenic calli and 21 miRNAs specific for non-embryogenic calli	Sabana et al. (2020)
C. Proteomics				
1.	Nutritional quality using MALDI-TOF/TOF-MS analysis	Mature endosperm	Proteins of classes 7S globulins and glutelin and receptor-like protein kinases were identified Studied the thermal stability of the proteins	Huang et al. (2016)
2.	Antioxidant properties of coconut proteins	Coconut cake	Various protein fractions (albumin, globulin, prolamin, glutelin-1, and glutelin-2) of coconut cake obtained All except albumin showed radical scavenging activity and chelating ability Peptides with antioxidant activity characterized	Li et al. (2018)
3.	ACE-inhibitory and antioxidant peptides of coconut	Coconut cake	Sequential digestion of coconut cake albumin protein fraction yielded bioactive peptides with angiotensin-I converting enzyme (ACE) inhibitory and antioxidant activities	Zheng et al. (2019)

(continued)

Table 10.7 (continued)

Sl. no.	Biological phenomena	Genotype(s)	Inference(s)	References
4.	Embryogenesis investigated using SDS-PAGE and MALDI-TOF/TOF MS	Stages of somatic and zygotic embryogenesis	Seven proteins common to somatic and zygotic embryogenesis identified	Lakshmi Jayaraj (2019)
5.	Cold stress using iTRAQ approach	Hainan Tall, Ben Di (BD) and aromatic coconut, Xiang Shui (XS)	Cold stress upregulated 193 and downregulated 134 proteins in BD. In XS, 140 and 155 proteins were up- and downregulated, respectively	Yang et al. (2020)
D. Metabolomics				
1.	Nut water at four different stages	Chowghat Orange Dwarf (COD) and Malayan Yellow Dwarf (MYD) using GC-MS and UPLC	Amino acid profile during various stages of nut maturity Metabolite profiling differentiated the varieties since COD has biomarkers (caffeic and myristic acids), whereas fumaric and stearic acid was present only in MYD	Kumar et al. (2021)
2.	Metabolomic changes during postharvest and storage period	Hainan-native coconuts using UPLC-MS/MS	Significantly upregulated metabolite biomarkers such as dibutylphthalate, L-leucine, (S)-malate, L-valine, and deethylatrazineetc and downregulated metabolites (gamma-aminobutyric acid zwitterion, acetoacetate, and keto-D-fructose, etc.) are identified	Zhang et al. (2020)

to ensure that the hybrids are evaluated and planted by coconut farmers. Great attention is required for the dispersal of coconut hybrids among farmers, acceptability versus availability of hybrids, economic and anthropological aspect of coconut seed nuts, and cultural coevolution between farmers and their coconut varieties and markets. The adoption of hybrids or varieties produced in breeding programs by the farmers is largely determined by their performance. Hence coconut breeders must put forth efforts to fully understand the basis of varietal preferences of farmers and other end users, and these factors have to be given importance while planning and executing the coconut breeding programs.

COGENT will continue to coordinate coconut breeding program worldwide in collaboration with national programs, partner organizations, farmers, and NGOs. It will be undertaken through prioritized research in coconut breeding involving testing the best hybrids already identified out of the research centers using a farmer's participatory approach, reinforcement of the dispersal of information regarding traditional and hybrids varieties to farmers through publishing catalogs

having consistent information, and breeding for characters such as tolerance to biotic and abiotic stresses, adverse growth conditions, and yield attributes and other important traits desired by coconut stakeholders. To establish an efficient and sustainable system for the multiplication and distribution of recommended hybrids, identifying the most suitable ecosystems where the hybrids perform best and technology transfer of suitable cultural management techniques are important to achieve the desired socioeconomic and environmental impact.

It is apparent that conventional breeding strategies of selection and propagation have been effectively utilized in the crop improvement programs of coconut. The application and use of molecular marker technologies in coconut have been largely restricted to examining the genetic diversity of germplasm lines. However, notable contributions such as the development of genetic maps for use in breeding are worth mentioning. It must be emphasized that large-scale implementation of genomics-based advancements in coconut breeding is severely lacking, as is evident from the scarce publications describing novel breeding strategies such as genome-wide association mapping studies, genotyping-by-sequencing, etc. Nevertheless, the initiatives in the genome sequencing front by independent groups in Asia have given an impetus to the generation of very comprehensive information regarding the genes of agronomic and economic importance. Also, efforts in the field of transcriptomics unfolding the molecular intricacies of biotic stress tolerance, embryogenesis, and abiotic stress tolerance are worth mentioning as it adds to the growing body of literature enriching the resources for coconut genomics. A relatively dense genetic linkage map and assembling the genome sequences of coconut into chromosome-like pseudomolecules utilizing the GBS approach is one such instance of moving forward using genomics technologies. It is anticipated that the ever-decreasing cost of genome sequencing would render resequencing of elite germplasm lines a possible strategy to perform large-scale GWAS analysis to develop appropriate genomic selection (GS) models in coconut. Harnessing of multiple omics technologies such as transcriptomics, proteomics, and metabolomics to complement the efforts of genomics would greatly aid in understanding the complex gene regulatory mechanisms underlying important traits such as oil yield, fatty acid composition, and resistance to biotic and abiotic stressors and in vitro recalcitrance. Applying these novel technologies would greatly reduce the long breeding cycle in coconut to develop new and elite varieties and hybrids.

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Chapter 11
Biotechnological Approaches for Genetic Improvement of Castor Bean (*Ricinus communis* L.)

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Abstract Castor bean (*Ricinus communis* L.) is an annual chiefly nonedible oilseed crop of commercial and industrial importance cultivated worldwide. There are emerging genetic improvement objectives including need for developing CMS system, resistance to biotic and abiotic stresses, ideal plant types for high-density planting and mechanical harvesting, and ricin-free castor bean seeds. Conventional breeding efforts and innovations including hybrid development using two-line system, selecting annual type from perennial nature, incorporating resistance against diseases, increasing harvest index, etc. have contributed immensely for increasing the productivity of the crop. However, in the absence of suitable genetic material with suitable traits, a few of the objectives such as resistance to gray mold disease, resistance to foliage feeders, ricin-free castor bean, etc. have not been achieved with the traditional approach. In this background, the success stories that have been witnessed in other crops as well as with the basic information that has been generated in castor bean suggest that biotechnological approaches employing genomics-assisted breeding and genetic engineering strategies have immense potential in the genetic improvement of castor bean. With this understanding, to create an updated information, the available literature on various aspects of biotechnological research such as genetic and genomic resources, genetic engineering tools, and techniques

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developed in castor bean has been reviewed, and appropriate strategies along with the future scope are presented that could be adopted for genetic enhancement of castor bean both for input and output traits.

Keywords Biotechnological approaches · Castor bean · Genetic engineering · Genomics-assisted breeding · Gene editing · Transgenics

11.1 Introduction

Castor bean (*Ricinus communis* L.) is an annual non-timber woody, chiefly nonedible, oilseed crop of commercial and industrial importance due to the unique ricinoleic acid in its seed oil (Rivarola et al. 2011; Xu et al. 2019) introduced worldwide (Foster et al. 2010) and belongs to spurge (Euphorbiaceae) family, which comprises approximately 6300 species of which many are economically important species including physic nut (*Jatropha curcas*), rubber tree (*Hevea brasiliensis*), and cassava (*Manihot esculenta*).

According to FAOSTAT (2019), the world harvested 1.408 million tonnes (mt) of castor oil bean, with the largest contribution by India (1.197 mt) followed by Mozambique (0.085 mt) and China (0.036 mt); from a total area of 1.186 million hectares (ma), spanning at least 46 countries, led by India (0.751 mha), Mozambique (0.224 mha), and Brazil (0.051 mha); and at an average productivity level of 1214.9 kg per hectare (kg/ha) topped by Mexico (3500 kg/ha), Syrian Arab Republic (2649.1 kg/ha), and Iran (Islamic Republic of Iran, 1947.4 kg/ha).

Castor is a diploid with 20 somatic chromosomes (2n = 2x = 20) with secondary associations observed during metaphase of meiosis (Richharia 1937; Jakob 1956; Jelenkovic & Harrington 1973), and the chromosomes are small with average size ranging from 1.19 to 2.12 μm, and average total length of diploid set is 32.15 μm (Paris et al. 1978; Vasconcelos et al. 2010). Regarding the origin of castor bean species, there is no consensus of opinions. While polyphyletic study suggests Palestine-South (West Asia), Iran-Afghanistan, Arabian Peninsula region, and Indo-China (Moshkin 1986), eastern Africa has been widely believed to be the center of origin of castor bean species (Weiss 1971). Though three separate species, viz., *Ricinus microcarpus*, *R. communis*, and *R. macrocarpus* (Weiss 2000a, b, c), and subspecies including *persicus*, *chinensis*, *africanus*, and *mexicanus* (Kulkarni and Ramanamurthy 1977; Moshkin 1986; Weiss 2000a, b, c) were reported, lack of sexual barrier among these suggests that they are only morphotypes adapted to specific regions. However, molecular phylogenetic studies (Xu et al. 2019, 2021) have thrown new light on the origin and spread of the cultivated species. Review of literature shows that genetics or inheritance of the many agro-morphological traits has been investigated in castor bean, viz., stem color (Solanki and Joshi 2001; Anjani et al. 2007; Lavanya and Gopinath 2008; Prabakaran and Balakishan 2012), waxy coating or bloom (Kulkarni and Ramamurthy 1977; Lavanya and Gopinath 2008),

plant height, nature of spike (Solanki and Joshi 2001; Lavanya and Gopinath 2008), capsule characteristics (Patwardhan 1931), and sex expression (Katayama 1948; Shifriss 1956; Zimmerman and Smith 1966).

Castor bean oil content ranges from 37 to 60 percent among germplasm, commercially released varieties have 48–50% (Wang et al. 2010), and the crop is able to yield up to 1400 kg of seed oil per hectare (Wan et al. 2019). Castor bean is unique among the vegetable oils having elite industrial and pharmaceutical applications due to the presence of an unusual hydroxy fatty acid, ricinoleic acid (>80%) (12-hydroxy-cis-9-octadecenoic acid), in its seed oil. Annually, approximately 408,000 tonnes of castor oil, directly as well as its derivatives, is consumed for industrial or pharmaceutical purpose worldwide (Lu et al. 2018). Weiss (2000a, b, c) has enlisted various medicinal and industrial applications of castor bean oil: laxative, cosmetics, paints, textile dyeing, varnishes, resins, synthetic polymer, caulks, hydraulic fluid, and high-quality lubricants for high-speed jet turbine engines and aeroengines. The castor seed oil is also unique in the sense that it is alcohol-soluble, highly viscous, and suitable for biodiesel production (Conceicao et al. 2007) and an eco-friendly source of fuel due to its reduced greenhouse gas emission as compared to other oils, including mineral oils (Jeong and Park, 2009; Tomar et al. 2017; Lu et al. 2018). Discovery of a natural mutant with low ricinoleic acid and high oleic acid (Rojas-Barros et al. 2004) came as a boon to biodiesel application castor bean seed oil.

11.1.1 Genetic Improvement

Floral Biology: Castor bean plant consists of several series of determinate branches each terminated by a flowering bud. Each bud takes 5–10 days to develop into a flowering panicle called raceme or spike. Basically the crop is monoecious with raceme-type inflorescence with male flowers arranged as lower whorls and female flowers arranged as later whorls. However, there are different sex forms based on the availability and distribution of male and female flowers: monoecious (the spike has basal 1/3 to 1/2 male flowers, while the top portion has female flowers), pistillate (occurs as a rare recessive mutant with the spike having female flowers throughout the central and lateral order spikes), staminate (where the spike will have only male flowers), and sex reversion either to monoecious or interspersed staminate flowers (where male flowers appear in between female flowers) as depicted in Fig. 11.1. Availability of distinct sex types has allowed adoption of different breeding approaches and developing two-line hybrid system.

11.1.2 Breeding

Castor is a highly cross-pollinated crop but with low inbreeding depression on selfing.

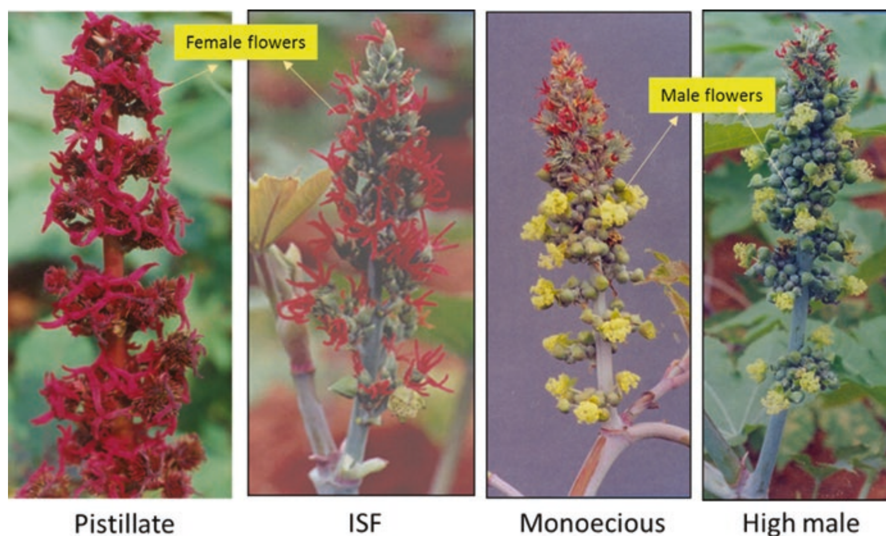


Fig. 11.1 Sex variants in castor

The genetic system is unique to other cross-pollinated crops due to its monotypic genus and sexual polymorphism. Commercial exploitation of heterosis is possible due to standard seed production technology based on a two-line breeding system, despite the lack of CMS system. At present, more than 80% area is occupied by public sector-based hybrids. However, its hybrid breeding for improving yield and high purity is still hampered by genetic instability of female and poor knowledge of sex expression mechanisms (Tan et al. 2016).

The major objectives of castor bean breeding include increased seed yield, incorporating resistance to biotic and abiotic stresses, plant types to suit mechanical harvesting, and to develop genotypes devoid of ricin and RCA. Genetic improvement of castor bean crop can be performed deploying two broad approaches: conventional breeding and innovative breeding. Among conventional breeding approaches, recurrent selection has been effectively employed (Auld et al. 2009; Chen et al. 2016). Innovative breeding approaches including mutation breeding (Lavanya et al. 2003), distant hybridization (Laosatit et al. 2017; Premjet et al. 2019), discovery of marker-trait association to effect marker-assisted selection (Senthilvel et al. 2017a, b, 2019), genotyping by sequencing and association studies (Yu et al. 2019; Xu et al. 2021), and genetic engineering (Sousa et al. 2017; Muddanuru et al. 2019) have also been adopted in castor bean improvement.

Biotechnological approaches for the crop improvement of castor bean can be grouped into two broad categories: genomics-assisted crop improvement and genetic engineering. While the former requires genetic and genomic resources, the prerequisites for the latter are functionally validated gene constructs and robust and efficient transformation and regeneration techniques and protocols. In addition, the other areas of omics including transcriptomics, proteomics, and metabolomics help gaining detailed insights into understanding varied molecular mechanisms,

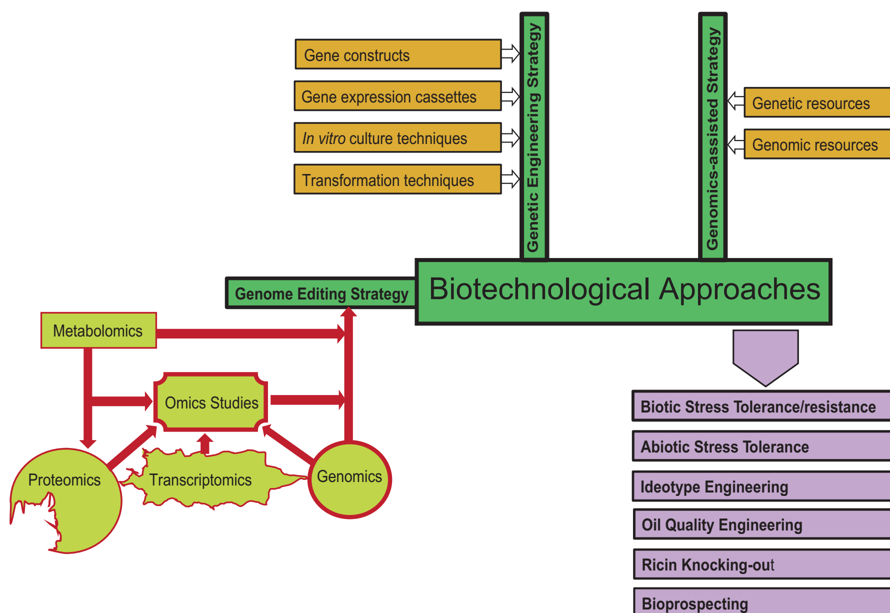


Fig. 11.2 A schematic diagram showing relationship among various biotechnological resources, tools, and techniques and their applications for genetic improvement of castor bean

candidate genes, pathways, and critical and rate-limiting steps underlying various biological processes. Such insights provide useful information required for formulating biotechnological strategies for crop improvement. The biotechnological approaches for genetic improvement of castor bean are schematically illustrated in Fig. 11.2 and described in the following sections.

In this chapter we make an attempt to summarize the recent developments in terms of biotechnological approaches, both genomics and genetic engineering based, employed in improving castor bean. A comprehensive review on the role of biotechnological interventions in castor bean has been published earlier (Sujatha et al. 2008), and some aspects have been highlighted by Severino et al. (2012). We provide an overall idea of the recent efforts made in adopting biotechnological approaches, in castor bean.

11.2 Genomics-Assisted Breeding Approach

11.2.1 Genetic Resources

According to Murray (2017), "Plant genetic resources have been defined as the genetic material of plants, which is of value as a resource for present and future generations of people."

11.2.1.1 Germplasm Stocks

Approximately, 15,000 castor bean germplasm accessions are being maintained in 30 germplasm centers including the 7 major centers: Nacional de Pesquisa de Algodao, Brazil (CNPA); Centro Nacional de Pesquisa de Algodao, Brazil (CNPA); National Bureau of Plant Genetic Resources of Indian Council of Agricultural Research (ICAR-NBPGR, ~ 4307 accessions); United States Department of Agriculture-Agricultural Research Service (USDA-ARS, ~ 117); N.I. Vavilov Institute of Plant Industry, Russia (VIR); Institute of Crop Germplasm Resources under Chinese Academy of Agricultural Sciences (ICGR-CAAS, ~ 2111 accessions); and Institute of Biodiversity Conservation, Ethiopia (IBC).

Worldwide characterizations of castor bean genetic resources have revealed considerable variation for morphological traits in castor (Webster 1994; Anjani 2012). However, molecular marker loci analyses have revealed low to moderate levels of DNA polymorphism (Foster et al. 2010; Allan et al. 2008; Qiu et al. 2010; Senthilvel et al. 2017a, b). Castor belongs to a monotypic genus *Ricinus*, and hence, attempts were made to create variability in castor bean through distant hybridization with cassava (*Manihot esculenta*) (Gedil et al. 2009), *Euphorbia lathyris* (Moshkin 1986), and *Jatropha* (DOR 2003) that led to realization of interspecific hybrids (*Ricinus communis* X *Jatropha curcas*) independently by Laosatit et al. (2017) and Premjet et al. (2019). However, these crosses have still not led to any usable genetic stocks.

ICAR-Indian Institute of Oilseeds Research (IIOR), India, maintains about 3400 germplasm collections of which 3036 were collected through explorations in India and 253 accessions were introduced from 36 countries (Anjani 2012). A core set of 165 accessions that represent agro-morphological variability present in the whole collection has been developed (Sarada and Anjani 2013), and molecular study showed low level of genetic relatedness and absence of population structure in the developed core set (Senthilvel et al. 2017a, b).

Precise phenotyping is a primary requirement to understand the genomics and genetic control of any trait. In castor bean handy tools and techniques have been developed for this purpose and are being employed for screening the germplasm against biotic stresses *Fusarium* wilt (Shaw et al. 2016, Fig. 11.3), gray mold disease (Prasad et al. 2016), root rot disease (Tomar et al. 2017), and leafhopper (Anjani et al. 2010). Both pot-based screening and field-based screening are followed to identify and confirm the resistance sources. Using these screening methods, many trait-specific germplasm accessions have been identified and registered as genetic stocks (Anjani 2012; Anjani et al. 2018a, b) including germplasm lines showing tolerance to drought (Parvathaneni et al. 2017), diseases (Anjani et al. 2004; Anjani 2010), early maturity, and high ricinoleic acid content (Anjani et al. 2018a, b). However, so far, genomic regions related to tolerance of these stresses have not been reported. Regarding cytogenetic stocks, there is a very limited availability (Alexandrov & Karlov 2016), and it includes naturally occurring haploid-based euploid stocks (Timko et al. 1980) and colchicine-induced polyploidy (Narain and Singh 1968) and tetraploidy (Baghyalakshmi et al. 2020).

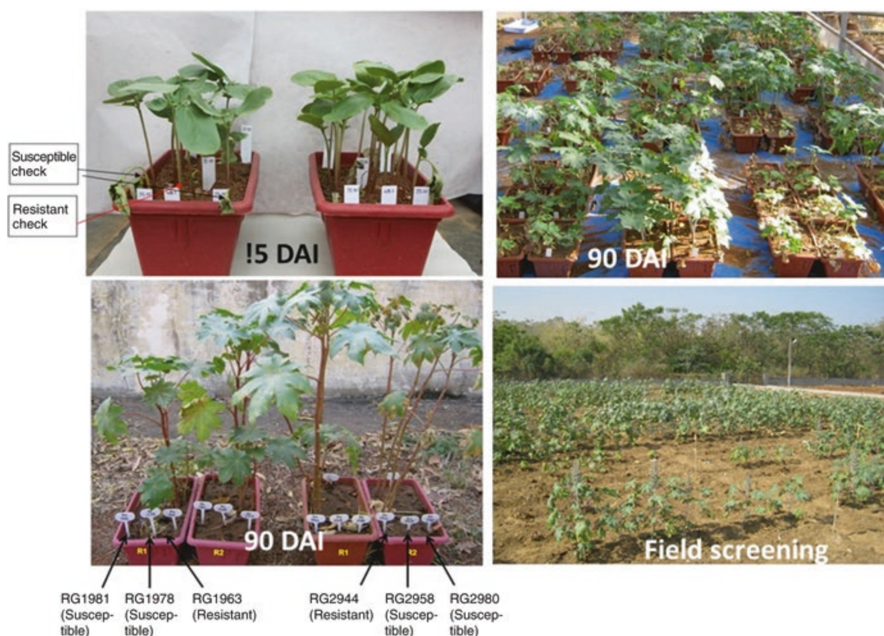


Fig. 11.3 Screening technique to identify resistant source against *Fusarium* wilt disease

11.2.2 Genomic Resources

As there is no comprehensive definition of genomic resources, taking clue from what has been referred to as genomic resources in literature and databases, we have considered "genomic resources" in a holistic way. Accordingly, the "genomic resources" is defined as biological material and/or information that can be used as a tool or basis for further study of genomes of the organisms with the objective of exploiting the whole organism, or tissue, cell, cell organelle(s), DNA, RNA, protein, or other biological molecules or substances of the organism, in the form of good(s) and/or service(s) for the benefit of mankind and/or environment. Since the genome sequence information published (Chan et al. 2010), many genomic resources of castor bean were developed including skeletal SSR linkage map (Liu et al. 2016), quantitative trait loci (QTLs) controlling plant height (Chen et al. 2014), *Fusarium* wilt resistance (Tomar et al. 2016), root rot resistance (Tomar et al. 2017), and seed size and weight (Yu et al. 2019).

11.2.2.1 Molecular Markers: Development and Utility in Genetic Diversity Studies

Whole genome and transcriptome sequencing efforts in castor have expedited the identification and development of a large number of sequence-based molecular markers, including SSRs and SNPs. Molecular markers have utility in many

structural, functional, and comparative genomic studies because of important attributes like codominance, high reproducibility, genome-wide distribution, chromosome-specific, and multi-allelic nature. Simple sequence repeats (SSRs) which are the markers of choice have now been utilized in many marker-based genotyping applications, including varietal identification, DNA fingerprinting, genetic diversity, phylogeny studies, QTL mapping, comparative mapping, and marker-assisted selection (MAS).

Genetic diversity is critical for selecting parental combinations for exploiting heterosis in castor hybrid development program (Thatikunta et al. 2016). Different types of DNA markers were used in castor genetic diversity studies, viz., simple sequence repeats (SSRs) (Allan et al. 2008; Bajay et al. 2009; Qiu et al. 2010; Bajay et al. 2011; Dhingani et al. 2012; Zubair 2014; Wang et al. 2017), expressed sequence tag SSRs (EST-SSRs) (Bajay et al. 2009; Qiu et al. 2010; Pranavi et al. 2011; Dhingani et al. 2012), amplified fragment length polymorphism (AFLP) (Allan et al. 2008), single nucleotide polymorphism (SNP) (Foster et al. 2010; Senthilvel et al. 2019), random amplified polymorphic DNA (RAPD) (Gajera et al. 2010; Dhingani et al. 2012; Machado et al. 2013), sequence-related amplified polymorphism (SRAP) (Lu et al. 2010; Agyenim-Boateng et al. 2019), target region amplification polymorphism (TRAP) (Samoes et al. 2017a; b), and inter-simple sequence repeats (ISSRs) (Gajera et al. 2010; Dhingani et al. 2012).

SSR markers have been also used for assessing molecular genetic diversity, relationship, population structure, and molecular variance in castor bean germplasm (Bajay et al. 2009; Qiu et al. 2010; Zubair 2014; Thatikunta et al. 2016; Samoes et al. 2017; Wang et al. 2017; Agyenim-Boateng et al. 2019). SNP-based marker system also has been employed for diversity studies (Foster et al. 2010; Senthilvel et al. 2019). A special type of DNA marker called target region amplification polymorphism (TRAP) also has been developed and utilized for diversity studies (Samoes et al. 2017).

Most of the studies reported limited genetic diversity among the germplasm lines when tested with different marker systems (Foster et al. 2010; Samoes et al. 2017; Wang et al. 2017; Lu et al. 2018). In a crop like castor bean where rich phenotypic variation is not explained by low genetic variability, molecular basis of phenotypic diversity and plasticity can be explained using relationship between phenotypic and epigenetic relationships (He et al. 2017). Among epigenetic modifications, cytosine DNA methylation is the major molecular mechanism. He et al. (2017) assessed 60 landraces from worldwide collection for epigenetic diversity using methylation-sensitive amplification polymorphism (MSAP) technique. Based on the polymorphic DNA-methylated loci, they found that population-level variation was medium, while it was high (3.80–34.31%) among accessions. They observed polymorphism of methylated loci in both organellar and nuclear genomes. Upon phylogenetic and population structure constructions, they found two clades that did not show geographical grouping, suggesting that epigenetic variation was a global phenomenon in castor bean. Results of this investigation provide foundation for further investigation as well as information to understand phenotypic and epigenetic diversity in castor bean.

Foster et al. (2010) discovered SNPs by genome sequencing of seven diverse cultivars and comparing these genome assemblies with that of reference genome of cultivar “Hale.” They used 48 SNP loci to study the population genetics using 676 genotypes comprising of worldwide germplasm collections and naturalized castor bean populations from Florida (USA). Based on Bayesian clustering, they observed that though population differentiation existed, it did not follow geographical pattern suggesting that few lineages exist but widely distributed globally. They reasoned that worldwide scenario of low-level geographical structuring was caused by mixing of genotypes and low genetic diversity. When they compared genetic diversity of world collection, they found that the natural population from Florida showed moderate to high levels. Presence of limited genetic variability was opined as an obstacle for ascertaining the population structure in castor bean (Foster et al. 2010). Wang et al. (2017) reported a moderately high level of genetic diversity in castor bean germplasm collection of the USA, based on analyses of cluster, population structure, and principal component.

Genetic variability in castor germplasm was found to be more within the population than among the populations (Foster et al. 2010, Agyenim-Boateng et al. 2019). Within a geopolitical nation, different regions vary with respect to genetic divergence of castor bean. For instance, wild castor material from Guangxi showed maximum genetic variability followed by those of West Guangdong, and Hainan material exhibited the lowest genetic diversity (Agyenim-Boateng et al. 2019). In most of the reports, there was a greater trend of correspondence between population structure, clustering, and geographical origin of the material (Qiu et al. 2010; Lu et al. 2010; Agyenim-Boateng et al. 2019). Summary of the molecular diversity studies in castor bean is presented in Table 11.1.

As discussed in the next section, genome sequence-based markers have been discovered in castor bean, and this is opening up new avenues for exploitation of markers in genomics-assisted breeding programs.

11.2.2.2 Genome Sequence-Based Resources

Genome Sequence-Based Studies

Genome sequence information provides insights into DNA-level basis of various biochemical, physiological, and genetic mechanisms governing traits of agronomic and economic importance in plants, apart from knowledge on genome evolution, size, organization, structure, comparative genomics, etc. In castor bean, nuclear (Chan et al. 2010) as well as mitochondrial and chloroplast (Rivarola et al. 2011) genomes have been sequenced, and the information is made available in the public domain (https://www.ncbi.nlm.nih.gov). Knowledge of genomics is important for biosecurity concerns as castor bean contains high levels of highly toxic substance called ricin with type-2 ribosome-inactivating mode of action (Endo & Tsurugi 1987, 1988; Parikh et al. 2008; Chan et al. 2010; Rivarola et al. 2011).

Table 11.1 Summary of DNA marker loci-based genetic diversity studies in castor bean

#	Marker type (number)	Genotypes (number)	Remarks	References
1	SSR (12)	38 accessions from Brazil castor germplasm collection	Each SSR locus had 2–5 alleles with an average of 3.3	Bajay et al. (2009)
2	SSRs	20 Nigerian castor genotypes	Revealed high average expected heterozygosity (0.74), PIC (0.68), Nei's gene diversity index (0.72)	Salihu et al. (2019)
3	SSR (14)	15 diverse castor genotypes	The polymorphic information content (PIC) ranged from 0.231 (SSR-8) to 0.684 (SSR-10) with an average of 0.413	Chaudhary et al. (2019)
4	SSR (11)	76 castor bean accessions	There is a conserved allelic richness among castor bean accessions	Bajay et al. (2011)
5	SSR (28)	72 accessions (68 accessions from Korea, 2 from Taiwan, and 1 each from Israel and Russia)	New SSR markers were validated with moderate level of diversity	Seo et al. (2011)
6	SSR (45)	144 inbred lines derived from castor bean core set	Modest gene diversity (0.382) observed in the germplasm collection	Senthilvel et al. (2017a, b)
7	SSR (14)	27 inbred lines from India	Genetic distance ranged from 0.04 to 0.62 with mean of 0.34	Rukhsar et al. (2017)
8	SSR (5)	60 genotypes obtained from Slovakia	Average diversity index of 0.826.	Vivodik et al. (2014)
9	EST-SSR (10)	14 genotypes from India	Genetic diversity is low	Ramana Rao et al. (2012)
10	EST-SSR (35)	51 accessions from Northeast India	Moderate genetic diversity is observed	Kanti et al. (2014)
11	EST-SSR (29)	33 accessions collected from Andaman and Nicobar Islands	Significant diversity was observed among the collected germplasm	Kanti et al. (2015)
12	EST-SSR (28)	27 Indian accessions	Moderate genetic diversity	Ramesh et al. (2016)
13	EST-SSR (25)	25 pistillate lines of castor	Low level of genetic variation was observed in castor at DNA level with SSR markers	Usha-kiran et al. (2016)
14	EST-SSR (35)	60 castor breeding lines	SSR allelic variation was low as indicated by the average number of alleles (2.8), gene diversity (0.53), and polymorphic information content (0.45)	Usha-kiran and Lavanya (2019)

(continued)

Table 11.1 (continued)

#	Marker type (number)	Genotypes (number)	Remarks	References
15	EST-SSR (118)	24 castor genotypes from across different countries	EST-SSRs showed allelic richness, 2–6 per locus, with an average of 2.97. Markers had moderate gene diversity (He, 0.41), and accessions showed geographic pattern of grouping	Qiu et al. (2010)
16	EST-SSR (130)	Initially 2 genotypes and later with 16 parental lines of 9 commercial hybrids	EST-SSR markers are useful for testing and confirming hybrid purity in commercial castor hybrid production	Pranavi et al. (2011)
17	EST-SSR (22)	574 US germplasm accessions	US germplasm collection exhibited moderately high level of genetic diversity based on cluster analysis, population structure, and principal component analysis. However there were both divergent and redundant accessions in the collections	Wang et al. (2017)
18	SSR (10), ISSR (24), RAPD (25)	8 castor genotypes	Clustering was different for SSR, ISSR, and RAPD. However clustering pattern was the same for RAPD and combined data of three classes of markers	Dhingani et al. (2012)
19	SSRs, AFLPs	200 accessions including 41 across the world	Compared to other plant species, castor beans have low genetic diversity worldwide. SSRs are more informative than AFLPs	Allan et al. (2008)
20	SNP (48)	676 germplasm and naturalized population from Florida	Worldwide 5 main groups existed, and population differentiation did not follow geographical pattern. Molecular variance was more within the population (74%) followed by among populations (22%) and among continents (4%)	Foster et al. (2010)
21	RAPD (200), ISSR (21)	22 castor genotypes	ISSR markers (5/21) were more polymorphic than RAPD markers (30/200). However, RAPD loci were allele-rich with 6.83 alleles per locus	Gajera et al. (2010)
22	SRAP (20)	81 castor bean varieties	SRAP displayed rich genetic diversity (genetic similarity coefficient, 0.352 to 0.929) among castor varieties. Four clusters agreed with the geographic origins or breeding centers	Lu et al. (2010)
23	RAPD (58)	15 castor cultivars	Fifty-six percent polymorphic bands and clustering ability proved suggested that RAPDs were more efficient markers for studying genetic dissimilarity in castor	Machado et al. (2013)
24	TRAP (70)	40 elite lineages of castor	TRAP markers augment the molecular markers for castor bean species	Samoes et al. (2017)

(continued)

Table 11.1 (continued)

#	Marker type (number)	Genotypes (number)	Remarks	References
25	SRAP (29)	Wild castor material	Moderate diversity occurred in wild castor material. There was a greater correspondence between population structure, clustering. and geographic origin. Maximum diversity was present in material from Guangxi followed by those of West Guangdong and Hainan	Agyenim-Boateng et al. (2019)
26	RAPD (30)	22 castor genotypes from India	Observed moderate genetic diversity within genotypes	Li et al. (2012a, b)
27	RAPD (8)	40 genotypes from Slovakia	Genotypes diversity ranged from 0.621 to 0.896	Vivodik et al. (2014)
28	RAPD (27)	13 genotypes from India	Average PIC value is 0.784 and diversity index value 0.798	Laksani et al. (2015)
29	RAPD (145), ISSR (42), SCoT (10)	33 accessions obtained from 7 different geographical regions of the world	High level of polymorphic markers (54%) was observed in RAPD markers as compared to ISSR (38%) and SCoT (21%). Genetic diversity is moderate in genotypes studied	Reddy et al. (2015)
30	ISSR (10)	39 Northeastern China genotypes	Total genetic diversity was high within the population and low among the population	Wang et al. (2013)
31	ISSR (16)	12 accessions from 6 regions of Iran	The genetic diversity was very low	Goodarzi et al. (2015)
32	AFLP (4), SSRs (7)	82 populations from Chiapas, Mexico	Estimated a high level of genetic diversity (71%) in the population	Quintero et al. (2013)
33	AFLP (21)	27 genotypes of Brazil	Low genetic diversity	Vasconcelos et al. (2016)
34	SNP (48)	152 accessions collected from USDA from 45 countries	Genetic diversity low with mean observed heterozygosity across population of 0.15	Foster et al. (2010)
35	SRAP (25)	50 Chinese accessions	Similarity coefficients ranged from 0.64 to 0.97	Mei-Lian et al. (2012)
36	TRAP (168)	40 genotypes developed at NBIO-UFRB, Brazil	A significant level of genetic dissimilarity	Simoes et al. (2017a, b)
37	AP-PCR (9) RMAPD (84)	31 accessions from domestic regions of China	Low genetic diversity	Dong et al. (2012)
38	AFLP (21), ISSR (16)	27 Brazilian germplasm accessions	Modest diversity among the germplasm was observed	Vasconcelos et al. (2016)

Chan et al. (2010) reported for the first time the draft nucleotide sequence for castor bean genome (4.6-fold coverage) and the first sequence report for Euphorbiaceous family member indeed. They highlighted the genes involved in oil biosynthesis and turnover as well as those involved in biosynthesis of ricin and RCA, the two endosperm toxic proteins. Organellar genome sequence-level diversity information of castor bean helps developing of breeding (Chan et al. 2010) as well as forensic (Rivarola et al. 2011) tools to address biosecurity concerns. Rivarola et al. (2011) have performed an extensive analysis of chloroplast sequence diversity of seven genetically and geographically diverse castor bean accessions and identified single nucleotide polymorphism (SNP) from the chloroplast genomes. Upon phylogenetic analysis using these chloroplast SNPs, they found two major novel clades and two distinct sub-clades within each of these two major clades that were hitherto not reported based on nuclear genome-specific DNA marker studies of population genetics. However, upon large-scale genotyping of worldwide collection of castor populations, they confirmed the previously reported low levels of genetic diversity (Lu et al. 2010; Qiu et al. 2010) within each sub-clade consisting of accessions from broad geographic origin. Thus, genetic diversity of castor bean germplasm worldwide is low both at genomic and chloroplast DNA sequence levels as reflected by other DNA-based marker analyses as detailed in Sect. 2.1.1.

Genetic Linkage Map

Chan et al. (2010) developed a genetic linkage map by using 4300 high-quality markers plus 120 SSR markers which were anchored onto 10 LGs after sequencing parents and population. The final genetic map spanned a total of 1547.41 cM with an average marker interval 0.35 cM genome. The first SSR-based genetic linkage map of castor bean consisting of 331 markers, distributed on 10 linkage groups (LGs), encompassing 1164.73 cM, with an average marker interval of 3.63 cM was constructed with 3 different F_2 populations derived from crosses between the YC2, YF1, and YF2 lines (Liu et al. 2016). Tomar et al. (2017) constructed genetic map with a F_2 population, containing 261 markers (76 RAPDs, 34 ISSRs, and 151 SSRs) assigned to 10 LGs with the total map length of 1833.4 cM and an average marker interval of 6.93 cM.

Genome-wide single nucleotide polymorphisms (SNPs, ~2,179,759) were discovered by whole genome sequencing of 14 diverse castor genotypes by Senthilvel et al. (2019). Of the discovered SNPs, 6000 high-quality SNPs were used to develop a genotyping array that represented 87.5% of the genome covered by 2492 scaffolds. Upon validating the array by genotyping 314 castor inbred lines, 5025 scorable SNPs with 100% reproducibility and 98% call rate were obtained. A consensus linkage map with an average inter-marker distance of 0.55 cM using 1978 SNP loci genotyped across recombinant inbred lines (RILs) from crosses JC12 × 48-1 and DCS9 × RG1139 has been constructed (Senthilvel et al. 2019).

Comparative Genomic Study

While commercially cultivated castor bean plant is an annual, its progenitors are perennial woody plants in habit (Xu et al. 2019). However, continuum of genetic variation during its domestication process remained largely unknown till recent time (Chan et al. 2010; Xu et al. 2019, 2021). Empowered with the advancement of genomic studies and their comparison, it has been possible to illuminate molecular evidence of evolution of annual castor genome. Two important studies have been reported regarding two aspects of this topic: origin of polyploidization by Chen and Cahoon (2010) and gene purification during domestication by Xu et al. (2019). Based on comparative genomic analysis, it is evident that castor bean shares an ancient event of hexaploidization with all the dicotyledonous plant species (Chan et al. 2010). Comparative genomic study by Xu et al. (2019) revealed the target genes of selection and genomic variation during the domestication process by conducting phylogenetic analysis using genome sequence of one accession each of cultivar (Hale or ZB306), landrace, and wild castor bean germplasm. They deciphered that level of DNA sequence variation between cultivar and wild castor bean accessions was high compared to the sequence variations between landrace and cultivated line as a result of which wild castor bean accession showed distinct phylogenetic grouping from landrace and cultivar accessions. Based on comparative genomic analysis, they could identify several candidate genes and key pathways related to perennial woody-to-annual castor bean transition during domestication, and they demonstrated that only 3 of the 16 oil biosynthesis-related genes were subjected to selection during domestication process indicating intense purification-oriented selections in both the gene pools: wild and domesticated.

In a comprehensive study, Xu et al. (2021) have used genotyping by sequencing (GBS) technique to resequence 505 worldwide accessions including wild accessions and have provided a de novo genome assembly at chromosome level. Based on analysis, they have established that the accessions from East Africa are the extant wild progenitors of castor bean and that the domestication occurred about 3200 years ago. Using the sequence information, they have performed genome-wide association studies (GWAS) coupled with quantitative trait loci analyses, to identify QTLs and candidate genes associated with plant architecture and seed-related traits. Genome-based studies carried out recently have provided not only insights into the molecular events during evolution of genome and selection during domestication but also serve as valuable resource for future genomic resources and tools, namely, candidate gene-trait associations, marker-trait associations, gene-pathway analyses, and understanding of gene-gene cross talks, all that are necessary for biotechnology-enabled genetic improvement of castor bean.

11.3 Genetic Engineering

Genetic engineering, also referred to as genetic modification or genetic manipulation, is a set of technologies that are used to manipulate the genetic makeup or expression of cells and is usually achieved through the transfer of genes within and across species boundaries to produce an improved or modified organism. Thus, it involves alteration of the genetic makeup of an organism using biotechnological approaches and involves recombinant DNA methods that are used to create gene constructs, new combinations of genes, and *cis*-elements like promoters and terminators. Genetic engineering could be used to either introduce a new functional unit(s) of expression or to knock down the existing gene(s). The major steps involved in developing a transgenic line include identification of the gene(s) that lead to manifestation of the trait, the precise expression pattern of the transgene by incorporating suitable regulatory elements like promoter and terminator, developing the gene construct(s) with the component traits, introduction of the gene construct into the plant to produce transformed cells, selection of the transformed cells and regenerating the plantlets from them, confirmation of the transgenicity of the regenerated plantlets, and selection of the transgenic lines that express the desired trait.

Castor cultivation is beset with many problems including biotic and abiotic stresses. In spite of the successful release of improved varieties through conventional approaches, owing to the lack of genetic variability in the germplasm, there are still some traits such as resistance to insect pests and diseases, abiotic stresses, presence of toxic proteins in the endosperm, etc. which are not addressed through this approach. Therefore, genetic engineering strategy has an immense potential in genetic improvement of castor bean crop. Through genetic engineering, it is possible to regulate the expression of existing genes of castor not only of nuclear genome but also of mitochondrial and chloroplast genomes. In addition, gene and gene combinations can be introduced to castor bean genomes from the foreign sources (exogenous) across the taxonomic barriers for de novo expression of these gene products that manifest in newer and desirable phenotypes.

11.3.1 Basic Requirements for Genetic Engineering

As stated above, genetic engineering approach requires identification of gene(s) to be introduced, selection of appropriate promoters and other cis-elements that impart the desired expression pattern for the selected genes, developing gene constructs, introducing them to the plant, and then selection of the manipulated transgenic plant. Of these steps, the most crucial and species specific is the availability of a procedure to introduce the gene construct into the plant. In this section, we discuss these requirements briefly with respect to castor bean.

11.3.1.1 Tissue Culture

Tissue culture and transformation protocols are the prerequisites for developing transgenic plants. Efficiency of producing transgenic plants ultimately relies on those of transformation, integration of transgene, and in vitro regeneration of transformed explants into complete plants and their acclimatization.

Explant Optimization

Establishment of suitable explant, basal media, growth regulators, conditions and duration of culturing and subculturing, and acclimatization of the developed plantlets are critical and crucial steps in the development of robust in vitro regeneration protocols in plants including castor bean.

Regarding explant optimization, Athma and Reddy (1983) have reported varied callusing and organogenic responses of different explants and observed shoots from the shoot tips and rhizogenesis from root explants, shoot, and leaf tissues. Plant regeneration that occurs in the seedling explants, especially the shoot apex and leaf axils, was reported to involve preexisting meristematic regions (Reddy et al. 1987b; Sangduen et al. 1987; Khumsub 1988; Sujatha and Reddy 1998; Malathi et al. 2006; Sujatha and Sailaja 2008). Hypocotyl explants derived from zygotic embryo axis produced more number of adventitious shoots when treated with thidiazuron (TDZ, 1 μM) than 6-benzylaminopurine (BA, 20 μM, Ahn et al. 2007). Further, cotyledonary explants (Ahn et al. 2008; Ganesh-Kumari et al. 2008) and embryonic tips (Li et al. 2015) have also been reported to be useful explants.

The other explants reported to be responsive to in vitro culture of castor bean include mature seed (Mohan-Ram and Satsangi 1963); de-coated mature seed (Satsangi and Mohan Ram 1965); fresh de-coated seed (Srivastava 1971; Johri and Srivastava 1972); endosperm (La Rue 1944); endosperm from germinated seed (Brown et al. 1970); shoot, cotyledon, hypocotyls, root, endosperm, and embryo (Khumsub 1988); cell suspensions (Cho and Choi 1990); cotyledons (Bahadur et al. 1991); epicotyl and cotyledons (Sarvesh et al. 1992); young stem (Genyu 1988); seedling explants (Athma and Reddy 1988); leaf (Reddy and Bahadur 1989a); shoot apex (Reddy et al. 1987b; Reddy and Bahadur 1989b; Molina and Schobert 1995); hypocotyl, leaf, and shoot tips (Reddy et al. 1986); seed and seedling-derived explants (Sangduen et al. 1987); embryo axis and shoot tips (Sujatha and Reddy 1998); hypocotyl (Sujatha and Reddy 2007); and cotyledon, hypocotyl, epicotyl, and leaf of seedlings (Ganesh-Kumari et al. 2008).

Media, Growth Regulators, and Culture Conditions

In majority of the reports, medium fortified with Murashige and Skoog (1962) salts, and B5 vitamins (MSB), had been used as the basal medium. Ganesh-Kumari et al. (2008) obtained green compact nodular organogenic callus on MSB supplemented

with 2.0 mg/L 6-benzyladenine and 0.8 mg/L α-naphthalene acetic acid (NAA). Multiple shoot proliferation was achieved by subsequent culturing of these green calli on MSB with 2.5 mg/L thidiazuron (TDZ), 0.4 mg/L NAA, and 15 mg/L glutamine. The elongated shoots were rooted on the medium containing MS salts, B5 vitamins, 0.3 mg/L indole-3-butyric acid, and 0.6 mg/L silver nitrate, and the plantlets were hardened in earthen pots containing sand, soil, and vermiculite in equal proportions.

Using embryonic tips as explants, Li et al. (2015) observed an optimal bud induction with MSB added with 0.35 mg/L BA and 0.25 mg/L IBA. The rooting and transplanting stages were also optimized and transplant survival rate was improved. They achieved a final regeneration efficiency of 68.3%. Ahn et al. (2007) showed that the pretreatment of explants in the dark increased the number of shoots regenerated per explant by 82% and 36% with TDZ and BA, respectively. Castor genotypes used in tissue culture include cv. TMV 6 (Ganesh-Kumari et al. 2008) and DPC-9 (Sujatha et al. 2008, 2009; Muddanuru et al. 2019). Usha-Kiran et al. (2020) have reported development of a regeneration protocol using the hypocotyl explants from 15-day-old seedlings. According to their protocol, seedlings raised on MS media supplemented with 0.25 mg/L thidiazuron (TDZ) gave better results when used as source of explants. They observed that among different media tried, shoot induction on MSB supplemented with BAP at 4.5 mg/L and 2-(N-morpholino)ethanesulfonic acid (MES) at 0.5 g/L gave better shoot induction, while the shoot elongation was better on MSB supplemented with 0.5 mg/L of BAP and GA_3 at 1 mg/L. They reported better rooting when treated with 1 mg/L IBA.

In an interesting study, to understand the molecular basis of organogenesis in cultured tissues of castor bean and thereby find answers to the recalcitrance, RNA-seq technique was adopted to identify the genes differentially expressed in hypocotyl explants of castor subjected to different concentrations of hormones under in vitro conditions (Puvvala et al. 2019). Genes that showed differential expression included components of auxin and cytokinin signaling, secondary metabolite synthesis, genes encoding transcription factors, receptor kinases, and protein kinases. In castor, many genes involved in auxin biosynthesis and homeostasis like WAT1 (Wall associated thinness), vacuolar transporter genes, and transcription factors such as short-root-like protein were downregulated, while genes like DELLA were upregulated accounting for regeneration recalcitrance. They also validated many of the differentially expressed genes using qPCR. These results could help in improving the in vitro response of castor bean and thus might help in transformation of the crop.

11.3.1.2 Selection Markers

The type of selection marker and antibiotic challenge used for selecting transformants influence regeneration efficiency and, thereby, the resultant transformation efficiency (Zhang et al. 2000; Kumaraswamy 2000; Penna et al. 2002). There are

many selectable markers genes including *bar*, *npt*II, *hpt*, and *gox* that code for enzymes which help transformed cells to grow in the presence of antibiotics or herbicides, as the case may be, by degrading antibiotics, namely, phosphinothricin, kanamycin, and hygromycin, and herbicides, namely, glyphosate, respectively. These selection agents help in selection of transformed cells from the chimeric tissue of the explant or callus (Zhang et al. 2000). Selectable markers play a significant role particularly in plant species with low transformation efficiency (Jones 2003). Hygromycin phosphotransferase (*hpt*II, Sujatha and Sailaja 2008; Sailaja et al. 2008; Sujatha et al. 2009; Lakshmidevi et al. 2018) and kanamycin phosphotransferase (*npt*, Sujatha et al. 2009) genes were used successfully in castor bean transformation studies.

11.3.1.3 Transformation Protocols

Limited success has been achieved in the genetic transformation of castor bean worldwide. In 2008, Sujatha and Sailaja reported *Agrobacterium tumefaciens*-mediated transformation of castor bean species. Transformation of castor bean using particle bombardment was reported by Sailaja et al. (2008) and successfully used by Sujatha et al. (2009) for producing castor bean transgenic plants against insect feeding. In a recent investigation, Sanches-Alvarez et al. (2019) have developed an innovative method to assess the gene constructs without actually developing transgenic plants. They have developed *Agrobacterium*-mediated transient gene expression system in the developing seeds of castor bean and have demonstrated that this system could be used for high-level, transient expression of the genes for 20 days.

In Vitro Culture-Based Transformation Techniques

The most popular methods of genetic transformation adopted to realize transgenic plants in different plant species are *Agrobacterium* or vector-mediated and gene gun or direct methods. In case of castor bean, researchers have used both *Agrobacterium*-mediated transformation (McKeon and Chen 2003; Sujatha et al. 2008, 2009; Ganesh-Kumari 2012) and particle bombardment/gene gun-mediated methods (Malathi et al. 2006; Sailaja et al. 2008; Sujatha et al. 2008, 2009) to obtain transgenic plants. Even though particle bombardment involves physical delivery of DNA coated on microparticles such as gold (Sujatha et al. 2009) directly into the plant cells of the chosen tissue for regenerating the plantlets, it is a random event and not precise with respect to the quantity or copy number of transgene. Consequently, transgene may get integrated with multiple copies of the insert. *Agrobacterium*-mediated method is relatively more precise with respect to copy number, though it is not precise with respect to the genomic site of integration.

The particle bombardment (Sailaja et al. 2008; Sujatha et al. 2009) and *Agrobacterium*-mediated transformation methods (Sujatha and Sailaja 2008) have

been used for the transformation of castor bean. Sailaja et al. (2008) reported development of stable transgenic plants using particle bombardment method with the efficiency of 1.4%. *Agrobacterium*-mediated floral bud transformation using vacuum infiltration (McKeon and Chen 2003), in vitro co-cultivation-based *Agrobacterium*-mediated methods (Malathi et al. 2006; Sujatha and Sailaja 2008; Li et al. 2015; Patel et al. 2015), particle gun bombardment (Sailaja et al. 2008) using embryo axes as explant, and *in planta* transformation method (Kumar et al. 2011) have been reported in castor. According to a report by Muddanuru et al. (2019), all the three methods, *in planta* (Kumar et al. 2011), *Agrobacterium tumefaciens* mediated (Sujatha and Sailaja 2008), and gene gun mediated (Sujatha et al. 2008), have been successful in creating transgenic castor against insect feeding, with the transformation efficiencies of 2.1%, 2.4%, and 1.1%, respectively.

Involvement of tedious procedures, heavy workload, long cycle, difficulty in regeneration system establishment, low transformation frequency, and high cost have been the undesirable factors that are discouraging the popularity and industrialization of preliminarily developed tissue culture-based transformation systems (Lu et al. 2018). Therefore, as recommended by Lu et al. (2018), the need of the hour is to develop tissue culture-independent transformation methods.

Tissue Culture-Independent Transformation Techniques

Compared to gene gun method, *Agrobacterium*-mediated transformation has tremendous advantages including low copy number and integrity of transgene, lesser problems of gene silencing, and better transgene expression (Gelvin 2003). However, cell wall of the plant acts as a physical barrier between *Agrobacterium* and the target plant cell that needs to be overcome (Gelvin 2003; Lu et al. 2018). Many strategies for establishing transformation protocols in plants in general and particularly in castor bean have dealt with the method of injuring the plant cell wall of the recipient. McKeon and Chen (2003) facilitated *Agrobacterium* infection by injuring castor bean flower buds before bringing it in physical contact with the bacterium harboring modified Ti plasmid. Akin to this, other supplementary procedures can be deployed to enhance the efficiency of *Agrobacterium*-mediated transformation of plant cells. These procedures include tissue rupturing by piercing (Lin et al. 2009) coupled with acupuncture-vacuum filtration (Bechtold et al. 1993; Lin et al. 2009; Lu et al. 2018), in situ transformation methods (Supartana et al. 2005), the ultrasonography (Liu et al. 2006), acupuncture (Supartana et al. 2005), ion beam (Wu et al. 2000), and carbon nanotube carrier (Burlaka et al. 2015; Kwak et al. 2019). These supplementary techniques need to be tried and refined in castor bean so as to exploit their potential to enhance transformation efficiency in a manner independent of tissue culture.

In Planta Transformation Techniques

In planta transformation protocols provide hope to genetically transform crop plants that are not amenable to in vitro culture (called recalcitrant species, Potrykus 1991). Besides, it also helps to avoid problems of chimeras arising out of tissue-culture-based methods (Kumar et al. 2011). One of the strategies of *in planta* transformation technique is to directly deliver competent cells of *Agrobacterium* to axillary or apical meristem where actively dividing cells lack thick meristem and become amenable for agro-infection as it is proved in soybean (Chee et al. 1989), sunflower (Rao and Rohini 1999), safflower (Rohini and Rao 2000b), peanut (Rohini and Rao 2000a), buckwheat (Kojima et al. 2000), mulberry (Ping et al. 2003), rice (Supartana et al. 2005), wheat (Supartana et al. 2006), maize (Chumakov et al. 2006), and capsicum (Kumar et al. 2009).

In planta transformation technique has been successfully validated using a *cry1AcF* gene (Kumar et al. 2011). Lakshmidevi et al. (2018) have optimized certain parameters of the *in planta* transformation protocol wherein they grew agro-infected (pricked) seedlings in soilrite for 2 days before selecting under hygromycin at 40 mg/l for 2 hours and planting putative transformants in the soil. Further, they successfully used this method to create 30 transgenic events with different gene constructs.

11.4 Biotechnological Approaches Against Biotic Stress Factors in Castor Bean

Castor bean crop production worldwide suffers from a number of biotic stresses including several insect pests, diseases, and weeds. Wherever suitable germplasm lines with the desirable traits are available, molecular markers associated with or linked to the trait are being identified to develop marker tool kits to aid in transferring such traits to agronomically superior genotypes, in marker-assisted breeding programs. Transgenic technology has been adopted where suitable germplasm lines with tolerance or resistance to these stress factors are not available. Here we summarize attempts made in castor bean to improve biotic stress tolerance using both molecular marker and transgenic technologies.

11.4.1 Transgenics with Insect Pest Tolerance or Resistance

Castor bean crop suffers heavy damage and up to 35–50% economic loss due to insect pests (Barteneva 1986; Kolte 1995). Among major insects, castor semilooper (*Achaea janata* L.) and tobacco caterpillar (*Spodoptera litura* Fabr), lepidopteran pests that voraciously feed defoliating completely, are serious problems in semiarid tropical regions of the world, particularly India (Narayanan 1959). Neither reliable gene source of resistance is available (Malathi et al. 2006) nor distant hybridization

between *Jatropha* and castor has proved successful due to the intercrossing barrier (Sathaiah and Reddy 1985; Reddy et al. 1987a, b). Furthermore, developments of resistance among insect pests to insecticides (Barton et al. 1987) have aggravated the problems of insect pest management in castor bean.

Transgenic castor bean lines have been developed with tolerance to these insect pests by deploying *Bacillus thuringiensis* (*Bt*) crystal protein genes such as *cry1Aa* (Muddanuru et al. 2019), *cry1Ab* (Malathi et al. 2006), *cry1AcF* (Kumar et al. 2011), and *cry1EC* (Sujatha et al. 2009). These transgenic lines have shown tolerance to tobacco caterpillar and semilooper. Sujatha et al. (2009) have reported development of insect-resistant transgenic lines using popular cultivar DCS-9 through deployment of chimeric *cry1EC* gene under improved 35S promoter, and eight transgenic events that showed resistance against both semilooper and tobacco caterpillar both under laboratory- and field-level bioassays were selected. The field-level evaluation was done up to fourth generation (T_4) where presence of transgene was confirmed using gene-specific PCR and southern analysis. Muddanuru et al. (2019) have reported that *Cry1Aa* insecticidal protein encoded by the deployed transgene accumulated in the range of 0.16 to 2.76 ng/gram of fresh leaf tissue in the transgenic lines. In the laboratory assay, they observed that larval mortality of *S. litura* and *A. janata* varied from 20% to 80% in different transgenic events and the surviving larvae showed weight loss of 27.9–78.1% for *A. janata* and 28.4–87.2% in the case of *S. litura.* Further, *w*hen they conducted field assay, transgenic event AMT-984 showed leaf damage of less than 25% in 43% of tested plants infested with *S. litura* and *A. janata* larvae. Rearing of *Samia cynthia ricini* (eri silkworm) is done by culturing the larvae on castor leaves. Assessment of toxicity of *Bt* proteins to *S. cynthia* indicated high toxicity of *Cry1Aa*, *Cry1Ab*, and *Cry1Ac* proteins to eri silkworm (Kumar et al. 2016). Bioassays against larva of *Samia cynthia ricini* (eri silkworm) using three castor transgenic events (AK1304-PB-1, AMT-894, and AK1304-PB-4) harboring *Cry1Aa* gene showed a weight reduction of 20.2–78.5% suggesting a potential threat of the transgenic events with this gene to ericulture if the transgene escapes through pollen (Muddanuru et al. 2019). Adopting the *in planta* transformation protocol, Kumar et al. (2011) infected 2-day-old seedlings with *Agrobacterium* strain *EHA105* carrying pBinBt8 plasmid containing *cry1AcF* and selected the transformants using kanamycin at 300 mg/L to obtain stable transgenic lines. They performed molecular and western analysis and confirmed the co-integration of *nptII* gene along with *cry1AcF*. Through bioassay they observed that T_1 generation showed resistance against larvae of *Spodoptera litura*, and the stability of the insert gene was confirmed in the T_2 generation plants.

11.4.2 Biotechnological Approaches for Disease Tolerance

Gray mold (*Botryotinia ricini*, Godfrey 1919), charcoal rot (*Macrophomina phaseolina*, Rajani and Parakhia 2009), and vascular wilt (*Fusarium oxysporum* f. sp. *ricini*) are the major diseases causing severe yield losses in castor bean (Tomar et al. 2017).

11.4.2.1 Biotechnology Against Gray Mold Disease

Castor gray mold caused by the necrotrophic fungus *Botryotinia ricini* (Godfrey) Whetzel, particularly its anamorphic phase known as *Amphobotrys ricini* (N.F. Buchw.), is one of the devastating diseases resulting in huge yield losses up to 100% (Godfrey 1919). However, there are a limited number of studies, and the effective disease management strategies are lacking (Soares 2012; Lakshmidevi 2017). Castor is a monotypic species, and reliable level of resistance for gray mold is not found in the germplasm (Anjani et al. 2004; Dange et al. 2005; Araujo et al. 2007) even though some low genetic variability is reported for gray mold tolerance (Anjani 2012). Therefore, genetic engineering is the obvious approach to explore the possibility of imparting tolerance or resistance to gray mold disease in castor bean. One of the better strategies is to combine multiple genes found to possess resistance to necrotrophic fungal pathogens. A phylogenetic analysis with the housekeeping genes indicated that *B. ricini* was closely related to *Botrytis cinerea* (Durgabhavani and Kumar 2009). This facilitated utilizing the success people have reported using genetic engineering approach against *B. cinerea*. Single-gene constructs have not really yielded transgenic plants with exploitable resistance against necrotrophic fungi prompting one to utilize multigene approach to tackle gray mold disease. In this direction, work has been initiated at ICAR-IIOR, and two multigene construct-based expression cassettes, each with distinct set of three genes, have been developed: one with tissue-specific promoters driving each gene independently and the other with a constitutive promoter driving all the three genes polycistronically. In the first gene construct, as gray mold in castor basically infects the inflorescence tissues, each of the chosen three genes was placed under inflorescence-specific promoters (*AtACS4*, *5*, and *7*; Wang et al. 2005) before tandemly cloning within a T-DNA-based binary vector (Durgabhavani et al. 2010; Durgabhavani 2014): *AtEBP* (*Arabidopsis thaliana* ethylene-responsive element binding protein), *ERF1* (ethylene response factor 1), and *BIK1* (*Botrytis*-induced kinase1) that are known to participate in signal transduction during interaction between the necrotrophic fungal pathogen and the host plant (Durgabhavani 2014). These gene constructs have been validated in tobacco model system by developing transgenic plants with individual gene constructs as well as by pyramiding the gene constructs in different combinations (Lakshmidevi 2017).

In the second gene construct, three genes were employed: chitinase (Konda et al. 2010), *RsAFP2* (*Raphanus sativus* antifungal protein2) protein2) (Konda et al. 2009), and *AceAMP1* (*Allium cepa* antimicrobial peptide1). All the three genes were placed under a constitutive promoter (CaMV 35S) with the coding sequence of each separated from the other by self-cleaving signal peptide sequence (2A class) so that they are transcribed together as a single polycistron but self-cleaved post-translationally into distinct gene products (Kumar 2020; Kumar et al. 2020).

Screening of germplasm lines using artificial screening methods has identified some lines that show partial tolerance to gray mold disease in terms of delayed onset of symptoms and restricted spread of disease. Genotype 48-1 is one such genotype. Efforts are on at ICAR-IIOR to identify the genomic regions associated with such

Fig. 11.4 Reaction of RILs of JC12 × 48-1 for gray mold under field conditions

partial resistance and then pool them up once such associations are established in different lines. To identify the putative QTL associated with gray mold resistance in 48-1, a set of 156 RILs of JC12 × 48-1 was evaluated for resistance to gray mold under natural epiphytotic condition (Fig. 11.4).

The disease severity data was used along with genotypic data from 1089 SNP markers in QTL analysis using QTL cartographer. A total of four QTLs (Table 11.2), two on linkage group (LG)-3 and one each on LG-5 and LG-9, was identified at LOD threshold of more than 2.5. Similar efforts are on to identify the genomic regions associated with partial resistance in other germplasm lines as well (Senthilvel, personal communication).

11.4.2.2 Biotechnology Against Charcoal Disease

Castor charcoal rot is a fungal disease caused by *Macrophomina phaseolina*, and its management through crop improvement using biotechnological approaches requires understanding of complex resistance mechanism. Tomar et al. (2017) developed a mapping population $F_{2:3}$ involving a cross between resistant genotype (JI357) and susceptible genotype (SKI338) and phenotyped in sick plot using randomized block design. Three novel quantitative trait loci (QTLs) that revealed 11.3–71.2% of phenotypic variation including one major QTL (LOD score 6.5) on linkage group 2 which explained 71.25 of phenotypic variation were identified. Since polygenes with additive and non-additive gene actions (Desai et al. 2001) are governing charcoal rot resistance, for transfer of the resistance loci, QTL information needs to be refined further (Tomar et al. 2017).

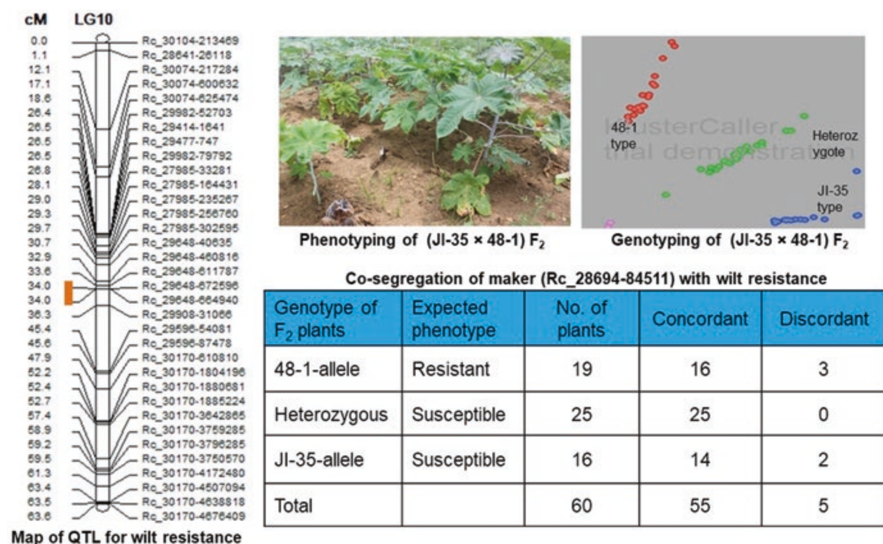
Table 11.2 QTLs associated with gray mold resistance in castor

No.	Linkage group	Marker	Position	LOD score	R^2
1	3	Rc_29929-1526434	34.8	4.02	0.17
2	3	Rc_28093-12497	83.1	3.83	0.13
3	5	Rc_29736-670976	92.9	2.65	0.08
4	9	Rc_29358-25528	48.1	7.77	0.25

11.4.2.3 Biotechnology Against *Fusarium* Wilt Disease

Castor *Fusarium* wilt was first reported from Morocco (Reiuf 1953), and Nanda and Prasad (1974) established that the causal agent is *Fusarium oxysporum* f. sp. *ricini*, a soilborne pathogen, while reporting it from India. *Fusarium* wilt could cause yield loss of up to 77 percent in castor bean (Pushpavati 1995). Several castor germplasm accessions have been reported to be resistant to this disease (Anjani et al. 2004; Lavanya et al. 2011) and can be exploited for castor breeding as genetic diversity exists among resistant genotypes (Anjani 2010).

Complexity associated with pathogen variability, difficulty in pyramiding genes from different sources conferring resistance to the pathogen (as phenotyping will not distinguish the two gene sources), and that of inheritance of resistance pattern to *Fusarium* wilt necessitate the development of molecular markers for screening and selection of resistant progenies in breeding population. In this regard, Zubair (2014) used a core set of 96 castor germplasm that comprised cultivated and wild forms maintained at ICAR-Indian Institute of Oilseeds Research, Hyderabad, India, and identified SSR marker RCM9109 associated with *Fusarium* wilt resistance trait which explained 19.88 percent of the total phenotypic variation. In an attempt to understand the inheritance pattern of wilt resistance, Shaw et al. (2018) used F_2 populations derived by crossing resistant inbred lines (48–1, CI-1, AP42, and AP48) with eight susceptible genotypes and concluded that the mode and nature of inheritance was influenced by gene interactions and genetic background. In the genotype 48-1, wilt resistance was governed by a single recessive gene, and using an F_2 population between JI-35 (susceptible) and 48-1 (resistant), a major QTL governing wilt resistance has been identified in LG-10. Co-segregation of the marker with trait has been established, and a KASP assay has also been developed to identify the homozygous plants carrying the resistance allele (Fig. 11.5). Using both association studies and biparental populations, markers associated with wilt resistance in different donor parents are being investigated at IIOR to develop maker tool kits that will enable pyramiding the loci conferring resistance to wilt pathogen (Senthilvel, personal communication). Further, several castor wilt-resistant monoecious lines have been identified (Manjunatha et al. 2020), and a germplasm accession RG-1624 is confirmed to be resistant to wilt through epiphytotic and multilocation experiments (Lal et al. 2020).



Genotype of F_2 plants	Expected phenotype	No. of plants	Concordant	Discordant
48-1-allele	Resistant	19	16	3
Heterozygous	Susceptible	25	25	0
JI-35-allele	Susceptible	16	14	2
Total		60	55	5

Fig. 11.5 Marker-trait co-segregation for wilt resistance in segregating population of JI-35 X 48-1

11.4.3 Biotechnology for Weedicide-Resistance Engineering

As is the case in cultivation of any field crop, weed infestation in castor bean crop causes decline or some time total loss in crop yield by competing for agricultural inputs and resources including space, light, water, and nutrients (Fartyal et al. 2018). Among weed management strategies, the most popular, time-saving, and economical is the chemical control by spraying certain class of weedicides (or herbicides) that does not adversely affect the normal germination, growth, development, and reproduction of crop plant species. If herbicide-tolerant genotypes are not available in germplasm collections, genetic engineering offers novel opportunity to build resistance in transgenic lines.

Potentials of glufosinate (Cai et al. 2014; Jalaludin et al. 2017; Sheng et al. 2017; Takano et al. 2019) and glyphosate (Cao et al. 2012; Chahal et al. 2017; Ortega et al. 2018) in weed control applications using transgenic approaches have been proved in various crop species including wheat (Cai et al. 2014), potato (Sheng et al. 2017), soybean (Chahal et al. 2017), and chili (Ortega et al. 2018). However, application of genetic engineering strategy for gaining herbicide resistance in castor bean was lacking till 2020. Zhao and his co-workers (2020) have reported for the first time the development of double herbicide-resistant transgenic lines of castor. They have deployed *EPSPS* (5-enolpyruvylshikimate-3-phosphate synthase) and *Bar* (phosphinothricin N-acetyltransferase) genes that impart resistance to herbicides glyphosate into castor cultivars CSR 181 (dwarf) and castor 2129 (tall) using *Agrobacterium*-assisted gene transformation method. They observed that transgenic lines of both the cultivars showed significant level of *EPSP* and *bar* gene

expressions and concomitantly enhanced resistance to glyphosate and glufosinate, respectively. Based on the foundational work of Zhao et al. (2020), further research focusing on genetic engineering of herbicide resistance and development of stable transgenic lines of castor bean is required to facilitate development of sustainable herbicide-resistant cultivars.

11.5 Biotechnological Approaches Against Abiotic Stress Factors in Castor Bean Crop

Castor bean is reported to have resistance to salt, drought, heavy metals, cold, and alkali (Mendes et al. 2015), and it is postulated to be mainly due its sturdy features including tallness and deep roots that enable the plant to reach deeper layer of soil (Ye et al. 2018). Continued breeding for agronomic gains has resulted in development of cultivars with reduced stem height leading to concomitant reduction in deep-rooting ability necessitating the exploration of genetic engineering to improve castor bean crop tolerance to these abiotic stresses (Dange et al. 2005; Severino et al. 2012).

11.5.1 Biotechnology for Imparting Drought Tolerance

Many studies have shown that lignin biosynthesis is enhanced under drought stress and increased accumulation of lignin can reduce plant cell wall water penetration and transpiration, thereby aiding maintenance of cell osmotic balance and protective membrane integrity. Besides, lignin biosynthesis extensively contributes to plant growth, tissue/organ development, lodging resistance, and the responses to a variety of biotic and abiotic stresses (Liu et al. 2018). Lignin production is through phenylpropanoid pathway (Dixon & Paiva 1995) and is catalyzed by phenylalanine ammonia-lyase (PAL) enzyme (Starr et al. 2014; Rao et al. 2018). Lu et al. (2019) adopted antisense as well as overexpression approaches with castor bean PAL (*RcPAL*) to investigate the functional role of PAL in castor bean. They observed that overexpression of RcPAL enhanced the PAL activity and concomitantly increased lignin content (14.44%) resulting in thick stem, deeper and thicker leaf blade, shorter internode, more green leaves, and reduced plant height; while the contrary results were evident when PAL was downregulated with antisense expression; confirming the role of RcPAL in lignin biosynthesis. Their results demonstrate the potential of *RcPAL* for genetic engineering of plant types.

Stress-associated proteins (SAPs) are believed to be produced in response to numerous stress factors in plants: biotic and abiotic stress conditions (Mukhopadhyay et al. 2004; Tyagi et al. 2014; Gao et al. 2016). Wang et al. (2020) reported a

comprehensive profiling of castor bean SAP (RcSAP) using high-throughput RNA-sequencing data. They discovered nine *SAP* genes (*RcSAP*) which showed a great variability in their structural and functional domains, with no common cis-elements. They also found that *RcSAP9* with an AN1-C2H2–C2H2 zinc finger domain was unique to castor bean among the plant species they included in their phylogenetic analysis. They further observed that *RcSAP* expression greatly varied across different tissues as well as under various abiotic stress conditions, namely, cold, drought, heat, salt, abscisic acid (ABA), and methyl jasmonic acid (MeJA), suggesting that *RcSAP* might be transcriptionally regulated in a manner independent of each other and at least partially independent of MeJA and ABA signaling pathways. Based on cytological studies, they observed that SAP proteins were found in different organelles. Based on their research findings, Wang et al. (2020) suggested that SAP might possibly impart ability of castor bean to adapt to diverse edaphic factors and abiotic stress conditions.

GRAS family proteins are unique in the sense that they have highly conserved carboxy-terminal (Pysh et al. 1999) and highly variable amino-terminal (Tian et al. 2004). In plant system, they play an incredible role during stress responses, growth, and development (Gulsen et al. 2010; Liu and Widmer 2014). Xu et al. (2016) identified 48 GRAS genes using castor bean genome sequence data and performed phylogenetic studies comparing with genomes of other plant species including model plant *Arabidopsis thaliana*. Based on tissue expression analysis under a range of abiotic stresses, they suggested that GRAS family proteins in castor bean play a regulatory role during abiotic stress responses and plant growth and development.

Improved drought tolerance and higher water use efficiency (WUE) are important traits for the crops that are grown under rainfed conditions. Aquaporins are key channels and regulators for water transportation and maintenance of cellular water status. A number of studies have provided substantial empirical evidence for the direct role for plant aquaporins in response to abiotic stresses and in situations that affect water availability. There are several reports of elevated expression of aquaporins (AQPs) leading to water stress tolerance in transgenic plants. Also, it has been observed that overexpression of aquaporin led not only to the improved drought tolerance and WUE but also altered other agronomic traits such as improved biomass yield and protein content in switchgrass (Zhang et al. 2020). Zou and co-workers (2015) made a genome-wide analysis of the AQP gene family using the genome sequence as well as transcriptome data and identified 37 AQP genes belonging to 5 classes. They also analyzed the expression pattern of these genes and identified the AQPs expressed differentially in tissues such as root, leaf, flower, seed, and endosperm. The results obtained in this study provide a clear idea of the AQP gene family, and this information could be used for developing transgenic lines for manipulating water acquisition and transport trait.

11.5.2 Biotechnology for Imparting Salt Tolerance

Salinity is one of the global edaphic problems that adversely affect the growth and yield of agricultural crops including castor bean. According to the Food and Agriculture Organization (FAO) estimate, nearly 6% of the world land amounting to over 22% of the world agricultural land is affected by salinity-related problems, and it is the chief cause that limited expanse of the fertile agricultural land on the planet earth (Patel et al. 2015). Soil salinity of 4–7 dS m^{-1} (40–70 mM) is tolerated by castor bean plant, and, beyond this range, increased salinity hampers chlorophyll content, and therefore photosynthesis, leading to reduced growth and hindered development; therefore, it is one of the major hurdles for castor cultivation (Severino et al. 2012).

Sodium exclusion by Na^+/H^+ antiporter coupled with confinement of sodium within large vacuoles so as to prevent it reaching toxic levels in the cytoplasm is the simplest of the numerous molecular adaptation mechanisms developed by plants against salinity stress (Apse et al. 1999; Jha et al. 2011a). Extensive researchers have established that *NHX1* are a class of proteins that are present in the vacuolar membrane system and they help creating proton gradient with the help of H^+-pyrophosphatases and H^+-ATPase, in the process of maintaining ion homeostasis in plant cells. As a foundational work, Apse et al. (1999) overexpressed *Arabidopsis thaliana NHX1* (*AtNHX1*) proteins and observed enhanced salt tolerance. This gene is shown to increase salt tolerance in cotton (He et al. 2005), buckwheat (Chen et al. 2008), and poplar (Jiang et al. 2012). Results of these investigations opened up a novel opportunity for exploiting *NHX1* genes from extreme salt-tolerant (i.e., halophyte) plant species as gene source for salt-tolerance engineering of crop plant species. An extreme halophyte plant species *Salicornia brachiata* is a potential candidate for mining salt-tolerant alleles, genes, and mechanisms (Jha et al. 2011b; Chaturvedi et al. 2012; Tiwari et al. 2014; Udawat et al. 2014; Patel et al. 2015).

Patel et al. (2015) have, for the first time, reported the ectopic expression of *Salicornia brachiata NHX1* (*SbNHX1*) genes that modulated physiological process leading to realization of enhanced salt tolerance in castor bean. They moved *SbNHX1* genes into castor bean genotypes CL7 and CL13 with the help of *Agrobacterium*. After confirming the stable integration of single copy of the transgene (*SbNHX1*), they evaluated the transgenic lines under salt-stress treatments with varying sodium chloride (NaCL) concentrations. Compared to non-transformants, they observed that in addition to elevated levels of salt tolerance, transgenic lines of CL7 and CL13 had enhanced levels of relative water content, K+/Na+ ratio, chlorophyll, and K+ content and declined contents of proline, MDA, Na+, and electrolyte leakage. These findings provide foundation for further research to engineer salt tolerance in castor bean so that marginal salty land can be brought under salt-tolerant castor bean cultivation.

11.5.3 Heavy Metal Tolerance in Castor Bean

Castor bean, being a nonedible and thus not entering the food chain and with its innate ability to produce high biomass under sub-optimal, harsh growing conditions on marginal lands, is considered as a potent crop for phytoremediation of soils with higher quantities of potentially toxic elements such as heavy metals. Molecular mechanisms underlying the tolerance to heavy metals in castor bean, though dependent on the genotype employed, are being understood. Some of the genes involved in conferring this tolerance are already identified, and they are known to be regulated at both transcriptional and posttranscriptional levels (reviewed by Yeboah et al. 2020). This has opened up new vistas for further manipulating this crop to increase its efficiency in phytoremediation as well as a source of genes and regulatory elements to manipulate other crops. Considering the ability of castor bean to act as a potent source of biofuel, Carrino et al. (2020) have opined that these characteristics make castor bean a perfect choice for sustainable biodiesel production along with environmental remediation. They have comprehensively reviewed this aspect with respect to phytoremediation of soils contaminated with different heavy metals. Tolerance to heavy metal as a genetic trait is known to be regulated by microRNAs. It is interesting to note that Celik and Akdas (2019) have investigated the expression pattern of seven heavy metal stress response-related miRNAs and the expression levels of target genes in both leaf and root tissues under three different concentrations of nickel stress. They observed that miR838 was the most responsive to the nickel stress and its target gene *Cu-Zn/SOD* was upregulated in both root and leaf tissues.

11.6 Biotechnology for Plant-Type Engineering in Castor Bean

Even though understanding of molecular mechanisms controlling agronomic traits in castor bean is limited, emerging research reports suggest that it is possible to engineer castor bean for various agronomic traits including plant height (Wang et al. 2021) and seed weight and size (Yu et al. 2019). Economically important woody plants are mainly of two types: timber and non-timber. While timber species are opted for tallness, non-timber plants are desired to be dwarf. Castor bean being a non-timber plant, dwarfism is its most desirable positive trait. Breeding castor bean for dwarfness requires understanding of molecular mechanism of genetic and physiological basis of dwarfness (Wang et al. 2021). Cytologically differential cell growth between tall and dwarf bulk segregants was observed in all the tissues: cambium, phloem, and xylem by them. When they analyzed bulk segregation for the trait in F_2 population advanced from a cross between tall and dwarf castor genotypes, two quantitative trait loci (QTLs) associated with plant tall-dwarf differentiation were found to involve 352 candidate genes. Wang and his team focused on one

of these candidate genes called *Rc5NG4-1*, and physiological and cytological investigation revealed that it encodes IAA transporter protein of the tonoplasts. They found that a single nucleotide polymorphism within the coding region distinguished tall castor plant from the dwarf. Tall phenotype had amino acid tyrosine (*Rc5NG4-1Y*), while dwarf had cysteine (*Rc5NG4-1C*) at position 218 of the IAA transporter. They further confirmed the functional significance of this mutation through heterologous expression in yeast. They observed that there was a significant difference in the capacity of *Rc5NG4-1Y* and *Rc5NG4-1C* to uptake indole-3-acetic acid (IAA). Therefore, this finding not only provides an insight into molecular basis of dwarfism in castor bean but also holds key to breeding for dwarfism in castor bean and other non-timber woody plants (Wang et al. 2021).

Lack of high-density genetic map is the major bottleneck to genomics-assisted crop improvement in castor bean. Using genome sequencing data of 200 individuals of recombinant inbred line (RIL) population, Yu et al. (2019) discovered 8896 high-quality genomic SNP markers, and they used these markers and RILs to construct a high-resolution map covering 1852.33 centimorgan (cM) genetic map and 996 scaffolds belonging to 10 linkage groups, totally covering 84.43% of the castor bean genome. Upon genome collinearity analyses within the castor bean genome as well as comparing it with cassava genome, they confirmed the quality of pseudo-chromosome scale assembly genome and iterated that castor bean had solitary position in Euphorbiaceae family. They identified 16 quantitative trait loci (QTLs) for seed weight and size and also identified the candidate genes in these regions. This work provides a framework for development and utilization of molecular markers for important agronomic traits as well as marker-assisted breeding in castor.

In a crop like castor bean where rich phenotypic variation is not explained by low genetic variability, molecular basis of phenotypic diversity and plasticity can be explained using relationship between phenotypic and epigenetic relationships (He et al. 2017). They assessed 60 landraces from worldwide collection for epigenetic diversity using methylation-sensitive amplification polymorphism (MSAP) technique. Based on the polymorphic DNA-methylated loci, they found that population-level variation was medium, while it was high (3.80–34.31%) among accessions. They observed polymorphism of methylated loci in both organellar and nuclear genomes. Upon phylogenetic and population structure constructions, they found two clades that did not show geographical grouping, suggesting that epigenetic variation was a global phenomenon in castor bean.

11.7 Biotechnology for Oil-Quality Engineering in Castor Bean

Caruncle is an elaiosome seen as a fleshy structure attached to the seeds of members of Euphorbiaceae family. Elaiosomes are known to be rich in lipids and proteins. Investigating the biochemical basis of accumulation of lipids in caruncle of castor

bean, Wan et al. (2019) reported that the mechanism involved in production and accumulation of triacylglycerol (TAG) in caruncle is entirely independent of and different from that of seed fatty acid anabolism. Further, based on transcriptome and transient expression analyses, they proved that selected genes involved in caruncle oil biosynthesis were able to produce and accumulate vegetable oil up to 20-fold more in leaves.

Castor bean oil with its unique hydroxyl fatty acid (12-hydroxyoctadecenoic acid or ricinoleic acid) occupies a special status among industrially and pharmaceutically important vegetable oil sources (Mutlu and Meier 2010; McKeon and He 2015). Ongoing research efforts worldwide suggest a huge potential for biotechnological intervention for furthering oil-quality engineering in castor bean by deployment of enzymes (Li et al. 2021), altering lipid anabolic pathway(s) (van Erp et al. 2011; Kim et al. 2011; Lee et al. 2015; Venegas-Caleron et al. 2016; Lin et al. 2019; Lunn et al. 2020), and exploring possibility of oil production in non-seed tissue (Wan et al. 2019). However, developing genetic engineering strategy to modify fatty acid composition requires detailed understanding of pathways and rate-limiting steps involved in producing desired fatty acid in natural system (Venegas-Caleron et al. 2016; Lin et al. 2019; Wan et al. 2019; Lunn et al. 2020).

Using castor genes for heterologous expression studies in *Arabidopsis thaliana*, mechanism of hydroxyl fatty acid (HFA) production has been widely investigated (Li-Beisson et al. 2013). Biochemical pathway leading to HFA production involves two important steps: production of HFA and its detachment from phosphatidylcholine (Weiss and Kennedy 1956; Weiss et al. 1960; Somerville et al. 2000). While the former step is catalyzed by oleate-12-hydroxylase, the latter is accomplished by phospholipase A (PLA, Lee et al. 2015). By performing heterologous expression of castor oleate-12-hydroxylase and plant phospholipase A (*PLA*) in transgenic line of *Arabidopsis thaliana*, it has been deduced that once released from PC, HFAs are either diverted to the pathway leading to synthesis of triacylglycerol (TAG) or other anabolic pathway(s) (Lee et al. 2015; Lin et al. 2019). Through experimental evidence in transgenic lines of *Arabidopsis thaliana*, it has been proved that by heterologous expression of castor bean genes, higher HFA content in seed oil can be realized in other oilseeds (van Erp et al. 2011; Kim et al. 2011; Lin et al. 2019). Upon heterologous expression of class III patatin-like PLA cDNAs (RcpPLAIIIβ) from castor, it was found that RcpPLAIIIβ plays a role in liberation of HFA from PC during synthesis of unusual fatty acids in developing seeds (Lin et al. 2019).

With growing interest in lipid biotechnology for producing castor bean lines with enhanced ricinoleic acid content in their seed oil, the availability of natural OLE-1 high-oleic castor mutant (Rojas-Barros et al. 2004), which produces low ricinoleic (12-hydroxyoctadecenoic) acid but accumulates high amount of its precursor (oleic acid, Rojas-Barros et al. 2005), and its well-characterized *FAH12* gene (Zhou et al. 2013) motivated Venegas-Caleron et al. (2016) to clone and sequence oleate desaturase (*FAD2*) and hydroxylase (*FAH12*) from mutant as well as wild type. Upon heterologous expression in yeast, they found that modifications at three positions in FAH12 protein of mutant (OLE-1) reduced its hydroxylase activity. Their findings provided insights into molecular mechanism of ricinoleic acid biosynthesis.

Findings of Venegas-Caleron et al. (2016) might also serve to provide framework for investigating mechanism involved in the biosynthesis of other unusual fatty acids in vegetable oils.

Realizing its industrial potential, one of the castor bean enzymes lipase (RcLipase) has been well characterized for its lipid hydrolysis property. According to Li and his co-workers (2021), castor lipase enzyme (RcLipase) performs its catalytic activity in 1,3-regioselective manner on two diverse substrates tripalmitic glycerides and trioleic glycerides, yielding 79.1% sn-2 palmitic acid and 21.3% oleic acid. RcLipase belongs to conservative serine group with serine-aspartic acid-histidine conserved at catalytic center and carries a conserved pentapeptide (GXSXG). Li et al. (2021) heterologously expressed this enzyme in methylotrophic yeast, *Pichia pastoris* GS115, and found that it exhibited the greatest catalytic activity and stability when extracted using solvents toluene and chloroform but was inhibited by copper and zinc ions. Therefore, overexpression of castor bean (Li et al. 2021) has potentiality for enhanced production of oleic acid-palmitic acid-glycerol oleate through plant genetic engineering approach as well as using bioreactor-based approach.

According to Lunn et al. (2020), plants producing special oils such as hydroxy fatty acids (HFAs) show poor agronomic suitability. Therefore, they iterated the need to develop innovative strategies for novel oil production in other oilseed crops, utilizing castor bean as source of special genes. Toward this goal, they genetically engineered *Arabidopsis thaliana* to enhance the efficiency of HFA transfer from phosphatidylcholine (PC), a rate-limiting step hitherto believed to be catalyzed by lysophosphatidic acid acyltransferase (LPCAT), in HFA incorporation onto TAG. When castor LPCAT (RcLPCAT) was co-expressed with castor phospholipid/diacylglycerol acyltransferase in transgenic *Arabidopsis thaliana*, HFA removal from PC, incorporation in diacylglycerol (DAG), and enhanced oil yield were obtained compared to the contrary results obtained when RcLPCAT alone was expressed. Based on their findings, it is clarified that phospholipase A2 enzymes (phospholipid/diacylglycerol acyltransferase) and not the RcLPCAT catalyze efficient removal of the HFA from PC and selective addition of HFA to DAGs. Further, genetic engineering of oilseeds including castor bean using phospholipase A2 enzymes can be explored as a practical option to introduce or enhance, as the case may be, novel fatty acids such as HFA as well as to achieve elevated oil accumulation in oilseeds.

11.8 Biotechnology for Utilization of Castor Bean Oil Cake/Meal

11.8.1 Castor Bean Oil Cake/Meal

The by-product obtained after extraction of oil from oleaginous material is called oil cake/meal, and it is economically important as it is rich in mineral, protein, and other nutrients. Several conventional and innovative oil extraction methods are

followed to obtain commercial oil from oilseeds. Type of the method used for oil extraction not only determines the recovery or yield of the oil but also those of corresponding oil meal and oil cake (Yusuf et al. 2015; Takadas & Doker 2017; Yusuf 2018).

Castor oil cake or meal, being a nonedible by-product, can be readily utilized as organic fertilizer and/or soil amendments to meet the requirements of plant nutrition and soil health management (Reddy 2005; Nagaraj 2009; Rothlisberger et al. 2012; Lewis et al. 2019). Nutrient content of castor seed meal also depends on whether it is decorticated or not. Protein content varies from 20.5% to 46% depending on the method of processing; carbohydrates and fiber may range from 26% to 49%, and mineral content from 10.5% to 15% (Annongu and Joseph 2008). The ash of the castor cake is rich in minerals and contains Ca (17%), P (20%), S (25%), Mg (6%), K (10%), and Fe (6%). Castor oil meal can supply plant nutrients, namely, 4.3 percent nitrogen (N), 1.8 percent phosphorus (P_2O_5), and 1.3 percent potassium (K_2O) on weight-by-weight basis (Reddy 2005; Nagaraj 2009; Rothlisberger et al. 2012), and it can be used as manure (Lewis et al. 2019). Castor bean meal, containing 35% crude protein and 25% fiber, can be utilized as a source of protein for livestock feeding (Lade et al. 2013b). In livestock, reproductive (Silva et al. 2015) as well as meat production (Oliveira et al. 2010; Diniz et al. 2010) can be enhanced particularly in ruminants (e.g., goats, Silva et al. 2015) by feeding castor meal as an alternative protein source. However, the castor bean oil cake/meal contains antinutritional factors such as ricin, ricinine, allergen and chlorogenic acid, lectins, oxalates, phytic acids, and tannins (Balint 1974; Taiwo et al. 2012; Lade et al. 2013b). Therefore, they need to be removed prior to utilization for livestock feeding (Anandan et al. 2005; Lade et al. 2013a; Sousa et al. 2017).

11.8.2 *Conventional Approaches for Removing Antinutritional and Toxic Factors in Castor Cake*

Traditionally various physical and chemical methods have been developed for detoxification of castor oil cake/meal in order to use the cake as animal feed. Physical methods, for instance, are autoclaving (Kodras et al. 1949; Rao et al. 1988; Anandan et al. 2005), boiling (Petrosyan and Ponomorov 1937; Perrone et al. 1966), steam treatment (Kodras et al. 1949; Punj 1988); heating (Tangl 1939), and use of ultraviolet rays (Balint 1972, 1973). Chemical methods include incubation of the meal in the presence of a mild alkali or acid followed by neutralization or mild oxidation with hydrogen peroxide (Kodras et al. 1949); extracting the press cake with halogens and alkalis followed by autoclaving (Massart and Massart 1942); treating with sodium chloride, sodium hydroxide, calcium hydroxide, and calcium hydroxide (Ambekar and Dole 1957; Fernandes et al. 2012) treating the press cake with hot water and chloroform (Rudolph 1942, 1943); 24-hour water soaking along with NaCl (2%) and $Ca(OH)_2$ (0.25%) (Lade et al. 2013a); hydrolysis using acids (Melo

et al. 2008) and enzymes (Le-Breton and Moule 1947); treatment with sodium ricinoleate, potassium permanganate, hydrogen peroxide, or halogens (Carmichael 1927, 1929); and reactive seed crushing (RSC, Dubois et al. 2013). Many of these methods though feasible at lab level or under organized sector, it may not be suitable at the farmer's level to detoxify the castor meal before using it as animal feed. Also, there will be a necessity to check the level of detoxification after every batch of meal is processed. Therefore, compared to physical and chemical treatment strategies, genetic improvement through biotechnological approaches provides economical, efficient, eco-friendly safe strategies (biotechnological approaches reviewed by Ashfaq et al. 2018; Kumaraswamy et al. 2020).

11.8.3 Advanced Approaches for Removing Antinutritional and Toxic Factors in Castor Cake

Emerging and evolving genomic and molecular information reiterate that ricin-free castor cannot be developed using classical mutation breeding approach as ricin family has more than 27 genes including putative genes, pseudogenes, and gene fragments (Chan et al. 2010). Genetic engineering approach employing RNA interference (RNAi) strategy is a viable option as explored by Sousa et al. (2017) using small interfering RNAs (siRNAs) technology to develop a ricin-free castor bean line TB14S-5D.

11.8.3.1 Genomic-Based Approaches

Mutation Breeding

Antinutritional factors reduce the nutritional value of oil cakes/meals by interfering with the digestion, absorption, and availability of nutrients (Nega and Woldes 2018). Through mutation breeding such antinutritional factors can be reduced or nullified in mutant lines and released as varieties (Clarke and Wiseman 2000). If different mutant lines are developed for different antinutritional factors, they can be utilized for gene pyramiding. Soybean mutant lines with low (Gillman et al. 2015; Yu et al. 2019a) and ultralow (Patent No. US20120317675A1) levels of trypsin inhibitors have been reported. This provides foundation for inducing random mutations using chemical or physical mutagens or site-directed mutations using genetic engineering approaches in castor bean. However, as stated earlier, owing to the number of genes coding for the toxic proteins and the problems in throughput estimation of ricin and RCA content, it becomes a difficult proposition to use mutation breeding to eliminate ricin and RCA content. Throughput methods of identifying mutants in specific loci such as Targeting Induced Local Lesions IN Genomes by Sequencing (TbyS) (Tsai et al. 2011), as used in many other crops (Irshad et al., 2019; Irshad et al.

2020), might open up new avenues to exploit induced mutations to identify castor bean lines with reduced or nil ricin and RCA.

Somaclonal Variations

When plants are subjected to tissue culture and are regenerated, there is a potentiality to induce somaclonal variations due to oxidative stress damage (Cassells and Curry 2001; Duncan 1997;Vazquez 2001; Tanurdzic et al. 2008; Ravindra et al. 2012). The genetic and molecular basis of somaclonal variations has been worked out (Krishna et al. 2016; Moniruzzaman et al. 2016). Once genetic variability is created, it is possible to screen and identify the genotype having significantly or completely reduced antinutritional and/or toxic (poisonous) factors in seed (Mujib et al. 2007). Some useful somaclonal variants have been successfully obtained in various crops, e.g., enhanced lysine content in rice (Sharpe and Schaeffer 1993), darker and stable skin color in sweet potato (Moyer and Collins 1983), neurotoxin-free *Lathyrus sativus* (Yadav and Mehta 1995), and fruits with fewer seeds in bell pepper (Bell sweet, Evans 1989). If a high-throughput tissue culture protocol for callus-mediated regeneration is developed in castor bean, then in vitro culture-induced genetic variations (Pina & Errea 2008) could be realized in this crop, and therefore, focused research in this regard needs to be undertaken to develop castor bean mutants with no or reduced toxic and/or antinutritional factors.

Gene Pyramiding

Gene pyramiding is one of the advanced breeding strategies to remove antinutritional factors (Hameed et al. 2018). Through gene pyramiding approach, it is possible to reduce or nullify antinutritional and/or toxic factors in oil meals/cakes by accumulating the favorable loci as it has been done or proposed for other traits in soybean (Anderson et al. 2019), brassica (Mei et al. 2020), rapeseed (Zhou et al. 2018), sunflower (Qi and Ma 2020), groundnut (Janila et al. 2016), sesame (Dossa et al. 2019), linseed (Prabha et al. 2017), castor (Singh et al. 2011), palm oil (Zhang et al. 2018), and coconut (Lantican et al. 2019). Molecular markers associated with or linked to candidate genes and genetic maps of such markers/traits in castor bean need to be developed and utilized for generating ricin-free cultivars.

Genetic Engineering

Genetic engineering offers an immense potential to alter the antinutritional and/or toxic factors in the vegetable oil cake/meal (Kajla et al. 2017; Petersen et al. 2018). Different possibilities of reducing ricin and RCA through biotechnological approaches have been reviewed by Ashfaq et al. (2018). The deployment of genetic engineering approach to knock out or silence the expression of genes related to

allergens and ricin would be highly beneficial. The genes that encode both ricin and RCA (*Ricinus communis* agglutinin) proteins are highly expressed during seed development, but the gene expression could be suppressed up to 10,000-fold with the proper choice of promoter and application of gene-silencing techniques (Chen et al. 2004, 2005). Ribonucleic acid interference (RNAi)-mediated silencing of ricin genes has been achieved at laboratory scale (Sousa et al. 2017). Ricin content being a relatively simply inherited trait and with knowledge of candidate genes governing the trait, efforts need to be made to map these candidate genes as well as to identify complete set of genes governing a particular phenotype (Chan et al. 2010; Sousa et al. 2017). In view of the fact that there are more than two dozen ricin homolog genes and putative pseudogenes (Chan et al. 2010), currently available knowledge on the genome and target genes needs to be utilized in strategizing biotechnological approaches for developing plants with no toxin (Rivarola et al. 2011; Chan 2018). For instance, inactivation of candidate genes could be achieved through transgenic approaches or mutagenesis (Ostergaard and Yanofsky 2004; Lloyd et al. 2005; Zhang et al. 2010; Wang et al. 2014; Chong and Stinchcombe 2019), including the deployment of CRISPR/Cas9 for genome editing (Ma et al. 2015; Lee et al. 2020; Si et al. 2020) and pyramiding of mutant alleles (Malav et al. 2016; Vigano et al. 2018; Chukwu et al. 2019) via molecular marker-empowered breeding approaches. For instance, in soybean antinutritional factor phytic acid has been removed by expressing phytase enzymes through genetic engineering (Clarke and Wiseman 2000). Seed sinapine (Kajla et al. 2017) and glucosinolate (Petersen et al. 2018) contents have been successfully altered in brassica through genetic engineering.

Though limited, attempts are underway in this regard, in castor, using the promoter of native ricin gene (Ashfaq et al. 2009, 2010), a set of gene-silencing constructs have been developed utilizing ihpRNAi, transitive RNAi, and artificial microRNA approaches to target the DNA segments common to ricin and *Ricinus communis* agglutinin (RCA) genes (Sai-Kumar et al. 2009; Soma-Sekhar et al. 2009, 2010). These constructs have been validated using tobacco (Soma-Sekhar et al. 2010), and it may provide means to genetically transform castor bean (Ashfaq et al. 2018). Sousa et al. (2017) explored the RNA interference (RNAi) concept to silence the ricin gene in castor seeds. RNAi is a posttranscriptional gene-silencing mechanism that regulates the expression of protein-coding genes. Constructs to express self-complementary RNA transcripts form a dsRNA, which is processed into small interfering RNAs (siRNAs). These siRNAs trigger a sequence-specific mRNA degradation, leading to gene silencing (Sousa et al. 2017). In a recent development, a Brazilian research group based in Embrapa resorted to RNAi (intron hairpin) for silencing ricin in castor bean (Sousa et al. 2017). Using this technique, a bio-detoxified line TB14S-5D has been developed, which is free from ricin. Non-detection of ricin protein in transgenic castor bean lines, lack of hemagglutination activity, and nontoxicity of the de-oiled meal from transgenic lines further established the effective silencing of ricin and RCA mediated by the intron hairpin RNAi strategy. This has ushered in a new era of utilizing the detoxified, protein-rich, de-oiled meal as a good animal feed.

11.9 Potential of Genome Editing in Castor Bean

Genome editing offers capability to design crops (Young et al. 2019; Bao et al. 2020). The most applied crop improvement tool of the twenty-first century will be genome editing with the first wave of its application being evident in soybean (Bao et al. 2020) and maize (Young et al. 2019). Genome editing can be accomplished by means of four types of genetic engineering tools: zinc finger nucleases (ZFNs, Urnov et al. 2005; Shukla et al. 2009; Townsend et al. 2009; Curtin et al. 2011; Baltes et al. 2014), transcription activator-like effector nucleases (TALENs, Christian et al. 2010; Zhang et al. 2013; Haun et al. 2014), clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas, Jansen et al. 2002; Barrangou et al. 2007; Zhang et al. 2016), and base editing system where nucleotide deaminase is fused with a Cas9-D10A nickase (nCas9, Chen et al. 2017; Li et al. 2017; Zong et al. 2017; Qin et al. 2020). Survey of research literature suggests that CRISPR/Cas9-mediated genome editing in castor bean is yet to be undertaken. Efficient and robust regeneration protocols or tissue culture-independent methods of genetic transformation in castor bean are needed to be developed for harnessing the potentials of genome editing technology to engineer designer cultivars in this crop. As a silver lining, two breakthrough techniques (Ellison et al. 2020; Maher et al. 2020) have been developed recently that allow genome editing without plant tissue culture procedure. This possibly could be employed in castor bean for bio-detoxification purpose as well as for meeting other breeding objectives (Vaikuntapu et al. 2020).

11.10 Omics Studies in Castor Bean

Omics is a powerful tool as it provides insights into comprehensive molecular mechanisms operating metabolic processes responsible for plant growth, development, and interaction with environment including dealing with biotic and abiotic stress factors (Wang et al. 2016; 2019). Understanding of the processes or pathways at molecular level helps in identifying critical genes involved in these processes and their regulatory mechanisms. This in turn offers candidate gene(s) that could be used to tinker these processes through genetic engineering and can help in meeting the breeding objectives. Omics studies, including proteomics, transcriptomics, and comparative genomics, have been taken up in castor bean, and they are summarized here.

11.10.1 Omics for Castor Bean Developmental Biology

Proteomics of nucellus during two developmental stages of castor bean seeds was studied by Nogueira et al. (2013) using GeLC-MS/MS approach, and 553 proteins,

mainly implicated in protein degradation, particularly peptidases related to programmed cell death were identified. But none of the proteins belonged seed storage class. These findings reiterated that the function of nucellus was to mobilize nutrients from the maternal tissue to the developing embryo and endosperm. They also identified, for the first time, isoforms of ricin in the tissues outside the seed endosperm. Further, proteome analysis during seed developmental process has led to identification of 1875 proteins of which 1748 were mapped to castor bean gene models (Nogueira et al. 2013). Based on functional annotation, they found that these proteins were involved in accumulation of seed storage proteins (SSPs), allergens, and toxins. Besides, they also identified few genes encoding seed storage proteins that were expressed during seed development.

Hybrid seed production in castor bean is dependent on the availability of distinct sex variants, pistillate lines that produce only female flowers in the spike and the monoecious lines that produce both male and female flowers on the spike. Seeds harvested on pistillate lines when they are grown in isolation along with the intended monoecious plants that act as pollen donors are hybrids. However the instability in pistillate trait leads to sex revertants that become either monoecious in nature or they produce interspersed staminate flowers and thus lead to selfed seeds on the spikes of pistillate plants. Therefore, it will be of great significance if the molecular mechanism behind sex reversion is understood in castor bean. To obtain some hints involved in sex expression and provide the basis for further insight into the molecular mechanisms of castor plant sex determination, differential gene expression analysis was carried out through the transcriptomes of apices and racemes derived from female and monoecious lines (Tan et al. 2016). More than 3000 of differentially expressed genes (DEGs) were detected at 3 developmental stages between the 2 sex types, and many of them were validated using qPCR technique. This study has provided some insights into the genes and pathways involved in manifestation of sex types in castor bean. In an interesting study, Parvathy et al. (2021) have reported that initially the flowers borne on the inflorescence were bisexual in nature which later changed to unisexual flowers (either female or male flower) depending on the genotype and temperature; and they opined that sex reversions as well as high sexual polymorphisms in castor bean were due to alterations in the floral developmental pathways.

Transcriptome analysis using high-throughput sequencing technologies has now shown that majority (almost 90%) of the eukaryotic genome is transcribed (Kung et al. 2013; Ariel et al. 2015) even though only 2% of the transcripts are translated and thus the concept of "junk DNA" is gradually fading off. The untranslated RNAs are termed noncoding RNAs (ncRNAs) which vary qualitatively and quantitatively across tissues and conditions and need to be empirically determined. The ncRNAs consist of a diverse range of transcripts, which vary in size, ranging from 20–30 nucleotides (nts) for small ncRNAs to more than 200 nts for long ncRNAs or lncRNAs, and they have been characterized in many plants (Rai et al. 2019).

MicroRNAs (miRNAs) are a class of small RNAs (sRNAs) that usually downregulate the gene expression posttranscriptionally by complementary binding to the cognate target mRNAs (messenger RNAs), facilitating their degradation or

blocking the translation process. MicroRNAs are known to play crucial roles in virtually every aspect of plant life including nutrient uptake, plant developmental process right from germination through reproduction, seed development and maturation, and adaptation to different biotic and abiotic stresses (Millar 2020). In castor bean, an attempt was made to identify miRNAs by deep sequencing the small RNA libraries prepared from five tissues, viz., root tips, leaves, developing seeds at two stages (at the initial stage and at the fast oil accumulation stage), and endosperm (Xu et al. 2013). With their efforts, Xu and co-workers identified 86 conserved miRNAs including 63 known and 23 novel ones, and they also identified variants/isoforms of 16 miRNAs. Combining the annotations and qPCR analysis, they annotated 72 novel miRNAs and 20 of them were validated, and they proposed the target transcripts of the novel miRNAs. As a fundamental work, Cassol et al. (2016) have identified a set of reference genes that could be used for qPCR analysis of both mRNAs and miRNAs under drought condition. This basic information should actually help other workers for taking up qPCR work with different tissues and under drought stress condition. Celik and Akdas (2019) have studied the expression pattern of seven heavy metal stress response-related miRNAs and the expression levels of target genes in castor bean when exposed to different concentrations of nickel metal, and they have found that this trait is regulated by miRNAs.

Apart from miRNAs there are many other noncoding RNA molecules that play a regulatory role in plant's life. Long noncoding RNAs (lncRNAs) are known to regulate processes through different modes (Rai et al. 2019) and are known to regulate plant development, disease resistance, nutrient acquisition, and other biological processes through chromatin remodeling, histone modification, pri-mRNA alternative splicing, or acting as "target mimicry" (Jha et al. 2020). Based on the importance of lncRNAs in different processes in plants, recently a database of lncRNA (PLncDB V2.0) has been developed (Jin et al. 2021). In castor bean, mining diverse RNA-seq data, 5356 lncRNAs have been catalogued, and the potential role of lncRNAs in regulating the development of endosperm and embryo has been demonstrated (Xu et al. 2018). This foundational study has opened up a new dimension in our understanding of the gene regulation in castor bean.

Using the genome sequence of castor, Han et al. (2020) have identified 34 genes responsible for autophagy, a process that helps in turning over damaged organelles or recycling cytoplasmic contents in the cell, and verified their expression pattern using transcriptomics as well as qPCR with different tissues during seed maturity and germination. They observed that autophagy genes (ATGs) were significantly upregulated during later stages of seed coat development and associated with the lignification of cell wall tissues. Their analysis further implicated ATGs in decomposition of storage oils during germination of castor seeds. This study has provided insights into understanding the role of autophagy in mediating seed development and germination.

11.10.2 Omics for Castor Bean Abiotic Stress Biology

Cold stress is one of the serious problems for cultivation of agricultural crops (Wang et al. 2016) including castor bean (Debnath et al. 2010) as it restricts the crop growth by inhibiting germination and low biomass (Jiang and Wen 2008; Wang et al. 2019). Imbibed seeds of a Chinese elite variety Tongbi 5, subjected to control condition (30 °C) and cold stress treatment (4 °C), were used to identify 127 differential abundance protein species (DAPS) based on isobaric tag for relative and absolute quantitation (iTRAQ) strategy (Wang et al. 2019). They found that these proteins were involved in imparting cold stress tolerance to imbibed castor bean seed by increasing of unsaturated fatty acid (UFA), by promoting protein synthesis (Kosmala et al. 2009), and by protecting cell against cold-induced damage (Wang et al. 2016).

11.10.3 Omics for Detecting Ricin

While biological assays, namely, cytotoxicity assay, real-time PCR, and time-resolved fluorescence, can provide presumptive evidence, mass spectrometric assays, namely, mass spectrometry-based ricin functional assay and mass spectrometry-based ricin functional assay, provide confirmatory evidence. Besides, growing biosecurity (Schieltz et al. 2011), forensic, and public health concerns (Bradberry et al. 2003; Audi et al. 2005; Guo et al. 2014) of ricin have led to the development of various investigative tools of proteomics to detect ricin. For instance, isobaric tag for relative and absolute quantitation (iTRAQ) (Schieltz et al. 2011; Wang et al. 2019), and off-line hydrophilic interaction chromatography (HILIC), were steps of peptide fractionation preceding the reverse-phase nanoLC coupled to a LTQ Orbitrap (Nogueira et al. 2013). Database search coupled to tandem mass spectrometry analysis further helps to rule out presence of any other proteins (Schieltz et al. 2011; Guo et al. 2014).

11.11 Future Perspectives

In the recent past, there are several new leads that have come up in castor bean research, and this has opened up further avenues to use the information in improving this crop through biotechnological approaches. In this section, we highlight such leads and offer opinions regarding how these developments could be used in castor bean improvement. There is an urgent need to mine elite genes and/or alleles from wild material of castor bean to aid genomics-assisted breeding as well as genetic engineering of castor bean against diseases and insect pests (Agyenim-Boateng et al. 2019). More research needs to be undertaken for augmenting scorable SNPs with high call rate and repeatability. Focused studies on genomics and

comparative genomics need to be undertaken on a worldwide consortium mode involving multidisciplinary studies and integrated approaches. Such studies provide not only insights into molecular events during evolution of genome and selection during domestication but also serve as valuable resource for candidate gene-trait associations, marker-trait association, gene-pathway analysis, and understanding of gene-gene cross talk. More research should focus on developing suitable marker tool kits to address biotic stresses such as charcoal rot, gray mold, and wilt and insect pests so that they could be used in routine breeding programs. High-resolution genetic map could be used for development and utilization of molecular markers associated with important agronomic traits such as branching, seed size and number, test weight, ability to tolerate stress conditions, nutrient use efficiency, ideal plant type, as well as marker-assisted breeding in castor.

Of late, omics research in castor bean has started contributing to our understanding of the molecular basis of cellular processes as well as the response of castor bean to different biotic and abiotic stress conditions. These efforts would provide us suitable genes and their regulators involved in the manifestation of traits and thus would provide molecular tools to manipulate the traits precisely. Omics studies should focus on (i) understanding the regulation of toxic protein (ricin and RCA) accumulation as they are known to be tightly regulated both spatially and temporally (Loss-Morais et al. 2013); (ii) mechanism of drought, salinity, and heavy metal tolerance; (iii) developmental milestones of the plant; and (iv) accumulation of seed oil.

Developing a reliable and efficient transformation procedure is essential to deploy the candidate gene(s) or gene constructs and test large number of transgenic lines for the expected phenotype. Since castor bean is recalcitrant to tissue culture, tissue culture-independent *Agrobacterium*-mediated transformation coupled with supplementary techniques such as piercing coupled with acupuncture-vacuum filtration (Lu et al. 2018), carbon nanotube carriers (Burlaka et al. 2015; Kwak et al. 2019), etc. needs to be tried and refined in castor bean so as to exploit their potentials to enhance *Agrobacterium*-mediated transformation efficiency in the crop. Once such a protocol is established, it could be used for genome editing, realizing bio-detoxified transgenics, herbicide-tolerant lines, fatty acid-modified lines, gray mold-resistant lines, etc. Bioprospecting the specific genes from microflora that might help in selective degradation of ricin and RCA in the mature seeds of castor bean might also open up new avenue for bio-detoxification through genetic engineering. Such genes could be expressed under promoters that are active in the endosperm and toward the final stages of castor seed maturation, so that the mature seeds of such transgenic lines could be free of ricin and RCA.

In essence, there should be incisive and intense research work to be carried out in castor bean to identify candidate genes involved/implicated in different developmental stages of the crop and in manifestation of the agronomic traits so that such genes could either be overexpressed or silenced, as the case may be, to design castor bean plant and meet the breeding objectives. This can be achieved only if there is integration of different approaches of molecular investigations coupled with genetic engineering of the traits.

11.12 Conclusions

Castor bean oil is the only plant source of a specialized fatty acid "ricinoleic acid" and, therefore, occupies a special status among industrially and pharmaceutically important vegetable oil sources. However, it is facing varying factors as bottlenecks in its production and value addition worldwide. Ongoing research efforts hint at a huge potential for biotechnological intervention in removing these bottlenecks. With the genome sequence available, this crop has become amenable for genomics-assisted selection, and already research efforts in this direction have started giving dividends. Genomics-assisted breeding and genetic engineering have been used by researchers to achieve limited yet promising successes in this crop. While the former strategy can be strengthened by genomic and genetic resources, the latter requires the robust transformation techniques. In spite of nonavailability of an efficient transformation protocol, there are reports of developing transgenic lines in this crop for insect resistance, weedicide tolerance, bio-detoxified lines, etc. Omics studies in the crop have started to offer new avenues to understand the molecular mechanism of the inherent hardiness of castor bean to withstand different types of abiotic stresses including drought and salinity, heavy metals in the soil, etc. Thus, there is enough evidence to show that biotechnological approaches are crucial to genetic improvement of castor bean crop. Genetic engineering could lead to development of transgenic lines useful for breeding against varied biotic and abiotic stress factors as well as ideotype engineering, particularly to suit high-density planting and mechanical harvesting. Emerging "tissue culture-independent" and *in planta* techniques hold key to success of genetic engineering in castor bean.

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Chapter 12
Genetic and Molecular Technologies for Achieving High Productivity and Improved Quality in Sunflower

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Abstract Globally, sunflower (*Helianthus annuus* L.) occupies a prominent position among edible oilseed crops, and its credit goes to the systematic breeding efforts performed in this crop, especially for oil content during the first half and exploitation of heterosis in the middle of the second half of the twentieth century. Today, sunflower ranks second biggest crop after maize cultivated through hybrid seed worldwide. Primarily, major breeding objectives in sunflower focused in seed and oil yield. However, with changing market demands, current breeding objectives in sunflower have shifted to oil quality along with productivity constraints prevalent in specific agro-climatic zone. The narrow genetic base of cultivated sunflower led main thrust for extensive utilization of both wild genetic resources and mutagenesis through conventional breeding for a long time now. This resulted in the creation of substantial genetic variability for different traits of interest, but progress in this field has been relatively slow and limited. The rapid advances in marker technology paved the way for molecular breeding in sunflower as a tool for acceleration of the

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breeding process. From the last three decades, a large number of random as well as gene-specific molecular markers have been developed and validated in sunflower, but their application depends on the research objectives. Moreover, recent large-scale DNA sequencing and high-throughput screening techniques transformed the way that crop breeding is performed. In present day, reverse genetics approaches have also become an important goal for researcher in many crops including sunflower. New molecular methodologies such as TILLING including EcoTILLING which entails detection of natural variation allowed to utilize induced as well as existing genetic variation for development of new varieties. In this chapter, we summarized information on available genetic resources, genetics of different traits together with validated molecular markers for their utilization in sunflower breeding programme. Finally, this chapter also reviewed the application of product-based alternative new breeding techniques, as the products developed in this manner could occur naturally over time.

Keywords Sunflower · *Helianthus annuus* L. · Breeding · Genetics and genomics

12.1 Introduction

Oilseed crops are major providers of calories for humans and livestock, and raw material for a wide range of industrial products. Over the coming decade, global crop production is projected to expand by 84 mt for oilseeds (OECD FAO (2019). Burgeoning human population and flourishing feed industry for poultry and livestock is expected to boost demand for oilseeds and their products resulting in significant changes in global oilseed markets. Sunflower (*Helianthus annuus* L.) is one of the main oilseed crops that is widely grown across the globe and ranks third in both oilseed produced and oilseed meal among protein feed sources.

12.2 Origin, History and Botany

The origin of sunflower is considered in North America and is believed to have domesticated by the Indians as early as 2300 BC based on archaeological evidence. From there, it was successfully introduced by Spanish explorers in Europe in the 1500s for ornamental and medicinal purposes (Heiser et al. 1969). Nonetheless, successful breeding efforts for increased seed and oil content in the former USSR during the middle of the twentieth century turned it into one of the most important oilseed crops. Yet, the discovery of cytoplasmic male sterility (CMS) in France (Leclercq 1969), followed by the identification of fertility restoration (Kinman 1970) system in this crop, enabled commercial hybrid seed production resulting in widespread popularity of sunflower in other parts of the world. Today, sunflower cultivation dispersed throughout the world and ranks with soybean,

rapeseed-mustard and groundnut as one of the four most important edible oil crops. The Russian Federation followed by Ukraine, the European Union (Romania, Bulgaria, Spain, France and Hungary), Argentina, Tanzania, China, Kazakhstan, Turkey, South Africa, the USA and India are major sunflower-producing countries. Among these major sunflower-growing countries, Hungary has the highest productivity (3.03 t/ha), while in India the productivity is lowest (0.82 t/ha) (FAOSTAT 2019).

Cultivated sunflower is an annual plant and belongs to the family Asteraceae, alternatively Compositae. This crop is often classified as thermo-sensitive but insensitive to photoperiod because it can flower through a wide range of daylengths (Robinson 1978). Sunflower inflorescence is a capitulum or head consisting of two types of flowers: an outer whorl of showy and generally yellow (pale or orange yellow) or reddish colour ray flowers, while central disc flowers are arranged in arcs radiating from the centre of the head. Ray flowers are normally sterile or pistillate, having a rudimentary pistil and vestigial style and stigma, but no anther. On the other hand, disc flowers are perfect (contains both stamen and pistil) and arranged in arcs radiating from the centre of the head that produce seeds generally termed as achene. New disc flowers emerge at regular intervals close to the centre of the head and move outward as the head diameter increases. Anthesis begins from the outer whorl rows of disc flowers and proceeds towards centre of the head. Generally, one to four rows of disk flowers open successively daily and complete head bloom within 5–10 days depending upon the genotype and prevailing environmental conditions (Dedio and Putt 1980).

Sunflower is a highly cross-pollinated crop which is attributed to the high level of self-incompatibility (sporophytic) due to protandrous nature of the disc flowers (Habura 1957; Fernandez-Martinez and Knowles 1978). Sunflower pollen lacks the buoyancy of grass pollen and is not conducive to wind dispersal but can move little by wind. Thus, insects particularly the eusocial bees (*Apis mellifera* L.) are the main pollinating agents.

12.3 Sunflower Genetic Resources

The process of transfer of desirable genes from the uncultivated or crop wild relative into cultivated germplasm is known as germplasm enhancement. Thus, conservation of available genetic resources including crop wild relatives is necessary for any crop improvement programme in the future (Campbell et al. 2010). Nonetheless, majority of breeding targets such as economic yield enhancement and resistance to (a)biotic stresses are everlasting, but some evolves rapidly due to the continuous change in food habits, adaptation to changing climatic condition and technological innovation driven by markets. Wherefore, effective collection and their conservation as well as utilization of diverse and rich germplasm are necessary to overcome these challenges (Terzic et al. 2020).

At world level, the Vavilov Research Institute for Plant Genetic Resources (Russia) is the oldest gene bank for sunflower. Yet, other gene banks located in

Table 12.1 List of major sunflower germplasm/gene bank

Country	Germplasm/gene bank
Argentina	National Agricultural Technology Institute [Instituto Nacional de Tecnologia Agropecuaria (INTA)], Cordoba
Bulgaria	Dobrudzha Agricultural Institute (DAI), General Toshevo
China	Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (CAAS), Wuhan
France	French National Institute for Agricultural Research [Institut National de la Recherche Agronomique (INRA)], Toulouse
Germany	Leibniz Institute of Plant Genetics and Crop Plant Research [Leibniz-Institutfür Pflanzengenetik und Kulturpflanzenforschung (IPK)], Gatersleben
India	ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad
Romania	National Agricultural Research and Development Institute (NARDI), Fundulea
Russia	Vavilov Research Institute of Plant Industry (VIR), Saint Petersburg
Serbia	Institute of Field and Vegetable Crops (IFVC), Novi Sad
Spain	National Institute for Agronomic Research [Instituto Nacional de Investigaciones Agronómicas (INIA)], Madrid
USA	USDA sunflower gene bank, US National Plant Germplasm System (NPGS), Ames, Iowa

America (the USA and Argentina), Europe (France, Serbia, Romania, Spain, Bulgaria and Germany) and Asia (India and China) also play an important role in maintaining the vast genetic variability of this crop (Table 12.1). In India, introduction, collection and maintenance of sunflower germplasm on a long-term basis are carried out by ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi, while germplasm management unit (GMU) of ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad, in close collaboration with NBPGR collects, evaluates and maintains sunflower germplasm on short- to medium-term basis and makes it available under All India Coordinated Research Project on Sunflower (AICRP-Sunflower) to various researchers particularly state agricultural universities across India.

There are 53 wild species in the genus *Helianthus*, including 14 annual and 39 perennials (Seiler and Jan 2014). Together with cultivated sunflower, wild *Helianthus* annual species are also diploid, with chromosome number ($2n = 2x = 34$), whereas perennial species include diploids ($2n = 2x = 34$), tetraploids ($2n = 2x = 68$) and hexaploids ($2n = 2x = 102$). Among diploid annual *Helianthus*, besides wild *Helianthus* (45), GMU of ICAR-IIOR, India, is also maintaining *H. argophyllus*; *H. neglectus*; *H. debilis* ssp. *cucumerifolius*, *silvestris*, *tardifolius* and *vestitus*; *H. niveus* ssp. *canescens*; *H. petiolaris* spp. *fallax* and *petiolaris*; and *H. praecox* ssp. *hirtus*, *praecox* and *runyonii* and utilizing them in its pre-breeding programme (Figs. 12.1 and 12.2). Moreover, there are also 13 perennial diploid species and 12 perennial species belonging to 6 each tetraploid and hexaploid species (Table 12.2). Annual wild *Helianthus* species and their interspecific derivatives with cultivated *Helianthus* together can serve as potential sources of novel genetic variability for

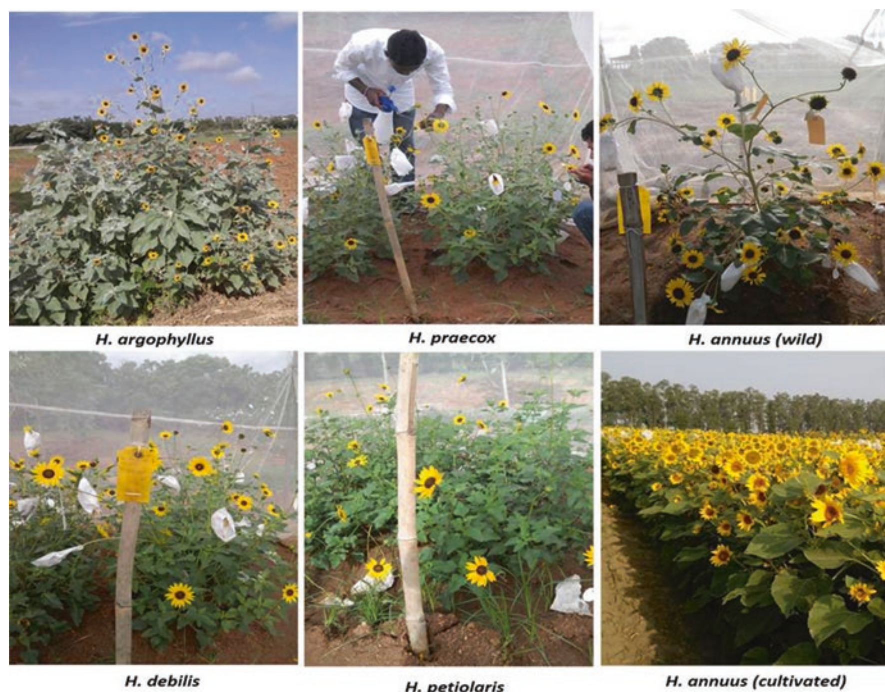

Fig. 12.1 Maintenance of wild relatives of cultivated sunflower

sunflower improvement purposes especially against new emerging challenges under climate change scenario.

Screening of wild species and their interspecific derivatives confirms the presence of desirable seed oil quality, host-plant resistance, cytoplasmic male sterility (CMS) and its fertility restorer genes (Thompson et al. 1981; Seiler 1992). For instance, silver leaf sunflower (*H. argophyllus* T. & G.) is closely related to wild *H. annuus* L. (Heiser et al. 1969) and displays common morphological characteristics such as general plant architecture and large leaves in contrast to other annual species with small leaves. Besides potential donor for fertility restoration genes, *H. argophyllus* is also a novel source of several desirable seed oil qualities (altered fatty acid composition), abiotic (salt and drought tolerance) as well as biotic stress traits such as resistance to downy mildew and some races of rust and tolerance to several insect pests including the sunflower beetle (*Zygogramma exclamationis*) and sunflower midge (*Contarinia schulzi*) (Thompson et al. 1981). In the past, interspecific derivatives developed from crosses between *H. annuus* and *H. argophyllus* resulted in development of numerous genetic pools containing useful traits from wild species into the cultivated background (Miller et al. 1992; Seiler 1992). For instance, dominant gene for all known races of downy mildew (Seiler 1991; Jan and Gulya 2006; Wieckhorst et al. 2010) including the new downy mildew resistance gene (Pl18) was introgressed from *H. argophyllus* (PI-494573) into cultivated

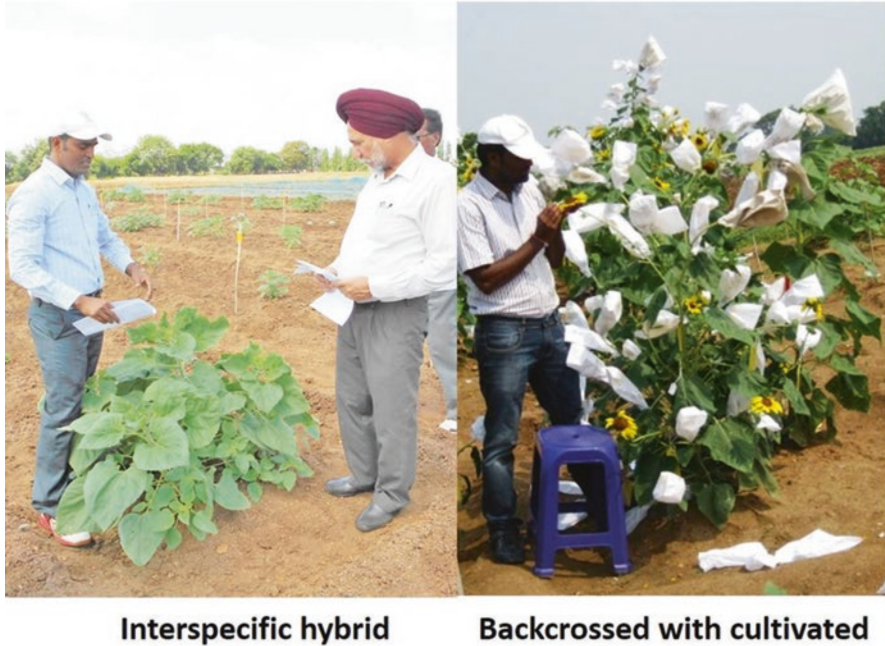

Fig. 12.2 Utilization of wild relatives in sunflower improvement

sunflower (Qi et al. 2016), resulting in the development of downy mildew-resistant germplasm HA-DM1. Moreover, silver leaf sunflower has also been reported as an important reservoir of useful genes for drought tolerance and resistance to parasitic weed orobanche (Jamaux et al. 1997; El Midaoui et al. 2003; Jan et al. 2008). Nonetheless, there is no use of these potential sources until these desirable genes are not exploited through introgression into cultivated sunflower to broaden the existing narrow genetic base and further enrich the existing varieties with desired agronomically important traits.

12.4 Genetics of Breeding Objectives in Sunflower

Basic directions in oilseed crop improvement programmes include high seed as well as oil yield with acceptable oil quality and resistance to prevalent biotic and abiotic constraints of specific production zone. As in other field crops, genetic gain for seed yield has nearly always been a main subject of research in sunflower. The main genetic base of modern sunflower breeding goes back to the work of Pustovoit at VNIIMK in Russia who initiated breeding for high-yielding, open-pollinated varieties (OPVs) with oil content of up to 50%. Kaya et al. (2012) extensively described different methodologies such as mass selection, Pustovoit's method for individual

Table 12.2 *Helianthus* species maintained at ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad, India

Species	Habit	No. of accessions	Desirable traits
Helianthus crop wild relatives (diploid)			
H. annuus (wild)	Annual	45	Rust and downy mildew (DM)
H. argophyllus	Annual	22	Rust, DM, brown stem canker (BSC) and *Orobanche cumana*
H. debilis ssp. *cucumerifolius*	Annual	18	–
H. debilis ssp. *silvestris*	Annual	15	Ornamental
H. debilis ssp. *tardifolius*	Annual	1	BSC
H. debilis ssp. *vestitus*	Annual	1	Ornamental
H. neglectus	Annual	14	–
H. niveus ssp. *canescens*	Annual	18	–
H. petiolaris	Annual	88	Rust and BSC
H. petiolaris ssp. *fallax*	Annual	1	Rust and BSC
H. petiolaris ssp. *petiolaris*	Annual	1	Rust
H. praecox	Annual	11	Rust and DM
H. praecox ssp. *hirtus*	Annual	1	Rust, DM and BSC
H. praecox ssp. *praecox*	Annual	1	Rust and DM
H. praecox ssp. *runyonii*	Annual	25	Rust and DM
H. angustifolius	Perennial	1	Rust
H. atrorubens	Perennial	4	Rust
H. cusickii	Perennial	1	–
H. divaricatus	Perennial	20	*Alternariaster* leaf blight (ALB), BSC
H. giganteus	Perennial	33	DM, BSC, *S. sclerotiorum*, *Verticillium dahlia*
H. glaucophyllus	Perennial	1	DM, PM
H. grosseserratus	Perennial	18	DM
H. maximiliani	Perennial	31	ALB, DM, BSC, *S. sclerotiorum*
H. microcephalus	Perennial	8	DM, powdery mildew (PM)
H. mollis	Perennial	11	ALB, DM, BSC, *S. sclerotiorum*
H. nuttallii spp. *rydbergii*	Perennial	57	DM
H. occidentalis ssp. *plantagineus*	Perennial	3	ALB, DM
H. paradoxus	Perennial	12	–
H. pumilus	Perennial	1	DM
H. salicifolius	Perennial	4	DM, BSC, *S. sclerotiorum*
H. simulans	Perennial	3	ALB, BSC
Helianthus crop wild relatives (tetraploid)			
H. ciliary	Perennial	1	DM and *P. macdonaldi*
H. decapetalus	Perennial	17	ALB, DM, BSC, PM
H. hirsutus	Perennial	11	Tolerant to ALB, resistant to DM and rust

(continued)

Table 12.2 (continued)

Species	Habit	No. of accessions	Desirable traits
H. laevigatus	Perennial	2	DM, *P. macdonaldi*
H. pauciflorus	Perennial	18	ALB, DM
H. smithii	Perennial	1	DM, PM, stem canker
Helianthus crop wild relatives (hexaploid)			
H. californicus	Perennial	1	Prohibited species in India
H. eggerti	Perennial	1	DM
H. resinosus	Perennial	8	ALB, PM and BSC
H. rigidus	Perennial	9	DM
H. strumosus	Perennial	13	*P. macdonaldi*
H. tuberosus	Perennial	18	ALB, DM, BSC, *S. sclerotiorum*

Source: Dudhe et al. (2015)

plant selection and heterosis breeding attempts performed over the period for cultivars development in sunflower.

Analysis of sunflower hybrid trials data suggests that with optimum plant architecture, it is possible to develop sunflower hybrids with a genetic potential for seed yields of 6 t/ha with 55% seed oil content (Skoric et al. 2007; Skoric 2012).Notably, seed yield is highly influenced by environment and genotype by environment interactions. Moreover, the genetics of seed yield is very complex because it is a quantitatively inherited trait which is governed by multiple genes with minor effects and also depends on several component traits (Table 12.3). Thus, the knowledge of mode of inheritance of seed yield and its contributing traits is essential for sunflower breeders to plan accurately for the genetic improvement of this crop.

Initially, Unrau and White (1944) proposed the exploitation of hybrid vigour or heterosis in sunflower because the manifestation of heterosis in hybrid depends on both additive and non-additive gene action, while open-pollinated varieties (OPVs) exploit additive gene action for the improvement of plant traits. Besides high seed yield potential, hybrids not only show better response to high input but also present uniformity in height and maturity compared to OPV and synthetic cultivars that facilitates harvest and easy possibility of cultural applications. Despite the presence of significant heterosis, a cost-effective system that would promote an efficient hybrid seed production remains always an issue in all the crops including sunflower. Putt (1964) continuously worked on the development of hybrid cultivars in sunflower at Morden Manitoba (Canada) but did not able to succeed for large-scale commercial hybrid production due to incomplete male sterility. Thus, OPVs were only source of sunflower production until the discovery of cytoplasmic male sterility (CMS) and its corresponding fertility restorer genes (Leclercq 1969; Kinman 1970). Today, sunflower ranks second biggest crop after maize cultivated through hybrid seed. However, sunflower hybrid production still mainly depends on a single CMS source (PET-1), derived from *Helianthus petiolaris* and a few fertility restoration genes. Thus, identification of new CMS sources in sunflower is highly relevant

Table 12.3 Genetics of seed as well as oil yield and their major contributing traits in sunflower

Character	Type of gene action	References
Days to flowering	Additive	Dua (1979), Alba et al. (1985), Shrikanth (1996), Ortis et al. (2005) and Tabrizi et al. (2012)
	Non-additive	Naik et al. (1988), Radhika et al. (2001), Sharma et al. (2003) and Manivannan et al. (2005)
	Dominance × dominance	Manjunath (1978) and Thakur (1992)
Days to maturity	Additive	Dudhe et al. (2011), Tabrizi et al. (2012) and Maryam et al. (2015)
	Non-additive	Dua and Yadava (1983), Harini (1992), Amenla (1996), Bajaj et al. (1997), Ashoka et al. (2000), Phad et al. (2002), Kaya and Atakisi (2004), Reddy and Madhavilatha (2005) and Patil et al. (2017)
Plant height	Additive	Miller and Hammond (1991), Ortis et al. (2005) and Tabrizi et al. (2012)
	Dominance	Velkov (1971), Manjunath (1978) and Singh et al. (1987)
	Partial dominance	Kongchuensin and Marinkovic (1984)
	Additive and dominance	Lay and Khan (1985), Goksoy et al. (2001) and Maryam et al. (2015)
	Dominance, complementary epistasis	Rao (1979)
	Non-additive	Sharma et al. (2003), Manivannan et al. (2005), Dudhe et al. (2011) and Patil et al. (2017)
	Dominance × dominance	Thakur (1992)
Head diameter	Additive	Alba et al. (1985), Sharma et al. (2003) and Tabrizi et al. (2012)
	Dominance	Manjunath (1978) and Maryam et al. (2015)
	Dominance × dominance	Thakur (1992)
	Additive and dominance	Goksoy et al. (2001)
	Non-additive	Naik et al. (1988), Manivannan et al. (2005), Reddy and Madhavilatha (2005), Dudhe et al. (2011) and Patil et al. (2017)
Stem diameter	Additive	Tabrizi et al. (2012)
	Additive and dominance	Maryam et al. (2015)
Seed yield/plant	Additive	Sharma et al. (2003) and Azam et al. (2014)
	Dominance	Manjunath (1978), Dua (1979), Thakur (1992), Goksoy et al. (2000) and Maryam et al. (2015)
	Partial dominance	Rao (1979)
	Non-additive	Alba et al. (1985), Reddy and Madhavilatha (2005), Dudhe et al. (2011), Seyed et al. (2013), Vikas and Supriya (2017) and Patil et al. (2017)
	Additive and non-additive	Tabrizi et al. (2012)
	Dominance, duplicate epistasis	Marinkovic et al. (2006)

(continued)

Table 12.3 (continued)

Character	Type of gene action	References
Test weight	Additive	Ortis et al. (2005), Tabrizi et al. (2012) and Maryam et al. (2015)
	Dominance	Dua (1979) and Singh et al. (1999)
	Dominance × dominance	Manjunath (1978) and Thakur (1992)
	Non-additive	Sharma et al. (2003), Kaya and Atakisi (2004), Reddy and Madhavilatha (2005), Dudhe et al. (2011) and Patil et al. (2017)
	Dominance and additive	Goksoy et al. (2001)
Oil content	Additive	Fick (1975), Manjunath (1978), Sharma et al. (2003) and Maryam et al. (2015)
	Dominance × dominance	Thakur (1992)
	Non-additive	Dua and Yadava (1983), Chidananda (1985), Kumar et al. (1998), Ortis et al. (2005), Reddy and Madhavilatha (2005), Dudhe et al. (2011) and Patil et al. (2017)
	Additive and non-additive	Tabrizi et al. (2012)

because the over-dependence on a single CMS source may threat hybrid seed production.

Since 1969, sunflower researchers had identified several new alternate sources of PET-1 CMS, but availability of effective fertility restoration genes (*Rf*) is still a major limitation, because most of restorers of PET-1 failed to restore fertility in the new CMS sources. The mechanism of CMS restoration is complex and is regulated by single to multiple genes (Table 12.4). Thus, knowledge of genetics of new *Rf* genes is highly desirable for diversification of CMS and their effective restoration to further utilize in heterosis breeding in sunflower (Crouzillat et al. 1991).

Sunflower vulnerability to various biotic stresses is highly unpredictable and a major limiting factor in its production stability. More than 30 species of pathogens belonging to fungi, bacteria, viruses and parasitic plants such as broomrape (*Orobanche cumana*) attack sunflower worldwide (Skoric 2016). Despite that biotic constraints tend to be geographically and environmentally restricted, fungi-caused diseases are quite serious that cause significant economic damage. Among the major biotic stresses attacking this crop worldwide, mainly *Alternariaster* leaf spot (*Alternaria helianthi*), downy mildew (*Plasmopara halstedii*), powdery mildew (*Golovinomyces cichoracearum*), rust (*Puccinia helianthi*) and sunflower necrosis disease (SND), are realized as major threat for sunflower productivity in India. Nevertheless, both chemical and agronomic management control measures are available for immediate control of these biotic stresses, but this is not always economically or physically feasible. Thus, host-plant resistance is the most reliable, eco-friendly and economical both for the grower and to the end users. In this direction, substantial progress has been achieved in finding the sources of resistance, the genetics of host-plant resistance and their incorporation into cultivated sunflower (Tables 12.2 and 12.5). Detailed description of biotic stresses in sunflower has been previously provided by Gulya et al. (1997), Kaya (2016) and Skoric (2016).

Table 12.4 Genetics of fertility restoration (*Rf*) of different cytoplasmic male sterility (CMS) sources in sunflower

Cytoplasm	Genes	Gene action	Reference(s)
PET-1	Monogenic and multigenic (1–3)	Dominant, complementary, cumulative, non-allelic	Kinman (1970), Enns et al. (1970), Vranceanu and Stoenescu (1971), Fick and Zimmer (1974), Vranceanu and Stoenescu (1978), Seiler and Jan (1994), Reddy and Thammiraju (1997), Yue et al. (2010), Sujatha et al. (2011) and Port et al. (2013)
ANL-1, GIG-1, MAX-1, PEF-1 and PET-2	Monogenic and digenic (1–2)	Dominant, complementary	Whelan (1980), Kukosh (1984), Iuoras et al. (1992), Kural and Miller (1992), Miller (1996), Horn and Friedt (1997) and Sujatha et al. (2011)
ANL-2, ANN-2, ANN-3, ANO-1, ARG, GIG-2, HA-89 (mutant), NEG-1 and PRP-1	Monogenic	Dominant	Jan (1990), Serieys (1994), Horn and Friedt (1997), Butta et al. (2005), Jan and Vick (2007), Feng and Jan (2008) and Sujatha et al. (2011)
ANN-4, FMS, IMS and RIG-1	Digenic	Dominant, complementary	Jan et al. (1994) and Jan et al. (2002a, b), Horn et al. (2002) and Chandra et al. (2010)

Over the past few years, the progression of broomrape (obligate root holoparasitic weed) became one of the major biotic issues in different sunflower-growing countries of Europe and Central Asia (Skoric and Pacureanu 2010). Fortunately, vast track of sunflower-producing regions are still free from broomrape infestation so far, but this parasitic weed has a great capacity for dispersion due to its very light weight and minute seed size. Moreover, single broomrape plant can produce thousands of seeds that are easily dispersed by wind and other agents, including sunflower seeds (Fernandez-Martinez et al. 2015). Despite the substantial progress made on breeding for broomrape resistance in sunflower (Cvejic et al. 2020), still there is need to continuously explore for new resistance sources and study their genetics. Moreover, researchers need to be conscious in the sunflower-producing countries such as India where this parasitic weed still does not exist.

12.5 Induced Mutation to Facilitate Sunflower Breeding

Plant breeders normally depend on hybridization to enrich the existing germplasm that could be used in future breeding programmes for the development of new varieties. In traditional breeding, breeders rely on numerous rounds of selection to fix the targeted traits (both qualitative and quantitative); wherefore, undiscovered desirable alleles that exist in natural plant populations got lost resulting in narrow genetic base of modern cultivars. As the market-driven breeding continues to grow at a rapid pace, crop varieties in major field crops including oilseed crops such as

Table 12.5 Estimated yield losses due to major diseases and the genetics of host-plant resistance

Disease	Losses (%)		Mode of inheritance	Reference(s)
	Potential	Actual		
Fungal				
Alternariaster blight (*Alternaria helianthi*)	10–40	90	Dominance	Kong et al. (2004)
			Polygenic	Agrawat et al. (1979), Islam et al. (1976), Mehdi et al. (1984), Carson (1985), Godoy and Fernandes (1985), Velazhahan et al. (1991) and Nagaraju et al. (1992)
Downy mildew (*Plasmopara halstedii*)	2–25	80	Monogenic dominant	Vranceanu and Stoenescu (1970), Zimmer and Kinman (1972), Miller and Gulya (1991), Molinero-Ruiz et al. (2003) and Zhang et al. (2017)
			Digenic	Rahim (2001) and Vear et al. (2003)
Powdery mildew (*Golovinomyces cichoracearum*)	–	–	Digenic	Rojas-Barros et al. (2005), Seiler (2008) and Supriya et al. (2017)
			Partially dominant	Jan and Chandler (1985)
Rust (*Puccinia helianthi*)	1–10	35	Monogenic dominant	Putt and Emilio (1955), Lambrides and Miller (1994), Qi et al. (2012), Gong et al. (2013) and Bulos et al. (2013)
Viral				
Sunflower necrosis disease (SND)	5–70	90	Polygenic	–
Parasitic weed[a]				
Broomrape (*Orobanche cumana*)	50–100[a]	–	Digenic recessive	Rodriguez-Ojeda et al. (2001), Akhtouch et al. (2002) and Fernandez-Martinez et al. (2004)
			Digenic dominant	Dominguez (1996)
			Monogenic dominant	Vranceanu et al. (1980), Pacureanu-Joita et al. (1998), Jan et al. (2002a, b), Perez-Vich et al. (2004) and Sayago et al. (2018)
			Monogenic, incomplete dominance	Guchetl et al. (2019)

Estimated yield losses data source: Sujatha 2006 (India)
[a]Losses due to broomrape infestation in European and Asian countries (Cvejic et al. 2020)

sunflower with novel traits like improved oil yield and its quality are needed to meet the current and near-future market demands. Nonetheless, breeders are continuously exploring the source of novel variation in crop wild relatives, but very limited amount of such genetic variation has been utilized from germplasm due to lengthy pre-breeding activities, cross incompatibility of most of the wild species due to different ploidy level and associated linkage drag. Thus, mutation techniques are

powerful tools to create novel variation particularly for traits with restricted genetic variability.

Alternatively, biotechnological tools such as antisense technique, RNAi, T-DNA knockouts, overexpression of genes, site-directed mutagenesis by zinc fingers and transposon tagging provide a way to create new genetic variation in plant improvement. Yet, there is scepticism against consuming transgenic crops which is almost absent with mutation-derived cultivars (Kumar et al. 2013). Thus, induction of variability by mutagenesis for breeding is highly desirable due to its adaptation to a wide spectrum of breeding objectives together with low cost and technology requirement (Zambelli et al. 2015).

Following the discovery of mutagenic actions of X-rays on fruit fly (*Drosophila melanogaster*) by Muller in 1927, plant breeders concentrated their efforts on the induction of novel mutants initially with physical and later on also with chemical mutagens and their utilization in crop improvement. However, chemical mutagens especially alkylating compounds such as ethyl methanesulfonate (EMS) have gained popularity and been most extensively used by researchers in field crops including sunflower due to its high efficacy, simple application and high changes of guanine-cytosine (G-C) to adenine-thymine (A-T), resulting in random point mutations (Till et al. 2007). Nevertheless, it is advisable to optimize the dose of either physical or chemical mutagen that produces a maximum mutation density without causing extensive sterility. Recently, Kumar et al. (2013) performed a kill curve analysis and advocated the application of 0.6% EMS treatment for 8 h, corresponding to the 50% survival rate, i.e. LD50 in sunflower (Fig. 12.3).

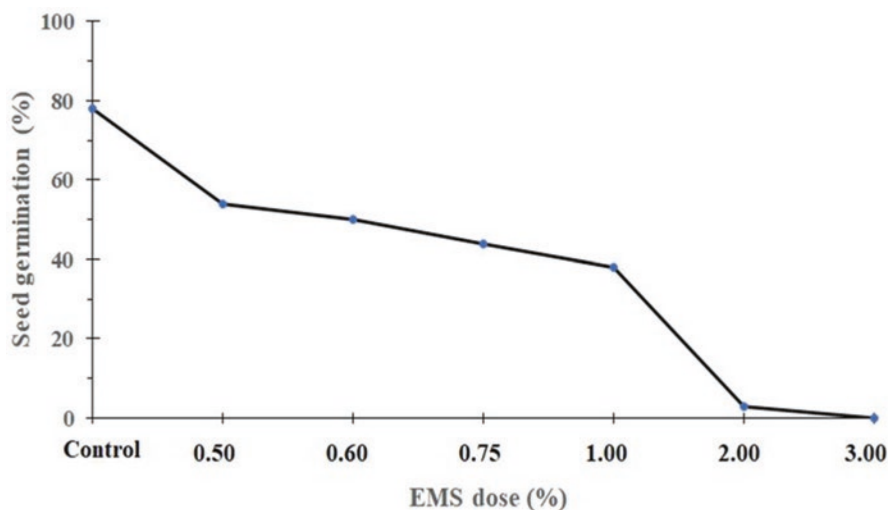

Fig. 12.3 Impact of EMS concentration on sunflower seed germination. (Source: Kumar et al. 2013)

Historically several mutants for morpho-physiological characters such as days to flowering (Cvejic et al. 2011), plant height (Fick 1978; Miller and Hammond 1991; Jambhulkar 2002; Cvejic et al. 2011), stem (Jambhulkar and Joshua 1996; Jambhulkar 2002a; Fambrini et al. 2006; Cvejic et al. 2011), leaf colour (Luczkiewicz 1975), petiole (Luczkiewicz 1975; Vranceanu et al. 1988), cotyledon (Hu et al. 2006), inflorescence (Luczkiewicz 1975; Fick 1976; Jambhulkar and Joshua 1996; Fambrini et al. 2003; Berti et al. 2005; Hu et al. 2006; Fambrini et al. 2007) and chlorophyll (Leclerq 1968; Mihaljicevic 1992; Jambhulkar and Joshua 1996) had been developed in sunflower. These morpho-physiological mutations are governed by either a recessive or dominant or additive gene (Jambhulkar and Shitre 2009). Nonetheless, mutagenesis has undoubtedly been the most successful procedure in bringing about genetic improvement for complex traits, but very limited success was observed in sunflower compared to self-pollinated crops including oilseed crops except for modifying seed oil quality parameters (Velasco and Fernández-Martínez 2002).

Among the fatty acids that exist in sunflower seed, unsaturated fatty acids, namely, oleic (C18:1; monounsaturated) and linoleic (C18:2; polyunsaturated) acids, represent up to 90%, while other fatty acid including saturated fatty acids [palmitic (16:0) and stearic (18:0)] accounts for the remaining 10% (Table 12.6). The credit of usefulness of induced mutation in sunflower goes to the pioneer work of Soldatov (1978), who obtained one of the first artificial high oleic mutations that led to obtain oleic versions of successful varieties. Subsequently, several researchers obtained induced mutants for altered fatty acid compositions using physical and chemical mutagens (Table 12.6).

Similarly, sunflower oil is also the richest source of tocopherols. Tocopherols represent a group of four [alpha (α)-, beta (β)-, delta (δ)- and gamma (γ)-tocopherol] naturally occurring lipid-soluble compounds collectively known as vitamin E. Tocopherols are not only essential micronutrients for humans and animals but also have several beneficial effects in plants (KamalEldin and Appelqvist 1996). The knowledge of tocopherol-related mutant genes such as *tph1*, *tph2* and t*ph1tph2* altering the concentrations of tocopherol has contributed to the development of sunflower cultivars with improved oil quality (Davey and Jan 2010).

12.6 Reverse Genetics: TILLING and EcoTILLING

Historically breeders have been mostly restricted to dominant mutations, but most of desirable mutations are indeed recessive and have been either lost or difficult to maintain during handling of segregating generations. With the advent of molecular marker technology, it becomes easy to identify the point mutation in genome. Targeting Induced Local Lesions IN Genomes (TILLING) is a technology to detect induced point mutation especially at single nucleotide polymorphism (SNP) levels in plants (McCallum et al. 2000; Till et al. 2003). This technology relies on the ability of a group of the endonuclease enzymes which can detect even rare recessive

Table 12.6 Sunflower-induced mutant showing altered fatty acid and tocopherol composition in seed oil

Characteristic	Standard composition	Mutant (altered characteristics)	Reference(s)
Mutant identified for altered fatty acid			
Palmitic acid (16:0)	7	275HP; fap1 low; CAS-5 (↑); CAS-12 (↑); HP line (↑); CAS-37 (↑)	Osorio et al. (1995), Vick and Miller (1996), Fernandez-Martinez et al. (1997), Miller and Vick (1999), Perez-Vich et al. (1999), Martinez-Force et al. (1999) and Fernández-Martínez et al. (2007)
Stearic acid (18:0)	3	M430 (↓); CAS-3 (↑); CAS-4 (↑); CAS8 (↑); CAS14 (↓); CAS15 (↑); CAS19 (↑)	Osorio et al. (1995), Vick and Miller (1996), Miller and Vick (1999), Fernandez-Moya et al. (2002) and Fernández-Martínez et al. (2007)
Oleic acid (18:1)	30	Pervenets (↑); M4229 (↑); CAS12 (↓); 29065 (↑); 29066 (↑); 29074 (↑); 29075 (↑); 29076 (↑); 29077 (↑); 29078 (↑); 29079 (↑); 29081 (↑); 29082 (↑); 39096 (↑); CAS-12 (↑)	Soldatov (1978), Vick and Miller (1996), Fernandez-Martinez et al. (1997) and Leon et al. (2013)
Linoleic acid (18:2)	60	CAS-5 (↑); F6 sel. (↑); 2698-1 (↑)	Osorio et al. (1995) and Fernández-Martínez et al. (2007)
Mutant identified for altered tocopherol			
α-Tocopherol	95	–	–
β-Tocopherol	3	LG-15 (↑); T589 (↑); IAST-5 (↑)	Cvejic et al. (2014)
γ-Tocopherol	2	LG-17 (↑); LG-24 (↑); T2100 (↑); IAST-1 (↑); IAST-540 (↑)	Cvejic et al. (2014)
δ-Tocopherol	0	IAST-4 (↑)	Cvejic et al. (2014)

mutations in the genome (McCallum et al. 2000). Recently, Kumar et al. (2013) developed TILLING genomic resource for cultivated sunflower and identified 26 induced mutations in 2 genes (FatA and SAD) involving in the accumulation of short- to medium-chain fatty acids. This approach can be used to develop markers for genomic-assisted selection strategy in sunflower breeding programmes, since mutations in important traits or genes such as in case of nutritional quality can be readily exploited by plant breeders without the legislative restrictions imposed on genetically modified organisms (GMO).

Similarly, larger collection of sunflower germplasm is also available in different gene banks (Table 12.1), but there have been relatively few comprehensive reports on the characterizations of this germplasm using molecular markers. A breeder would ideally like to know the relative value of all the alleles for genes of interest in the available germplasm. Thus, such information can be gathered by performing 'allele mining' experiments which seek to identify naturally occuring allelic

variants at loci or genes controlling agronomically important traits. In this context, a strategy based on Targeting Induced Local Lesions IN Genomes (TILLING), called EcoTILLING, was developed for detecting multiple types of polymorphisms in germplasm collections (Comai et al. 2004). EcoTILLING allows natural alleles at a locus to be characterized across many germplasms, enabling both SNP discovery and haplotyping. After the confirmation of SNPs/haplotype in the candidate genes by EcoTILLING, functional markers (FMs) can be developed for the target traits for use in breeding programmes for (i) more efficient fixation of alleles in populations, (ii) controlled balancing selection, (iii) screening for alleles in natural as well as breeding populations, (iv) combination of FM alleles affecting identical or different traits in plant breeding and (v) construction of linked FM haplotypes.

12.7 Molecular Marker and Biotechnology Resources

Despite the overly sensitive nature of agronomic traits, classical methods in conventional breeding based on agronomic traits played a tremendous role in the crop improvement including sunflower. In the late twentieth century, molecular technologies became equally competitive because these technologies are free from the environmental influence (genotype by environment interactions) as well as more efficient (Chander et al. 2021). Contrarily, lengthy survey of seed-to-seed cycle makes agronomic trait-based approaches more costly, time-consuming and labour-intensive which encouraged researchers to identify alternative methods such as DNA-based marker analysis (Nadeem et al. 2017). The advantages of molecular marker technique lie in their rapidity and it is free from phenological stage specificity. Advances in marker technology especially medium-throughput PCR-based makers simplified the genotyping process and further reduced the requirement of amounts of tissue samples which allows the analysis of single seeds and/or seedlings (Nadeem et al. 2017). Owing to continuous invention in genotyping technologies, high-throughput sequence-based SNPs marker techniques, such as KASP (Kompetitive allele-specific PCR) or gene chip microarray, emerged as an attractive option nowadays because they not only allowed large-scale identification of SNP-based diversity within genomes but also display low genotyping error rate, and complete amenability to automation, resulting in drastic reduction in cost per data point (Mammadov et al. 2012; Thomson 2014).

In the past, the large number of molecular markers has been identified in different crops that linked to quantitative traits in specific populations (i.e. segregating as well as immortal mapping populations). For effective utilization of molecular marker in breeding programme, these identified markers should be validated in different genetic backgrounds and possibly other environments to ensure widespread utility (Rauf 2019). In sunflower, several molecular markers validated for number of economically important traits such as host-plant resistance (rust, downy mildew, *Sclerotinia sclerotiorum* and broomrape resistance), quality [high oleic acid and tocopherol content (vitamin E)] and fertility restoration have been developed to facilitate sunflower breeding (Table 12.7). These markers can be utilized to

Table 12.7 Validated markers available for different desirable traits in sunflower

Name of marker [linked gene]	Linkage group	Reference(s)
Rust (*Puccinia helianthi*) resistance		
SCT06 (950 b) [*R1*]	8	Qi et al. (2011)
ORS-333, SFW-00211 and SFW-01272 [*R2*]	9	Qi et al. (2011, 2015b)
ORS-316 [*R4*] and ORS-799 and ORS-45 [*R4u*]	13	Qi et al. (2011) and Qi and Ma (2020)
ORS-316 and ORS-630 [*R5*]	2	Qi et al. (2011)
ORS-728 and ORS-45 [*R11*]	13	Qi et al. (2012)
CRT-275 and ZVG-53, NSA-001392 [*R12*]	11	Gong et al. (2013) and Qi and Ma 2020)
ORS-316 [*R13a* and *R13b*]	13	Qi et al. (2011)
ZVG-61 and ORS-581 [*RHAR6*]	13	Bulos et al. (2013)
NSA-002798 [*PlARG*]	13	Qi and Ma (2020)
SFW01920, SFW00128, SFW05824 NSA_008457 [*R15*]	8	Ma et al. (2018)
Downy mildew (*Plasmopara halstedii*) resistance		
OPAC-20 [*Pl2*]	8	Brahm and Friedt (2000)
ORS-675, ORS-716 and ORS-662 [*PlArg*], ORS-509, ORS-605, ORS-610, ORS-1182, ORS-1039 [*PlARG*]; NSA-007595 and NSA-001835 [*PlARG*]	1	Imerovski et al. (2014), Solodenko (2018) and Qi et al. (2017)
4W2 [*Pl1*]	8	Najafabadi et al. (2015)
ORS-1008 [*Pl3*], HT-636; ORS-328 [*Pl16*]; RS-1008 and Hap-3 [*Pl5*, *Pl16*]	1	Liu et al. (2012), Solodenko (2018) and Mirzahosein-Tabrizi (2017)
SFW-01497 and SFW-06597 [*Pl8*]	1	Qi et al. (2017)
SNP SFW-04052 and SSR ORS-963 [*Pl17*]	4	Qi et al. (2015a)
CRT-214 and ORS-203; ORS-781 [*Pl18*]	2	Qi et al. (2016) and Solodenko (2018)
NSA-003564 and NSA-006089 [*Pl19*]	4	Zhang et al. (2017)
SFW-04358 and S8_100385559 [*Pl20*]	8	Ma et al. (2017)
11 SNPS, 4 co-segregated with *Pl35*	1	Qi et al. (2019)
Sclerotinia sclerotiorum resistance		
ORS-337 [QTL for stem and leaf lesion]; HA432 [QTL for speed of fungal growth]	4	Micic et al. (2005)
ORS-1129 [QTL for stem and leaf lesion], ORS-889 [QTL for speed of fungal growth]	10	Micic et al. (2005)
ORS-588 [QTL for stem lesion], ORS-811 [QTL for stem lesion and speed of fungal growth]	17	Micic et al. (2005)
Broomrape resistance		
(Or5) Markers linkage group 3	3	Tang et al. (2003) and Imerovski et al. (2013)
CRT392, CRT314, ORS1036, ORS1040 [*Or3*]	3	Tang et al. (2003)
C12Q1/6895 and C12Q1/6881 [*OrDEB2*]	4	Gao et al. (2019)
High oleic acid		

(continued)

Table 12.7 (continued)

Name of marker [linked gene]	Linkage group	Reference(s)
NI-3F/N2-IR [*A12*-oleate desaturase]	14	Nagarathna et al. (2011) and Tilak et al. (2017)
F4-R1 N1-3F/N2-1R [*FAD2-1D*]	14	Dimitrijevic et al. (2017)
HO_Fsp_b [*Ol*]	14	Premnath et al. (2016)
High tocopherol content		
ORS716 [*tph1tph1*]	1	Vera-Ruiz et al. (2006)
ORS312, ORS599; γ-TMT-F1/F2/R24; F9/R24 [*tph2tph2*]	8	Garcia-Moreno et al. (2006) and Garcia-Moreno et al. (2012)
γ-TMT-F9/R24d [*tph2tph2*]	14	Garcia-Moreno et al. (2012)
Restoration of fertility (*Rf*)		
HRG-01 and HRG-02; 67N-04_P, PPR621.5R and PPR621.5M [*Rf1*]	13	Markin et al. (2017) and Horn et al. (2019)
ORS-1114 [*GIG2-Rf4, Rf6*]	3	Feng and Jan (2008) and Liu et al. (2013
67N04_P HRG02 [*Rf1*]	14	Horn et al. (2019)
ORS-316 [*Rf7*]; ORS-191, HT-32 [*Pl34*]	13	Talukder et al. (2019)

characterize, diversify and transfer genes between sunflower inbred lines within cultivated germplasm and from wild species without excessive linkage drag.

12.8 Genetic Engineering: New Breeding Techniques to Facilitate Sunflower Improvement

Innovations in molecular and computational plant biology capabilities have generated a wealth of scientific information, and their applications such as molecular markers greatly improved the efficiency of crossing and selection in plant breeding. Despite the several successful examples, marker-assisted selection is still time-consuming and faces several limitations to develop improved cultivars. On the other hand, genetic engineering (GE) techniques increase the precision of making changes in the genomes and are being implemented to speed up plant breeding but drew widespread public controversy. The product of GE techniques was familiarly termed as genetically modified organism (GMO) which depends on robust genomics platforms and on plant transformation technologies. Recent advances in GE techniques have addressed most of doubts because final product developed by using these techniques is indistinguishable from the conventional plant breeding products, but the lack of clarity at process-based regulatory issues undermines confidence in these new technologies. For example, introgression of desired traits from wild relatives into cultivated species is one of the major breeding objectives in most of the crop improvement programmes. However linkage drag seriously hampers introgression

of the trait of interest due to simultaneous transfer of undesirable traits from wild species. Thus, to circumvent linkage drag in traditional breeding, concentrated efforts are required to develop large population size with multiple generations of backcrossing, but sometimes it becomes almost impossible when genes which support undesirable traits are closely linked with the gene of interest. Cis-genesis overcomes such problems which relies on the addition of only the gene of interest, while keeping unwanted genes behind in the wild germplasm. This technique is equivalent to transgenesis, but final product is the same likewise as conventionally bred plants, because the gene under transfer originated from the same gene pool while later relies on one or more genes from any non-plant organism or from a donor plant that is sexually incompatible with the recipient plant (Rommens 2004; Schouten et al. 2006; Jacobsen and Schouten 2008). Thus, cis-genesis overcomes limitations of conventional breeding which relies on tedious and lengthy backcrossing to restore the recurrent parent genotype (Lusser et al. 2012). The European Food Safety Authority GMO Panel had reviewed this approach and concluded that cis-genic plants have a risk level similar to conventionally bred plants, albeit controversial option (EFSA 2012; Delwaide et al. 2015).

So far, this approach has been successfully tested in various agriculturally important plant species including field crops such as cereals and potato (Cardi 2016). The transfer of resistance (R) genes for late blight of potato (*Phytophthora infestans*) from *Solanum bulbocastanum* to cultivated potato (*Solanum tuberosum*) through conventional breeding techniques using bridge crosses and successive backcrosses took almost 50 years. Nonetheless, marker-free potato plants containing late blight resistance genes from *S. stoloniferum* (Rpi-sto1) and *S. venturii* (Rpi-vnt1.1) were produced within a few years through cis-genic breeding approach using *Agrobacterium*-mediated transformation followed by PCR-based selection of transformed plants (Haverkort et al. 2016). Similar strategy can be attempted in sunflower for introducing biotic and abiotic resistance into elite susceptible lines, especially when the focus is on stacking multiple resistance genes.

12.9 Progress in Sunflower Hybrid Development in India

The first attempt of utilization of heterosis in sunflower was in Canada during the 1950s; however, it became successful only after the discovery of PET-1 by Leclercq (1969) and the corresponding *Rf* gene for fertility restoration (Kinman 1970). Since the release of the first CMS-based hybrids (Fransol and Relax) in 1974, the productivity of sunflower hybrids reached 4 t/ha in France and suggested that potential yield would be possible to realize 6 t/ha (Skoric et al. 2007). Nonetheless, sunflower is considered a very new crop in India, and the first attempt to cultivate this crop was made in the early 1970s with the introduction of five open-pollinated varieties (OPVs) [VNIIMK-8931 (EC-68413), Peredovick (EC-68414), Armavirskii-3497 (EC-68415), Armaverta (EC-69874) and Vashod (Sunrise)] from Russia (former USSR), but very soon, it adapted to different climatic and soil conditions. Despite

Table 12.8 Details of public sector bred sunflower hybrids in India during the past two decades (2001–2020)

Name of hybrid	Parentage		Institute/ university	Seed yield (t/ha)	Oil content (%)
	Female	Male			
BSH-1 (first sunflower hybrid in 1980)	*CMS-234A*	*RHA-274*	*UAS, Bengaluru*	*1.0–1.2* (***1.5–1.8***)	*41*
From 2001 to 2010					
KBSH-41	CMS-234A	RHA-95C-1	UAS, Bengaluru	1.4–1.6 (**2.0–2.5**)	39–41
KBSH-42	CMS-851A	RHA-95C-1	UAS, Bengaluru	1.4–1.6 (**2.0–2.5**)	38–41
KBSH-44	CMS-17A	RHA-95C-1	UAS, Bengaluru	1.5–1.8 (**2.2–2.8**)	36–38
KBSH-53	CMS-335A	RHA-95C-1	UAS, Bengaluru	1.0(R) and 2.2(I) **[2.0(R) and 2.7(I)]**	38
TUNGA (RSFH-1)	CMS-103A	R-64-NB	UAS, Raichur	1.2–1.8 (**2.0–2.8**)	39–41
KSFH-437 (Phule Raviraj)	CMS-17A	R-437	UAS, Dharwad	1.8–2.0	34
RSFH-130 (Bhadra)	CMS-104A	R-630	UAS, Raichur	1.2–1.5 (**1.8–2.0**)	39–42
NDSH-1	CMS-234A	RHA-859	ANGRAU, Hyderabad	1.2–1.6 (**1.8–2.4**)	40
HSFH-848	CMS-91A	RHA-298	CCS HAU, Hisar	1.5–2.0 (**2.5–3.0**)	41–42
DRSH-1 (PCSH-243)	ARM-243	RHA-6D-1	IIOR, Hyderabad[a]	1.2–1.5 (**2–2.5**)	43
LSFH-35	CMS-234A	RHA-1-1	MAU, Parbhani	1.2–1.6 (**2.0–2.4**)	39–41
PSFH-118	CMS-10A	P-61-R	PAU, Ludhiana	1.5–2.0 (**2.4–2.8**)	40
CO-2	COSF-1A	CSFI-99	TNAU, Coimbatore	1.8(K) and 2.2(R/S) **[2.0(K) and 2.2(R/S)]**	38–40
From 2011 to 2020					
KBSH-78	CMS-1103A	RHA-92	UAS, Bengaluru	1.7–2.3 (**2.54**)	39–41
DSFH-3	CMS-234A	RHA-IV-77	UAS, Dharwad	1.8–2.0 (**2.0–2.5**)	37–39
RSFH-1887	CMS-38A	R-127-1	UAS, Raichur	1.2–1.6 (**1.8–2.5**)	38–40
NDSH-1012 (Prabhat)	NDCMS-30A	R-843	ANGRAU, Hyderabad	1.0–1.2 (**2.0–2.5**)	40–41
LSFH-171	CMS-17A	RHA-1-1	MAU, Parbhani	1.8–1.9 (**2.0–2.4**)	34–35

(continued)

Table 12.8 (continued)

Name of hybrid	Parentage		Institute/ university	Seed yield (t/ha)	Oil content (%)
	Female	Male			
PSH-1962	CMS-67A	P-93R	PAU, Ludhiana	2.05 (**2.37**)	41.9
PSH-996	CMS-11A	P-93R	PAU, Ludhiana	1.95 (**2.5**)	37–38
PSH-2080	CMS-67A	P-160R	PAU, Ludhiana	2.44	43.7
PDKVSH-952	CMS-302A	AKSF-6R	PDKV, Akola	1.8–2.0	36.8
COH-3 (CSFH-12205)	COSF-6A	IR-6	TNAU, Coimbatore	2.28 (**2.4**)	42

Note: R and I represent rainfed and irrigated conditions, respectively. ANGRAU, Acharya N.G. Ranga Agricultural University; CCS HAU, Ch. Charan Singh Haryana Agricultural University; IIOR, Indian Institute of Oilseeds Research, [a]formerly known as Directorate of Oilseeds Research; MAU, Marathwada Agricultural University; PAU, Punjab Agricultural University; PDKV, Panjabrao Deshmukh Krishi Vidyapeeth; TNAU, Tamil Nadu Agricultural University; UAS, University of Agricultural Sciences. Values in parenthesis are potential seed yield

the good adaptation of OPV, new interest arose in the utilization of heterosis, in order to obtain higher yields in sunflower. Thus, to develop hybrids for diverse situations, four CMS (CMS-2A, CMS-124A, CMS-204A and CMS-234A) and two restorer (RHA-266 and RHA-274) lines were introduced from the USA. Within 5 years, the first public sector sunflower hybrid (BSH-1) was released for commercial cultivation in southern India (Seetharam 1980). Since 1980, about 30 hybrids and 19 OPVs have been released by various agricultural universities and/or public sector research institutes (Sujatha et al. 2019). Nonetheless, significant progress has been made in sunflower improvement during the past four decades; the productivity of the present-day hybrids is only doubled (Table 12.8) when compared to the first sunflower hybrid BSH-1 (1.2–1.3 t/ha) in 1980. Being an oilseed crop, oil yield is one of the major objectives; nonetheless, a substantial improvement in oil yield was obtained during this period owing to higher seed yield, but no change in the oil content was noticed.

12.10 Concluding Remarks

Since sunflower plays a prominent role in edible oil industry worldwide, it is expected that research will continue to sustain the growth in productivity of this crop in the future. In the past, classical genetics in conjunction with statistics played a pivotal role to achieve the substantial genetic gains in field crops including sunflower. In post-genomic era, genetic and genomic tools have utmost potential for

systematic reshuffling of the genome; therefore, application of these innovative tools in breeding programme is highly desirable for broadening the genetic base of cultivated sunflower. Despite the substantial progress, most of the diversity within the germplasm pool is yet unexplored due to lack of activity in the characterization and transfer of valuable genes from related species. Wherefore, only a small proportion (less than 2%) of the desirable genes were introgressed from wild relatives, and majority of these genes relate to biotic resistance in sunflower (Hubner et al. 2019). Historically, breeders are selecting multiple desirable traits in segregating populations, but nowadays, gene pyramiding became feasible, especially with the advent of molecular markers since it can accommodate multiple desirable loci/genes in a single line. Finally, the recent released sunflower genome assembly may help to speed up the identification of candidate genes for different important commercial traits resulting in early release of cultivars at both global and regional levels.

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Chapter 13
Genomic Cross Prediction for Linseed Improvement

Frank M. You, Chunfang Zheng, Sampurna Bartaula, Nadeem Khan, Jiankang Wang, and Sylvie Cloutier

Abstract Crossing between two or more parents is a fundamental way to generate superior genetic variants through genetic recombination and transgressive segregation in modern crop breeding. The selection of parents and crosses is the first key step for the success of crop breeding. The traditional method for screening parents and crosses is primarily based on phenotypic performance and genetic differences between parents and the breeders' empirical expertise. With the availability of genome-wide molecular markers and other genomic information, computer simulation offers a computational approach to simulate genetic recombination events between parents and progeny segregation of crosses and to generate segregation populations of any virtual crosses for various breeding schemes. Genomic selection (GS) enables to estimate breeding values (BVs) of the segregation individuals of crosses. Therefore, the integration of computer simulation and GS leads to an advanced genomic tool, named genomic cross prediction, to predict the genetic performance of different types of crosses by evaluating BVs and genetic variances of their segregation populations, enhancing the potential of success in crossbreeding. This chapter overviews the strategies and methods of genomic cross prediction and illustrates its application potential in crops, especially flax linseed breeding.

Keywords Flax · Linseed · Quantitative trait loci (QTL) · Genome-wide association studies (GWAS) · Genomic prediction · Molecular breeding · Cross prediction

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13.1 Introduction

Plant breeding by artificial selection has been exceptionally successful in developing new varieties that have contributed to the growth of modern societies and in satisfying the demand for plant-based products since the beginning of the domestication of plants, some 10,000 years ago (Fedoroff 2010). Phenotypic selection is the foundation of traditional breeding. Breeders select superior progenies to achieve genetic enhancement of target traits based on their expertise and the observed crop phenotypes. However, the success of this type of selection process depends on the genetic complexity of target traits. Most agronomic and economic traits are quantitative with complex genetic backgrounds and readily affected by environments; therefore, the evaluation of progeny populations of crosses needs to be performed in multiple years and locations. With the development of quantitative genetics and biostatistics, some statistical techniques have been used in plant breeding. For example, the best linear unbiased predictor (BLUP) that uses the progeny's phenotypic data and pedigree information was proposed to estimate breeding values (BVs) for assessing and selecting superior individuals (Henderson 1975). Since then, BLUP has been commonly used for genetic assessment in plant and animal breeding. Advances in molecular genetic methods have uncovered widespread genetic diversity in genomics since the 1990s. The vast numbers of molecular markers have been developed, allowing breeders to use markers to aid with breeding. Especially biparental populations-based quantitative trait locus (QTL) mapping has identified many large-effect QTLs. Marker-assisted selection (MAS) was then proposed (Lande and Thompson 1990) to improve the traits that are controlled by genes or QTLs with relatively large effects. As most of the economically important traits of crops are influenced by polygenes, each with a small effect (Riedelsheimer et al. 2012; Xu et al. 2012), the use of MAS in breeding practices is limited. Therefore, an improved method, genomic selection (GS), was introduced (Meuwissen et al. 2001). GS is considered an extension of MAS that uses genome-wide markers in a genotyped and phenotyped training population to build a statistical prediction model to predict BVs called genomic estimated breeding values (GEBVs) of unphenotyped individuals (Meuwissen et al. 2001). This landmark study laid the foundation for both plant and animal breeding to predict GEBVs of individuals and thus identify superior genotypes among selection candidates according to their genomic information.

Crossbreeding through crossing two or more parents is the fundamental method to generate superior genetic variants through genetic recombination between parents and their progeny's transgressive segregation in modern crop breeding. However, selecting parents to make crosses and predict the potential genetic performance of the crosses is the first critical step for the success of crossbreeding. This chapter introduces a new genomics-based strategy, named genomic cross prediction, which makes full use of information of genome-wide molecular markers and consensus genetic maps and integrates computer simulation and GS to simulate virtual crosses and predict the genetic performance of the virtual crosses to assist

breeders in selecting parents and crosses in plant breeding effectively. A case study will be described to demonstrate the methods of genomic cross prediction in flax breeding.

13.2 Strategy of Genomic Cross Prediction

13.2.1 Genomic Cross Prediction

The fundamental objective of a breeding program is to develop superior cultivars for specific target traits under a wide range of environmental conditions. Crossbreeding using different types of crossing schemes such as single-, double-, or backcrosses, followed by progeny selection such as pedigree selection and single-seed descent, is the most commonly used method in plant breeding. Breeders routinely make many crosses every year and evaluate their progeny populations in the fields or the greenhouses. However, mostly very few of them outperform the check cultivars. Thus, parent evaluation and cross selection are critically important for crossbreeding. The traditional method for screening parents and crosses is usually based on parents' phenotypic performance and the difference between parents and breeders' empirical expertise. But the accuracy and efficiency of parent selection are impeded by unknown genetic structures, allelic makeup of the potential parents, and genetic performance of progeny populations. In practical breeding programs, making crosses using all potential genetic resources is impractical due to the extensive resources that would be required. However, the limited number of crosses will narrow the probability of finding the best recombinants.

Computer simulation is an efficient research tool used in various disciplines, including plant breeding, which can generate data that is unable or difficult to obtain from empirical experiments based on some theoretical considerations and/or empirical data. It is usually used to compare different methods and verify/validate proposed theoretical assumptions and models. Two types of computer simulation methods, deterministic and stochastic, are implemented in different simulation software tools for plant breeding studies. Deterministic simulation relies on genetic models derived from quantitative genetics theory with a set of parameter values and initial conditions, resulting in the deterministic output. DeltaGen (Jahufer and Luo 2018) is one of the software tools implementing the deterministic simulation and has been used to predict genetic gain and cost per selection cycle for different breeding strategies in forage species with empirical data. In contrast, stochastic simulation integrates some inherent variation and randomness of gene-to-phenotype relationship within the quantitative genetics framework (Hoyos-Villegas et al. 2019), leading to an ensemble of different outputs even with the same set of parameter values and initial conditions. Due to the nature of complex quantitative traits, stochastic simulation is more commonly used in plant breeding studies, for example, for strategic comparison of different breeding strategies (Wang et al. 2003,

2009) and evaluation of genomic selection in the breeding programs (Iwata and Jannink 2011; Lin et al. 2016; Sekine and Yabe 2020). With the availability of genome-wide molecular markers and the development of consensus genetic maps, computer simulation offers a computational approach to simulate genetic recombination between parents. It can generate segregation populations of numerous virtual crosses and generate segregation populations for crosses based on genome-wide markers on parents for various breeding schemes. On the other hand, GS provides an effective genomic approach to predict GEBVs of segregation populations, making it possible to evaluate the usefulness of crosses through the performance of their progeny populations.

GS has been studied in many crops, including flax, and emerged as one of the most promising breeding methods to improve genetic gains over conventional practices. GS has also been implemented in some practical breeding programs of crops, such as wheat (Crossa et al. 2013; Sun et al. 2020) and barley (Schmid and Thorwarth 2014), among others (Crossa et al. 2011). GS has demonstrated its potential for agronomic, abiotic, and biotic stress-related traits (You et al. 2016a; He et al. 2019a; Lan et al. 2020; Khan et al. 2021). The predictive ability of GS is largely based on the use of statistical models, the density of markers, and the relationship between training and testing populations (Desta and Ortiz 2014; Lipka et al. 2015). One of the fundamental features of GS is the use of high-density genome-wide markers. However, it usually results in low genomic predictive ability (GA) due to background noise and uncorrelated markers, along with possible high costs generated by genotyping of such a large number of markers in test populations (He et al. 2019a; Lan et al. 2020). The use of quantitative trait loci (QTLs) identified by genome-wide association studies (GWAS) can significantly improve predictive ability (He et al. 2019a; Lan et al. 2020). For instance, the predictive ability of pasmo resistance in flax was generated as high as 0.92 for 500 QTLs compared to 0.67 for 52,347 random SNPs (He et al. 2019a). Similarly, when QTLs were adopted in GS models, the predictive ability was 0.89 and 0.73 for seed yield (YLD) and days to maturity (DTM), respectively, in flax biparental populations. In contrast, it was 0.84 and 0.44 for YLD and DTM, respectively, when 17,277 genome-wide random SNPs were used (Lan et al. 2020). Similar results were also obtained for drought stress tolerance traits (Khan et al. 2021). Therefore, GS can be further fine-tuned by using a marker screening procedure to accelerate the rate of genetic gains in GS. The identification is one of the marker selection methods to select appropriate marker sets that have genetic correlation markers and breeding selection traits in the training population. The prediction ability of GS using QTL identified from the training population as markers relies on whether these QTLs also exist in the test population. Overall, the use of selected markers has the potential to enhances the predictive ability and reduces the number of markers likely to minimize genotyping costs, especially for selecting large breeding populations.

Therefore, the integration of computation simulation and GS offers an advanced genomic tool to predict the performance of different types of crosses, assisting breeders in making decisions in crosses to be made and increasing the potential of success in crossbreeding. Here we name this new genomic tool “genomic cross prediction.” The genomic cross prediction was initially proposed by Bernardo (2015) and applied

to cross-evaluation of inbred lines in maize. In this pioneering study, a genetic resource panel of 284 diverse inbreds was phenotyped for several traits (flowering time, kernel composition, and disease resistance) and genotyped at 28,626 genome-wide SNPs in a previous study (Schaefer and Bernardo 2013). Then a similar procedure PopVar was described and used in barley (Mohammadi et al. 2015). This procedure has been implemented in the R package PopVar (Tiede et al. 2015a). A similar method was also used in wheat with single traits and a selection index for multiple traits (Yao et al. 2018). In this study, 57 wheat lines were used as a training population and genotyped with 7588 selected markers. The results showed that parental selection with the "usefulness" (definded in 13.2.3) resulted in higher genetic gain than midparent GEBVs. A selection index incorporating yield, extensibility, and maximum resistance as a new trait improved both yield and quality, while more genetic variance was retained in the selected progenies than the individual trait selection.

13.2.2 Procedure of Genomic Cross Prediction

We summarize the procedure of genomic cross prediction in Fig. 13.1. In this new procedure, the marker screening via GWAS has been integrated to improve the predictive ability of BVs of progenies using GS models. This procedure is described in detail as follows:

1. Genotyping and phenotyping of the genetic panel that includes genetic resources for potential parents. This panel is used as a training population to construct an optimal GS model for GEBV estimation of progenies.

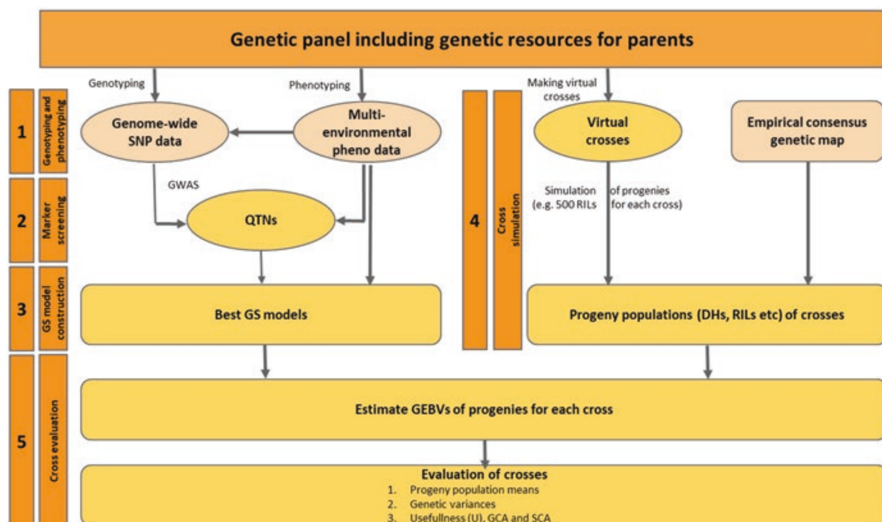

Fig. 13.1 A strategy of genomic cross prediction for crop improvement

2. Marker selection. Marker selection can be useful to reduce redundant markers from a large set of genome-wide random markers. The simultaneous use of several single- and multi-locus GWAS models to identify all potential large- and small-effect QTNs as markers (Lan et al. 2020) may be useful for genomic cross prediction in order to remove some of the SNPs unrelated to the traits of interest, especially in the case that the parents used to make crosses are selected from the training population for GS models.
3. GS model construction and cross-validation of models. Multiple GS models for a specific trait and a training population are compared and cross-validated to find an optimal GS model.
4. Cross simulation. Virtual crosses between all or a subset of parents in the genetic panel are made, and a certain number of progenies (e.g., 500) for each cross are simulated based on an empirical consensus genetic map; a specified crossing scheme such as single-, double-, or backcross; and a progeny advancing method, such as pedigree, single-seed descent (SSD) to generate doubled haploid (DH), or recombination inbreed line (RIL).
5. Cross-evaluation. The optimal GS models for the trait per se will be used to predict GEBVs of individuals in each progeny population. For each cross, some genetic parameters for the selection of parents and crosses will be calculated based on GEBVs of individuals. As a result, the potentially best parents and the top crosses will be selected to make crosses in practical breeding.

The prerequisites of genomic cross prediction include a panel of diverse genetic resources including potential parents and a consensus genetic map with high-density genome-wide markers. The genetic panel can be phenotyped over multiple years and locations and genotyped using different genotyping approaches, such as genotyping by sequencing. The accuracy of the genomic cross prediction depends mostly upon these two conditions. A large and diverse genetic panel as a training population helps to obtain a high predictive ability GS model. In particular, including the parents to be evaluated in the training population benefits the identification of QTLs shared by both the training population and the parents to be evaluated, increasing the genetic relationship between the training population and the progeny populations and thus promoting the predictive ability of progeny GEBVs. High-density genome-wide SNPs in the training population are also beneficial to the identification of all potential QTLs associated with the target traits and their genetic interactions (epistasis), which helps the simulation of true recombination events and the development of an optimal GS model. A high-density genetic map facilitates the estimation of genetic distances for identified QTLs. Ideally, the markers in the genetic map are a subset of the SNPs identified from the genetic panel.

13.2.3 Genetic Parameters for Cross-evaluation

According to the theories of quantitative genetics, a good cross should have a high progeny mean and large genetic variance that may have a high chance to obtain superior individuals from progenies. Thus, the performance for a cross can be measured by the progeny population mean and its genetic variance. Schnell and Utz (1975) defined a genetic parameter, usefulness (U) of a cross as

$$U = \mu \pm \Delta G = \mu \pm ih\sigma_g, \quad (13.1)$$

where μ is the mean of the progeny population derived from a cross, ΔG is a genetic gain, i is the standardized selection intensity based on the selected proportion (e.g., $i = 2.063$ for 5% or $i = 1.755$ for 10%), h is the square root of the trait heritability, and σ_g is the standard deviation of genetic variance of the progeny population. Zhong and Jannink (2007) further simplified this definition by setting $h = 1$ in the formula 13.1, resulting in

$$U = \mu \pm i\,\sigma_g \quad (13.2)$$

This new formula has a simpler property that expresses which crosses would generate progenies with higher genotypic values and thus is used in our procedure.

Midparent GEBVs of a cross can also be obtained from GS to evaluate whether midparent GEBV is adequate for predicting U.

13.3 Software Tools for Genomic Cross Prediction

13.3.1 Software Tools for Data Analysis

Genomic cross prediction involves both empirical and simulation data. Software tools are demanded to complete various genetic model development, data generation, and analysis. Based on the strategy of the genomic cross prediction diagrammed in Fig. 13.1, four categories of software tools are required: (1) GWAS to identify QTNs associated with the target traits or other marker screening methods, (2) GS model construction to find the optimal model to predict GEBVs of progeny populations, (3) genetic simulation to simulate virtual crosses and their progeny populations based on genomic data of parents, and (4) GEBV estimates of progeny populations and evaluation of cross performance. Table 13.1 lists some software tools that can be used for genomic cross prediction.

GWAS is usually used to identify QTNs related to the traits with a genetic panel that consists of potential genetic resources for parent selection. Two types of statistical models are available for QTL detection: single-locus models such as general linear model (GLM) (Price et al. 2006) and mixed linear model (MLM) (Yu et al. 2006) popularly used in the early QTL analyses and multi-locus models such as

Table 13.1 Software tools related to genomic cross prediction

Name	Description	Reference
GWAS		
mrMLM 4.0.2(R)	Implement six different multi-locus models, including mrMLM, FASTmrEMMA, ISIS EM-BLASSO, pLARmEB, pKWmEB, and FASTmrMLM	https://cran.r-project.org/web/packages/mrMLM/index.html
MVP 1.01(R)	Include single-locus models GLM and MLM and a multi-locus model FarmCPU	Liu et al. (2016)
Genomic selection		
rrBLUP 4.6.1(R)	Include a fast maximum-likelihood algorithm for mixed models	Endelman (2011)
BGLR 1.08(R)	Construct Bayesian regression models and GBLUP for continuous and categorical traits	Perez and de los Campos (2014)
Sommer 4.1.2(R)	GBLUP, rrBLUP	Covarrubias-Pazaran (2016)
Genetic simulation for breeding programs		
PedigreeSim 2.0 (R)	Simulate pedigreed populations for diploid and tetraploid species	Voorrips and Maliepaard (2012)
ADAM-plant (standard-alone Fortran program)	Simulate populations of various breeding schemes for both self- and cross-pollinated crops	Liu et al. (2019)
AlphaSim 0.13.0 (R)	Simulate plant and animal breeding programs	Faux et al. 2016
QuLine 2.5 (standard-alone)	Simulate breeding programs for cereal and leguminous crops, including male/female master selection, parent selection, single, backcross, top or double cross, and different progeny selection methods such as doubled haploid, marker-assisted selection etc.	Wang and Dieters (2008)
QuLinePlus0.0.10 (standard-alone)	Extension of QuLine for simulation of breeding populations of cross-pollinated crops	Hoyos-Villegas et al. (2019)
Blib	A generalized and powerful Fortran library to develop applications for various genetic modeling, simulation, and prediction in plant breeding	Personal communication with Dr. J Wang)
PopVar 1.3.0	Using phenotypic and genotypic data of a set of candidate parents to predict the mean, genetic variance, and superior progeny value of all or a subset of pairwise biparental crosses, and perform cross-validation to estimate genome-wide predictive ability of multiple statistical models	Tiede et al. (2015b)
MareyMap (1.3.6) (R)	A utility tool to calculate genetic distance between all markers on a physical map using a training genetic map	Siberchicot et al. (2017)

(R): R package

mrMLM (Wang et al. 2016; Li et al. 2017). The multi-locus models have a high statistical power to identify large and minor-effect QTNs that are genetic features of most complex quantitative traits. This provides us with a new option to directly use QTNs as markers in genomic cross prediction rather than genome-wide random markers to predict breeding values. The mrMLM package implements six different mixed models (mrMLM, FASTmrEMMA, ISIS EM-BLASSO, pLARmEB, pKW-mEB, and FASTmrMLM) (Wang et al. 2016; Li et al. 2017; Ren et al. 2017; Tamba et al. 2017; Zhang et al. 2017; Wen et al. 2018) (Table 13.1) that are complemental (He et al. 2019b; Lan et al. 2020). Thus, the combined results of these models are recommended to obtain as many associated markers as possible for GS model construction and cross simulation in genomic cross prediction.

Many genomic prediction models have been developed to increase genomic predictive ability, for example, ridge regression, best linear unbiased prediction (rrBLUP), genomic best linear unbiased prediction (GBLUP), Bayesian regression, partial least squares regression, and machine learning methods (Wang et al. 2018). These models may be roughly grouped into two groups based on the assumptions for statistical distributions of the marker effects. The models, such as RR-BLUP and GBLUP, assume that all markers contribute to the variation of the trait, while models such as BayesA and BayesB assume a specific variance for each marker. Thus, the first group of models is expected to be useful for complex quantitative traits with a polygenic architecture, while the second group of models is suitable for traits that are controlled by a small number of genes or QTL with large-effect architectures (e.g., Jannink et al. 2010; Spindel et al. 2015). Some studies have shown the better performance of BayesB than GBLUP or rrBLUP for traits controlled by a few genes with large effects (Daetwyler et al. 2010; Jannink et al. 2010; Thavamanikumar et al. 2015). However, if QTNs were used in the construction of GS models, similar predictive ability was obtained from different models, and rrBLUP or GBLUP is thus recommended because of their simplicity and computational efficiency (He et al. 2019a). Several R packages, including rrBLUP (Endelman 2011), BGLR (Perez and de los Campos 2014), and sommer (Covarrubias-Pazaran 2016), are available for GS model constructions (Table 13.1).

Making virtual crosses and simulating their progeny populations based on predefined breeding schemes are one of the steps for genomic cross prediction. Computer simulation has been widely used to simulate breeding schemes to save time and investigate problems that cannot be solved only by empirical data. Some software tools have been implemented to simulate various breeding schemes, such as single, double, and three crosses or backcrosses followed by different selection strategies for HD lines, RILs, etc. These tools include AlphaSim (Faux et al. 2016), pSBVB (Zingaretti et al. 2019), PedigreeSim (Voorrips and Maliepaard 2012), ADAM-plant (Liu et al. 2019), and QuLine or QuLinePlus (Wang and Dieters 2008). Notably, Blib developed by Dr. Jiankang Wang's laboratory (Chinese Academy of Agricultural Sciences, Beijing, China) is a universal library that has various functions to develop different applications of genetic model-ing, simulation, and prediction in plant breeding, including simulation of cross progeny populations.

Table 13.2 Components of a pipeline package of genomic cross prediction

Step	Module	Description
1	QTL mapping	Identify QTNs from the training population using a set of statistical models, especially multi-locus models
2	GSMoldeler	Construct and evaluate genomic prediction models using the training population that includes parents to be evaluated and the QTNs identified in Step 1 as markers
3	GeneticMapConversion	Generate a new genetic map covering all markers (QTNs) using a consensus genetic map as a training data set, using MareyMap (Siberchicot et al. 2017)
4	CrossSimulator	Simulate various virtual crosses and their genomic values of progeny populations using Blib
5	GEBVEstimator	Estimate GEBVs of progeny populations generated in Step 4 using GS models constructed in Step 2
6	CrossEvaluator	Evaluate performance of parents and crosses through analysis of progeny populations for the results in Step 5

13.3.2 A Pipeline Package of Genomic Cross Prediction

Although some third-party software tools are available for GWAS, GS modeling, and cross simulation, a pipeline program to integrate all these analyses is needed. PopVar is an earlier but practical pipeline program that includes GS modeling for multiple statistical models, cross simulation, and evaluation for a set of candidate parents and for all or a subset of pairwise biparental crosses (Table 13.1) (Mohammadi et al. 2015). We have developed a pipeline package that implements the strategy proposed in Fig. 13.1 and integrates all steps and eases the entire data analysis process. In particular, marker screening through GWAS and cross-evaluation by calculating genetic parameters of the cross are added to this pipeline. This pipeline package contains five program modules and is implemented in five separate pipeline programs (Table 13.2):

1. GWAS pipeline (a Perl program) that integrates all single-and multi-locus GWAS models implemented in R packages mrMLM and MVP (Step 1)
2. GS modeling and evaluation pipeline (a Java program) that integrates ten different GS models implemented in R packages rrBLUP, BGLR, and sommer (Step 2) and calculates GEBVs of simulated progeny populations (Step 5)
3. Genetic map conversion pipeline that integrates MareyMap (Siberchicot et al. 2017) to estimate the genetic distance of all markers used for genetic simulation based on a consensus genetic map as a training data set (a Perl program)
4. Cross simulation pipeline that combines the Blib library-based applications to simulate various crosses of breeding programs and generate genotypes of progeny populations (a Perl program) (Step 4)
5. Cross-evaluation pipeline to analyze various genetic parameters of crosses, including genetic means and variances, usefulness, etc. (Step 6)

13.4 Genomic Cross Prediction for Linseed Improvement

Pedigree analyses have shown that Canadian flax cultivars have a relatively narrow genetic base (You et al. 2016b). To broaden the narrow genetic base of Canadian flax cultivars, a core collection of 407 accessions has been selected from the world flax collections (Diederichsen et al. 2013; Soto-Cerda et al. 2013). These accessions originate from 39 countries in different ecological regions covering America, Europe, Asia, Oceania, and Africa and include some of the recently developed flax cultivars and superior breeding lines. They represent the majority of genetic variation in the world collection. This collection has been then fully phenotyped during 4 years and at 2 locations for more than 27 various traits, such as seed and fiber yield, seed and fiber quality, and disease resistance (You et al. 2017). A total of ~1.7 million SNPs have also been identified using a genotyping by sequencing (GBS) approach (He et al. 2019b). To date, crossbreeding is still a major breeding approach in flax breeding. To make full use of this phenotypic and genomic information in flax crossbreeding, as a case study, we applied the genomic cross prediction method to evaluate the performance of potential crosses and assist cross selection. Our idea was to use the well-phenotyped and well-genotyped core collection as a training population to develop optimal GS models, simulate progeny populations of all virtual crosses based on genomic data of parents, and then use the developed GS models to predict GEBVs of all progenies of crosses. Parent and cross performance were evaluated based on the estimates of general specific ability (GCA) of parents and the usefulness of the crosses.

13.4.1 Materials and Methods

13.4.1.1 Training Population and Phenotypic and Genomic Data

A total of 290 linseed accessions were extracted from the flax core collection as a training population. These accessions are potential parents in linseed breeding, including193 cultivars, 59 breeding lines, 13 landraces, and 25 unknown lines originating from 34 countries. A set of 258,708 SNPs in 290 linseed accessions extracted from the whole collection (He et al. 2019b) was extracted for analyses.

Five representative breeding target traits in linseed breeding selection, including seed yield (YLD), days to maturity (DTM), oil (OIL), linolenic acid (LIN), and powdery mildew resistance (PM), were chosen for the case study. The first four traits were phenotyped in 4 years (2009–2012) at two locations (Morden and Saskatoon, Canada), while PM was field evaluated for 5 years (2009–2013) in the PM nursery at Morden, Manitoba, Canada, which have been previously described in detail (You et al. 2017).

13.4.1.2 Identification of Quantitative Trait Nucleotides (QTNs)

QTNs of the five traits were detected using six multi-locus statistical models implemented in the R package mrMLM (Zhang et al. 2020), including mrMLM (Wang et al. 2016; Li et al. 2017), FASTmrMLM (Zhang and Tamba 2018), FASTmrEMMA (Wen et al. 2018), pLARmEB (Zhang et al. 2017), ISIS EM-BLASSO (Tamba et al. 2017), and pKWmEB. All individual phenotypic data sets from different years and locations were independently analyzed for GWAS, and then all detected nonredundant QTNs were combined for the downstream analyses. Significant QTNs were identified based on a cutoff value of the log of odds (LOD) score greater or equal to 3.0. The details of GWAS have been previously described (He et al. 2019b).

13.4.1.3 Construction of Genomic Selection Models

To select the optimal GS prediction models for different traits, ten GS models, including RR-BLUP, GBLUP, BayesA, BayesB, BayesC, BLL, BLR, RFR, RKHS, and SVR, were used to construct prediction models. The models were assessed using predictive ability with the fivefold cross-validation scheme (He et al. 2019a). Predictive ability was defined as a Pearson's correlation coefficient between predicted values and actual observed values. The optimal models were chosen to construct prediction models for traits using the data of all individuals in the training population to predict GBEVs of the crosses' progenies. To examine the impact of different marker sets on predictive ability, two different marker sets – all genome-wide random SNP markers and QTNs identified for each trait – were used to construct separate models.

13.4.1.4 Virtual Crosses and Simulation of Progeny Populations

All 290 linseed accessions were used to make possible virtual single crosses with a partial diallel cross scheme, i.e., a total of 41,905 (290 × 289/2) crosses were virtually made. For every single cross, 500 DH and 500 RIL individuals derived from two parents were simulated based on an additive model. Their genotypes were generated based on QTN markers in two parents and their genetic recombination in individuals. The consensus genetic map of flax (Cloutier et al. 2012) was used as a training data set to estimate genetic distance between neighboring markers for all QTNs. Since the consensus genetic map was SSR marker-based, we first anchored SSR markers to the flax scaffold sequences (Wang et al. 2012) and then mapped them to the pseudo molecule-scale flax reference sequence (You et al. 2018). Subsequently, the R package MereyMap (Chakravarti 1991; Siberchicot et al. 2017) was used to convert the physical distance of QTNs on chromosomes to genetic distance (cM). The optimal GS model for each trait was then used to predict the GEBVs of DH or RIL individuals for each cross and each trait.

13.4.1.5 Evaluation of Virtual Crosses

For each cross, GEBVs of all 500 progenies (DH or RIL individuals) were estimated using the GS model, and then its population mean (μ) and genetic variance (σ_g^2) were calculated. The usefulness (U) of a cross was calculated at the selected proportion of 5% based on formula 13.2: $U = \mu + i\,\sigma_g$ for YLD, OIL, and LIN to seek greater U values and $U = \mu - i\sigma_g$ for DTM and PM to seek smaller U values, where μ is the mean GEBVs of progenies, i is the standardized selection intensity 2.036, and σ_g is the standard deviation of genetic variance of the progenies.

GCA of a parent was defined as the average performance of this parent crossing with all other 289 parents, whereas U of a cross represents the specific performance of progeny populations by crossing two parents.

In the Canadian linseed breeding program, breeders select superior individuals of high seed yield and oil and linolenic content but short growth periods and high resistance to diseases (small ratings). Thus, selection for a parent or a cross is comprehensive for all major target traits, not only for a single trait. To comprehensively evaluate parents and crosses for all five traits, here we made an index trait that is a linear combination of all five traits with a specified weight for each trait, i.e., index trait $I = w1 \times x_{YLD} + w2 \times x_{DTM} + w3 \times x_{OIL} + w4 \times x_{LIN} + w5 \times x_{PM}$, where $w1$, $w2$, $w3$, $w4$, and $w5$ are the economic weights for YLD, DTM, OIL, LIN, and PM, respectively, with $w1 + w2 + w3 + w4 + w5 = 1$, and x is the GCA value of a parent or the U value of a cross for a single trait. Because the traits have different scales and units as well as different selection directions (high values for YLD, OIL, and LIN but low values for DTM and PM), the GCA values of parents or the U values of crosses were converted to relative values:

$x'_{Trait} = 1 - x_{Trait} / \max\left(x_{Trait}\right)$ for DTM and PM or

$x'_{Trait} = x_{Trait} / \max\left(x_{Trait}\right)$ for YLD, OIL, and LIN. As such, $I = w1 \times x'_{YLD} + w2 \times x'_{DTM} + w3 \times x'_{OIL} + w4 \times x'_{LIN} + w5 \times x'_{PM}$, with $0 \le I \le 1$. According to the relative importance of five traits in breeding, as an example, we assigned 0.4, 0.15, 0.15, 0.15, and 0.15 to $w1$, $w2$, $w3$, $w4$, and $w5$, respectively.

All computations for this study were performed using the pipeline package as described in 13.3.

13.4.2 Results and Discussions

13.4.2.1 Identification of Quantitative Trait Nucleotides (QTNs)

A total of 450, 317, 496, 313, and 235 nonredundant QTNs were identified using six multi-locus models for YLD, DTM, OIL, LIN, and PM, respectively, which had a range of large or minor QTN effects (Table 13.3). More QTNs were detected from YLD and OIL than from DTM, LIN, and PM. These outcomes of QTNs indicated varying genetic complexity or background for different traits.

Table 13.3 . Summary of phenotypic performance and quantitative trait nucleotides (QTNs) identified using multi-locus models for five traits from the training population of 290 linseed accessions.

Trait	Unit	Acr[a]	Mean ± s[b]	Min – Max	No. of QTNs	R^2 of QTNs (%)
Seed yield	t/ha	YLD	0.83 ± 0.28	0.17 – 1.36	450	1.13 – 38.30
Days to maturity	days	DTM	97.73 ± 3.64	89.06 – 109.71	317	0.63 – 17.66
Oil content	%	OIL	42.61 ± 1.71	37.74 – 50.69	496	0.80 – 23.57
Linolenic acid content	%	LIN	54.83 ± 5.30	5.02 – 66.07	313	0.10 – 17.86
Powdery mildew	0–9	PM	4.71 ± 1.26	2.50 – 8.00	235	0.72 – 14.40

[a]Acronym
[b]Means and standard deviation of traits over 4 years and two locations except for PM which is 5 years and one location

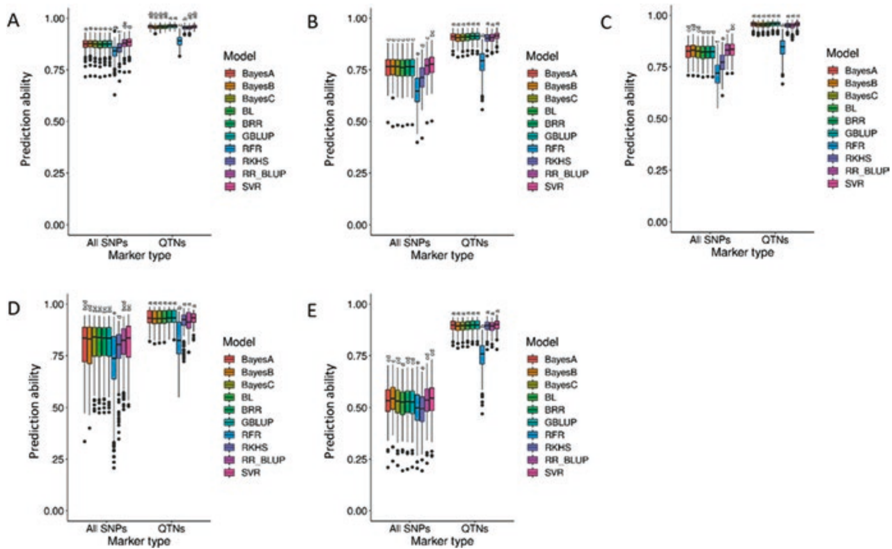

Fig. 13.2 Comparisons of predictive ability for ten genomic selection models with two different types of markers (all SNPs and QTNs). A fivefold cross-validation was used to estimate the predictive ability. Different letters represent statistical significance at a 5% probability level. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

13.4.2.2 Optimal GS Models

To choose the optimal GS models, ten different GS models were compared using a fivefold cross-validation approach. Also, for each model, two types of markers, all genome-wide random markers (all SNPs) and QTNs identified for each trait, were used to construct models. The results were depicted in Fig. 13.2. Significant differences in predictive ability using two types of markers were observed for all five

Table 13.4 Predictive ability (*r*) using RR-BLUP obtained with the trait-specific QTNs and the All SNPs datasets for five traits using a five-fold cross-validation scheme. The population sizes in model evaluation are 217 and 72 for the training and test populations, respectively.

Trait	Unit	Acronym	*r* ± *s* for datasets	
			All SNPs	QTNs
Seed yield	t/ha	YLD	0.88 ± 0.03	0.95 ± 0.01
Days to maturity	days	DTM	0.76 ± 0.06	0.90 ± 0.02
Oil content	%	OIL	0.83 ± 0.04	0.95 ± 0.02
Linolenic acid content	%	LIN	0.80 ± 0.11	0.92 ± 0.04
Powdery mildew	0–9	PM	0.53 ± 0.08	0.89 ± 0.03

s standard deviation. For each trait, the predictive ability between All SNPs and QTNs is statistically significant at a 0.01 probability level

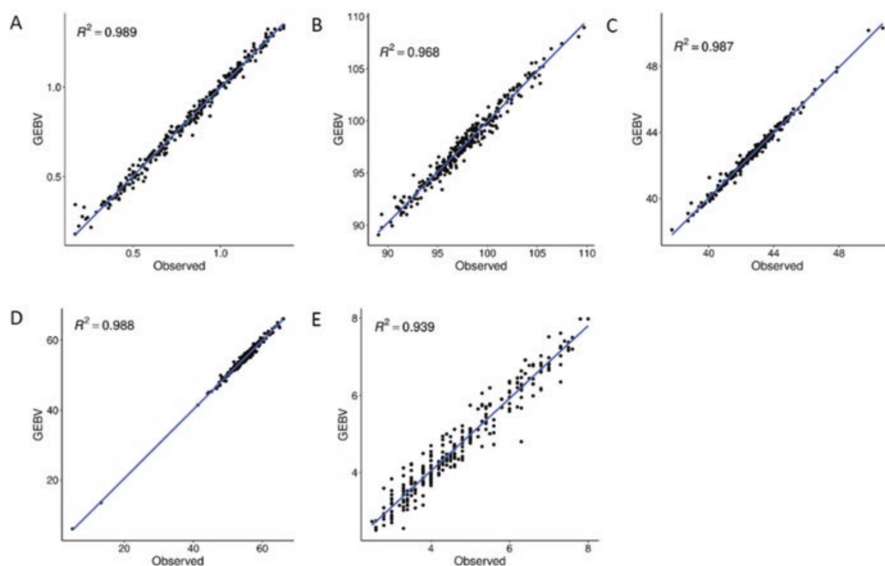

Fig. 13.3 Relationship between the general combining abilities (GCAs) and the GEBVs of parents of parents single crossed for five traits. (a). seed yield (t/ha); (b). days to maturity (days); (c). oil content (%); (d) (d). linolenic acid content (%); (e). powdery mildew resistance

traits. The GS models using QTNs generated consistently and significantly greater predictive ability than those using the genome-wide random markers (Table 13.4, Fig. 13.2). In particular, for PM, the predictive ability of the GS models using all SNPs was 0.53, compared to 0.89 when using the QTN dataset. For the QTN markers, except for RFR with significantly less predictive ability (0.75–0.89), all other nine GS models showed similarly high predictive ability (0.89–0.96) for all traits. For the genome-wide random SNP markers, ten models performed to be slightly different. Both RKHS and RFR had a low predictive ability, while the other eight models had no significant difference. Thus, in the following analyses, the RR-BLUP model with QTNs as markers was chosen to predict GEBVs of progeny populations of crosses because RR-BLUP has high computation efficiency. The RR-BLUP models constructed using respective QTNs for five traits generated very high model

R^2 (0.94–1.00) and prediction ability for themselves. The GEBVs of 290 accessions were highly correlated with the observed trait values for all five traits ($R^2 = 0.94$–0.99) (Fig. 13.3). The predictive abilities for all five traits were greater than 0.90, being 0.95, 0.90, 0.95, 0.92, and 0.89 for YLD, DTM, OIL, LIN, and PM, respectively (Table 13.4). Overall, the RR-BLUP models with QTNs of single trait per se as markers demonstrate high predictive ability and can be used to estimate GEBVs of progeny populations.

13.4.2.3 General Combining Ability (GCA) of Parents

In this study, we evaluated two progeny advancing methods, DH and RIL. The results show that the outcomes from both methods are highly similar ($R^2 = 0.993$–0.997) for all five traits. Here on end, only the results obtained from DH populations are displayed.

GCAs of 290 parents were calculated for all 41,905 single crosses. A consistently high linear relationship, i.e., close to 1, was observed between GCAs and GEBVs of the parents (Fig. 13.4), suggesting that GEBVs of parents estimated using GS models with QTN markers can effectively predict the GCAs of the parents.

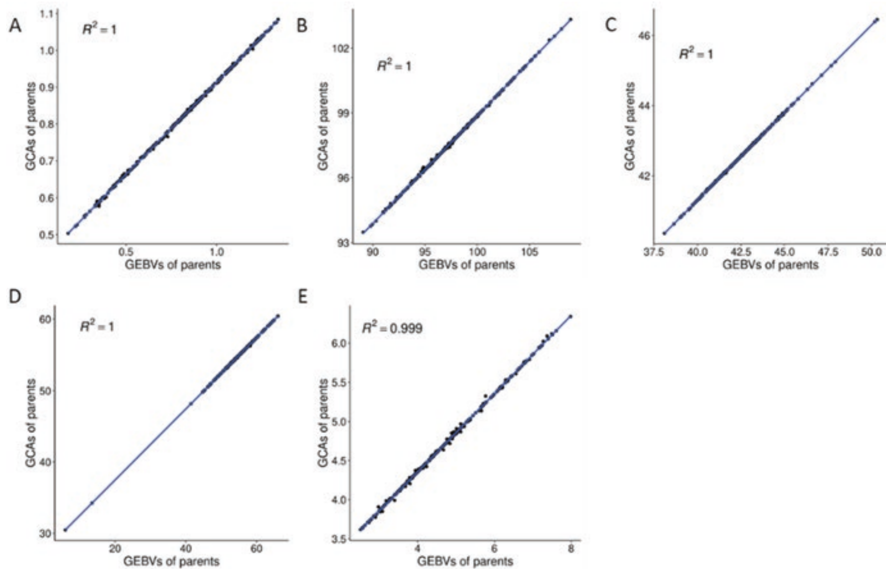

Fig. 13.4 Relationship between the general combining abilities (GCAs) and the GEBVs of parents of single crosses for five traits. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

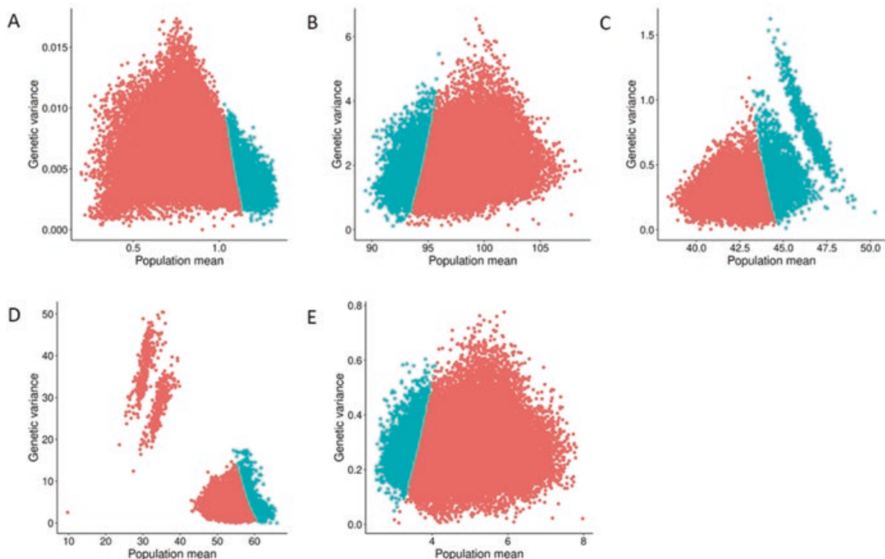

Fig. 13.5 Relationship between the population means and the genetic variances of progeny populations of single crosses for five traits. The blue dots represent the top 10% of crosses based on the usefulness *(U)* of the crosses at a 10% selection rate. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

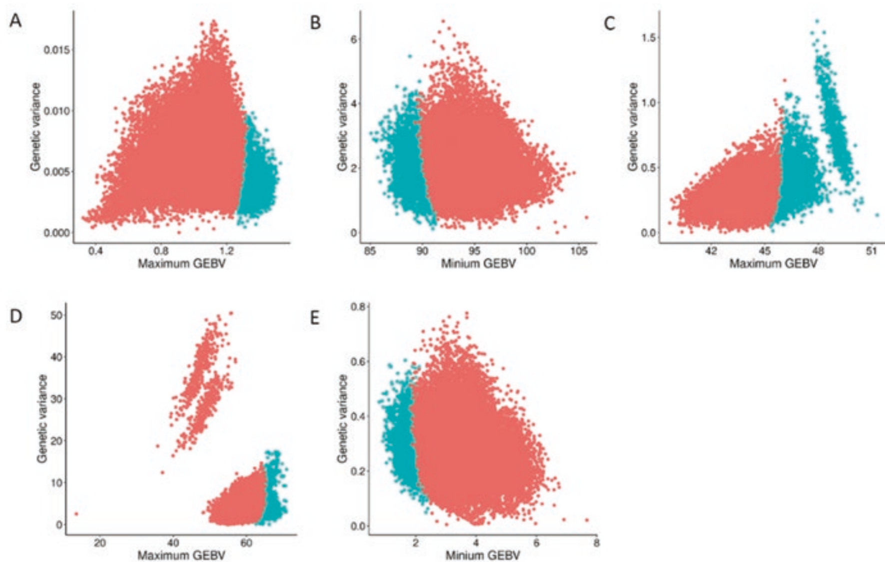

Fig. 13.6 Relationship between the minimum/maximum GEBVs and the genetic variance of progeny populations of single crosses for five traits. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

13.4.2.4 Usefulness of Crosses

Us of 41,905 single crosses were calculated based on the 500 DH individuals simulated for each cross. *Us* were not linearly correlated with genetic variances of progeny populations. The top 10% of crosses (blue dots in Fig. 13.5) had high population means for YLD, OIL, and LIN or low population means for DTM and PM, but with the exception of OIL (Fig. 13.5c) they did not have the maximum genetic variation (Fig. 13.5a, b, d, and e). This was also true for the relationship between the minimum and/or maximum GEBV values and the genetic variance in the progeny populations the minimum or maximum values in prog-eny populations (Fig. 13.6). In linseed breeding programs, early maturing, disease resistant (minimum values) individuals with high YLD, OIL and LIN (maximum values) are selected.

13.4.2.5 Relationship of GCAs with *Us*

Significant linear relationship ($R^2 = 0.93–0.98$) between GCAs and *Us* was observed for all five traits (Fig. 13.7), showing that high GCA of a parent are high performant in crosses with other parents and results in superior crosses. Midparent value is often used to predict the performance of a cross. Fairly high correlations between midparent GEBVs and *Us* ($R^2 = 0.93–0.98$) were also observed (Fig. 13.8). The best

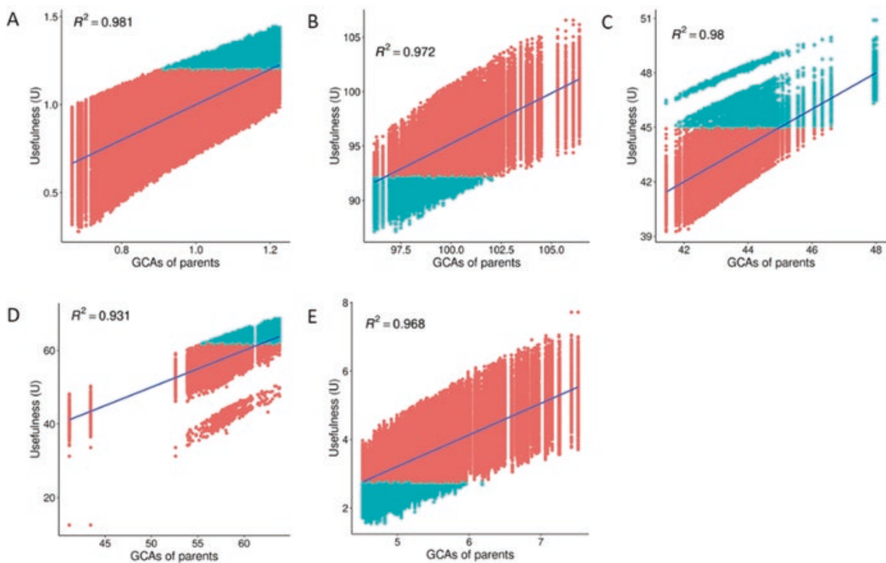

Fig. 13.7 Relationship between the general combining abilities (GCAs) of parents and the usefulness *(U)* of the corresponding crosses between parents for five traits. The blue dots represent the top 10% of crosses based on the usefulness of crosses at a 10% selection rate. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

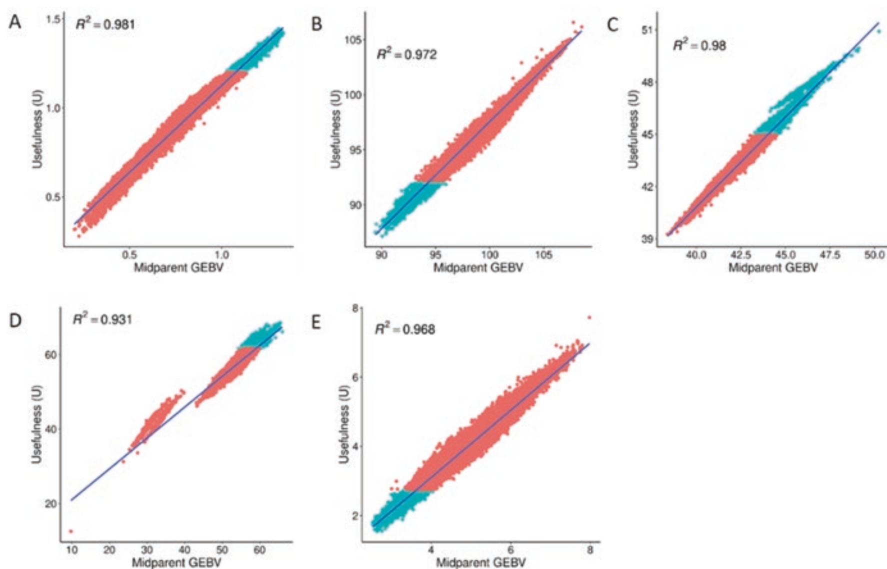

Fig. 13.8 Relationship between the midparent GEBV values and the usefulness *(U)* of single crosses for five traits. The blue dots represent the top 10% of crosses based on usefulness *(U)* of crosses at a 10% selection rate. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

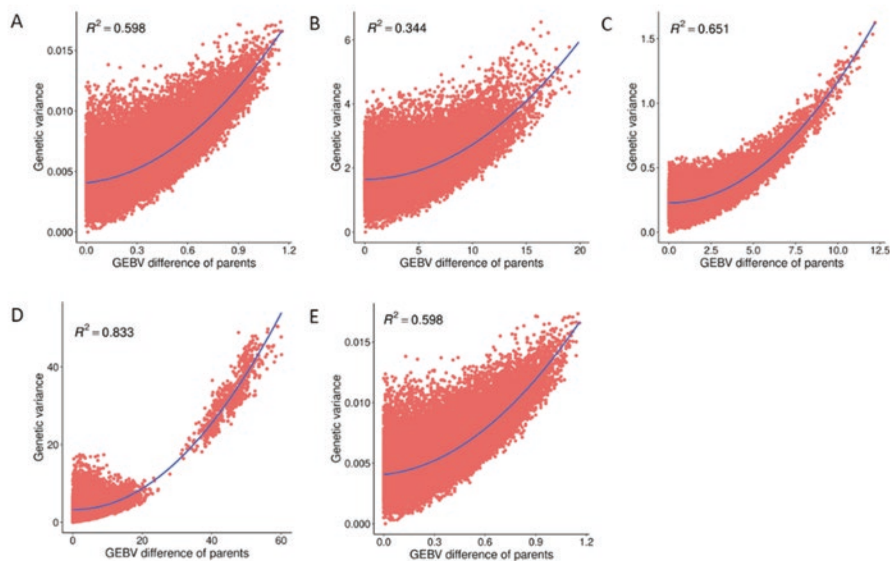

Fig. 13.9 Relationship between the GEBV differences of the two parents and the genetic variances of progeny populations of single crosses for five traits. The blue dots represent the top 10% of crosses based on usefulness *(U)* of crosses at a 10% selection rate. (**a**). seed yield (t/ha); (**b**). days to maturity (days); (**c**). oil content (%); (**d**). linolenic acid content (%); (**e**). powdery mildew resistance

crosses consistently had the best mid-parent GEBVs, confirming that it is indeed a good indicator of cross performance.

13.4.2.6 Differences Between Parents with Genetic Variance of Progeny Populations

The GEBV differences between the two parents reflected the genetic variation of progeny populations to some extent, varying in different traits (R^2 = 0.27–0.83) (Fig. 13.9). However, large parent differences between parents did not generate population mean (Fig. 13.5a, b, d and e). Once again, OIL performed differently from the other four traits, with the top crosses having a high genetic variance of populations, parent difference and parent GEBVs (Fig. 13.5c, 13.6c, and 13.9c). In particular for LIN, two low-LIN parents (Linola989 and CDC Gold) as parents with 5.02% and 13.26% LIN, respectively, resulting in large genetic variance in progeny populations (Fig. 13.9d), but did not generate superior crosses (superior population means) (Fig. 13.5d).

13.4.2.7 Evaluation of Top Parents and Crosses

Genomic cross prediction aims to predict superior parents of high GCAs and superior crosses of high *Us*. Table 13.5 lists the top 10% accessions (29 out of 290 accessions) with the highest GCAs for each trait, which have the best potential to improve traits in linseed crossbreeding. We observed that mostly unique subsets of high GCA accessions were obtained for different traits. However, 23 accessions were superior for two traits and the accession CN97907 (a USA cultivar) for three traits LIN, OIL, and PM. Using the 5-trait selection index, 25 out of the 29 parents were selected.

In the top 10% crosses (4090 out of 41,905 crosses), there were 51, 78, 212, 87, 60, and 214 parents involved in these crosses for YLD, DTM, OIL, LIN, PM, and the index trait, respectively. All 29 parents of the top 10% GCA were part of these crosses for all traits and the selection index trait (Table 13.6).

Table 13.5 Flax accessions of the top 10% general combining ability (GCA) values in the 290 potential linseed accessions for five traits and the selection index

Trait	Unit	Acronym	Accessions with top 10% general combining ability values		
Seed yield	t/ha	YLD	CAN_C_CDCMons	USA_C_CN97377	RUS_C_CN32542
			CAN_C_PrairieGrande	**USA_C_CN97873**	**CAN_C_CN19005**
			CAN_C_CN52732	ARG_C_CN97334	CAN_C_CN101413
			CAN_C_PrairieThunder	**CAN_C_CN19004**	CAN_C_CN33388
			USA_C_CN33992	UKR_U_CN101378	CAN_C_CN33385
			RUS_U_CN101348	USA_B_CN97670	CAN_C_CN18973
			USA_B_CN101286	**USA_C_CN18994**	RUS_C_CN97520
			CAN_C_CN18981	**USA_C_CN97444**	UNK_C_CN30861
			USA_B_CN100785	**ETH_B_CN19007**	**CAN_C_Shape**
			CAN_C_CN37286	**CAN_C_CN97392**	
Days to maturity	days	DTM	ARG_C_CN98014	RUS_L_CN97605	**IND_C_CN98157**
			DEU_C_CN97886	IND_C_CN98982	FRA_L_CN98742
			NLD_C_CN97616	FRA_C_CN97350	HUN_C_CN97300
			RUS_C_CN97484	ARG_C_CN97341	USA_B_CN98566B
			USA_C_CN97444	USA_B_CN97665	**CAN_C_CN97392**
			FRA_C_CN98794	USA_B_CN98566	**RUS_L_CN97483**
			IND_C_CN98135	CAN_C_CN97671	**RUS_C_CN97529**
			UNK_C_CN100547	IND_C_CN98364	**IND_U_CN98569**
			TUR_L_CN96958	TUR_U_CN101331	ARG_C_CN98027
			AFG_U_CN100952	**IND_C_CN97306**	

(continued)

Table 13.5 (continued)

Trait	Unit	Acronym	Accessions with top 10% general combining ability values		
Oil content	%	OIL	**RUS_B_CN101137**	**RUS_B_CN101307**	RUS_B_CN101279
			CAN_C_Shape	RUS_CN101402	PAK_C_CN97096
			CAN_B_CN101596	PAK_C_CN97092	RUS_B_CN101132
			IND_C_CN98157	**IND_C_CN97306**	USA_C_CN97396
			CAN_C_CN19003	IND_L_CN98242	FRA_B_CN98806
			USA_C_CN97907	IND_L_CN98240B	USA_C_CN98821
			CAN_C_PrairieBlue	CAN_B_CN101463	**IND_U_CN98569**
			IND_C_CN98363	**PAK_C_CN97064**	**CAN_C_CN19005**
			FRA_C_CN98734	FRA_C_CN98807	FRA_B_CN98741
			IND_C_CN98250	**AFG_U_CN101338**	
Linolenic acid content	%	LIN	**RUS_B_CN101137**	**UNK_U_CN100841**	CZE_C_CN100805
			RUS_B_CN101307	NZL_B_CN100797	FRA_C_CN98708
			CAN_B_CN101463	NZL_B_CN100797B	**CAN_B_CN101600**
			FRA_B_CN100863	CAN_B_CN101472	CAN_B_CN101454
			IND_C_CN100799	ETH_C_CN96988	SUN_C_CN100827
			RUS_C_CN96846	**CHN_C_CN101016**	USA_C_CN97393
			RUS_C_CN96845	USA_C_CN19160	ARG_C_CN97214
			CAN_C_CN19004	**USA_C_CN33992**	**FRA_C_CN18989**
			CAN_B_CN101448	**USA_C_CN97907**	CZE_C_CN98704
			CAN_C_PrairieBlue	CAN_B_CN101471	

(continued)

Table 13.5 (continued)

Trait	Unit	Acronym	Accessions with top 10% general combining ability values		
Powdery mildew	0–9	PM	RUS_C_CN97475	USA_C_CN33399	USA_C_CN98231
			USA_C_CN98541	**USA_C_CN97873**	TUR_C_CN96962
			ARG_C_CN97953	HUN_C_CN97287	TUR_U_CN100828
			GEO_U_CN101367	FRA_C_CN98773	**PAK_C_CN97064**
			IRN_L_CN97129B	FRA_L_CN98710	**UNK_U_CN100841**
			ETH_B_CN19007	**AFG_U_CN101338**	POL_B_CN98733
			MAR_C_CN98193	**USA_C_CN97907**	**CHN_C_CN101016**
			USA_B_CN97679B	TUR_U_CN100837	**RUS_C_CN97529**
			USA_C_CN18994	**FRA_C_CN18989**	USA_B_CN97679
			RUS_L_CN97483	**CAN_B_CN101600**	
Selection index			*ARG_C_CN97334*	*USA_C_CN97873*	*CAN_C_PrairieGrande*
			CAN_C_CDCMons	*CAN_C_PrairieThunder*	*CAN_C_CN19004*
			CAN_C_Shape	*USA_C_CN33992*	*USA_C_CN97377*
			USA_B_CN100785	*RUS_U_CN101348*	*CAN_C_CN18973*
			CAN_C_CN33385	*CAN_C_CN33388*	*CAN_C_CN101413*
			USA_C_CN18994	*UNK_C_CN30861*	*USA_B_CN97670*
			USA_C_CN97740	*USA_C_CN33399*	*CAN_C_CN19005*
			UKR_U_CN101378	*ETH_B_CN19007*	USA_C_CN97642
			RUS_L_CN97483	*CAN_C_CN37286*	*RUS_B_CN101132*
			CAN_C_CDCSorrel	TUR_U_CN101385	

Acron Acronym. Each accession name consists of three parts: country code (such as *CAN* Canada), development status (*C* cultivar, *L* landrace, *B* breeding line, *U* unknown), and CN numbers (unique accession identifier). For each trait, accession names are ordered by GCAs. The accessions that are in the top 10% subsets of at least two single traits are in bold type font, and the top 10% accessions for the selection index that are common to those for single traits are italicized.

s: standard deviation. Each accession name consists of three parts: country code (such as CAN: Canada), development status (C: cultivar; L: landrace; B: breeding line), and CN numbers (actual accession ID). For each trait, accession names are ordered by GCAs. The accessions that are in the top 10% subsets of at least two sing traits are bold, and the top 10% accessions for the index trait that are common to those for single traits are italicized.

Table 13.6 Best crosses for five traits and the selection index

Trait	Unit	Acronym	No. parents in top 10% crosses	Cross No.	Parent1	Parent2
Seed yield	t/ha	YLD	51	1	USA_C_CN97377	CAN_C_CDCMons
				2	USA_C_CN97873	CAN_C_PrairieGrande
				3	USA_C_CN97377	RUS_C_CN32542
				4	CAN_C_PrairieGrande	CAN_C_CDCMons
				5	USA_C_CN97873	USA_C_CN97377
				6	CAN_C_CN101413	CAN_C_CDCMons
				7	USA_C_CN97873	CAN_C_CN19005
				8	USA_C_CN97873	CAN_C_CN52732
				9	CAN_C_CDCMons	RUS_C_CN32542
				10	USA_C_CN97873	RUS_C_CN32542
Days to maturity	days	DTM	78	1	ARG_C_CN98014	RUS_L_CN97605
				2	IND_C_CN98157	RUS_L_CN97605
				3	ARG_C_CN98014	DEU_U_CN97886
				4	FRA_C_CN97350	RUS_L_CN97605
				5	ARG_C_CN98014	RUS_C_CN97484
				6	DEU_U_CN97886	IND_C_CN98157
				7	IND_C_CN98982	RUS_L_CN97605
				8	ARG_C_CN98014	IND_C_CN98982
				9	RUS_L_CN97605	FRA_L_CN98742
				10	ARG_C_CN98014	IND_C_CN98157

(continued)

Table 13.6 (continued)

Trait	Unit	Acronym	No. parents in top 10% crosses	Cross No.	Parent1	Parent2
Oil content	%	OIL	212	1	RUS_B_ CN101307	RUS_B_ CN101137
				2	RUS_B_ CN101279	RUS_B_ CN101137
				3	RUS_B_ CN101307	CAN_C_Shape
				4	RUS_B_ CN101137	CAN_C_Shape
				5	RUS_B_ CN101307	RUS_B_ CN101279
				6	RUS_B_ CN101137	CAN_B_ CN101596
				7	RUS_B_ CN101137	PAK_C_ CN97096
				8	RUS_B_ CN101307	RUS_U_ CN101402
				9	RUS_B_ CN101137	RUS_U_ CN101402
				10	RUS_B_ CN101307	PAK_C_ CN9709
Linolenic acid content	%	LIN	87	1	RUS_B_ CN101137	FRA_C_ CN98708
				2	FRA_C_CN98708	RUS_B_ CN101307
				3	NZL_B_ CN100797	FRA_C_ CN98708
				4	FRA_C_CN98708	CZE_C_ CN100805
				5	UNK_U_ CN100841	FRA_C_ CN98708
				6	FRA_C_CN98708	NZL_B_ CN100797B
				7	UNK_U_ CN100841	RUS_B_ CN101137
				8	FRA_C_CN98708	CAN_B_ CN101600
				9	CAN_B_ CN101463	FRA_C_ CN98708
				10	CZE_C_ CN100805	RUS_B_ CN101137

(continued)

Table 13.6 (continued)

Trait	Unit	Acronym	No. parents in top 10% crosses	Cross No.	Parent1	Parent2
Powdery mildew	0–9	PM	60	1	RUS_C_CN97475	USA_C_CN98541
				2	USA_C_CN98231	RUS_C_CN97475
				3	ARG_C_CN97953	USA_C_CN98541
				4	USA_C_CN33399	RUS_C_CN97475
				5	USA_C_CN98231	USA_C_CN98541
				6	USA_C_CN97873	PAK_C_CN97064
				7	HUN_C_CN97287	USA_C_CN98541
				8	TUR_C_CN96962	PAK_C_CN97064
				9	USA_C_CN97873	USA_C_CN98231
				10	USA_C_CN98541	FRA_L_CN98710
Selection index			214	1	ARG_C_CN97334	USA_C_CN97873
				2	USA_C_CN97377	USA_C_CN97873
				3	UNK_C_CN30861	USA_C_CN97873
				4	CAN_C_PrairieGrande	USA_C_CN97873
				5	USA_C_CN18994	USA_C_CN97873
				6	USA_C_CN33399	USA_C_CN97873
				7	USA_C_CN97873	USA_B_CN97670
				8	USA_C_CN97642	USA_C_CN97873
				9	CAN_C_Shape	USA_C_CN97873
				10	UKR_C_CN30860	USA_C_CN9787

13.5 Conclusions

With the availability of genome-wide molecular markers of potential breeding parents and other genomic information of biparental populations such as consensus genetic maps, a new genomics-based breeding tool, named genomic cross prediction, has been proposed to help breeders in assessing parents and choosing the potentially best crosses to make in breeding programs. The rationale of this genomic tool relies on the facts that the progeny populations of any number of virtual crosses can be easily simulated based on the genomic information of parents and the GS models built based on genome-wide QTLs identified from the parent panel can be effectively used to predict GEBVs of progenies. The evaluation of this genomic tool in maize, barley, wheat, and flax demonstrates its potential in breeding, offering an effective and low-cost breeding assisting tool. In particular, the case study in linseed demonstrates that the GEBVs of parents and midparent GEBVs are two good indicators for evaluating GCAs of parents and the usefulness of crosses, respectively. The comprehensive index traits combining several breeding target traits may benefit to the selection of parents and crosses with a superior comprehensive performance of several target traits.

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Chapter 14
Biotechnological Interventions for Improving Cottonseed Oil Attributes

Dharminder Pathak and Manjeet Kaur Sangha

Abstract Cotton, though mainly cultivated for its natural fibre (lint), is one of the chief edible oilseed crops globally. Cottonseed, the major by-product of cotton plant, is an excellent source of oil and protein. The fatty acid profile of cottonseed oil is dominated by unsaturated fatty acids (linoleic acid and oleic acid). Among the saturated fatty acids, palmitic acid is the predominant one. Breeding efforts in cotton have primarily focused on yield, fibre quality enhancement and tolerance to stresses. Genetic improvement of cottonseed oil attributes has never received the desired attention. Though hundreds of QTLs for yield and component traits, fibre quality and stress tolerance have been mapped, not many investigations on QTL mapping of cottonseed oil attributes have been taken up. The development of ultra-low gossypol cottonseed through the use of RNAi technology is a landmark achievement and holds great potential to enhance nutritional security. Many recent investigations have provided proof of concept of genome editing through CRISPR/Cas9 system in complex polyploid cotton. Using this technology, development of non-transgenic upland cotton mutants possessing high oleic acid has been reported very recently, which is a step towards the production of cottonseed oil with desirable functionality.

Keywords *Gossypium* · Cottonseed oil · Edible oil · Oleic acid · Palmitic acid · Cadinene synthase · Gossypol · QTL mapping · Antisense technology · RNA interference · CRISPR/Cas9

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14.1 Introduction

The word 'cotton' is used to represent four cultivated species of the genus *Gossypium*. These are *Gossypium arboreum* ($2n = 2x = 26$), *G. herbaceum* ($2n = 2x = 26$), *G. hirsutum* ($2n = 4x = 52$) and *G. barbadense* ($2n = 4x = 52$). Of these, *G. hirsutum* also known as American cotton or Upland cotton is the leading cotton species occupying greater than 98% of the global cotton acreage (Kranthi 2019). Asiatic cottons comprising *G. arboreum* and *G. herbaceum* are mainly cultivated in India, Pakistan, Bangladesh, China, Iran, Myanmar, Thailand, etc. whereas *G. barbadense* (Egyptian cotton or Pima cotton) – prized for its extra-long staple fibre – is grown in Egypt, Sudan, the USA, China, Uzbekistan, India, Israel, Peru, Turkmenistan, etc. Eight genome groups, viz. A, B, C, D, E, F, G and K, have been recognized among the diploid cotton species. Hendrix and Stewart (2005) reported more than threefold variation in the size of these genomes. Allotetraploid cotton species originated from the hybridization between two diploid cotton species – a New World D-genome species (resembling *G. raimondii*) and an Old World A-genome species (resembling *G. herbaceum*) about 1–2 million years ago. Designations of various *Gossypium* genomes and chromosomes have recently been reviewed by Wang et al. (2018a). After the rediscovery of Mendelian principles in 1900, cotton was one of the first crops subjected to genetic analysis (Balls 1906; Shoemaker 1908).

Cotton, one of the most important cash crops, is grown in greater than 80 countries by nearly 28.67 million farmers. It was cultivated on an area of 32.65 million ha worldwide during 2018–2019 with India, China, the USA, Brazil, Pakistan and Uzbekistan contributing about 80% to the global cotton production of 25.694 million metric tonnes (www.icac.org). Cotton is primarily cultivated for lint (long fibres) which is the principal natural fibre used in the textile industry worldwide. Cottonseed, the major by-product of cotton plant, is an excellent source of oil. In fact, cotton is the fifth largest source of vegetable oil globally (Chen et al. 2021). Cottonseed constitutes at least 60% of the seed cotton (lint plus seeds) by weight. Besides oil (16%), the other important by-products of cottonseed include hull (27%), linters (8%) and meal (45%) (Mageshwaran et al. 2015). Availability of cottonseed for the extraction of edible oil and other products is not likely to be a constraint owing to the perennial demand for cotton lint by the textile industry.

14.2 Composition of Cottonseed Oil

Cottonseed is not only a source of fibres and linters but also of valuable edible oil, meal and minerals for livestock, poultry, etc. (Yu et al. 2012; He et al. 2013). Crude cottonseed oil is dark, reddish-brown in colour with a strong distinctive taste. The dark colour of crude oil is due to the presence of polyphenolic compound gossypol in it. The refined oil is clear with a light golden colour. The reverted flavour of

deodourized cottonseed oil is typically described as nutty or nut-like and is appropriate at higher levels of oxidation than other vegetable oils. Its properties make it an excellent choice for cooking and salad oil, margarines, shortenings, specialty fats and oil products. Cottonseed oil is also a potential raw material for the energy industry (Shang et al. 2016). The genetic variability for oil content in cotton is widely reported in the literature. Several studies on variation in oil content in cotton have reported values ranging from 13.6% to 30.2% (de Cavalho et al. 2010; Khan et al. 2010).

The cottonseed oil is grouped with unsaturated vegetable seed oils, viz. safflower, corn, soybean, rapeseed and sunflower (Bert et al. 2003). Its fatty acid profile generally consists of 65–70% unsaturated and 30–35% saturated fatty acids. The unsaturated component has 18–24% monounsaturated (oleic acid) and 42–52% polyunsaturated (linoleic acid) and 26–35% saturated (palmitic acid and stearic acid) (Table 14.1). Typically, cottonseed oil is composed of about 58% linoleic acid (C18:2), 15% oleic acid (C18:1), 26% palmitic acid (C16:0), 3% stearic acid (C18:0), 1% myristic acid (C14:0), 0.6% palmitoleic acid as well as 0.17% linolenic acid (C18:3) (Radcliffe et al. 2004). High content of palmitic acid makes the oil stable and suitable for high-temperature frying applications, but being a saturated fatty acid, it raises the content of low-density lipoprotein cholesterol that is atherogenic (Cox et al. 1995). The oil also contains high content of linoleic acid, an essential fatty acid that has to be provided through diet. In the body, it is converted into alpha-linolenic acid which is then converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), otherwise obtained from fish oil and some other plant sources, that are essential for the brain functioning. Linoleic acid however, being oxidatively unstable, tends to decrease the shelf life of cottonseed oil making it rancid and imparting off-flavour to the oil. The commercial cottonseed oil is partially hydrogenated to raise its oleic acid content needed for deep-frying

Table 14.1 Typical fatty acid composition in different forms of cottonseed oil

Fatty acid	Cottonseed cooking oil	[a]Partially hydrogenated
Myristic (14:0)	0.8	0.9
Palmitic (16:0)	24.4	22.5
Palmitoleic (16:1)	0.4	0
Stearic (18:0)	2.2	5.5
Oleic (18:1)	17.2	50.0
[b]Linoleic (18:2)	55.0	20.3
[b]Linolenic (18:3)	0.3	0.3
Summary		
% Saturates	27	29
% Monounsaturates	18	50
% Polyunsaturates	55	21

Source: Cottonseed Oil Quality Utilization and Processing. Technical Bulletin from ICAR-Central Institute for Cotton Research, Nagpur, India (www.cicr.org.in)

[a]Partially hydrogenated cottonseed oil (iodine value, approximately 80)

[b]Essential fatty acids; linolenic is an omega-3 fatty acid.

applications in the food industry. However, extensive hydrogenation of the oil is done for margarine production. The drawback of extensive hydrogenation is the formation of trans-fatty acids, which though are preferred by confectionary industry for providing good taste but have been demonstrated to raise LDL cholesterol levels, like the saturated fatty acids (Ascherio and Willett 1997). Stearic acid is a neutral saturate in terms of lipoprotein metabolism and cardiovascular disease (Orsavova et al. 2015). Oleic acid has the same LDL lowering effect as linoleic acid, but it is less susceptible to oxidation. Cottonseed oils with enhanced levels of oleic acid (20% raised to 77% mainly at the expense of linoleic acid) and of stearic acid (2% raised to 38% at the expense of both oleic and linoleic acid) have been produced (Anonymous 2001). Recently Gao et al. (2020) modified fatty acid composition of cottonseed oil by transformation with *FAD3* and *D6D* genes resulting in 30% ALA (alpha-linolenic acid) and 20% GLA (gamma-linolenic acid) contents, while the oil content remained unchanged. The *FAD3* and *D6D* genes were derived from *Brassica napus* and *Echium plantagineum*, respectively. The major aim of transformation was to produce cottonseed oil containing ALA and GLA (which have higher oxidative stability than LC-PUFAs) that would act as precursors of LC-PUFAs: EPA and DHA.

The oil also contains unusual fatty acids in it: the cyclopropenoid fatty acids, malvalic acid and sterculic acid. Their presence in poultry diet causes deleterious effects like pink colour and rubbery texture of yolk and depression in egg production (Phelps et al. 1965; Shenstone et al. 1965). They also increase level of stearic acid and decrease oleic acid in animal tissues that would increase atherogenic lipid profile (Matsumori et al. 2013).

Due to high concentration of unsaturated fatty acids, the refined and processed cottonseed oil has been considered as a promising substitute at 50% of soybean oil in diet for broilers without affecting their performance (Yang et al. 2019). Because of its low cost and flavour stability as compared to other oils, cottonseed oil is widely used in processed foods (Dowd et al. 2010). Cottonseed oil is generally taken as the standard for evaluating flavour and odour of other oils. Cottonseed oil inherently has high level of antioxidants, tocopherols, that contribute to its long shelf life. Vit E is retained at high levels in fried products, preserving their freshness and shelf life. Tocopherols protect lipids against peroxidation (Salimath et al. 2021).

Apart from free fatty acids, the non-glyceride components constitute about 2% of the crude oil. The main non-glyceride components are phospholipids, tocopherols, sterols, resins, carbohydrates, pesticides and gossypol and other pigments. Refining removes most of these components. Gossypol is the major yellow polyphenolic binaphthyl dialdehyde pigment found in lysigenous glands of the cotton plant. The glands in the green parts of the plant contain gossypol, hemigossypolone and heliocides, while those in roots contain gossypol, gossypol-6-methyl ether, gossypol-6,6-dimethyl ether plus hemigossypol, desoxyhemigossypol, hemigossypol-6-methyl ether and desoxyhemigossypol-6,6-dimethyl ether (Rathore et al. 2020). In petals and the seed kernels, the glands contain mainly gossypol (Sunilkumar et al. 2006). In addition to gossypol, other related pigments present in seed are gossypurpurin, gossycaerulin, gossyfulivin and gossyverdurin. Considerable

variations in gossypol content have been reported from variety to variety even within the same species (Pandey 1998). In seed, gossypol is present in two forms: free gossypol (0.4–1.5%) and bound gossypol (2.0–4.0%) (Pons and Eaves 1967). Gunstone (2013) compared fatty acid composition of various edible oils. According to this report, there are two major oils that are rich in palmitic fatty acid: palm oil (46%) and cottonseed oil (27%). Cottonseed oil is also rich in linoleic acid along with corn, linola, soybean and sunflower oil, whereas groundnut oil is rich in both oleic and linoleic acids. Olive and canola oils are mainly oleic acid oils.

The oil without gossypol is pale yellow in colour and rich in vitamin E and can be used directly for cooking and for *vanaspati* (vegetable hydrogenated oil) production. Gossypol reacts mainly with lysine residue of the meal protein and decreases its nutritive value through reduction in availability of this amino acid to the body. In non-ruminants (pigs, birds, fish and rodents), it is released from the bound form during digestion, and the free gossypol is absorbed, which is biologically active and causes toxicity to animal (Gadelha et al. 2014). In high concentrations, gossypol causes many acute clinical problems like respiratory distress, reduction in body weight gain, anorexia, anaemia and weakness (Gadelha et al. 2014). It also affects male and female reproductive systems (Randel et al. 1992). Gossypol can be removed by various chemical treatments, viz. solvent extraction, ferrous sulphate treatment and calcium hydroxide treatment. These methods inactivate free gossypol or transform it from a free to a bound state. Microbial fermentation is a promising method since gossypol is biodegraded during this process (Kumar et al. 2021).

Absence of gossypol glands in certain plant parts (stem, petiole, carpel wall) was reported to be governed by a single recessive gene (gl_1) and did not influence level of gossypol in cotton seeds (McMichael 1954). Later on, McMichael (1960) reported that two more genes designated gl_2 and gl_3 in the double homozygous condition resulted in almost complete absence of lysigenous glands containing gossypol. Glandless cotton varieties were developed and commercially released for cultivation in the USA. However, due to their vulnerability to pests and other factors, these varieties were not commercially successful.

14.3 Enhancing Cottonseed Oil Attributes Through Biotechnological Interventions

DNA-based markers have found a variety of applications in plant breeding such as mapping of economically important genes/quantitative trait loci (QTLs), construction of high-density genetic linkage maps, germplasm characterization, marker-assisted selection, etc. Hundreds of QTLs for seed cotton yield, its component characters, various fibre traits, stress resistance/tolerance, etc. have been mapped in cotton. However, limited studies on QTL mapping of cottonseed nutrients including oil and component fatty acids have been conducted. Song and Zhang (2007) identified a significant QTL (designated as *qOP-D8-1*) linked with kernel oil content in

cotton. Four QTLs (*qOC-18-1*, *qOC-LG-11*, *qOC-18-2* and *qOC-22*) for cottonseed oil content were reported by Alfred et al. (2012). In another study by Yu and coworkers (2012), 17 QTLs located on 12 chromosomes were found to be associated with seed oil content in cotton. Liu et al. (2015) identified 15 QTLs for coarse oil, 8 QTLs for linoleic acid, 10 QTLs for oleic acid, 13 for palmitic acid and 12 for stearic acid content in upland cotton. QTLs for coarse oil were detected on 15 chromosomes and explained 2.0% through 39.8% of the phenotypic variation. Eleven of the 15 QTLs associated with coarse oil were detected in one environment, whereas 2 QTLs (*qCO21.1* and *qCO23.1*) were detected in two environments and *qCO07.1* and *qCO12.1* in three environments. Of the eight QTLs identified for linoleic acid, none was detected in more than one environment. These eight QTLs explained 2.2% to 8.0% of the phenotypic variation. The ten QTLs identified for oleic acid explained 2.0% to 15.4% of the phenotypic variation. One QTL (*qOA18.1*) was detected in two environments, whereas rest of the QTLs could be detected in one environment only. All the 13 QTLs for palmitic acid were detected in one environment only and explained 4.2% to 13.3% of the phenotypic variation for this trait. Similarly for stearic acid, 12 QTLs explaining 4.4% to 22.7% phenotypic variation were detected. One of the QTLs (*qSA14.1*) was detected in two environments, whereas rest of the QTLs could be detected in one environment only. In a more recent study, Shang et al. (2016) identified 24 QTLs associated with cottonseed oil content using composite interval mapping. Nine of these QTLs were detected in at least two environments or two mapping populations.

It is evident that genetics of cottonseed oil and its component fatty acids is complex and genotype × environment interactions play an important role in the expression of these traits. For marker-assisted transfer of QTLs associated with cottonseed oil and component fatty acids, it will be fruitful to focus on stable and major QTLs.

Gossypium sturtianum (C_1), one of the diploid wild Australian cotton species, possesses glanded foliage, but the seeds are devoid of glands; hence they do not contain gossypol (Fryxell 1965). This cotton species belongs to tertiary gene pool and is very difficult to hybridize with upland cotton. Several attempts were made to transfer the glandless seed trait from *G. sturtianum* to *G. hirsutum*. However, the interspecific derivatives manifested several defects and could not compete with the commercial cultivars. After the conventional plant breeding approaches were unsuccessful in producing a cotton plant having glanded foliage and seeds without gossypol, scientists resorted to the use of biotechnological tools available at that time such as antisense technology in which activity of the target gene is downregulated. Here, gene encoding (+)-δ-cadinene synthase (involved in biosynthesis of gossypol) was chosen for silencing. However, these research efforts did not produce the desired results (Rathore et al. 2020 and relevant references therein).

RNA interference (RNAi) was initially identified as a response to pathogen attack in the nematode *Caenorhabditis elegans* (Fire et al. 1998). RNA interference is a mechanism of post-transcriptional gene silencing and has been widely used as a

reverse genetics strategy to silence/reduce the expression of selected gene(s) in order to produce the desired phenotype. Abdurakhmonov et al. (2011) have reviewed RNAi as functional genomics approach for improving cottonseed and oil quality, disease resistance, tolerance to abiotic stresses, fibre quality, etc. Liu et al. (2002) reported the disruption of *FAD2* and *SAD1* genes in cotton and consequent increase in the content of oleic and stearic acid, respectively. Using this technology, Dr. Keerti Rathore's laboratory at TAMU (USA) selectively silenced the δ-cadinene synthase gene (that catalyses first step in the synthesis of cadinene) using seed-specific promoter leading to 97% reduction in seed gossypol levels. No alterations were observed in the gossypol and related terpenoid content in rest of the plant body (Rathore et al. 2020). In 2019, the genetically engineered event (TAM66274) with ultra-low gossypol content in cottonseed was approved in the USA as food and animal feed.

The post-genomic era is witnessing the rise of genome-editing-based precision breeding. Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated enzyme (Cas) is a three-component system consisting of crRNA, tracrRNA and Cas9 (an endonuclease protein) used for inducing targeted genetic alterations (Datsenko et al. 2012; Jinek et al. 2012). In plants, the use of this system for genome editing was first published in 2013 (Li et al. 2013; Shan et al. 2013). Several investigations on the use of this technology in cotton have been reported since the last few years (Chen et al. 2017; Gao et al. 2017; Janga et al. 2017; Li et al. 2017; Wang et al. 2017, 2018b; Long et al. 2018; Zhu et al. 2018). Many of these initial studies explored the possibility of editing a complex polyploid genome of cotton and provided proof of concept of genome editing in cotton. Using CRISPR/Cas9, visual marker genes (*DsRED*, *phytoene desaturase*, *GFP* or *Cloroplastos alterados*) were edited which resulted in the appearance of albino phenotype. These successful demonstrations involving CRISPR/Cas9 have set up the stage for utilization of this genome editing technology for enhancing several attributes of cotton including seed nutritional quality. Very recently, Chen et al. (2021) reported the development of non-transgenic upland cotton mutants possessing high oleic acid using CRISPR/Cas9 editing system. To accomplish this task, *GhFAD2-1A/D* homologs were targeted for editing/knockout as fatty acid desaturase (FAD2) is responsible for conversion of oleic acid to linoleic acid through the addition of a double bond. Substantial increase in oleic acid content along with corresponding reduction in linoleic acid level was recorded in the *GhFAD2-1A/D* cotton knockouts. One of the edited cotton lines possessed 77.72% oleic acid as compared to average 13.94% in the wild-type parent. Similarly, linoleic acid content in the same *GhFAD2-1A/D* cotton knockout decreased from 58.62% to 6.85%. The results were encouraging as no changes in total oil content, stearic acid content and other economically important traits such as fibre length, fibre strength and fibre fineness were detected in the cotton mutant lines as compared to wild type. These non-transgenic mutant cotton lines with high oleic acid would serve as invaluable genetic resource for the transfer of high-oleic acid character in elite cotton backgrounds.

14.4 Future Prospects

The post-genomic era is witnessing the rise of genome-editing-based precision breeding and has opened new vistas for the development of non-transgenic cotton cultivars with desirable seed oil composition.

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Chapter 15
Advances in Classical and Molecular Breeding in Sesame (*Sesamum indicum* L.)

Manjeet Singh, Surjeet Chahar, Ram Avtar, Anoop Singh, and Neeraj Kumar

Abstract Sesame (*Sesamum indicum* L.) is an important but underexploited oilseed crop of tropical and subtropical region having potential to sustain agriculture under changing climatic conditions. Sesame oils have high nutritional and industrial values due to its desirable fatty acid compositions and high amount of antioxidant components, viz., sesamin and sesamolin. Despite this, still sesame is not grown on large acreage due to unavailability of high-yielding cultivars with inbuilt resistance to various biotic and abiotic stresses. Therefore, serious efforts are necessary to develop cultivars having high adaptive potential to the diverse climatic situations along with high yield potential. Classical plant breeding methods impart considerable improvement in sesame, but still a huge gap is left between realized and actual yield potential of sesame. Therefore, efforts should be made toward modern molecular techniques like marker-assisted plant breeding and omics and modern bioinformatics tools to develop climate adaptive, high yield potential along with excellent oil quality cultivars in sesame.

Keywords Sesame · *Sesamum indicum* L. · Nutritional values · Antioxidant · Resistance · Yield potential · Cultivars

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15.1 Introduction

Many developing countries of the world are facing the problem of malnutrition as major part of population is vegetarian and the availability of good-quality nutritional food is limited. Oilseeds are next to cereals in importance which contain superior quality protein, essential fatty acids, vitamins, and minerals. In India, usually nine oilseeds, i.e., soybean, sesame, castor, niger, rapeseed-mustard, groundnut, safflower, sunflower, and linseed, are cultivated. Out of which, sesame (2n = 26) is of great importance and widely known as "Queen of Oilseeds" due to high resistance to rancidity and oxidation of sesame oil (Sarwar et al. 2013), and for the same reason, sesame seeds are also known as the "seeds of immortality" (Bedigian and Harlan 1986). Tocopherol confers the resistance to oxidative deterioration in sesame oil, bioavailability of which is in turn enhanced by sesamol (Wu 2007).

The oil content is abundant in seeds (32.8–62.7%) (Uzun et al. 2008; Couch et al. 2017). Sesame oil contains significantly high amount of unsaturated essential fatty acids [linoleic acid (37–47%), oleic acid (35–43%)], while the saturated fatty acid content [stearic acid (5–10%), palmitic acid (8–11%), behenic acid, and arachidic acid] is usually low. The seeds also contain 4.3–20.5% carbohydrates, 4.2–6.9% ash, 2.7–6.7% fiber content, and 14.1–29.5% proteins as well as vitamin E, minerals, lignans (sesamolin and sesamin), and tocopherols (Fukuda et al. 1985; Kamal-Eldin et al. 1992; Ashri 1998; Unal and Yalcin 2008; Hassan 2012, Couch et al. 2017). In addition, sesame seeds are also rich in a variety of minerals including potassium (K), magnesium (Mg), phosphorous (P), calcium (Ca), and sodium (Na) (Nzikou et al. 2009; Couch et al. 2017). Sesame oil is often considered as a good protein source because of its balanced amino acid composition, particularly methionine and tryptophan, and is therefore beneficial to patients suffering from kwashiorkor (Pathak et al. 2014a, b). The various chemical compositions of sesame seed are given in Table 15.1.

Sesame is being cultivated from ancient times and its oil was used for spiritual purposes (Weiss 1983). It belongs to division Phanerogams, class Dicotyledonae, subclass Gamopetalae, series Bicarpellatae, order Persoriales, family Pedaliaceae, and genus *Sesamum*. It is also named as gingelly, beniseed, sim-sim, and til (Shah 2013). It is grown throughout the tropical and subtropical region from 25°N to 25°S. The origin of sesame can be traced back to Africa as several wild relatives of sesame exist in Africa (Sani et al. 2014). India as well as China, Central Asia, Near East, and Abysinia has been recognized as sesame diversity centers in classical studies which is not at all surprising considering the genotypic variability of sesame in India (Zeven and Zhukovsky 1975; Hawkes 1983; Laurentin and Karlovsky 2006).

Sesame is mostly self-pollinated short-day plant, with the probability of 5–68% of cross-pollination (Langham 1944; Ashiri 2007). Generally, its flower opens at morning and withers after 4–6 h of anthesis. While stigma receptivity lasts for 14–24 h after flower opening (Abdel et al. 1976; Yermanos 1980) and varies with genotype (Langham 2007). Anther dehisce just after opening of flower and pollen grains remains viable for 24 h after dehiscence at 24–27 °C (Yermanos 1980). Plants

Table 15.1 Sesame seed chemical compositions

Constituents	Concentration (per 100 g dry seeds)
Fat	49.7 g
Saturated fatty acid	7 g
Monounsaturated fatty acid	18.8 g
Polyunsaturated fatty acid	21.8 g
Energy	573 kcal (2400 kJ)
Carbohydrate	23.4 g
Protein	17.7 g
Dietary fiber	11 g
Moisture	4.7 g
Sugar	0.3 g
Minerals	
Potassium	468 mg
Zinc	7.8 mg
Magnesium	351 mg
Sodium	11 mg
Phosphorous	629 mg
Iron	14.6 mg
Calcium	975 mg
Vitamins	
Vitamin A	9 IU
Thiamine (B1)	0.79 mg
Riboflavin (B2)	0.25 mg
Niacin (B3)	4.52 mg
Pyridoxine (B6)	0.79 mg
Folate (B9)	97 μg
Vitamin E	0.25 mg

Source: https://ndb.nal.usda.gov/ndb/foods/show/3620

are erect to semi-erect decided by branching types lanceolate to ovate, having pointed leaves with entire or serrate margins and round stem. Flowers are axillary, solitary, short pedicellate and zygomorphic with five fused sepals. Corolla is pendulous, campanulate, and tubular, and one petal is longer than others. Each flower has five (one sterile and four fertile) didynamous and epipetalous stamens with dorsifixed filaments. The gynoecium is multicarpellary with bifid stigma, long style, and a superior ovary. Seeds are very small (4 × 2 mm with 1-mm-thick hilum), pearl shaped, ovate, small, and slightly flattened. Indeterminate growth habit is observed in most of the varieties showing continuous growth of new leaves, regular flowering, and formation of capsules, and as long as the environmental conditions are favorable though, distinct varieties and strains differ considerably in size, growth, form, flower color, seed size, and seed color.

Sesame (*Sesamum indicum* L.) is a valuable *Kharif* oilseed crop, mostly grown in light sandy soil as rainfed crop in the arid and semiarid tropics of southern parts of Haryana, Rajasthan, Madhya Pradesh, Gujarat, and Uttar Pradesh. The growth

period could be 70–150 days based on the variety and the environmental conditions (Ashri 1998). Sesame seed requires temperature around 20 °C for germination, and more than 23 °C favors good growth and high yields. The crop rarely requires redundant irrigation factually due to its high susceptibility toward the moisture stress. Sesame production is progressively endorsed because of relatively simple cultivation as it can be grown on various kinds of soil, tolerance to high temperatures, less labour-intensives and flexibility to fits in crop rotation schemes (Langham 2007; Dossa et al. 2017a, b). The overall sesame production is below the expectation worldwide mostly due to absence of effective pest control methods, occurrence of biotic and abiotic stress, and notably lack of a pertinent breeding program (Duhoon 2004; Ram et al. 2006). The statistics have shown a decline in production of sesame despite the fact that it is a highly self-sufficient crop (Anthony et al. 2015; FAOSTAT 2015). Declining production and low yield are also of great concern because of its economic importance and high medicinal value. The commercial varieties of sesame are susceptible to biotic and abiotic stress factors including photosensitivity and experience early senescence (Rao et al. 2002). Various studies have elucidated the presence of biotic and abiotic stress-resistant genes in wild species of sesame (Joshi 1961; Weiss 1971; Brar and Ahuja 1979; Kolte 1985).

Late maturing cultivars are proclaimed to have high oil contents than the early maturing counterparts. Amount of oil content also varies according to the location of capsules on the same plant such as the seeds on the main stem from the basal capsules carry more oil than those placed toward the apex and on side branches (Mosjidis and Yermanos 1985). Similarly, brown and white seeded cultivars often have high oil content than black ones, indicating a probable genetic linkage between seed coat color and oil content. Black seed coats are usually thicker than brown- and white-colored coats.

Global sesame production and area coverage were 5,531,948 tons and 9,983,165 hectare, respectively, in 2017 (FAOSTAT 2019). Prime sesame producing countries are Tanzania, India, Nigeria, China, and Ethiopia (FAOSTAT 2019). Worldwide sesame seed consumption in 2018 was reported to be USD 6559.0 million and is approximated to extend up to USD 7244.9 million by 2024, at 1.7% CAGR (compound annual growth rate). India and China are among the top producers of sesame globally, and average yield is highest in China (1223 kg/ha) trailed by Nigeria (729 kg/ha) and Tanzania (720 kg/ha) (FAOSTAT 2020). India was the largest exporter of sesame seeds with annual production recorded 850,000 tons, and total area under cultivation was 1951,000 hectares in 2015–2016. West Bengal was top producer trailed by Madhya Pradesh, Rajasthan, and Uttar Pradesh (Anonymous 2016).

Sesame seeds are used to produce high-quality edible oil also used in fish canning and the production of butter substitutes like margarine (Uzo 1998). Varieties of steroids present in sesame oil increase the insecticide potency of pyrethroids which are used as commercial and household insecticide. Seeds as well as the leaves of sesame are used to prepare food delicacies such as variety of porridges and are also consumed with fried groundnut. Consumption of sesame in the form of porridge is well known from the tribal populations which are eaten by the Fulani tribe mixed with the millet and by Tiv people with the boiled yam (Uzo 1998). Dried sesame leaves after pulverization are used in soups as it is a rich source of minerals and

proteins. The seeds are often consumed as a candy and as snacks mixed with sweetener and groundnuts or with partially cooked cowpea. The seeds are used in bread and confectionery industry as well. Sesame flour is more compatible with wheat flour than other oilseeds for producing bread with good loaf volume and crumb texture. The brown and black varieties are fermented to produce local brew beer (Burukutu). Sesame oil is used as a substitute of other solvents in paints, liniments, ointments, and cosmetics (Mbaebie et al. 2010).

Commercially sesame semidrying oil is used as an alternate of olive oil, corn oil, and cotton seed oil for restraining bacterial infection on umbilical cord of infants. Studies have also revealed the anticancer and neuroprotective properties of sesame oil to cure hypoxia or brain damage (Hibasami et al. 2000; Miyahara et al. 2001; Cheng et al. 2006). Antioxidants have been proclaimed to possess health promoting effects like reducing hypertension and cholesterol levels in humans (Noguchi et al. 2001; Sankar et al. 2005). The cake left after oil extraction is rich in phosphorus and calcium and thus used as animal feed. Other uses of sesame seed and oil for medical purposes include the treatment of sores, ulcers, diarrhea, and dysentery. Historically, it is used for customary marriage rites of some tribes in Nigeria. The dried stems of plants are tied together to be made broom, while the ashes of dried shrub are used for the production of black soap. The plant residue is also plowed into the soil to enrich it. The dehulled press cake of sesame is used for the treatment of malnutrition in children because of the presence of globulin as a principal protein in it (Abdullahi 1998).

15.2 Classical Breeding in Sesame

Sesame is often described as the oldest oilseed crop cultivated in ancient times both for its edible seed and mainly for its oil. Sesame oil is esteemed as a best vegetable oil because of its high nutritional quality and stability to oxidative rancidity (Biswas et al. 2018). Despite this, sesame is mainly grown under marginal and submarginal land and also known as poor's farmer crop. Sesame is an underexploited oilseed crop, yet still it holds tremendous potential for enhanced food value (Manjeet et al. 2020). Sesame is one such crop that justifies imperative and instant consideration of the scientific communal. The sesame plant type is not well adapted to current farming schemes since of its unstipulated growth habit causing varying ripening of capsules, their varying susceptibility to different stresses, and unavailability of non-shattering varieties suited for mechanical harvest (Ashri 1998). The important breeding objectives of sesame are high yield potential along with high oil content; yield stability through tolerance against different abiotic stresses, viz., drought, salinity, heavy metal stress, water logging, and temperature extremities; resistance to biotic stresses like phyllody and charcoal rot, *Alternaria* blight, leaf curl virus, and several insect pests; and good confectionary quality to meet industrial demands (Ashri 1998; Islam et al. 2016; Sinha et al. 2020). India is a center of origin and diversity for sesame, and several of important germplasm contain economically important traits that are largely underexplored for use in sesame improvement

programs (Bisht et al. 1998; Bedigian 2003). However, a systematic screening of these germplasm or their characterizations is still totally lacking. Hence, germplasm evaluation and pre-breeding are still a key approach for sesame improvement. The important breeding objectives in sesame will be discussed in the following heads.

15.2.1 High Seed Yield

In spite of being a great source of very healthy edible oil in terms of presence of huge amounts of polyunsaturated fatty acids and several antioxidants, sesame is cultivated on very small acreage due to availability of poor yielding dehiscent varieties with low harvest index. Lack of improved varieties is the major reasons behind low seed yield in sesame (Pathak et al. 2014a, b). So, productivity enhancement by accumulating desirable alleles into single genetic background is the prime objective for sesame breeders. Sesame seed yield is depending upon its several component traits like number of primary and secondary branches per plant, number of capsules per plant and capsule length, seed weight, and number of seeds per capsule (Teklu et al. 2014; Mustafa et al. 2015; Ramazani 2016; Shakeri et al. 2016;). As yield is a complex trait with low heritability, therefore, indirect selection for yield through its abovementioned attributing traits may enhance productivity in sesame. Another very important yield attributing trait in sesame is harvest index, which directly related with high yield in sesame (Day et al. 2002). However, to improve harvest index in sesame, plant type should be of medium plant height with high density capsule bearing starting from 15 to 20 cm above the ground (Tripathy et al. 2019).

15.2.2 Early Maturity and Short Plant Stature

Early maturity and short plant stature are the two important agronomic traits in sesame which makes it fit for cultivation for farmers (Uzun and Çagırgan 2006). Early maturity not only helps in reducing crop cultivation cost but also provides enough times for succeeding crop. Short plant stature is very helpful to breed lodging-tolerant sesame and an important component toward mechanical harvesting in sesame (Ashri 1998).

15.2.3 High Oil Content

High oil content is another important breeding objective as oil is chief produce for oilseed crops. Among different oilseed crops, sesame has high oil content (∼55%) which makes it suitable as a key oilseed crop (Yadava et al. 2012). Sesame oil content and its quality are varying with the genotype, color (black to white), and size of the seed. Oil content in sesame gene pool varies from 35% to 63% indicating the

presence of sufficient genetic variation for oil content in sesame which is a prerequisite for breeding cultivar with high oil content. Also, genotypes with white seed coat color possess higher oil content than the dark seed coat-colored genotypes. Oil content in sesame is oligogenic to polygenic; therefore, breeders should pay attention on recurrent selection schemes to develop high oil content genotypes (Velasco and Fernández-Martínez 2002; Islam et al. 2016).

15.2.4 Fatty Acid Compositions of Oil

The worth and usefulness of an oilseed crop for both dietary and manufacturing purposes mainly depend upon the fatty acid composition of its oil (Dyer et al. 2008). Varietal development with desirable fatty acid compositions could augment the usefulness of the oil for definite comestible purposes. Sesame geneticists and breeders prefer to select those lines which exhibit high oil content with high polyunsaturated fatty acids like oleic, linoleic, and linoleic acid. Beside this, the presence of several antioxidant compounds like minerals, vitamins, phytosterols, tocopherols, and unique class of lignans such as sesamin and sesamolin adds further nutritional value in sesame. These components mainly help in scavenging of reactive oxygen species and are very helpful for recoverable patients. Hence, breeding for these quality components traits also becomes important for sesame breeders. The high PUFA compositions along with high antioxidant components like tocopherol, sesamin, and sesamolin are desirable for high-quality export value in sesame (Hwang et al. 2005; Gupta 2015). For confectionary purposes, cultivars should have white seed color, bold size, and appealing shape. Beside this, germplasm should be screened for required texture and seed coat thickness and oil flavor using specific descriptors (Tripathy et al. 2019).

15.2.5 Shattering Resistance

Capsule shattering often leads to heavy yield losses in sesame. Most of the sesame varieties are of shattering type, and almost all of the fields are harvested by hand which leads to approximately 60% yields loss (Langham 2007). There is a prerequisite to reorient breeding approach to reduce the high cost of manual harvesting and yield loss due to shattering. Development of new high-yielding cultivars with semi-indehiscent capsules is a possible option to fit mechanized farming.

15.2.6 Abiotic Stress Tolerance

Sesame is usually grown under marginal to submarginal land and faces several types of environmental extremities. However, only limited efforts have been made to develop genotype with high yield potential and improved tolerance to abiotic

stresses. Sesame withstands water scarcity to some extent because of its extensive root system but may experience huge yield losses under different environmental stresses such as flooding, salinity, heavy metals stresses, and temperature extremities. This crop is considered moderately salt tolerant and can give gainful output on saline soils. Salinity affects water potential and causes ion imbalance and toxicity in living cells; this altered water status leads to initial growth reduction and reduction in productivity and may lead to death of plant. Stress affects all the major metabolic processes such as germination, seedling growth and survival, accumulation of photosynthetic pigments, photosynthesis, and respiration processes which leads to water scarcity, nutrient imbalance, and oxidative stress in salt affected plant. Abbasdokht et al. (2012) revealed that germination percentage, shoot length, shoot dry weight, root length, and germination rate decreased as the salinity concentration increased in sesame. There was no significant difference between the cultivars up to 0.16 ds.m^{-1} salinity levels; however, there were significant differences between the cultivars beyond 0.16 ds.m^{-1}, and they also conclude that selection within cultivars for salt tolerance could be possible at germination stage. Bahrami and Razmjoo (2012) concluded that germination and seedling growth were strongly inhibited by 12.05 dSm^{-1} among the ten cultivars they studied.

Sesame is usually cultivated under rainfed conditions where precipitation is irregular. It is regularly subjected to mild to severe water deficit stress. Vegetative stage is most sensitive to drought stress (Boureima et al. 2011). Drought stress is the main constraint in production potential of the crop in the semiarid regions (Boureima et al. 2012). Drought affects the plant metabolism, growth development, and yield. Different cultivars respond differently to drought stress with some cultivars being highly resistant and others more susceptible (Boureima et al. 2011). Due to extensive rooting system, sesame can overcome drought although it experiences substantial yield losses if drought occurs when it is cultivated on marginal and rainfed areas. Sesame seed yield is more affected by drought than any other morphological characters. Kim et al. (2007) investigated the drought effect on yield, and its component traits in sesame found that water stress significantly decreased sesame yield by decreasing the number of seeds per capsule. Dossa et al. (2019) identified 543 sesame core abiotic stress-responsive genes using meta-analysis of 72 RNA-Seq datasets from drought, water logging, salt, and osmotic stresses using contrasting sesame genotypes. You et al. (2018) performed transcriptomic analysis to study the expression profiling of stress-responsive genes in different tissue and development stage under various abiotic stresses. They found that the genes, namely, *SiGolS* and *SiRS*, were significantly regulated by drought, salt, osmotic, and water logging stresses but slightly affected by cold stress. Wang et al. (2018) studied the transcriptomic profiling of *SibZIPs* gene. Their results indicated that this gene exhibited considerable changes against abiotic stresses, including salt, drought, water logging, osmotic, and cold. Li et al. (2017) studied *SiWRKY* gene expression patterns and revealed that 33 and 26 *SiWRKYs* gene expression was strongly responded to water logging and drought stress, respectively. Drought tolerance in sesame is associated with wax depositions, root length, transpiration rate (Sun et al. 2010), and higher activities of few antioxidant enzymes like superoxide dismutase, catalase,

polyphenol oxidase, and peroxidase (Fazeli et al. 2007) as well as antioxidant metabolites content, viz., carotenoids (Kadkhodaie et al. 2014), and higher levels of ABA, proline, arginine, lysine, aromatic and branched chain amino acids, GABA, saccharopine, 2-aminoadipate, and allantoin under drought stress (You et al. 2019). Water logging is another important stress, and sesame crop is highly susceptible to flooding, as the crop undergoes immediate senescence and declines within 2–3 days of exposure to waterlogging stress (Anee et al. 2019). High rainfall during monsoon often leads to yield losses in sesame, and there is a need to develop improved cultivars that could survive the waterlogging stress. Anee et al. (2019) observed that lipid peroxidation as well as hydrogen peroxide (H_2O_2) and methylglyoxal contents increased while leaf relative water content, proline content, and chlorophyll and carotenoid contents decreased under prolonged water logging stress in sesame. Beside this, glutathione and oxidized glutathione contents increased under waterlogging, while the GSH/GSSG ratio and ascorbate content decreased. Ascorbate peroxidase, monodehydroascorbate reductase, glutathione peroxidase, and glyoxalase I activity increased under water logging, while dehydroascorbate reductase, glutathione reductase, and catalase activity showed decreasing trend. Wang et al. (2012) found strong association between cell wall modification and growth pathways, glycolysis, fermentation, mitochondrial electron transport, and nitrogen metabolism with waterlogging tolerance in sesame. These traits should be under consideration when breeding for flooding tolerance in sesame. Salinity often limits sesame cultivation especially in arid and semiarid regions. Sesame cultivars show a considerable variation in the degree of salt tolerance (Bekele et al. 2017). Zhang et al. (2019) compared salt tolerant and sensitive genotype of sesame and revealed that tolerant genotype has higher seed germination percentage, more plant survival rate, as well as better growth rate than susceptible one. Their transcriptome study revealed strongly induced salt-responsive genes in sesame mainly related to amino acid metabolism, carbohydrate metabolism, biosynthesis of secondary metabolites, plant hormone signal transduction, and oxidation-reduction process, while metabolomics investigation revealed amino acid metabolism and sucrose and raffinose family oligosaccharide metabolism impart salt tolerance in sesame. Several antioxidant enzymes, viz., superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase as well as malondialdehyde and proline content, have been found closely related to salt tolerance in sesame (Koca et al. 2007). In addition to this, seed germination percentage, root and shoot length, root to shoot length ratio, and seedling fresh weight are also associated with salinity tolerance particularly at seedling stage (El Harfi et al. 2016). Heavy metal stresses also adversely affect sesame yield considerably. Heavy metal stress tolerance in sesame was associated with accumulation of more dry mass during early growth phase and nitrate reductase activity. Considerable genetic diversity exists in collected sesame germplasm which could exploit to breed cultivar tolerance to abiotic stresses. In addition to this, sesame crop wild relatives have been reported to have agronomically desirable alleles for stress tolerance including both biotic and abiotic stresses.

15.2.7 Biotic Stress Tolerance

Biotic stresses such as pathogens, insects, and weeds adversely affect sesame crop which cause unpredicted losses in productivity and production due to lack of proper management practices including unavailability of resistant varieties (Girmay 2018). Hence, development of resistant cultivars helps in sustainability and in improving yield stability in sesame. The important diseases, their causal organism, and estimated yield losses are presented in Table 15.2. Plants face a combination of different biotic stresses, and to mitigate the effects, they evolved complex signaling pathways. In general, disease resistance in crop plants is two types: whether a hypersensitive or incompatible type is governed by few genes and which is often known as oligogenic or vertical resistance. Second one is partial resistance which is governed by many genes and popularly known as quantitative or horizontal resistance (Singh et al. 2020; Beebe and Corrales 1991; Vale et al. 2001). In sesame, genetics of disease resistance varied as it is was monogenic to oligogenic against phyllody and *Alternaria* blight, while it was polygenic against charcoal rot (El-Bramawy and Shaban 2007; Eswarappa et al. 2011; Shindhe et al. 2011; Lokesha et al. 2013; Renuka and Lokesha 2013). However, breeding for single disease resistance is often not much effective; hence there is a need to develop cultivars with multiple resistances to the above biotic stresses including both disease and

Table 15.2 Major disease and insect pests and their estimated yield in sesame

Major diseases	Causal organism	Yield losses	References
Phyllody	Phytoplasma like organism	Up to 80%	Ganem Junior et al. (2019)
Dry root rot	*Rhizoctonia bataticola* Taubenh	80–100%	Renganathan (2020)
Phytophthora blight	*Phytophthora parasitica* var. sesame Dastur	Up to 100% loss when infection occurs severely at seedling stage	Kumari et al. (2019)
Alternaria leaf blight	*Alternaria sesame* Kawamura (Mohanty and Behera)	20–40% yield losses	Pawar et al. (2019)
Charcoal rot	*Macrophomina phaseolina*	5–100%	Deepthi et al. (2014a, b)
Leaf curl virus disease	Gemini virus	–	Manjeet et al. (2020)
Insect pests			
Common name	Scientific name	Yield losses	References
Leaf Webber or roller and capsule borer	*Antigastra catalaunalis* Duponchel (Lepidoptera: Crambidae)	Up to 90% yield losses	Pandey et al. (2018)
Gall fly	*Asphondylia sesami* Felt (Diptera: Cecidomyiidae)	Up to 100% in susceptible genotypes and under favorable conditions	Adam et al. (2020)

Fig. 15.1 Symptoms of sesame phyllody in the field. (**a**) The entire sesame inflorescences are replaced by short twisted leaves closely arranged on top of the stem with very short internodes, but leaves on the lower part of infected plant did not exhibit any visible symptoms. (**b**) Floral virescence and dark exudates appear on foliage floral parts. (**c**) Floral virescence

insect pest. The symptoms of various diseases, viz., phyllody, charcoal rot, and leaf curl virus disease, are shown in Figs. 15.1, 15.2, and 15.3.

15.3 Sesame Classical Breeding Methods

Sesame is known as a predominantly self-pollinated crop. Hence, the selection methods employed for breeding self-pollinated plants are equally effective in sesame. Existence of genetic variation is a precondition for hybridization and accumulation of desirable alleles into single genetic background through selection. Domestication, plant introduction, mass, and pure line selection are the important breeding method applicable for existing genetic variability. Meanwhile, creation of genetic variation through crossing contrasting genotypes followed by pedigree, bulk, and single seed descent selection method is another important breeding scheme for sesame breeders. Recurrent selection and diallel mating selective schemes are the two most effective although time-consuming breeding methods in sesame. However, transferring one or two genes of agronomically important traits

Fig. 15.2 Field symptoms of sesame charcoal rot. (**a**) Infected sesame plant showing charcoal rot symptoms on lower portion of the stem. (**b**) Severe charcoal rot infection leads to stem breakage. (**c**) Root portion showing typical charcoal rot symptoms and devoid of lateral and finer roots

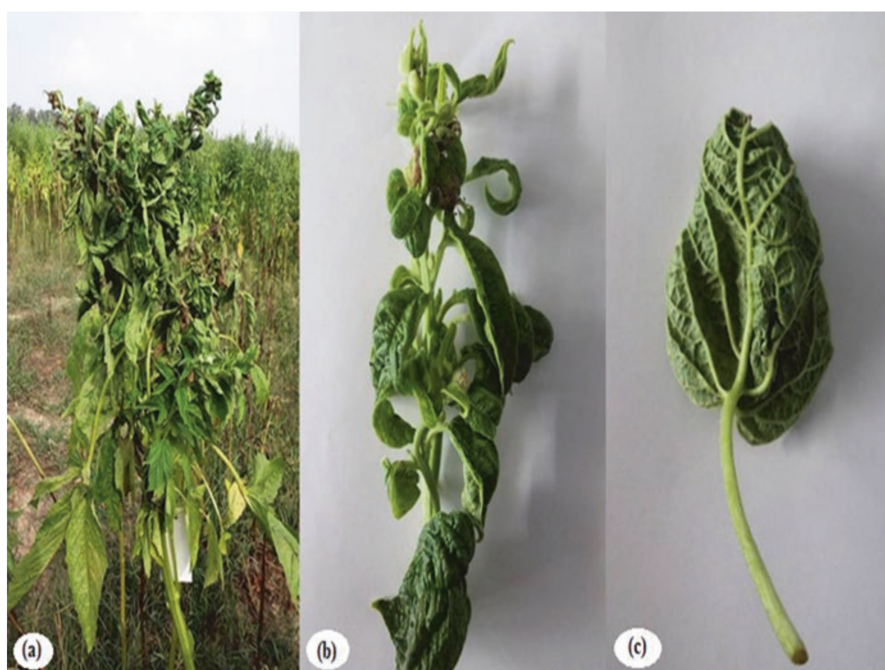

Fig. 15.3 Symptoms of sesame leaf curl virus disease. (**a**) Severely infected sesame plant showing leaf curling and plant stunting. (**b**) Close look of sesame twin with severe curling along with thickening of leaves. (**c**) Underside of an infected sesame leaf showing vein swelling and upward curling along with leaf thickening

from known source to high-yielding, well-adapted genotype is possible through backcrossbreeding schemes. Mutation breeding is another key approach for creating new desirable allele which is totally lacking in germplasm. Mutation breeding is generally employed for quality traits, while backcross method is often used for transferring disease resistance alleles and for transferring genes responsible for male sterility and fertility restoration. Baydar (2005) applied pedigree selection method to improve the ideal type of sesame and revealed that the bicarpels, mono- to tricapsule, with a greater number of branches were considered as ideal plant types in breeding for high-yielding varieties. Ismail et al. (2013) performed pedigree selection in sesame for seed yield per plant and compared yield after two selection cycle. The average yield after two selection cycle of selected families surpassed as compared to their parents. The plant geneticists and breeders are always interested in identifying gene/allele source responsible for desirable agronomic traits and determining the genetic basis of agronomic traits in order to design proper breeding approaches for development of new cultivars. Information about inheritance pattern of any particular trait is indispensable in deciding most appropriate breeding method for the development of elite genotype/variety. The information about nature of gene action for different agronomic traits in sesame is given in Table 15.3, while the gene/allele source for different agronomic traits is given in Table 15.4.

We at CCS Haryana Agricultural University, Hisar, India, have been maintaining about 550 germplasm lines, and these were evaluated for various agronomic traits during *Kharif*, 2014. The range of variation for different agronomic traits revealed substantial and potential variability in different genotypes. Among these germplasm lines, four lines, viz., TC 180, TC 26, TC 104, and TC 174, were promising for seed yield per plant, while the genotypes, viz., TC 302, TC 354, TC 301, TC 183, F 45, and TC 353, were promising for early flowering and maturity. For the development of short stature cultivars, the genotypes, viz., TC 302, TC 24, TC 325, TC 327, TC 167, TC 342, TC 27, and TC 45, were having desirable short plant height. These genotypes might be useful for development of high-yielding variety in sesame. Two high-yielding sesame varieties, viz., HT 1 and HT 2 (HT 9713), were developed at CCS HAU for farmer's cultivation. (Fig. 15.4). The variety HT 1 possesses medium to long height and dark green-colored leaves, resistant to phyllody and leaf curl virus with an average yield of 2.4 q/acre. The variety HT 2 is a short stature, early maturing, high-yielding variety developed from pedigree selection of population developed from the cross HT 1 × HT 15, and it possessed both high yield and disease resistance as it showed resistant reaction to all the major diseases (phyllody and leaf curl virus) as well as to leaf roller/capsule borer.

15.3.1 Heterosis Breeding in Sesame

In spite of many efforts through classical breeding methods, viz., mass, pedigree, backcross and recurrent selection, still there have not been accomplished a foremost yield revolution in sesame productivity. Therefore, heterosis breeding could have potential to break yield plateau in sesame by exploiting the advantage of heterosis

Table 15.3 Gene action responsible for seed yield and its component traits as well as other important agronomic traits in sesame

Trait	Gene action	References
Hairiness	Single dominant gene	Yol and Uzun (2011)
Number of capsules per leaf axil	Monogenic (with one capsule per leaf axil character was dominant to three capsules)	
Seed coat color	Oligogenic (two major genes V and B)	Pandey et al. (2013)
Seed yield per plant	Polygenic with the importance of both additive and nonadditive gene action along with duplicate epistasis and complementary epistasis	Gaikwad et al. (2010, 2009), Sharmila et al. (2007), Raikwar (2018), Rajput et al. (2017), Dasgupta et al. (2018), Elaziz and Ghareeb (2018), Anyanga et al. (2016), Solanki and Gupta (2003), and Tripathy et al. (2016a, b)
Number of seeds per capsule	Polygenic along with duplicate epistasis; both additive and nonadditive gene action were important	Gaikwad et al. (2010), Gaikwad et al. (2009), Aladji et al. (2014), Dasgupta et al. (2018)
1000-seed weight	Polygenic with both additive and nonadditive gene effects along with duplicate epistasis	Gaikwad et al. (2010), Dasgupta et al. (2018) and Elaziz and Ghareeb (2018)
	Additive gene effects	Rajput et al. (2017)
	Polygenic with both additive and nonadditive gene effects along with both duplicate and complementary epistasis	Sharmila et al. (2007)
Capsule length	Polygenic with the preponderance of both additive and nonadditive gene effects; both duplicate and complementary epistasis present	Gaikwad et al. (2010), Aladji-Abatchoua et al. (2014), Sharmila et al. (2007), Rajput et al. (2017) and Dasgupta et al. (2018)
	Polygenic with preponderance of additive gene effects	Gaikwad et al. (2009)
Number of primary branches	Polygenic with both additive and nonadditive gene action along with complementary and duplicate epistasis	Gaikwad et al. (2010) and Aladji-Abatchoua et al. (2014)
Number of capsules on main axis	Both additive and nonadditive gene action along with duplicate and complementary epistasis	Gaikwad et al. (2010, 2009)
Number of capsules per plant	Mostly polygenic, both additive and nonadditive gene actions important, mostly governed by duplicate epistasis	Gaikwad et al. (2010, 2009), Aladji et al. (2014), and Elaziz and Ghareeb (2018)
Days to 50% flowering	Mostly governed by additive gene action	Gaikwad et al. (2010, 2009)

(continued)

Table 15.3 (continued)

Trait	Gene action	References
	Both additive and nonadditive gene effects	Aladji et al. (2014), Rajput et al. (2017) and Sumathi and Muralidharan (2009)
Days to maturity	Both additive and nonadditive gene actions, duplicate epistasis	Gaikwad et al. (2010, 2009), Aladji-Abatchoua et al. (2014), Rajput et al. (2017), and Sumathi and Muralidharan (2009)
Days from flowering to capsule maturity	Both additive and nonadditive gene actions	Aladji-Abatchoua et al. (2014)
Plant height	Polygenic inheritance with both additive and nonadditive gene effects	Gaikwad et al. (2010, 2009), Aladji et al. (2014), Raikwar (2018), Rajput et al. (2017) and Dasgupta et al. (2018)
Harvest index	Additive gene effects	Tripathy et al. (2019)
Leaf chlorophyll content	Nonadditive	
Capsules bearing nodes	Additive	
Photoperiod response	Polygenic inheritance	
Photosynthetic rates	Additive gene action	
Oil content	Polygenic inheritance with both additive and nonadditive gene effects	Rajput et al. (2017)
	Preponderance of nonadditive gene effect	Tripathy et al. (2016a, b)
Alternaria leaf spot resistance	Additive gene effects	El-Bramawy and Shaban (2007)
Fusarium wilt	Both additive and nonadditive	
Charcoal rot	Both additive and nonadditive	
Monostem/shy branching	Monogenic to oligogenic with complementary epistasis	Sumathi and Muralidharan (2009)
Capsule shape	Governed by dominant epistasis; long capsule is dominant over dense capsule	Yol (2017)
Shattering resistance	Governed by two pairs of genes with duplicate dominant epistasis and duplicate recessive epistasis	Kotcha et al. (2012)
Genetic male sterility	Governed by single recessive genes	Liu et al. (2013)
Sesame gall midge	Nonadditive gene effects	Ubor et al. (2015)
Powdery mildew tolerance	Governed by two independent recessive genes with complementary epistasis	Rao et al. (2012)
Sesamolin content	Additive and nonadditive	Khuimphukhieo et al. (2020)

Table 15.4 Agronomically important gene/allele source within cultivated gene pool in sesame

Genetic sources	Trait	References
$C_{3.8}$	Drought tolerance	Abdelraouf and Anter (2020)
Darab 14, Shaban and Yekehsaud		Asadi et al. (2020)
TEX-1		Song et al. (2020)
shi165, lc162, mc112, lc164, icn115, icn141, mt169, dwf172, cc102, 38–1-7, and Birkans		Boureima et al. (2012)
Sistan and TN238		Khammari et al. (2013)
LC 164, LC 162 and BC 167 and 32–15		Boureima et al. (2016)
KC50658 and Oltan		Abbasali et al. (2017)
C8.4 and C8.8	Salinity tolerance	Anter and El-Sayed (2020)
BD 6980 and BD 6985	Water logging tolerance	Saha et al. (2016)
ZZM1501, ZZM2113, ZZM2147, ZZM2208, ZZM3342, ZZM3379, ZZM3410, ZZM4780, ZZM4781, 09-P65, Liaopinzhi 3, and Luozhi 15		Wang et al. (2011)
P5(NM59), C6.3, C1.10, and C3.8	Charcoal rot resistance	Shabana et al. (2014)
1.6, C1.10, C3.8, C6.3, C6.5, and C9.15	*Fusarium* oxysporum resistance	
Zhongzhi No. 13	Charcoal rot resistance/ tolerance	Wang et al. (2017)
PKDS-91		Deepthi et al. (2014a, b)
87,008		Farooq et al. (2019)
Sesame lines No., 33, 3, 15, 64, 40, 63, 14, 39, 4, 16, 13, 80, 58, and 79		Bedawy and Moharm (2019)
Potak-e-Mousian, MahalliIranshahr and Safiabad line 3		Garmaroodi and Mansouri (2014)
ORM 7, ORM 10, and ORM 17		Thiyagu et al. (2007)
T6, Dashtestan 2, Darab 1, AT1, and AT2		Zaker et al. (2020)
Chinese, Varamin 2822, and PotkeMusian		Sadeghiy Garmaroudi et al. (2003)
JLS 110–12, HT 9913, T 78, and KMR 60	Multiple disease resistance (phyllody, charcoal ro, and sesame leaf curl virus)	Manjeet et al. (2020)
HuRC-4 and HuRC3	Multiple disease resistance (resistant to bacterial blight, *Fusarium* wilt, and phyllody)	Belay (2018)

(continued)

Table 15.4 (continued)

Genetic sources	Trait	References
RJS78, RJS147, KMR14, KMR79, Pragati, IC43063, and IC43236 and two wild spp., i.e., *Sesamum alatum* and *Sesamum mulayanum*	Phyllody resistance	Singh et al. (2007)
KAU-05-2-12, PC-14-2 and Kanakapura		Mahadevaprasad et al. (2017)
NS98002-04, NS98003-04, NS99005-01 and NS01004-04		Akhtar et al. (2013)
RT-273	*Alternaria* blight resistance	Lokesha and Naik (2011)
NS 11204	Sesame leaf curl virus resistance	Sarwar et al. (2006)
TSP 933229 and TR 3821512	High oleic acid	Baydar et al. (1999); Were et al. (2006a, b)
Majengo, Stewa,		
TSP 932410 andTSP 932,403	High linoleic acid	
Webuye, Kisumu301, ug1, Koyonzo		

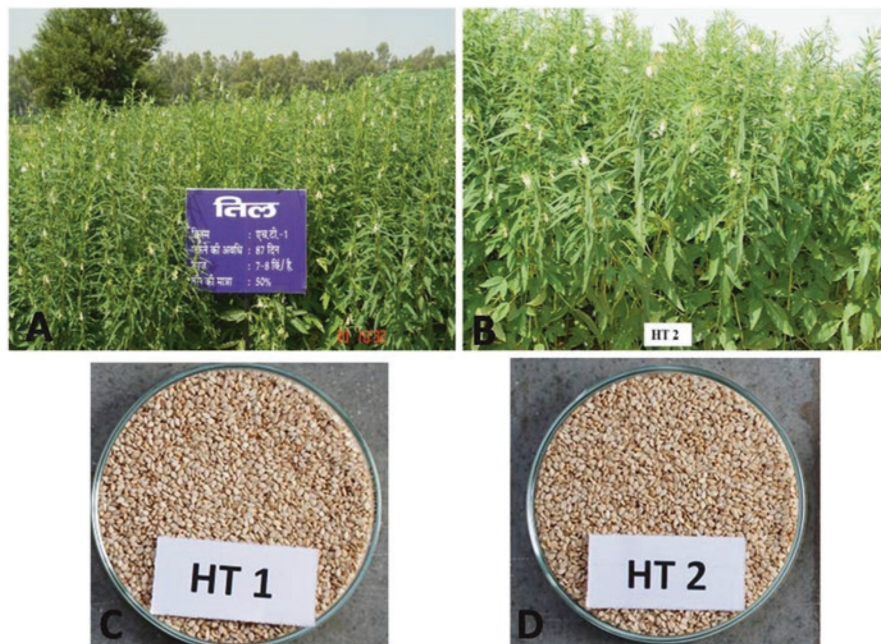

Fig. 15.4 Sesamum varieties developed by CCS, HAU, Hisar. A. Variety HT I possessing resistance to phyllody and leaf curl virus with an average seed yield of 2.4 q/acre. B. HT 2, a dwarf, early maturing and high-yielding variety. C. White-colored bold seeds of variety HT1. D. White-colored bold seeds of variety HT2

or hybrid vigor phenomenon (Mothilal and Ganesan 2005; Monpara and Pawar 2016). Heterosis is the term used for superiority of F_1 hybrids as compared to their parents. Heterosis was already exploited in many important oilseed's crops including rapeseed-mustard, groundnut, and soybean to some extent. Recently, several workers reported the existence of noteworthy heterosis in certain cross combinations of sesame (Murty 1975; Sasikumar and Sardana 1990; Jiarong 1991; Quoada and Layrisse 1995; Uzun et al. 2004; Sumathi and Muralidharan 2008; Banerjee and Kole 2010; Jadhav and Mohrir 2013; Parimala et al. 2013; Saravanan and Nadarajan 2002; Lal Jatothu et al. 2013; Vavdiya et al. 2013; Hassan and Sedeck 2015;). Hence, there is urgent need to exploit this heterosis to capitalize on seed yield and oil content in sesame. Effective male sterility-fertility restoration system might provide opportunity to develop efficient system for commercial hybrid seed production in sesame. Several workers reported genetic male sterility in sesame like corolla recessive genic male sterile mutant (Langham 1947), greenish anther color at pollen dehiscence-associated male sterility (Osman and Yermanos 1982), short anther's filaments and cold night temperature-based recessive genetic male sterility (Brar 1982), and mutagen-induced monogenic recessive genetic male sterility (Rangaswamy and Rathinam 1982). Cytoplasmic male sterility was also identified in sesame wild relative *Sesamum malabaricum* (Prabakaran et al. 1995; Bhuyan and Sarma, 2003; Prabakaran 1998). This CMS system was used to develop 36 cross combinations with high heterotic effects (77–540%) for many seed and oil yield (Tripathy et al. 2019). Several experimental F_1 cross combinations have been developed in India which exhibited heterotic effect of 31.0–44.3% in seed yield and 13–48% in oil yield over commercial pure line variety TKG 22 (Gangaiah 2008).

We at CCS HAU evaluates several lines in different cross-combinations for exploiting heterosis in sesame through cytoplasmic genetic male sterility system (CMS) and also to develop resistant lines against insect-pests and diseases. Also, the possibility for exploitation of inter-specific hybridization was explored at CCS HAU by exploiting *Sesamum malabaricum*. The genotypes, viz., IC 043144–1 and JJK/MIS10–67 of *S. malabaricum*, were crossed with HT 1, HT 2, HT 9316, HT 9907, HT 9913, TKG 22, MT 11–8-2, LT 210, HTC 1, and KMR 60. The 49 newly developed F1 hybrids were evaluated for seed yield and its component traits. Out of these cross combinations, eight showed higher seed yield over local check HT 2. Highest seed yield per plant was observed in hybrid HT 20 × HT 2 (7.3 g), followed by HT 45 × RT 125 (6.7 g), CST 2001–9 × HT 2000 (6.5 g), OC 201 × HT 9316 (6.0 g), OC 251 × HT 2000 (6.0 g), T 78 × HT 2 (4.5 g), KMR 41 × RT 125 (4.4 g), RH 54 × HT 9316 (4.0 g), and local check HT 2 (3.6 g) during *Kharif*, 2015. This indicates the potential of F_1 hybrids for breaking yield plateau in sesame. For charcoal rot resistance breeding at CCS HAU, 24 germplasm lines, viz., NIC 7837, NIC 7875, HT 1, NIC 17274, HT 2, NIC 17849, HT 15, SI 2174–1, SI 3296, IS 92–2, and HT 9913, were found moderately resistant.

15.4 Molecular Breeding in Sesame

Crop breeding together with improved agronomic practices resulted in the Green Revolution in the 1960s with spectacular yield gains, particularly for staple crops like wheat and paddy in developing countries (Lenaerts et al. 2019). However, crop yield augmentation has been slowing down more in recent times. Changes in climatic patterns, arable land, and water accessibility now endow with further challenges for ensuring yield steadiness across varied environment. Changing climatic conditions affect farming and foodstuff formation in multifaceted ways. It influences food production straightforwardly by altering in agroclimatic environment and on another way by distressing development and allocation of income and thus demands for agricultural outcomes (Shetty et al. 2013). Advanced biological techniques in plant breeding like genomics, proteomics, bioinformatics tools, molecular breeding, and plant tissue culture and genetic engineering have already led to significant impacts on several important crops including rice, wheat, rapeseed-mustard, soybean, maize, potato, sorghum, and pearl millet (Varshney et al. 2005). Sesame is an underexploited crop of tropical and subtropical region of the world. As sesame is the crop of developing countries, major efforts for sesame improvement were made only through classical plant breeding methods (Gupta 2015). However, under changing climatic conditions and evergreen increasing human populations, efforts should be directed toward the use of recent biotechnological techniques to boost up the sesame production and productivity. The molecular breeding work in sesame began very late with only one genetic map published and no information on QTL mapping before 2013 (Dossa et al. 2017a, b). However, over the last decade, some noteworthy advancement has been made in sesame breeding programs to use advanced molecular biology techniques including plant tissue culture techniques; highly informative molecular marker techniques like SNPs; high density linkage and genetic maps; omics studies including genomics, proteomics, transcriptomics, and metabolomics; and advanced bioinformatics tools (Tripathy et al. 2019). In addition, the draft of sesame genome triggered functional analyses of candidate genes related to important agronomic traits (Wei et al. 2017a, b; Zhang et al. 2013a, b). With these invaluable efforts, sesame has some important genomic resources and platforms for improvement, and presently, sesame has shifted from an "orphan crop" to a "resource-rich crop." Among different advanced techniques, molecular marker technologies have considerably accelerated the classical sesame breeding programs in enhancing the genetic gain and minimizing the breeding cycles in many crop species (Dossa et al. 2017a, b). Molecular marker technologies in sesame are witnessing significant progress, and it is clear that sesame is no longer far behind large crops in this field. Various kinds of molecular markers have been developed and used to sesame genotyping including randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or simple sequence repeat (SSR), and inter-simple sequence repeats (ISSR) employed mainly for genetic diversity analysis at DNA level. The next class of markers concerned mostly of expressed sequence tags-SSR (EST-SSR), cDNA-SSR, genome

sequence-SSR (gSSR), and chloroplast SSR (cpSSR) which were mainly employed for association mapping, germplasm characterizations, and molecular breeding in sesame (Dar et al. 2017; Dixit et al. 2005; Wei et al. 2014a, b; Kizil et al. 2020; Cui et al. 2017; Li-Bin et al. 2008;Wei et al. 2011; Zhang et al. 2013a, b; Kumar and Sharma 2011). Recently, with the discovery of next-generation sequencing technology (NGS), another class of molecular markers emerged. SNPs are more useful as genetic markers than many other simple markers because they are the most abundant and stable form of hereditary difference in most genomes (Uncu et al. 2016; Wei et al. 2014a, b). Therefore, high-throughput methods available for SNP detection and genotyping have been used in sesame research including restriction site-associated DNA sequencing (RAD-seq), specific length amplified fragment sequencing (SLAF-seq), RNA-Seq, whole-genome sequencing (WGS), genotyping by sequencing (GBS), and insertion/deletions (Indels) (Uncu et al. 2016). Using these marker techniques, several important genes and QTLs were mapped and validated in sesame till now (Table 15.5). Different types of molecular markers have been developed and used successfully for genetics and breeding activities in *Sesamum indicum.* The following sections provide brief information related to different types of molecular markers used in sesame based on their detection method.

15.4.1 RFLP (Restriction Fragment Length Polymorphism)

RFLP was the first molecular marker and the barely marker system based on hybridization. Polymorphism occurs among individuals of same species as a result of insertion/deletion (InDels), translocation, duplications, inversions, and point mutations. RFLP begins with the isolation of pure genomic DNA, after which isolated DNA is treated with restriction enzymes resulting in a large number of fragments varying in length. Agarose or polyacrylamide gel electrophoresis (PAGE) is used to study the polymorphism among genomic DNA (Kundan et al. 2014).

15.4.2 RAPD (Randomly Amplified Polymorphic DNA)

Williams et al. and Welsh and McClelland independently developed RAPD technique (Welsh and Mcclelland 1990; Williams et al. 1990). Simple, short (ten nucleotides), and random primers were used for PCR amplification of genomic DNA. When two hybridization sites are similar and in the opposite direction, PCR amplification takes place. Sharma et al. studied the characterization and analysis of genetic variance in Indian sesame (*Sesamum indicum* L.) genotypes. To find out the extent of genetic diversity between 60 sesame varieties in diverse geographical regions of India, 20 phenotypic (qualitative and quantitative) traits and 200 RAPD markers were used. In accessing the diversity, 14 RAPD markers were found to be useful. Among the population, high level of genetic variability (HT = 0.1991) and

Table 15.5 Important gene(s)/QTLs responsible for different agronomic traits mapped in sesame

Traits	Genes/QTLs	References
Flowering time	SiDOG1 (SIN_1022538) and SiIAA14 (SIN_1021838)	Wei et al. (2015)
Seed yield	Qgn-1, Qgn-6	Mei et al. (2017)
Seed coat color	QTL-1, QTL11-1, QTL11-2, QTL13-1	Zhang et al. (2013a, b)
	qSCa-8.2, qSCb-4.1, qSCb-8.1, qSCb-11.1, qSCl-4.1, qSCl-8.1, qSCl-11.1, qSCa-4.1, and qSCa-8.1	Wang et al. (2016)
1000-seed weight	Qtgw-11	Wu et al. 2014
Flowering times	SiDOG1 (SIN_1022538) and SiIAA14 (SIN_1021838)	Wei et al. (2015)
Capsule length	Qcl-3, Qcl-4, Qcl-7, Qcl-8, and Qcl-12	Wu et al. (2014)
First capsule height	Qfch-4 and Qfch-12	
Plant height	Qph-6 and Qph-12	Wei et al. (2015)
	SiDFL1 (SIN_1014512) and SiILR1 (SIN_1018135)	
	Qph-8.2, Qph-3.3	Wang et al. (2016)
Semidwarf sesame plant phenotype	QTL (qPH-3.3), gene[SiGA20ox1(SIN_1002659)]	Wang et al. (2016) and Wei et al. (2016)
Capsule length and capsule number	Qcl-3, Qcl-4, Qcl-7, Qcl-8, and Qcl-12	Wu et al. (2014)
	SiLPT3 and SiACS8	Zhou et al. (2018)
Number of capsules per axil	SiACS (SIN_1006338)	Wei et al. (2015)
Mono flower vs. triple flower	SiFA	Mei et al. (2017)
Determinate trait in sesame	geneSiDt (DS899s00170.023)	Zhang et al. (2016)
Oil content	SIN_1003248, SIN_1013005, SIN_1019167, SIN_1009923SiPPO (SIN_1016759), SiNST1 (SIN_1005755)	Wei et al. (2015)
Biotic and abiotic stresses		
Drought tolerance	TF (transcription factor) families (AP2/ERF and HSF)	Komivi et al. (2016), Dossa et al. (2016)
Water logging tolerance	qEZ09ZCL13, qWH09CHL15, qEZ10ZCL07, qWH10ZCL09, qEZ10CHL07, and qWH10CHL09	Wang et al. (2016)
Drought, salinity, oxidative stresses, charcoal rot	Osmotin-like gene (SindOLP)	Chowdhury et al. (2017)

(continued)

Table 15.5 (continued)

Traits	Genes/QTLs	References
Phytophthora blight resistance	SIN 1019016	Asekova et al. 2021
Charcoal rot tolerance	qCRR12.2, Qcrr8.2, and Qcrr8.3	Wang et al. (2017)
Sesamin production	SiDIR (SIN_1015471), SiPSS (SIN_1025734)	Wei et al. (2015)
Leaf length and width	qLS15–1	Sheng et al. (2021)
Leaf growth and development	SIN-1004875, SIN-1004882, and SIN-1004883	
Leaf position	qLP2.1 and qLP9.1	Rao et al. (2014)
Basal leaf shape	qBLS1.1 and q BLS3.1	
Leaf angle	qLA1.1 and qLA7.1	
Corolla color	qCC9.1, qCC5.1, and qCC5.2	
Capsule hair density	qCHD6.1	
Capsule hair length	qCHL1.1 and qCHL6.1	
Capsule shape	qCS3.1	
Stem hairiness	q SH1.1 and qSH7.1	
Oil content	SIN_1003248, SIN_1013005, SIN_1019167, SIN_1009923 SiPPO (SIN_1016759) SiNST1 (SIN_1005755)	Wei et al. (2015); He et al. (2020)
Protein content	SiPPO (SIN_1016759)	
Sesamin and Sesamolin content	SiNST1 (SIN_1005755)	
Fatty acid composition	SiKASI (SIN_1001803), SiKASII (SIN_1024652), SiACNA (SIN_1005440), SiDGAT2 (SIN_1019256), SiFATA (SIN_1024296), SiFATB (SIN_1022133), SiSAD (SIN_1008977), SiFAD2 (SIN_1009785)	
Seed fatty acid compositions	SLG01_ 4,079,562, SLG01_20113237, SLG02_6286222, SLG04_8808724, SLG05_7707991, SLG08_6007683, SLG10_15546794, SLG11_ 4,907,057, SLG12_7532529, SLG12_16076994, SLG12_ 16,077,007, SLG12_16077015, and SLG12_16077027	
Sesamin production	SiDIR (SIN_1015471), SiPSS (SIN_1025734)	
Dominant GMS geneMs	SBM298 and GB50	Li et al. (2014)
Recessive GMS	SiMs1	Zhao et al. (2013)

(continued)

Table 15.5 (continued)

Traits	Genes/QTLs	References
Seed potassium concentration	QTL-qK-1	Teboul et al. (2020)
Seed zinc concentration	QTL-qZn-5; qZn-6	
Seed iron concentration	QTL-qFe-6	
Seed magnesium concentration	QTL-qMg-2	
Black seed coat development	SIN_1018961 and SIN_101895; SIN_1006242 and SIN_1016759/PPO, SIN_1026689 and SIN_1006025, SIN_1025056	Dossou et al. (2020)
Internode length and plant height	SiDWF1	Miao et al. (2020)

within population less variability (HS = 0.0749) were observed. Among the sesame population, mean coefficient of gene differentiation (GST = 0.6238) was 62.38% and 37.62% within the population. The above information suggests that the Indian sesame lines are genetically different, which should be used to improve the sesame crop (Sharma et al. 2014). Dar et al. reported the assessment of genetic variance in sesame using 22 RAPD, and 18 SSR primers were used for the study of 47 diverse sesame accessions cultivated in different agroclimatic regions of India. One hundred ninety-one polymorphic bands were observed with RAPD primers while SSR gives 64 bands. Maximum PIC was reported with SSRs (0.194) compared to RAPD (0.186). In describing genetic variation between the varieties studied, RAPD primer RPI-B11 and SSR primer S16 were the most informative (Dar et al. 2017).

15.4.3 AFLP (Amplified Fragment Length Polymorphism)

The combination RFLP and RAPD markers results in the development of AFLP markers, in which digestion of genomic DNA is followed by PCR amplification. AFLP is a cost-effective technique, in which there's no need of former sequence information. In AFLP, two restriction enzymes (a frequent cutter and a rare cutter) are used. After restriction digestion, oligonucleotide fragments were used for PCR amplification (Vos et al. 1995). Laurentin and Karlovsky studied the genetic variance in a sesame germplasm set using AFLP. Great genetic variability was studied within the 32 sesame associations from the Venezuelan Germplasm Collection which represents genotypes from 5 diversity centers (India, Africa, China-Korea-Japan, Central Asia, and Western Asia). Out of the 457 AFLP markers recorded, 93% were polymorphic. The Jaccard similarity coefficient ranged from 0.38 to 0.85 between pairs of accessions. According to geographical origin, five groups of genetic diversity study discovered that only 20% of the total diversity was due to

diversity among groups that used Nei's coefficient for population differentiation. Similarly, only 5% of the total diversity is accredited to differences between groups through analysis of molecular variance (AMOVA). This study showed that 32 sesame associations were genetically highly variable and did not show a link between geographical origin and AFLP patterns. This suggests that there was a large gene flow among diversity centers (Laurentin & Karlovsky 2006).

15.4.4 SSR or Microsatellites (Simple Sequence Repeats)

SSRs are short tandem repeats of one to six nucleotides having simple sequence length polymorphism, which are present profusely in the genome of different taxa. Microsatellites are distributed throughout the whole genome, viz., nuclear and mitochondrial as well as chloroplast genes. They are also present in the protein coding genes and expressed sequence tags (ESTs). Due to the presence of different numbers of repeats in microsatellite regions, high polymorphism is easily detected by PCR (Kalia et al. 2011). Zhang et al. studied the development and validation of genic-SSR markers in sesame by RNA-seq. In this study, 75 bp and 100 bp paired RNA seq were used to sequence 24 cDNA libraries, and 42,566 uni-transcripts were collected from more than 260 million filtered readings. The total length of uni-transcript was 47.99 Mb, and 7324 SSRs (SSRs ≥15 bp) and 4440 SSRs (SSRs ≥18 bp) were acknowledged. On a usual, there was one genic-SSR per 6.55 kb (SSRs≥15 bp) or 10.81 kb (SSRs≥18 bp). A total of 2164 genic-SSR markers have been developed in sesame using transcriptomic sequencing. Two hundred seventy-six of 300 validated primer pairs successfully yielded PCR amplicons in 24 cultivated sesame accessions (Zhang et al. 2012). Park et al. reported the genetic diversity, phylogenetic conditions, and population structure of 227 connections of sesame seed collections collected from 15 countries in 4 different continents. Among sesame accessions, a total of 158 alleles were detected, with an average of 11.3 alleles per locus. The average polymorphism content value was 0.568. It indicates a high genetic variance at 14 loci both among and within the population. UPGMA and the unweighted pair group method formed four robust clusters among the 277 core collection accessions of sesame. Similar patterns were obtained using country-based dendrograms and model-based analysis, as certain geographically distant connections were grouped in the same cluster (Park et al. 2014). Surapaneni et al. (2014) studied the genetic characterization of 68 Indian sesame cultivars and 3 related wild species using 102 SSR markers. By constructing the genomic libraries, 62 novel sesame-specific microsatellites were isolated from the study. The content of polymorphic information in the markers of the markers ranged from 0.43 to 0.88 with an average of 0.66. All connections were grouped into two large clusters with a genetic similarity between 0.40 and 0.91 by UPGMA cluster analysis. A high percentage of variation (87.1%) was observed within the population by AMOVA analysis. An overall F_s of 0.11 among the populations indicated low population differentiation. The study reveals that the development of SSR markers will be

constructive for genetic analysis, linkage mapping, and selection of parents in future breeding programs. Uncu et al. (2015) used a pyro-sequencing approach for the development of genomic SSR markers. They approached successfully in identifying 19,816 nonredundant SSRs, 5727 of which were identified in a coting assembly that covers 19.29% of the sesame genome. Molecular genetic diversity and population structure in a collection of world affiliations were analyzed using a subset of the newly identified SSR markers. The results of two analyses almost overlapped and suggested a correlation between genetic similarity and geographical closeness. Iqbal et al. (2018) reported on the calculation of the genetic diversity of sesame genotypes using morphological traits and SSR gene markers. To access the molecular genetic diversity at the molecular level of 70 genotypes from ecogeographic regions of the world, 235 gene markers were developed by mining expression sequence tag data from the NCBI database. The PIC content ranged between 0.36 and 0.82 with an average of 0.61. Neighbor-joining (NJ) analysis discovered that the five main groups and grouping were independent of geographic origin. Stavridou et al. (2021) studied the characterization of genetic diversity present in a varied sesame landrace set using seven expressed sequence tag-simple sequence repeat (EST-SSR) markers coupled with a high-resolution melting (HRM) analysis. The PIC value of 0.82 indicates that the selected markers were highly polymorphic. The sesame genotypes were classified into four major clades based on the principal coordinate analysis and dendrogram reconstruction of molecular data.

15.4.5 ISSR (Inter-simple Sequence Repeat)

Zietkiewicz et al. (1994) developed the ISSR marker technique. ISSR is based on the amplification of DNA segments situated in between two identical but oppositely oriented microsatellite repeat regions, at a distance which allows amplification. Parsaeian et al. (2011) conducted a research to study the genetic variations between 18 genotypes of sesame taken from diverse agroclimatic parts of Iran along with 6 exotic genotypes from the Asian countries by means of combined agro-morphological and ISSR marker traits. Total 13 ISSR primers were chosen for molecular analysis revealed 170 bands, of which 130 (76.47%) were polymorphic. On the basis of ISSR profiles, the generated dendrogram divided the genotypes into seven groups. A nonsignificant co-phenetic correlation was observed in the Mantel test by studying genetic variation in sesame using agro-morphological traits and ISSR markers.

15.4.6 SNP (Single Nucleotide Polymorphism)

Single base pair changes present in the sequence of an individual's genome are known as SNPs. SNPs are results of transition or transversion, and in plants, SNP frequency ranges between 1 SNP in every 100–300 bp. On the basis of different

molecular mechanism, diverse types of SNP genotyping assays have been developed, and among them, allele-specific hybridization, invasive cleavage, primer extension, and oligonucleotide ligation are most important (Sobrino and Carracedo 2005). Several recent high-throughput genotyping methods such as chip-based NGS, GBS, and NGS and allele-specific PCR make SNPs the most attractive markers for genotyping (Agarwal et al. 2008). Uncu et al. (2016) reported an identification and mapping of high-throughput SNPs in the sesame genome with genotyping by sequencing (GBS) analysis. SNPs preferred through a high stringency filtering protocol (770 SNPs) for better map precision were used in concurrence with SSR markers (50 SSRs) in linkage analysis. This results in 13 linkage groups spanning a total genetic distance of 914 cM with 432 markers (420 SNP, 12 SSR). Wei et al. studied the three kinds of markers (SNPs, InDels, and SSRs) used for DNA fingerprinting of 151 sesame cultivars released in China. The 140 polymorphic markers used (47 SNPs, 47 InDels, and 46 SSRs) bare a narrow range of genetic variations. Of the 151 cultivars, 3 cultivars (AH03, AH04, and AH05 from Anhui Province) were considered synonymous cultivars due to their high coefficients of genetic similarity (> 98%). To distinguish all sesame cultivars overall, 15 SNPs, 14 InDels, and 9 SSRs were sufficient (Wei et al. 2017a, b).

15.5 Plant Tissue Culture in Sesame

Sesame is prevalently self-pollinated; however, the hybrids by conventional crosses with wild types were difficult to produce because of the sexual incompatibility (Tiwari et al. 2011; Kulkarni et al. 2017). Protoplast fusion and somatic hybridization using tissue culture techniques are effective strategies to overcome sexual incompatibility. Interspecific hybrids have been successfully developed between cultivated variety of sesame and its wild relatives *S. occidentale* and *S. radiatum* through ovule and ovary culture by Dasharath et al. (2007). Rajeswari et al. (2010) have standardized an efficient protocol to produce hybrids of a cross between *Sesamum indicum* and *S. alatum* using ovule culture. The developed hybrids were resistant against phyllody disease. In vitro culture has been largely investigated in sesame which may result in somaclonal variations. Somaclonal variations are induced during callus induction and callus proliferation (Hoffman et al. 1982). Repeated and prolonged subculturing of calli enhances the frequency of gross chromosomal aberrations and gene mutations (Sanal and Mathur 2008). Regenerants resulting from such cultures are liable to carry heritable variations for seedling vigor, growth, capsule dehiscence, placental thickness, seed dormancy, seed size, yield, oil content, and oil quality (Bairu et al. 2006; Ram et al. 1990). Sesame is exceedingly recalcitrant for in vitro regeneration.

Attempts have been made for direct as well as indirect regeneration and callus induction of sesame in tissue cultures using various explants such as cotyledons and/or hypocotyl (Younghee 2001; Baskaran and Jayabalan 2006; Were et al. 2006a, b; Chakraborti and Ghosh 2009; Yadav et al. 2010; Al-Shafeay et al. 2011;

Shashidhara et al. 2011; Rao and Honnale 2011; Honnale and Rao 2013; Pusadkar et al. 2015), shoot tips (Lee et al. 1985; George et al. 1987; Baskaran and Jayabalan 2006), de-embryonated cotyledon (Seo et al. 2007; Lokesha et al. 2012; Malaghan et al. 2013; Chowdhury et al. 2014; Pusadkar et al. 2016), embryo (Saravanan and Nadarajan 2005), and nodes (Gangopadhyay et al. 1998). Cotyledon or hypocotyl has proven significantly successful as an explant for plant regeneration by somatic embryogenesis (Younghee 2001; Baskaran and Jayabalan 2006; Yadav et al. 2010; Honnale and Rao 2013). All these studies achieved varying degree of success in terms of callus growth and regeneration. Callus induction and shoot regeneration frequency were significantly enhanced by supplementing cytokinins and auxins with nutrient media (Baskaran and Jayabalan 2006, Wadeyar and Lokesha 2011; Honnale and Rao 2013; Zamik et al. 2017; Gayatri and Basu 2020). Auxins and cytokines alone as well were found capable of promoting regeneration in cultures (Baskaran and Jayabalan 2006; Yadav et al. 2010). Genotypes, explant type, type of growth regulators, concentration of growth regulators, age of the explants, and the developmental stage of explants were revealed as crucial factors that governed in vitro shoot regeneration and somatic embryogenesis (Mary and Jayabalan 1997; Venkatachalam et al. 1999; Seo et al. 2007; Malaghan et al. 2013; Zimik and Arumugam 2017). However, some cotyledonary explants were also found to undergo necrosis after supplementing with cytokinins (Baskaran and Jayabalan 2006; Were et al. 2006a, b). Auxin and cytokinin treatment has been found to induce rooting in cotyledon culture of sesame (Were et al. 2006a, b; Seo et al. 2007; Zimik et al. 2017). ABA and $AgNO_3$ were also reported to enhance shoot regeneration if supplemented with plant growth regulators (Seo et al. 2007, W deyar and Lokesha 2011). $AgNO_3$ inhibits ethylene which is produced during in vitro culture (Chi and Pua 1989) and responsible for recalcitrant behavior of tissues in culture (Chi and Pua 1989; Shashidhara 2005). $AgNO_3$ have also been reported to enhance conversion of somatic embryos to plants (Honnale and Rao 2013; Xu et al. 1997). Abscisic acid promotes seed maturation and inhibits seed germination (Zeevaart and Creelman 1988). The impact of ABA is broadly studied in somatic embryogenesis. ABA treatment prevents the precocious germination of embryos (somatic) and generation of secondary embryo (Choi and Jeong 2002; García-Martín et al. 2005).

15.6 Concluding Remarks

Due to increasing health awareness, people are more concerned about nutrition quality of food. Sesame is a very promising crop of future prospects due to its high-quality oil for nutritional and industrial purposes. Sesame is an underutilized and poor farmer's crop and is fit for sustainable agricultural development. Improvement of the sesame crop can be achieved by various methods such as conventional and molecular breeding methods to obtain agronomically elite lines. Breeding efforts so far made in the country have not resulted in any substantial and significant breakthroughs in terms of productivity and yield stability. Data so far available from yield

trials carried out at CCS HAU clearly show that none of the high-yielding varieties are available till date with incorporating multiple stress tolerance. The future of sesame as a commercial crop totally now totally depends upon developing high-yielding strains with inbuilt resistance to various biotic and abiotic stresses to overcoming the present yield barriers. Unluckily, the on hand germplasm materials have not yet been fully and thoroughly exploited in sesame crop improvement program. Recent years have witnessed a continuously increasing number of functional genes discovered for key agronomic traits in sesame, thanks to the availability of omics tools. In this regard, the future strategies in the sesame breeding are the functional validation of these gene resources through genetic engineering approaches and marker-assisted breeding.

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