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

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2.1 Introduction

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

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

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

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

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

2.2 Germplasm and Genetic Resources

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

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

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

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

2.3 Varietal Development

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

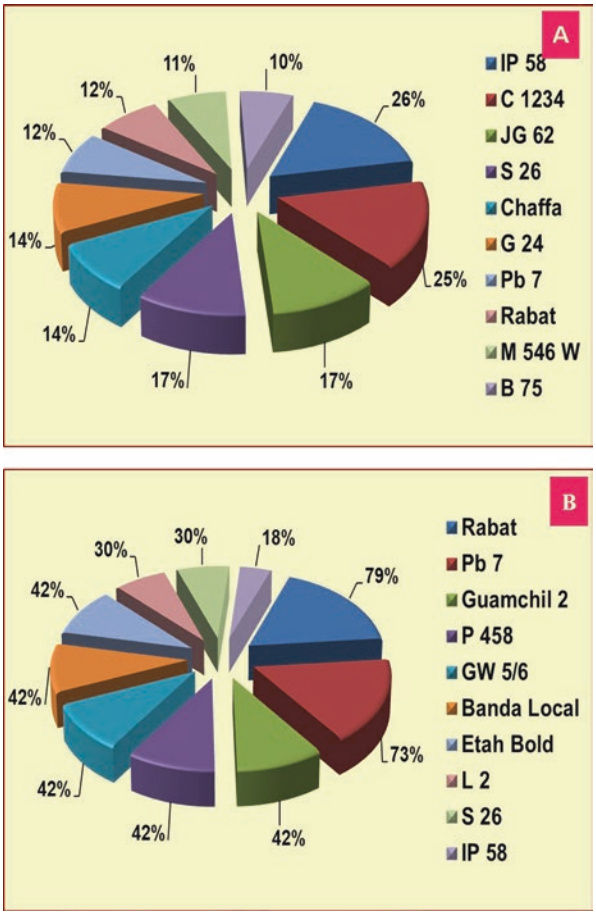

Fig. 2.1 (**a**) Per cent utilization of major parents in development of *desi* chickpea varieties. (**b**) Per cent utilization of major parents in development of *kabuli* chickpea varieties

landraces; hence most of the varieties were developed via selection and purification of existing landraces. Varieties like Dahod yellow, Chaffa, Annegri-1, Ujjain 21, BR 78 and Gwalior 2 are selection from the local germplasm/landraces. During the 1980s, major emphasis was laid on breeding for disease resistance. Systematic breeding programme led to the identification and development of disease-resistant/disease-tolerant donors/varieties against major diseases particularity *Fusarium* wilt and *Ascochyta* blight. As a result, varieties like KWR 108, H 82-2, GPF 2, Vijay, JG 11, Vishal, Gujarat Gram 1, Gujarat Gram 2, GNG 663, JG-16, KPG 59, Digvijay, Rajas, BGM 547, BGD 128, GNG 1581 etc. were evolved exhibiting potential in minimizing the wilt incidence. In the early 1980s, *Ascochyta* blight outbreak caused substantial damage to chickpea crop in northern states like Punjab, Haryana, north-west Rajasthan and Jammu region. Hence, the emphasis was laid to develop

Table 2.1 Donors identified for major biotic and abiotic stresses

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

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

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

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

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

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

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

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

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

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

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

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

2.4 Major Constraints

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

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

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

2.4.1 Biotic Stresses

2.4.1.1 *Fusarium* Wilt

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

2.4.1.2 *Ascochyta* Blight

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

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

2.4.1.3 *Botrytis* Grey Mould

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

2.4.1.4 Pod Borer

Pod borer (*Helicoverpa armigera*) is the major insect pest infesting chickpea crop, predominantly causing damages across Asia, Africa, Australia and some other chickpea-growing regions. Being a polyphagous insect, pod borer is known to cause damage to more than 182 plant species. The development of cultivars resistant or tolerant to *H. armigera* could be integrated in the pest management strategy particularly in the developing countries (Fitt 1989; Sharma and Ortiz 2002). More than 14,000 chickpea germplasm accessions screened under field conditions at ICRISAT for resistance towards *H. armigera* (Lateef and Sachan 1990) led to the identification and release of moderately resistant/tolerant chickpea cultivars (Gowda et al. 1983; Lateef 1985; Lateef and Pimbert 1990). Still complete resistance against pod borer is far from reach, as different chickpea cultivars express differential inhibition activity of gut proteinases of *H. armigera*, indicating that *H. armigera* is adapted to a wide range of host protein inhibitors (Singh et al. 2008).

2.4.1.5 Bruchids

Significant level of storage losses occurs in the Mediterranean region and in India by storage pest bruchids (*Callosobruchus chinensis*) where infestation levels approach 13% (Mookherjee et al. 1970; Dias and Yadav 1988) to total loss (Weigand and Tahhan 1990). Till date there is no report of resistance in the cultivated chickpea, though wild chickpea accessions have shown some resistance to bruchids (Singh et al. 1994, 1998). Owing to crossing barrier, it has not been possible to transfer this trait to the cultivated background. Thus, it is advised to go for chemical control measures (Duke 1981). Recent studies in legume crops indicated that seed storage in three-layered polythene bag resulted in effective control of bruchids and their further spread (Vales et al. 2014; Sudini et al. 2015).

2.4.1.6 Weeds

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

2.4.2 Abiotic Stresses

2.4.2.1 Drought

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

2.4.2.2 Heat Stress

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

period was found to be sensitive to heat stress conditions; if temperature rises above the threshold level, it would affect the pod formation and seed set causing reduced grain yield (Summerfield et al. 1984; Wery et al. 1993; Wang et al. 2006; Basu et al. 2009; Kumar et al. 2013). Moreover, high temperature has been observed to cause adverse effects on seed germination, respiration, membrane stability, photosynthesis, hormone level, nutrient absorption, protoplasmic movement, quality of seeds, fruit maturation, fertilization, materials transport, withering, burning of lower leaves, desiccation of poorly developed plants, stunting flower and pod abortion, reduced root nodulation, nitrogen fixation and seed yield (Chen et al. 1982; Saxena et al. 1988; Kurdali 1996; Wahid and Close 2007). Although chickpea is more tolerant to heat stress compared to other cool season legume crops (Summerfield et al. 1984; Erskine et al. 1994; McDonald and Paulsen 1997; Patrick and Stoddard 2010), acute heat stress could lead to high-yield losses and crop failure (Devasirvatham et al. 2012). Large genetic variations have been observed for heat tolerance in chickpea as revealed in multi-location screening of reference set against heat stress in India (Krishnamurthy et al. 2010). A field screening technique for heat tolerance has been standardized, and several sources of heat tolerance were identified (Gaur et al. 2014). A heat-tolerant variety JG 14 was released in India and found promising under both normal and late planting conditions in central, southern and eastern states.

2.4.2.3 Cold Stress

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

2.5 Genomic and Transcriptomic Resources

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

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

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

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

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

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

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

2.6 Linkage Maps, Physical Maps and Functional Maps

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

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

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

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

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

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

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

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

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

2.8 Genomics-Assisted Breeding (GAB) for Trait Improvement

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

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

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

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

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

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

2.9 Rapid Generation Advancement/Speed Breeding

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

2.10 Future Research Priorities

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

2.10.1 Germplasm Characterization

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

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

2.10.2 Trait Identification and Germplasm Enhancement

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

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

2.10.3 Regaining Chickpea Area in Northern India

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

2.10.4 Varieties for Vegetable Purpose

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

2.10.5 Kabuli Chickpea Varieties for Export and Domestic Consumption

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

2.10.6 Machine-Harvestable Chickpea for Reducing Cost of Cultivation

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

2.10.7 Herbicide-Tolerant Varieties

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

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

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

2.10.8 Varieties with Better Nutrient Acquisition Efficiency

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

2.10.9 Nutritionally Rich Varieties

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

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

2.10.10 Integrated Breeding

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

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

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