

# Chickpea

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## Abstract

The narrow genetic base of cultivated chickpea warrants systematic collection, documentation and evaluation of chickpea germplasm and particularly wild *Cicer* species for effective and efficient use in chickpea breeding programmes. Limiting factors to crop production, possible solutions and ways to overcome them, importance of wild relatives and barriers to alien gene introgression and strategies to overcome them and traits for base broadening have been discussed. It has been clearly demonstrated that resistance to major biotic and abiotic stresses can be successfully introgressed from the primary gene pool comprising progenitor species. However, many desirable traits including high degree of resistance to multiple stresses that are present in the species belonging to secondary and tertiary gene pools can also be introgressed by using special techniques to overcome pre- and post-fertilization barriers. Besides resistance to various biotic and abiotic stresses, the yield QTLs have also been introgressed from wild *Cicer* species to cultivated varieties. Status and importance of molecular markers, genome mapping and genomic tools for chickpea improvement are elaborated. Because of major genes for various biotic and abiotic stresses, the transfer of agronomically important traits into elite cultivars has been made easy and practical through marker-assisted selection and marker-assisted backcross. The usefulness of molecular markers such as SSR and SNP for the construction of high-density genetic maps of chickpea and for the identification of genes/QTLs for stress resistance, quality and yield contributing traits has also been discussed.

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### 3.1 Introduction

Chickpea (*Cicer arietinum* L.) is cultivated in almost all parts of the world covering Asia, Africa, Europe, Australia, North America and South America continents. It is known by various common or local names in different countries like *Hamas*, *Hommos*, *Humz*, *Nakhi* and *Melanch* in Arabian countries; *Keker* in the Netherlands; *Kichererbse* in Germany and Belgium; *Ceseror* and *Cicerolle* in France, *Ceci* in Vatican City and Switzerland, *Simbra* in Ethiopia; *Lablabi* in Turkey; *Garbanzo* or *Garbanzobean* in Spain; *Gravanço* in Portugal; and *Ovetichie* in Russia. Similarly, in India, chickpea is known by various names like *Chana* or *Gram* or *Bengal gram* or *Chani* in Haryana, Rajasthan, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Bihar, Jharkhand, etc.; *Chhole* in Punjab, Jammu and Kashmir and Delhi; *Chola* in West Bengal; *Harbara* in Maharashtra; *Boot* in Orissa; *Sanagulu* in Andhra Pradesh; *Kadale* in Karnataka; *Kadalai* in Tamil Nadu; and *Kadala* in Kerala, indicating its widespread cultivation and knowledge of utilization.

Chickpea, a member of Fabaceae, is a self-pollinated true diploid ( $2n=2x=16$ ) with genome size of 738 Mbp (Varshney et al. 2013a). It is an ancient cool season food legume crop cultivated by man and has been found in Middle Eastern archaeological sites dated 7500–6800 BC (Zohary and Hopf 2000). Its cultivation is mainly concentrated in semiarid environments (Saxena 1990). Chickpea is the second most important food legume crop after common bean (FAOSTAT 2011). It is grown in more than 50 countries on an area of 13.2 m ha, producing approximately 11.62 m tonnes annually. India ranks first in the world's production and area by contributing around 70.7 % to the world's total production (FAOSTAT 2011). It is one of the most important food legume plants in sustainable agriculture system because of its low production cost, wider adaptation, ability to fix atmospheric nitrogen and fit in various crop rotations (Singh 1997) and presence of prolific tap root system. Chickpea can fix atmospheric nitrogen up to 140 kg/ha through its symbiotic association with *Rhizobium* and meets its 80 % requirement (Saraf et al. 1998). It also helps in enhancing the

soil quality for subsequent cereal crop cultivation by adding organic matter for the maintenance of soil health and ecosystem. Deep and tap root system of chickpea is known to help in opening up of the soil to the deeper strata, ensuring better texture and aeration of the soil for next crop.

It is a rich source of quality protein (20–22 %) to the predominantly vegetarian population in Indian subcontinent, other South Asian countries and the Middle East. It has the highest nutritional compositions and free from anti-nutritive components compared to any other dry edible grain legumes, and thus, it is considered a functional food or nutraceutical. Besides proteins, it is rich in fibre and minerals (phosphorus, calcium, magnesium, iron and zinc), and its lipid fraction is high in unsaturated fatty acids (Williams and Singh 1987). It has no anti-nutritional factors (Mallikarjuna et al. 2007) and contains higher amounts of carotenoids like  $\beta$ -carotene than genetically engineered 'golden rice' (Abbo et al. 2005). This plant holds a good repute in 'Ayurvedic' and 'Unani' systems of medicine. In India, acid exudates from the leaves were used medicinally for aphrodisiac, bronchitis, cholera, constipation, diarrhoea, dysentery, snakebite, sunstroke and warts. It also has the property to act as hypo-cholesteremic agent; germinating chickpea is believed to reduce the blood cholesterol level. Sprouted seeds are eaten as a vegetable or salad. Young leaves and stems and green pods are eaten like vegetables. Leaves yield an indigo-like dye. The dried seeds may be used in soups or after grinding as flour. Grain husks, stems and leaves may be used in livestock feed. In the USA and Europe, chickpeas are marketed dried, canned or in various vegetable mixtures. Mashed chickpea mixed with oils and spices (hummus) is a popular hors d'oeuvre in the Mediterranean Middle East. Vavilov (1926) supported the idea of Southwest Asia and the Mediterranean region being the primary centres of origin, with Ethiopia as the secondary centre. van der Maesen (1987) suggested that Anatolia in Turkey was the area where chickpea was believed to have originated. Two types of chickpea cultivars are recognized globally – *kabuli* and *desi*. The *kabuli* types are generally grown in the Mediterranean region including Southern Europe, Western Asia and Northern Africa, and the *desi* types are grown mainly in Ethiopia and Indian subcontinent. *Desi*

chickpeas are characterized by flowers of varying colours, angular to round seeds with dark seed coat, anthocyanin pigmentation on stem or other plant parts, rough seed surface and with erect, semierect or semi-spreading growth habit, whereas *kabuli* types generally have owl- or ram-shaped beige-coloured seeds, white flowers, smooth seed surface, lack of anthocyanin pigmentation and semi-spreading to erect growth habit (Pundir et al. 1985). Of the total production, the *desi* and *kabuli* chickpeas contribute around 80 % and 20 %, respectively. *Kabuli* type is mainly grown in temperate regions, while the *desi* type chickpea is grown mostly in the semiarid tropics (Malhotra et al. 1987; Muehlbauer and Singh 1987).

## 3.2 Systematics, Genetic Relationships and Crop Gene Pool

### 3.2.1 Systematics

The *Cicer* genus belongs to family Leguminosae, subfamily Papilionaceae and tribe Cicereae Alef. The *Cicer* genus currently comprises 43 species, out of which 9 are annual and 34 are perennial species (Muehlbauer et al. 1994). Most of these species are found in West Asia and North Africa, covering Turkey in the north to Ethiopia in the south and Pakistan in the east to Morocco in the west. Of the 9 annual *Cicer* species, *C. arietinum* is the only cultivated species. The eight other annual *Cicer* species are *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum*, *C. judaicum*, *C. bijugum*, *C. cuneatum*, *C. chorassanicum* and *C. yamashitae*. The wild annual progenitor of chickpea has been identified as *C. reticulatum* L. (Ladizinsky and Adler 1976), and the perennial progenitor is proposed as *C. anatolicum* (Tayyar and Waines 1996). The *Cicer* species, including cultivated and wild, have been classified into four sections based on their geographical distribution, life cycle and morphological characteristics (van der Maesen 1987). The 8 annual species, namely, *C. arietinum*, *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum*, *C. bijugum*, *C. judaicum*, *C. yamashitae* and *C. cuneatum*, were grouped in *Monocicer* section, *C. chorassanicum* and *C.*

*incisum* (perennial species) in *Chamaecicer* section, 23 perennial species in *Polycicer* section and the remaining 7 woody perennial species in *Acanthocicer* section. The distribution of different *Cicer* species is given in Table 3.1.

### 3.2.2 Genetic Relationships

The knowledge of genetic relationships between the cultivated and its wild relatives is a prerequisite to exploit related species for the introgression of useful traits from wild to cultivated background, to track the evolution of cultivated species and also to establish the relatedness among the species within the genus. Before making use of wild forms in a better way, there is need to understand crossability relationships, chemotaxonomic relationships and cytogenetical affinities among the wild species and cultigens (Hawkes 1977). Interspecific hybridization, seed storage protein profiles, isozymes, karyotype and molecular markers have been used as criteria to study species relationships in the genus *Cicer* (Kaur et al. 2010a).

### 3.2.3 Crop Gene Pool

Harlan and de Wet (1971) proposed the concept of gene pools and their uses in crop improvement. The genus *Cicer* is classified into three gene pools (primary, secondary and tertiary) based on crossability with cultigens. The experimental evidences permitted to define the gene pool of chickpea (Ladizinsky and Adler 1976; Ahmad et al. 1987). The accumulated evidence of experimental hybridization to the gene pool approach would save the misdirected efforts in attempting wide crosses. Various researchers proposed different pools for different species. van der Maesen et al. (2007) proposed recent classification in which primary gene pool consists of cultivated species and landraces. The secondary gene pool consists of the progenitor species, *C. reticulatum* and *C. echinospermum*, the species that are crossable with *C. arietinum* but with reduced fertility of the resulting hybrids and progenies; nevertheless, both are cross-compatible with the cultigen and do not need in vitro interventions to produce hybrids. The tertiary gene pool

**Table 3.1** Distribution of different annual and perennial *Cicer* species

Species	Distribution
<b>Annual species</b>	
<i>C. arietinum</i>	Mediterranean region to Burma, Ethiopia, Mexico, Chile
<i>C. chorassanicum</i>	N and C Afghanistan, N and NE Iran
<i>C. bijugum</i>	SE Turkey, N Syria, N Iraq
<i>C. cuneatum</i>	Ethiopia, SE Egypt, NE Sudan, Saudi Arabia
<i>C. echinospermum</i>	Turkey, E Anatolia, N Iraq
<i>C. judaicum</i>	Palestine, Lebanon
<i>C. pinnatifidum</i>	Cyprus, N Iraq, Syria, Turkey, USSR
<i>C. reticulatum</i>	E Turkey
<i>C. yamashitae</i>	Afghanistan
<b>Perennial species</b>	
<i>C. acanthophyllum</i>	Afghanistan, Pakistan, USSR
<i>C. anatolicum</i>	Turkey, Iran, Iraq, Armenia
<i>C. atlanticum</i>	Morocco
<i>C. balcaricum</i>	Caucasus (USSR)
<i>C. baldshuanicum</i> , <i>C. flexuosum</i> , <i>C. grande</i> , <i>C. incanum</i> , <i>C. korshinskyi</i> , <i>C. laetum</i> , <i>C. mogoltavicum</i> , <i>C. paucijugum</i> , <i>C. rassuloviae</i> , <i>C. songaricum</i>	USSR
<i>C. canariense</i>	Canary islands, Tenerife, La Palma
<i>C. fedtschenkoi</i>	USSR, N and NE Afghanistan
<i>C. floribundum</i> , <i>C. heterophyllum</i> , <i>C. isauricum</i>	Turkey
<i>C. graecum</i>	Greece
<i>C. incisum</i>	Greece, Turkey, Iran, Lebanon, USSR
<i>C. kermanense</i>	SE Iran
<i>C. macrocanthum</i>	Afghanistan, India, Pakistan, USSR
<i>C. microphyllum</i>	E Afghanistan, Tibet, India, Pakistan, USSR
<i>C. montbretti</i>	Albania, Bulgaria, Turkey
<i>C. multijugum</i> , <i>C. rechingeri</i>	Afghanistan

Species	Distribution
<i>C. nuristanicum</i>	Afghanistan, India, Pakistan
<i>C. oxyodon</i>	Iran, Afghanistan, N Iraq
<i>C. pungens</i>	Afghanistan, USSR
<i>C. spyroceras</i> , <i>C. stapfianum</i> , <i>C. subaphyllum</i>	Iran
<i>C. tragacanthoides</i>	Iran, USSR

consists of all the annual and perennial *Cicer* species that are not crossable with cultivated species.

The *Cicer* species possess wealth of useful genes for biotic and abiotic stresses and hold promise for enhancing seed yield through introgression of wild genes into cultivated species (Singh et al. 1982a, b, 2005, 2014; Singh and Ocampo 1993). Large inter-accessions as well as intra-accessions variation has been observed in different *Cicer* species for resistance to *Botrytis* grey mould (Kaur et al. 2007). Verma et al. (1990) studied the crossability of cultivated chickpea, used as female, with *C. echinospermum*, *C. judaicum* and *C. bijugum*, used as males. All the F<sub>1</sub>s were fertile and were successfully advanced to later generations. All the crosses were developed under field conditions using mixture of plant growth hormones at the time of emasculation and pollinations. The success was attributed to the use of growth hormones and large number of pollinations and, thus, indicated that there is a need to reclassify *Cicer* species. The recent studies (Sandhu et al. 2005; Kaur et al. 2013) indicated that *C. pinnatifidum*, a valuable source for several biotic and abiotic stresses, can be crossed successfully with cultivated chickpea for the transfer of high level of resistance to *Botrytis* grey mould and *Ascochyta* blight (Kaur et al. 2013). These studies further confirm findings of Verma et al. (1990).

### 3.3 Assessment of Gene Flow for Crop Improvement

Due to long evolutionary process, high-yielding genes might have been eroded from populations or become silent resulting in average grain yield of most of the present-day cultivars. It is also true that in most of the cases, high-yielding varieties developed through hybridization in the last 40–50 years have utilized limited variability as the derivatives of

already utilized plant genetic resources were considered in making crosses for the improvement of targeted traits. Therefore, the assessment of gene flow and constraints in flow has been discussed below.

Due to domestication bottlenecks, the genetic base of various legume crops including chickpea became narrow (Spillane and Gepts 2001). The bottleneck is perpetuated further by various reproductive isolation factors preventing gene flow. The domesticated plants may be carried by the cultivators to sites far removed from its original habitat. During transfer between latitudes, there may be further narrowing of the genetic base, because the population would not be well adapted to the new day-length conditions, and so only a small number of the genotypes would survive. There may be other chance occurrences that narrow the genetic base such as disease epidemics, which may decimate populations. Post-domestication, the crops evolved under human selection but continued to possess a breadth of genetic variation in order to overcome challenges from changes in biotic and abiotic milieu. The advent of modern plant breeding resulted in the creation of plant varieties that optimized adaptation at the cost of adaptability. The narrowed genetic base of germplasm is often evident from plateau in yield gains as have been observed in several other crop plants. Historically, instances of more disastrous consequences have also been observed. Often quoted instances include the *Ascochyta* blight epidemics caused by *Ascochyta rabiei* in North India in 1980 and 1982 (Singh et al. 1982b, 1984b). During the process of evolution, chickpea like other crops are subjected to genetic bottlenecks and subsequent founder effect that resulted in narrow genetic base. The progenitor species *C. reticulatum* (Ladizinsky and Adler 1976) is narrowly distributed in Southeastern Turkey and harbours limited adapted variation compared to wheat and barley (Berger et al. 2003). During domestication, it is likely that only a small proportion of diversity of wild population is sampled. Subsequent gene flow between new cultivars and its wild progenitors might be restricted by breeding barriers and nature of domestication event (Cooper et al. 2001). Shifting from autumn to spring season probably to avoid *Ascochyta* blight has reduced the genetic diversity and selection to suit post-rainy season cropping has further narrowed the genetic base.

The replacement of landraces by elite cultivars developed through hybridization using closely related parents caused another bottleneck. With changing climatic conditions and evolution of new pathogens, selection will be rigorous for chickpea germplasm to withhold biotic and abiotic stresses, which will further narrow down the genetic base. The pedigree analysis of 86 chickpea varieties released in India through hybridization and selection revealed that top ten ancestors contributed more than 35 % to their genetic base and about 41 % varieties have 'Pb7' as one of the ancestors in their pedigree (Kumar et al. 2004, 2008, 2009). Because of the limited genetic variability within the primary gene pool, the genetic improvement of chickpea by classical breeding involving inter-varietal crosses has met with limited success (Singh and Ocampo 1993). Thus, the situation warrants an urgent need to broaden the genetic base of cultivated varieties through genetic enhancement by involving unadapted germplasm, exotic germplasm and landraces in hybridization (Duvick 1995). Since the genetic variability within the primary gene pool is limited, there is a need for introgression of alien genes through pre-breeding efforts for widening the base of cultivated gene pool (Verma et al. 1990; van Rheenen et al. 1993; Nadarajan and Chaturvedi 2010).

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### 3.4 Level of Diversity in Crop Germplasm

The sum total of hereditary materials present in a crop species and its wild relatives is referred to as germplasm. This is also known as genetic resources or gene pool. In other words, germplasm is a collection of genetic resources for an organism which include inbred lines, landraces, open pollinated varieties, exotic accessions, wild species, cultivars and breeding stocks. These types of germplasm can carry unidentified variation that may be a valuable resource for breeders and other researchers. Germplasm can be collected from centres of diversity, gene banks, gene sanctuaries, farmer's field, markets and seed companies. Genetic pool represents the entire genetic variability or diversity within a crop species. Agricultural practices have gradually dispersed the local tradi-

tional varieties and crop wild relatives (CWRs) leading to a loss of indigenous diversity. However, CWR and landraces (LR) are the two major components of agro-biodiversity that offer the widest range of diversity for breeders in crop improvement programmes. The CWRs and locally adapted traditional crop varieties contain vital sources of useful genes. These invaluable resources are threatened by the climate change as well as by a range of other human-induced pressures and socio-economic changes, while the value of CWR and LR for food security is widely recognized. A systematic strategy for the conservation of the highest priority CWR and LR resources is required at global level.

### 3.4.1 Crop Wild Relatives

The crop wild relatives (CWRs) provide the broadest range of genetic diversity in grain legumes, including chickpea, and have the ability to provide the wide range of germplasm resources for the incorporation of various traits of agro-economic importance (Singh et al. 2013). Many species are being extinct because natural habitats are being lost due to increased human pressure and ecological threats. There is an urgent need for systematic exploration and sample of the genetic diversity in wild relatives that was partially captured during the domestication. The *ex situ* conservation was pioneered by Vavilov (1926), and subsequent explorations resulted in large collections in gene banks. However, these gene banks are dominated by cultivated forms of crops. Crop wild relatives are also important from phylogenetic perspective, applied in interspecific crosses to increase the diversity, as the majority of allelic variation is predicted to occur outside of the crop itself. In spite of the existence of vast collections of CWR, their use for crop improvement has been limited as assessing the genetic diversity was a challenge until now in most of the legumes including chickpea. Selections for higher yield and quality characteristics during domestication have resulted in narrowing of the genetic variation in cultivated chickpea. The wild *Cicer* species do not consist of useful variation for morphological characteristics and protein content, but they are rich sources of resis-

tance to various biotic and abiotic stresses (Singh et al. 1998; Croser et al. 2003; Sandhu et al. 2006; Mallikarjuna et al. 2007; Kaur et al. 2013), yield QTLs (Singh and Ocampo 1997; Singh et al. 2005) and biochemical traits (Kaur et al. 2010b).

### 3.4.2 Landraces

A variable population, which is identifiable and usually has a local name, is designated as landrace. It lacks 'formal' crop improvement and characterized by a specific adaptation to the environmental conditions of the area of cultivation and is associated with the traditional uses, knowledge, habits, dialects and celebrations of the people who developed and continue to grow it (Lorenzetti and Negri 2009). The LR belongs to the people who developed it and feel to be its owner (in a specific human context). They are maintained because of their better quality than commercial varieties and better yield performance/persistence under difficult pedo-climatic conditions. It is estimated that less than one third of them is already marketed as niche, typical product (Negri 2003). However, most of them are highly threatened because they are cultivated primarily by ageing farmers (Negri 2003; Galluzzi et al. 2010). The lack of traditional information severely hampers the possibility of conserving and using these LR effectively. There is an urgent need to make an inventory and focus should be given to the priority species. Landraces as an agro-biodiversity resource is not only critical for future food security but is a vital component of our biodiversity and cultural heritage. The extent of loss of crop genetic diversity is associated with the loss of landraces which is very difficult to quantify accurately. This erosion of our agro-biodiversity resources is likely to be critical for future food security. It has been recognized in a number of international legal platforms, including the Convention on Biological Diversity and the International Treaty on Plant Genetic Resources for Food and Agriculture. In general, it has been noticed that the average landrace maintainer was found to be 65 years old and there was little evidence of the next generation being willing or able

to continue the family role as maintainers. Hence the need for research in this area and the production of a corresponding inventory is necessary. Any new variation will not replace the old varieties that have been lost. Rich diversity in the form of landraces of chickpea has been reported in Bundelkhand and part of Chhattisgarh in India.

### 3.4.3 Commercial Cultivars

In the 1970s, most of the commercial varieties were developed through selection from landraces. The major emphasis was given on increasing yield potential. During the 1980s, the focus was laid on breeding for disease resistance. Consequently, several varieties (Avarodhi, JG 315, Pusa 209, GNG 16, Pusa 212, Pusa 240, Pusa 244, Pusa 256, Pusa 413, ICCV 10, ICCV 37, Phule G 5, Phule G 12, etc.) resistant/tolerant to *Fusarium* wilt were developed and released for their cultivation in different regions of the country (Chaturvedi et al. 2003). Similarly, genotypes tolerant to *Ascochyta* blight, namely, C235, Gaurav, H 75-35, BG 261, PBG 1, GNG 146 and PBG 5, were developed for North West Plain Zone (Delhi, Punjab, Haryana, North Western Rajasthan and Western UP) and G 543 and PBG 5 for the state of Punjab (Sandhu et al. 2004). However, during the 1990s, the major thrust was given on breeding for multiple disease resistance and high-input responsive varieties. Sources for drought tolerance (RSG 44, RSG 963, RSG 888, ICC 4958, ICCV 10, Vijay, GL 769, GPF 2, PDG 3, PDG 4, Phule G 5), cold tolerance (ICCV 88506, ICCV 88503, Phule G 96006, ICC 8923, PDG 84-10, GL 28008, GL 28028) and salt tolerance (CSG 88101, CSG8962) were identified for their use in breeding programme. As a result, multiple disease-resistant varieties, namely, Bharati, Pusa 372, Pusa 362, BG 391, KWR 108 and GNG 1581 against wilt and root rot and GNG 469 against *Ascochyta* blight and root rot, and high-input responsive variety like DCP 92-3 were released for cultivation. Rice fallows (about 11.0 m ha) in Eastern India (eastern UP, Bihar, West Bengal, Orissa, Jharkhand and Assam) and Central India (eastern MP and Chhattisgarh) provide opportunities for horizontal expansion of

area under chickpea. This requires the development of varieties amenable for late planting to popularize rice-chickpea sequential cropping system. As a result of concerted breeding efforts, varieties like KPG 59, Pant G 186, BGM 547, RSG 963, Rajas and Pusa 372 were developed for late sown condition in Eastern India. Similarly, recently developed varieties like JG 14, Vaibhav, JSC 55 and JSC 56 have great potential for adaptation in late sown condition and rice fallow of Central India due to short duration, drought and heat tolerance traits.

The development of short-duration varieties like ICCV 2, JG 74, Vijay, JG 11, JG 16, JAKI 9218 and KAK 2 was the major catalyst for the expansion of chickpea area in Southern and Central India. In spite of reduction in duration, the yield potential of these early maturing varieties remains almost unaffected, thus improving per day the productivity of the crop. Presently emphasis has been laid on the development of extra-large seeded *kabuli* chickpea varieties (>50 g/100 seed weight). Some of the promising varieties, viz. Phule G 0517, IPCK 02, MNK 1 and PKV Kabuli 4-1, have been released for Maharashtra, Madhya Pradesh, Andhra Pradesh and Karnataka, and a medium bold seeded variety L 552 has been released for Punjab state. A major breakthrough has been witnessed in developing large seeded *kabuli* varieties with high-yield potential such as KAK 2, BG 1003, BG 1053, JGK 1, Phule G 95311, IPCK 2002-29, IPCK 2004-29, L 555 and HK 05-169 (Chaturvedi et al. 2010). Similarly, prominent large seeded *desi* varieties, viz. BG 256, Phule G 5, BGM 391, K 850, Radhey, Gujarat Gram 2 and L 556, were also developed.

## 3.5 Production-Related Problems

### 3.5.1 *Fusarium* Wilt

Chickpea wilt occurs in 32 countries across 6 continents in the world (Nene et al. 1991; Singh and Sharma 2002). It is caused by *Fusarium oxysporum* f. sp. *ciceris*. The yield losses caused

by it vary from 10 to 90 % (Jimenez-Diaz et al. 1989; Singh and Reddy 1991). At present there are eight distinct physiological races of *Fusarium oxysporum*, viz. 0, 1A, 1B/C, 2, 3, 4, 5 and 6, out of which four races that have been identified as 1, 2, 3 and 4 are prevalent in India (Haware and Nene 1982) and races 0, 5 and 6 are reported from Spain (Jimenez-Diaz et al. 1989). Breeding for *Fusarium* wilt is of prime importance because of the nature of the pathogen – it can persist in soil year after year even in the absence of the host (Haware et al. 1996). Due to the presence of such a number of races, it is very difficult to develop a cultivar which shows stability to the disease across different regions. Most of the resistance against *Fusarium* wilt is of vertical type (Sharma et al. 2005) as is mostly governed by a single major gene. Chickpea genotypes differ in the development of initial symptoms of wilt, indicating different degrees of resistance which is controlled by the segregation of a single gene (Upadhyaya et al. 1983). Such individual genes which are part of oligogenic resistance mechanism delay the onset of disease symptoms, and such a phenomenon is called as late wilting. The yield losses are significantly less in the case of genotypes which show the phenomenon of late wilting due to the combination of two recessive genes for resistance. Resistance to race 0 is due to two independent genes (Rubio et al. 2003), while resistance is digenic or trigenic for race 1A, 2 and 4. Resistance to race 3 and 5 is controlled by a single gene (Sharma et al. 2005). Singh et al. (2012a) have also reported resistance against *Fusarium* wilt in the indigenous chickpea germplasm.

### 3.5.2 *Ascochyta* Blight

*Ascochyta* blight is the most important foliar disease of chickpea in many parts of the world including India. It is caused by *Ascochyta rabiei* and has a devastating effect on the chickpea production by causing yield losses ranging from 10 to 100 % (Nene and Reddy 1987; Singh 1990). It is reported in 37 countries all over the world. Among the most affected regions are the Indian

subcontinent and Mediterranean region as their prevailing climatic conditions are conducive for the pathogens. *Ascochyta* disease epidemics are common and had occurred in India, Pakistan, the USA, Northwest Pacific, Australia and Syria in the past (Malhotra et al. 2003). *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). The main reason for the epidemics is the nature of pathogen which is unstable and continuously evolves new races which break down the host resistance and, thus, reduces the life of cultivars under production systems. The development of more virulent pathotypes has been reported in Syria (Reddy and Kabbabeh 1985), Italy (Stamigna et al. 2000) and Pakistan (Jamil et al. 2000). The genetics of resistance to *Ascochyta* blight is mainly digenic in nature, and both recessive and dominant genes are involved in its control (Bhardwaj et al. 2010). This has forced the breeders to devise a different approach for breeding cultivars with durable resistance. Under new breeding strategy, plant breeders have shifted to gene pyramiding in elite lines instead of incorporating vertical resistance. An alternative strategy of deploying different lines possessing resistance against different races of the pathogen prevalent in different regions can also be effective for minimizing yield losses.

### 3.5.3 *Botrytis* Grey Mould

*Botrytis* grey mould (BGM) is a second major foliar disease of chickpea and is 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 of resistance to BGM in India (Singh and Reddy 1991), but in recent years, derivative lines from the interspecific crosses of *C. arietinum* and *C. pinnatifidum* had shown high level of genetic resistance to the BGM (Kaur et al. 2013). This resistance can be incorporated into elite lines to develop high-yielding chickpea cultivars with



durable resistance. According to Mallikarjuna (unpublished results), the resistance introgressed from wild *C. echinospermum* to cultivated chickpea was found to be monogenic in nature, indicating that resistance to BGM can be easily incorporated to elite lines from the interspecific derivatives.

### 3.5.4 Pod Borer

Among the various insects that attack chickpea crop, pod borer (*Helicoverpa armigera*) is the most damaging insect. It is quite prevalent in Asia, Africa, Australia and some other chickpea-growing regions. As pod borer is a polyphagous insect that attacks on 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 were screened under field conditions at ICRISAT for resistance towards *H. armigera* (Lateef and Sachan 1990). This resulted in the identification and release of moderately resistant/tolerant chickpea cultivars (Gowda et al. 1983; Lateef 1985; Lateef and Pimbert 1990). Still a complete resistance against pod borer is far from reach as different chickpea cultivars show differential inhibition activity of gut proteinases of *H. armigera*, which indicate that *H. armigera* is adapted to a wide range of host protein inhibitors (Singh et al. 2008).

### 3.5.5 Bruchids

Apart from field, chickpea is also damaged in the storage and one such insect is bruchids (*Callosobruchus chinensis*). It causes loss of grains in the Mediterranean region and in India, where infestation levels approach 13 % (Mookherjee et al. 1970; Dias and Yadav 1988) to total loss (Weigand and Tahhan 1990). Till now 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, chemical methods are advised for the control of bruchids (Duke 1981).

### 3.5.6 Cold Tolerance

If temperature ranges between 0 and 12 °C, then this stress can be defined as chilling without snow cover (Wery et al. 1993). If below 10 °C, the susceptibility of reproductive phase of chickpea to chilling temperatures increases (Sandhu et al. 2005; Srinivasan et al. 1999; Nayyar et al. 2005b). When crop is sown in autumn or in early spring, it is exposed to freezing stress during vegetative growth in WANA region, Europe and Central Asia (Singh et al. 1994). Usually chickpea grown in winter season is more productive than the traditionally grown spring season in the Mediterranean region (Singh and Hawtin 1979). This is due to long growing season and better moisture availability. Problem encountered by winter season crop is flower drop and pod abortion, which leads to major yield loss, when mean temperature of the day falls below 15 °C (Savithri et al. 1980; Srinivasan et al. 1999; Clarke and Siddique 2004; Nayyar et al. 2005a). Chaturvedi et al. (2009) reviewed the work on cold tolerance in chickpea extensively and highlighted the importance of cold tolerance at reproductive stage in chickpea. The deployment of genes for cold tolerance will protect crop from getting damaged and reduce yield losses. Cold tolerance was found to be controlled by at least five genes with both additive and nonadditive gene effects and was dominant over susceptibility (Malhotra and Singh 1990, 1991). Selfing generations would result in reduced dominance and epistatic effects, which would be better for the selection of cold tolerance in chickpea. However, Bhardwaj and Sandhu (2009) reported that cold tolerance is under the control of a single recessive gene. Screening of wild *Cicer* species showed promising traits for cold tolerance (Berger et al. 2012), but till now there are no reports of introgression of cold tolerance from wild to the cultivated chickpea.

### 3.5.7 Drought Tolerance

Drought is the second most important abiotic stress that contributes immensely to the losses in chickpea production globally. Most of the time, it is terminal drought that has an adverse effect on the crop productivity (Khanna-Chopra and Sinha 1987). In order to counter this drought stress, development of early maturing cultivars will make judicious use of the available soil moisture efficiently and produce relatively higher yields. Critical review made by Upadhyaya et al. (2012) illustrates efficient methods for phenotyping with respect to drought in chickpea and pigeon pea. Drought tolerance may be under polygenic or oligogenic control with additive and nonadditive gene effects. Root traits have been given most importance in recent years because lines with longer root systems have shown better drought tolerance. As chickpea is grown under receding moisture conditions, the long root trait plays an important role in countering drought. Apart from this, early flowering is considered to be very important for drought escape. There a single recessive locus involved in controlling early flowering (Kumar and van Rheenen 2000). Genotypes with recessive allele in homozygous condition escape drought and give a good yield, and such alleles could be easily transferred to the drought-susceptible lines using suitable breeding method for developing drought-tolerant cultivars. Apart from this, wild *Cicer* species have been screened, and 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, the line ICC 4958 is currently considered as potential donor for drought tolerance. Some chickpea cultivars with improved drought tolerance have been released using ICC 4958 as one of the donors in South India and Kenya (Gaur et al. 2012a).

### 3.5.8 Heat Tolerance

Most of the time, heat stress occurs in combination with/overlapping with drought stress (Toker and Canci 2009). It is very difficult to make distinction between whether the crop is under heat or drought stress; thus, less progress has been made on heat tolerance (Malhotra and Saxena

1993). Chickpea is grown in post-rainy period in South Asia, which results in the exposure of crop to drought along with high temperature. It is predicted that climate change would result in temperature rise by 3–4 °C over current levels by 2050 (Basu et al. 2009). Chickpea is usually grown in winter season in Northern India. But it experiences a high temperature (>35 °C) during the reproductive phase. Most sensitive organs of plant to heat are flowers (Wery et al. 1993; Toker and Canci 2006). During the flowering or reproductive period, if temperature rises above the threshold level, it would adversely affect the pod formation and seed set and, thus, results in reduced grain yield (Summerfield et al. 1984; Wang et al. 2006; Basu et al. 2009; Kumar et al. 2013). Adverse effects of high temperature occur in 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 (Saxena et al. 1988; Kurdali 1996; Chen et al. 1982; Wahid and Close 2007). In comparison to other cool season legume crops, chickpea is more tolerant to heat stress (Summerfield et al. 1984; Erskine et al. 1994; McDonald and Paulsen 1997; Patrick and Stoddard 2010). However, acute heat stress in chickpea could lead to high-yield losses and crop failure (Devasirvatham et al. 2012). Large genetic variations have been observed for heat tolerance in chickpea when reference set was screened against heat stress at several locations in India (Krishnamurthy et al. 2010). A field screening technique for heat tolerance has been developed and several sources of heat tolerance were identified (Gaur et al. 2014a).

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### 3.6 Traits of Importance for Base Broadening

All studies on assessing genetic diversity in chickpea using molecular markers indicate that chickpea has a narrow genetic base. A large variability is seen in chickpea germplasm for

morphological traits, but it could be a reflection in expression of a limited number of mutant genes, as a single mutant gene may cause marked changes in the appearance of the plant (Gaur and Gour 2003). The narrow genetic base of chickpea is a major concern for plant breeding programmes as the genetic variability is a key factor that contributes to genetic gain from selections. Thus, broadening the genetic base of chickpea is very much needed for improving effectiveness of breeding efforts. The genetic variability in the cultivated chickpea can be enhanced by gene introgression from the wild species and by inducing genetic changes through induced mutagenesis. The wild species of chickpea constitutes a valuable genetic resource, particularly for resistance to biotic and abiotic stresses and the nutritional quality traits. Sources of resistance are available in the cultivated species for several biotic (*Fusarium* wilt, *Ascochyta* blight) and abiotic (drought, heat) stresses (reviewed by Gaur et al. 2010). There is a need to diversify sources of resistance being used in the breeding programmes by bringing new sources of resistance from the wild species. There are several biotic stresses, such as dry root rot, *Botrytis* grey mould, phytophthora blight and pod borer, for which high levels of resistance are not available in the germplasm of cultivated species and can be introgressed from the wild species or induced through mutagenesis. Over a dozen mutants have been directly released as varieties (Gaur et al. 2007), while many others have been used as parents in crossing programmes. Mutants with novel traits, such as cymose inflorescence with more than three flowers per node (Gaur and Gour 2002), brachytic growth habit (Gaur et al. 2008) and determinate growth habit (Hegde 2011), have been identified in chickpea and have potential for developing new plant types in chickpea. Singh et al. (2014) have also identified several useful agro-morphological traits and important biotic parameters in various wild annual *Cicer* species and suggested their introgressions for widening the genetic base of cultivated gene pool.

In present day, when farm labourers are becoming expansive day by day, the farmers are demanding chickpea varieties with traits such as suitability to mechanical harvesting and toler-

ance to herbicides to enhance mechanization of chickpea cultivation (Sandhu et al. 2010; Gaur et al. 2012a). Large genetic variations have been observed for postemergence herbicide tolerance in chickpea (Gaur et al. 2013a) which can be utilized for the development of herbicide-tolerant cultivars. Several diseases, such as dry root rot, collar rot, wet root rot and stem rot, were minor hitherto and are becoming potential threat to chickpea cultivation in many parts of the world including India. There is a need to strengthen research efforts on identifying useful sources of resistance and breeding for enhancing resistance to these diseases. The other traits which have not received much attention in the past, but are important under current scenario of growing environments and consumer requirements, include nutrient use efficiency, especially phosphorus, and nutritional quality traits (protein, iron, zinc,  $\beta$ -carotene, oligosaccharides, etc.).

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### 3.7 Interspecific Hybridization in Crop Species

The crop wild relatives are species closely related to crops, including crop progenitors, identified as critical resources that are vital for wealth creation, food security and environmental stability (Meilleur and Hodgkin 2004; Stolton et al. 2006; Maxted et al. 2008). Historically, the commercial use of wild relatives started in the late nineteenth century (Prescott-Allen and Prescott-Allen 1988), and in the middle of twentieth century, the value of CWR was widely recognized and breeding efforts to explore the potential of wild relatives were initiated in many crop species. The use of wild relatives increased in the 1970s and 1980s (Hodgkin and Hajjar 2008) and in the mid-1980s. Prescott-Allen and Prescott-Allen (1988) asserted that the achievements were substantial enough to recognize the potential of wild relatives.

The proportion of wild or weedy relatives in gene bank holdings has significantly increased in a span of 21 years starting from 1983 (Plucknett et al. 1987) to 2004 (<http://singer.grinfo.net/>). Summarizing the use of wild relatives for the improvement of major crop species in the last 20 years, Hajjar and Hodgkin (2007) have listed the

number of traits. They showed that the extent of utilization varies from crop to crop. Tomato takes lead with 55 traits followed by rice and potato with 12 traits each. Using CWR, wheat was improved for nine traits and sunflower for seven traits. Millet was featured on the list with three traits and maize and chickpea with two traits each.

Wild species have contributed substantially to crop improvement for many characters including seed yield in different crop plants (Stalker 1980). Similarly in chickpea, the productivity genes/alleles have been introgressed from *C. echinospermum*, *C. reticulatum* (Singh and Ocampo 1997; Singh et al. 2005) and *C. pinnatifidum* (Sandhu et al. 2006; Singh et al. 2012a, b, c). However, the transfer of specific genes is frequently associated with the transfer of large alien chromosome segments having undesirable traits (Tanksley and Nelson 1996). Owing to linkage drag, the genes for primitive or wild traits are often introduced along with desirable traits. Breaking linkages with unwanted type and restoring the genotype associated with accepted agronomic background may take a long time. The first report on interspecific crosses involving *C. arietinum* on one hand and *C. reticulatum* and *C. cuneatum* on the other hand was published by Ladizinsky and Adler (1976). The cross between *C. arietinum* and *C. reticulatum* was achieved successfully. Subsequently, wide hybridization was attempted between *C. arietinum* and *C. echinospermum* by various workers (Pundir and Mengesha 1995; Singh and Ocampo 1993). van Dorrestein et al. (1998) attempted crosses involving *C. arietinum*, *C. bijugum* and *C. judaicum*. Due to the use of in vitro technique, success has been made in achieving hybrids between *C. arietinum* and *C. bijugum* and *C. arietinum* and *C. judaicum*. Badami et al. (1997) also reported successful hybridization between *C. arietinum* and *C. pinnatifidum* using embryo rescue technique. No success has been reported in hybridization between *C. arietinum* and *C. microphyllum*. The crossability and further generation advance studies showed that the species like *C. pinnatifidum* and *C. judaicum* can be crossed with cultivated chickpea (Verma et al. 1990, 1995; Sandhu et al. 2006, 2007). The derived populations showed

wide variation for different agro-morphological traits. Further, F<sub>5</sub> population of an interspecific cross with *C. pinnatifidum* showed that some of the derived lines were superior in yield performance than the recommended check variety and some of the lines also showed resistance against *Botrytis* grey mould.

### 3.8 Barriers to Interspecific Hybridization

Cross-incompatibility, inviability of F<sub>1</sub> hybrid and its progenies are the most common barriers to wide hybridization. Cross-incompatibility between parent species arises when pollen grain does not germinate or pollen tube does not reach to the ovary or male gamete does not fuse with female gamete (Chowdhury and Chowdhury 1978). Post-fertilization barriers in legumes such as production of shrivelled hybrid seed with reduced germination (hybrid inviability), production of dwarf and weak F<sub>1</sub> plants (hybrid weakness) and death of F<sub>1</sub> plants at critical stage of development (hybrid lethality) have been observed by various workers in *Cicer* (Ahmad et al. 1988). This may be attributed to disharmonies between genomes of parental species between genome(s) of one species and cytoplasm of other, or between genotype of F<sub>1</sub> zygote and the genotype of maternal tissues. These barriers were found in varying degrees in most of the interspecific crosses (Al-Yasiri and Coyne 1966; Biswas and Dana 1976; Chowdhury and Chowdhury 1977; Machado et al. 1982; Chen et al. 1983; Gopinathan et al. 1986). Reciprocal crosses should be attempted, if there is disharmony between genome of one species and cytoplasm of the other. The application of flower and fruit setting hormones to pollinated buds (GA<sub>3</sub>, indole-3-acetic acid, 6-benzyl amino purine, etc.) and pre-pollination foliar spray of x-aminocaproic acid (immunosuppressant) were also found useful in overcoming inviability of wide crosses. With the advent of in vitro techniques, viz. embryo rescue and ovule culture, ambit of crossable species is greatly enhanced (van Rheenen 1992). In many instances, the hybrid zygote from wide cross died after few

days. Growth regulators in such cases increase the embryo survival. These embryos further rescued by culturing them on artificial medium. Murashige (1977) and Raghvan (1980) have presented a good discussion on embryo culture for crop breeding. The procedure of embryo culture has been detailed by Hadley and Opeshaw (1980). This technique has facilitated the production of many viable hybrids in different legume crops (Ahn and Hartmann 1978; Cohen et al. 1984; Chen et al. 1990; Sharma and Satija 1996; Gomathinayagam et al. 1998).

Hybrid sterility generally arises when chromosomes do not pair in  $F_1$ , and therefore, gametes receive different number of chromosomes leading to sterility. Studies have shown that in some of the interspecific hybrids, sterility was of segregational type (as per Stebbins 1966 classification) and was mainly due to interchange, inversion and possible duplication and deficiency type of structural heterozygosities in the  $F_1$  individuals (Biswas and Dana 1976; Karmarkar and Dana 1987). Chromosome doubling of the parental species before crossing sometimes increases the chances of obtaining a viable hybrid. Colchicine-induced allopolyploids were raised from most of the semi-fertile and completely seed sterile  $F_1$  hybrids that had high pollen fertility and seed set (Pande et al. 1990). Some of the allopolyploids have been used as bridge species in wide crosses.

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### 3.9 Molecular Markers, Genome Mapping and Genomics as an Adjunct to Breeding

#### 3.9.1 Molecular Markers

Molecular markers which are abundant and have high level of polymorphism and can be subjected to high-throughput analysis are desired for applications in genomic studies and crop improvement (Sharma et al. 1995). Isozymes were the first molecular markers used in chickpea genetic studies, but these markers were small in numbers and showed very low level of polymorphism in the cultivated species. Nevertheless, these markers were used in developing the first linkage map of

chickpea (Gaur and Slinkard 1990a, b) and establishing phylogenetic relationships among annual *Cicer* species (Kazan and Muehlbauer 1991; Ahmad et al. 1992). The genetic maps developed from isozyme markers were further expanded using restriction fragment length polymorphisms (RFLP) and randomly amplified polymorphic DNA (RAPD) markers (Simon and Muehlbauer 1997). The use of these markers was restricted, because of some limitations associated with them. The extensive use of molecular markers in chickpea genetics and breeding started only after the development of simple sequence repeat (SSR) markers. The multi-allelic and codominant nature of these markers made them ideal for genomic studies and for use in plant breeding. The SSR markers have been developed from sequence information obtained from various sources, including genomic libraries (Hüttel et al. 1999; Winter et al. 1999; Sethy et al. 2006a, b; Nayak et al. 2010), bacterial artificial chromosome (BAC) libraries and BAC-end sequences (Lichtenzveig et al. 2005; Choudhary et al. 2006; Thudi et al. 2011), tentative unique sequences (TUS) (Hiremath et al. 2011) and expressed sequence tags (ESTs) (Coram and Pang 2005; Varshney et al. 2005, 2009a; Gujaria et al. 2011; Hiremath et al. 2011). Over 2,000 SSR markers are now available for chickpea molecular analysis. The recently published draft genome sequence of chickpea identified over 48,000 SSRs suitable for PCR primer design for use as genetic markers (Varshney et al. 2013a).

Diversity arrays technology (DArT), which utilizes the microarray platform to analyse DNA polymorphisms, is a high-throughput genome analysis method enabling a rapid and economical approach for screening a large number of marker loci (Jaccoud et al. 2001). In chickpea, 15,360 DArT markers were generated from 94 diverse genotypes and 5,397 of these were found polymorphic (Thudi et al. 2011). The level of polymorphism observed for DArT markers was comparable to other crops like sorghum and cassava. Single nucleotide polymorphism (SNP) markers are the new class of markers and have become the preferred choice of markers because of their abundance, codominant nature and amenability to

high-throughput analysis. Several thousand SNPs have been identified from transcriptomic analysis in chickpea (Coram and Pang 2005; Varshney et al. 2009b; Gujaria et al. 2011; Hiremath et al. 2011). The draft genome sequence of chickpea is now available (Varshney et al. 2013b) and identified 76,084 SNPs in 15,526 genes. Of these, 27,117 SNPs were identified within the cultivated species and 54,178 SNPs between the cultivated species and its progenitor *C. reticulatum*.

### 3.9.2 Genome Mapping

All the markers developed in chickpea showed low level of polymorphism within the cultivated species as compared to between the cultivated and wild species. For this reason, the initial studies on genome mapping in chickpea used interspecific mapping populations. The first linkage map of chickpea was developed by Gaur and Slinkard (1990a, b) using isozyme markers and interspecific crosses of *C. arietinum* with *C. reticulatum* and *C. echinospermum*. The DNA-based molecular markers, such as RAPDs and RFLPs, were later integrated to this map by Simon and Muehlbauer (1997). These initial studies used F<sub>2</sub> populations for the development of linkage maps. The first mapping population of recombinant inbred lines (RILs) was developed from the interspecific cross *C. arietinum* (ICC 4958) × *C. reticulatum* (PI 489777) and has been considered as the reference mapping population for genome mapping in chickpea. The genotyping of this mapping population gave the first large genetic map of chickpea consisting of 351 markers and covering a total distance of 2,077.9 cM (Winter et al. 2000). This map was further expanded by later studies using this reference mapping population. Nayak et al. (2010) developed a map with 521 markers and spanning 2602.1 cM. Thudi et al. (2011) developed a comprehensive genetic map including 1,291 markers and spanning 845.56 cM. Recently, Hiremath et al. (2011) developed a genetic map comprising 1,328 marker loci for this reference population. Several studies have been conducted on the development of genetic maps of chickpea based

on intraspecific mapping populations (Cho et al. 2002; Flandez-Galvez et al. 2003a, b; Cobos et al. 2005; Radhika et al. 2007; Anuradha et al. 2011; Garg Tosh 2012). Because of limited polymorphism in the cultivated chickpea, maps developed from intraspecific mapping populations had fewer markers (<250 markers) and less genome coverage (<800 cM). Consensus genetic maps using both interspecific and intraspecific populations were also developed. A consensus map based on five interspecific (*C. arietinum* × *C. reticulatum*) and five intraspecific populations was developed by Millán et al. (2010). This map had integrated 555 marker loci. BAC and binary bacterial artificial chromosome (BIBAC) libraries have been developed (Rajesh et al. 2004; Lichtenzweig et al. 2005; Zhang et al. 2010) and used in the development of a physical map of chickpea (Zhang et al. 2010). This physical map comprised 1,945 contigs, spanning about 1,088 Mb. Efforts have also been made to assign linkage groups to specific chromosomes using flow cytometry and PCR-based primers that amplify sequence-tagged microsatellite site markers (Tekeoglu et al. 2002; Zatloukalová et al. 2011). The linkage group 8 (LG8) was assigned to chromosome H, LG5 to chromosome A, LG4 to chromosome E and LG3 to chromosome B. In other cases the chromosomes could not be sorted out separately, so LG1 and LG2 were jointly assigned to chromosomes F and G, and LG6 and LG7 were jointly assigned to chromosomes C and D.

### 3.9.3 Molecular Mapping of Genes/Quantitative Trait Loci Controlling Agronomically Important Traits

Several RIL mapping populations have been developed in chickpea at ICRISAT (Gaur et al. 2014b) and several other institutes. Molecular markers have been identified for the genes/QTLs linked to resistance to several diseases, including *Fusarium* wilt (Sharma et al. 2004, 2005; Cobos et al. 2005; Gowda et al. 2009; Garg Tosh 2012; Sabbavarapu et al. 2013), *Ascochyta* blight (Millán et al. 2003; Rakshit et al. 2003; Collard

et al. 2003; Udupa and Baum 2003; Cho et al. 2004; Iruela et al. 2006; Lichitenzveiz et al. 2006; Anbessa et al. 2009; Kottapalli et al. 2009; Aryamanesh et al. 2010; Garg Tosh 2012; Sabbavarapu et al. 2013), *Botrytis* grey mould (Anuradha et al. 2011) and rust (Madrid et al. 2008); salinity tolerance (Vadez et al. 2012); traits related to drought tolerance (Chandra et al. 2004; Molina et al. 2008; Rehman 2009; Rehman et al. 2012; ICRISAT unpublished results), growth habit and podding (Rajesh et al. 2002; Radhika et al. 2007; Gowda et al. 2009; Aryamanesh et al. 2010; Garg Tosh et al. 2012); phenology (Aryamanesh et al. 2010; Rehman 2009; Rehman et al. 2012); and seed characteristics (Gowda et al. 2009). Several of these studies have been summarized in earlier reviews (Varshney et al. 2007; Upadhyaya et al. 2011; Gaur et al. 2012b, 2014b).

### 3.9.4 Marker-Assisted Breeding

Marker-assisted breeding can greatly improve the precision and efficiency of breeding programmes. Recent advances in the development of molecular markers and identification of molecular markers linked to genes/QTLs controlling traits of breeders' interest have encouraged applications of marker-assisted backcrossing (MABC) in chickpea improvement. A 'QTL-hotspot' containing QTLs for several root and drought tolerance traits was transferred from the drought-tolerant line ICC4958 to a leading *desi* chickpea cultivar JG 11 through MABC (Varshney et al. 2013b). Varshney et al. (2013b) reviewed the status of genomic resources available for chickpea improvement and suggested ways to go for genomic-assisted breeding in chickpea. Multilocation evaluations of introgression lines (ILs) in India, Ethiopia and Kenya led to the identification of lines with significantly higher yield than JG11 at each location and in each growing condition (rainfed/irrigated) (Gaur et al. 2013b). MABC is also being used for introgressing resistance to various diseases in chickpea. ICRISAT is pyramiding resistances to two races of *Fusarium* wilt (*foc1* and *foc3*) from WR315 and 2 QTLs for *Ascochyta* blight resistance from

ILC3279 line into C214. Marker-assisted recurrent selection (MARS) has also been initiated in chickpea for the improvement of yield, particularly under moisture stress conditions. Two good-by-good crosses (JG 11×ICCV 04112 and JG 130×ICCV 05107) are being used at ICRISAT to implement MARS (Gaur et al. 2014b). QTLs were identified specific to these crosses by genotyping in F<sub>3</sub> and phenotyping of F<sub>3:5</sub> progenies. A set of eight lines were selected for each cross using OptiMAS 1.0 to pyramid superior alleles of the favourable QTLs identified. Superior lines will be developed by accumulating favourable alleles through successive intercrossing using genotypic selection. In addition to MARS, genome-wide selection (GWS) or genomic selection (GS) has been proposed as a potential approach for improving complex traits governed by many genes/QTLs. In this approach, both phenotyping and genotyping data are used to predict genomic estimated breeding values (GEBVs) of progenies and superior progenies are selected based on GEBVs.

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### 3.10 Conclusions

The narrow genetic base of cultivated chickpea warrants systematic collection, documentation and evaluation of chickpea germplasm, particularly wild annual *Cicer* species for effective and efficient use in chickpea breeding programmes. Researchers have clearly demonstrated that desirable alien genes conferring resistance against biotic and abiotic stresses can be successfully introgressed from unexploited wild annual *Cicer* species to the cultivated chickpea. The valuable genetic resources present in the primary gene pool comprising progenitor species can be successfully utilized for genetic enhancement. However, most of the wild species possessing high degree of resistance to multiple stresses are present in the secondary and tertiary gene pools, where hybridization with cultivated species is often limited due to reproductive barriers. There are indications that useful traits which are not available in cultigens may be recovered in the segregating generation of crosses involving wild *Cicer* species. Further the molecular markers can be effectively used to

monitor alien gene introgression into the elite cultivars. Besides major genes, the transfer of agronomically important traits into elite cultivars has been made easy and practical through marker-assisted selection. Molecular markers such as SSR and SNP are useful for the construction of high-density genetic maps of chickpea which will be very useful to identify genes/QTLs for stress resistance, quality traits and other yield contributing characters.

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