4 Chickpea

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4.1 Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated true diploid ($2n=2x=16$) cool season leguminous crop that ranks second among food grain legumes in the world after common bean (FAOSTAT, 2011). It is grown in a wide range of environments in over 50 countries in subtropical and temperate regions of the world, mainly in the Indian subcontinent, West Asia, North Africa, the Americas and Australia (FAOSTAT, 2011). Based on seed shape, size and colour, two distinct forms of cultivated chickpea are known (Cubero, 1975); namely, the *desi* type, characterized mostly by pink flowers, angular. brown, small seeds with a high percentage of fibre, primarily grown in South Asia and Africa; and *kabuli* type, having white flowers and owl-head-shaped, beige, large seeds with a low percentage of fibre, grown in Mediterranean countries. A third type, designated as intermediate or pea-shaped, is characterized by medium to small size and round, pea-shaped seeds. Kabuli types are grown in about two-thirds of chickpea-growing countries, but desi type predominates in chickpea production and accounts for about 85%, while kabuli accounts for about 15% of the world chickpea production.

It is grown primarily for its protein-rich seeds. In addition, chickpea seeds are also rich in minerals (calcium, potassium, phosphorus, magnesium, iron and zinc), fibre, unsaturated fatty acids, and β-carotene (Jukanti, Gaur, Gowda, & Chibbar, 2012). Owing to its high nutritional qualities, chickpea is considered one of the most nutritious food grain legumes for human consumption, with potential health benefits. For example, high fibre content in chickpea has the ability to lower the cholesterol level as well as prevent blood sugar levels from rising too rapidly after a meal, thus making it a healthy food for diabetic patients (McIntosh & Miller, 2001; Pittaway et al., 2006). Further, chickpea does not contain any antinutritional factors except the raffinose-type oligosaccharides, which cause flatulence (Williams & Singh, 1987) and can be neutralized by boiling or mere soaking in water (Queiroz, de Oliveira, & Helbig, 2002). Chickpea plant is an efficient symbiotic nitrogen fixer, improving soil fertility by fixing atmospheric nitrogen, meeting up to 80% of its nitrogen requirement and playing an important role in crop diversification and sustainability of farming systems. However, chickpea is cultivated mostly in marginal lands under rain-fed conditions, with low and unstable productivity (Kumar & van

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Rheenen, 2000). Development of high-yielding, early-maturing cultivars that fit well into the short cropping season is one of the major objectives of chickpea improvement programmes. But the narrow genetic base of cultivated chickpea is one of the major obstacles to sustaining and improving its productivity and renders the crop vulnerable to new biotic and abiotic stresses. The narrow genetic base of chickpea is particularly due to the restricted distribution of its wild progenitor, *Cicer reticulatum*, the founder effect associated with domestication, the shift from winter to summer cropping and the replacement of locally adapted landraces by the genetically uniform modern varieties (Abbo, Berger, & Turner, 2003). Plant genetic resources comprising landraces, obsolete varieties and crop wild relatives are the reservoirs of natural genetic variations, but general reluctance of the breeders to use exotic germplasm has severely restricted the introgression of useful variation present in the exotic germplasm. This chapter will provide information about the nature and extent of chickpea genetic resources conserved across gene banks globally, the pattern of diversity in cultivated and wild *Cicer* species, and various approaches including genomic tools to promote utilization of genetic resources to broaden the genetic base for sustainable chickpea crop production.

4.2 Origin, Distribution, Diversity and Taxonomy

Chickpea is one of the earliest grain crops domesticated in the Old World at Tell el-Kerkh (tenth millennium BC) in Syria, Cayönü (7250–6750 BC), and Hacilar (ca 6700 BC) in Turkey, and Jericho (8350–7370 BC) in the West Bank. The earliest to date is Tell el-Kerkh, where both *Cicer arietinum* and its immediate progenitor *Cicer reticulatum* were clearly identified. Since Tell el-Kerkh is at a considerable distance from the native lands of the wild chickpea, *C. reticulatum* in southeast Turkey, it is suggested that the domestication took place somewhat earlier than that (Tanno & Willcox, 2006). However, the cultivation of chickpea is well documented from 3300 BC onwards in Egypt and the Middle East (van der Maesen, 1972). Most probably, it originated in an area of present-day southeastern Turkey and Syria, where three wild annual *Cicer* species are found, namely, *C. bijugum*, *C. echinospermum* and *C. reticulatum*, closely related to chickpea. From here, chickpea spread with human migration toward the West and South via the Silk Route (Singh et al., 1997). Four centres of diversity have been identified in the Mediterranean, Central Asia, the Near East and India, as well as a secondary centre of origin in Ethiopia (Vavilov, 1951).

Presently, *Cicer* species occur from sea level to over 5000 m near glaciers in the Himalayas. The cultivated species *C. arietinum* is found only in cultivation and cannot colonize successfully without human intervention. The wild *Cicer* species occur in weedy habitats (fallow or disturbed habitats, roadsides, cultivated fields of wheat, places not touched by man or cattle), mountain slopes among rubble and also naturally in inhospitable areas of the Himalayas in India (Chandel, 1984).

Globally, chickpea is grown on about 13.2 million hectare area with a production of 11.62 million metric tons and an average productivity of 880.4 kg/ha (FAOSTAT, 2011). The developing countries account for 90% of the global chickpea cultivation

and South and Southeast Asia (SSEA) contribute about 79% of the global chickpea production. India is the principal chickpea-producing country, with a 68% share in the global chickpea area and production. Other countries producing substantial amounts of chickpea include Australia, Pakistan, Turkey, Myanmar, Ethiopia, Iran, Mexico, Canada, USA, Morocco and Yemen (FAOSTAT, 2011). Chickpea is the only domesticated species under the genus *Cicer*, family Fabaceae and subfamily Papilionoideae. Earlier, the genus *Cicer* was classified in the tribe Vicieae Alef., which was later reported to belong to its own monogeneric tribe, Cicereae Alef. (Kupicha, 1981). The tribe Cicereae is closer to the tribe Trifolieae, which differs from the former in having hypogeal germination, tendrils, stipules free from the petiole, and nonpapillate unicellular hairs. The genus *Cicer* currently comprises 44 species, including 35 wild perennials, 8 wild annuals and the cultivated annual (Muehlbauer, 1993; van der Maesen, 1972) (Table 4.1). The infragenic classification of genus *Cicer* includes two subgenera: *Pseudononis* and *Viciastrum*, four sections, *Monocicer*, *Chamaecicer*, *Polycicer* and *Acanthocicer*, and 14 series (van der Maesen, 1987).

The subgenus *Pseudononis* is characterized by small flowers (normally 5–10 mm), subregular calyx, hardly gibbous base, with sublinear, nearly equal teeth. It comprises two sections, *Monocicer* (annuals, with firm erect or horizontal stems branched from the base or at middle) and *Chamaecicer* (annuals or perennials, with thin, creeping, branched stem, and small flowers). The section *Monocicer* is the most important section for chickpea improvement and includes eight annual species, namely *C. arietinum, C. reticulatum, C. echinospermum, C. judaicum, C. bijugum, C. pinnatifidum, C. cuneatum* and *C. yamashitae*. This section is further subdivided into three series, *Arietina* (characterized by imparipinnate leaves, with none to small arista), *Cirrhifera* (leaves ending in a tendril, with short arista) and *Macro-aristae* (leaves imparipinnate, long arista). The second section, *Chamaecicer*, includes one annual species, *C. chorassanicum,* and one perennial species, *C. incisum*, and is divided into two series, *Annua* and *Perennia* (Kazan & Muehlbauer, 1991; Muehlbauer, Kaiser, & Simon 1994).

The subgenus *Viciastrum* (perennials, characterized by medium large flowers, calyx strongly gibbous at the base, with unequal teeth) comprises two sections, *Polycicer* and *Acanthocicer*. *Polycicer* (leaf rachis ending in a tendril or a leaflet, never a spine) contains 23 perennial species and is divided into two subsections, *Nano-polycicer* (with creeping rhizome, short stem, imparipinnate leaves, weak and short arista) and *Macro-polycicer* (with short rhizome, non-creeping, stems ascending to 75 cm, firm arista longer than pedicel). *Macro-polycicer* is further divided into six series: (i) *Persica* (inflorescences 1–2 flowered, flowers 14–15 mm, calyx teeth 2–4 times the tube, stipules 14–15 mm, half as large as the leaflets, which are in 2–12 pairs); (ii) *Anatolo-persica* (inflorescences 1–2 flowered, flowers 20–27 mm, calyx teeth short, stipules smaller than the largest leaflets, which are in 4–9 pairs); (iii) *Europaeo-anatolica* (inflorescences 2–5 flowered, bracts foliolate, stipules small or up to half as large as the leaflets, which are in 4–8 pairs); (iv) *Flexuosa* (inflorescences 1–2 flowered, bracts minute, stipules much smaller than the leaflets, which are in 4–13 pairs); (v) *Songarica* (inflorescences 1–2 flowered, bracts minute,

Table 4.1 List of Various *Cicer* Species

S. No.	Species	S. No.	Species
Cultivated species			
1	*Cicer arietinum* (Chickpea)		
Annual wild *Cicer* species			
1	*Cicer reticulatum*	5	*Cicer pinnatifidum*
2	*Cicer echinospermum*	6	*Cicer chorassanicum*
3	*Cicer judaicum*	7	*Cicer cuneatum*
4	*Cicer bijugum*	8	*Cicer yamashitae*
Perennial wild *Cicer* species			
1	*Cicer acanthophyllum*	20	*Cicer macracanthum*
2	*Cicer anatolicum*	21	*Cicer microphyllum*
3	*Cicer atlanticum*	22	*Cicer mogolatvicum*
4	*Cicer balcaricum*	23	*Cicer montbretii*
5	*Cicer baldshuanicum*	24	*Cicer multijugum*
6	*Cicer canariense*	25	*Cicer nuristanicum*
7	*Cicer fedtschenkoi*	26	*Cicer oxyodon*
8	*Cicer flexuosum*	27	*Cicer paucijugum*
9	*Cicer floribundum*	28	*Cicer pungens*
10	*Cicer graecum*	29	*Cicer rassuloviae*
11	*Cicer grande*	30	*Cicer rechingeri*
12	*Cicer heterophyllum*	31	*Cicer songaricum*
13	*Cicer incanum*	32	*Cicer spiroceras*
14	*Cicer incisum*	33	*Cicer stapfianum*
15	*Cicer isauricum*	34	*Cicer subaphyllum*
16	*Cicer kermanense*	35	*Cicer tragacanthoides*
17	*Cicer korshinskyi*		*Cicer tragacanthoides* var. *tragacanthoides*
18	*Cicer laetum*		*Cicer tragacanthoides* var. *turcomanicum*
19	*Cicer luteum*		

stipules more or less equal to the largest leaflets, which are in 2–18 pairs) and (vi) *Microphylla* (inflorescences 1–2 flowered, bracts minute, stipules smaller than or equal to the largest leaflets, which are in 7–10 pairs). Section *Acanthocicer* (perennials, with branched stems with woody base, persistent spiny leaf rachis, spiny calyx teeth, and large flowers) encompasses nine perennial species and is divided into three series: *Pungentia* (foliate or small spiny stipules), *Macrocantha* (long spiny stipules) and *Tragacanthoidea* (small, triangular, incised stipules).

4.2.1 Gene Pool

In the genus *Cicer*, 43 wild species are classified into three gene pools based on their crossability status, with the cultivated chickpea following the Harlan and de Wet (1971) gene pool concept. The primary gene pool consists of cultivated chickpea, its landraces and the progenitor species, *C. reticulatum*, the species that are freely crossable with cultivated chickpea with regular gene exchange. The secondary gene pool

consists of *C. echinospermum*, a species that is crossable with cultivated chickpea, but with reduced fertility of the resulting hybrids and progenies. The tertiary gene pool consists of remaining six annual and 35 perennial species that are not readily crossable with cultivated chickpea and require specialized techniques for gene transfer into the cultivated background.

4.3 Erosion of Genetic Diversity from the Traditional Areas

The major factors responsible for genetic erosion include replacement of the traditional varieties, indigenous species and landraces with genetically uniform, high-yielding, modern cultivars resulting in loss of about three-quarters of the genetic diversity of agricultural crops, climate change posing serious threats on crop germplasm, intensive recent development activities, habitat destruction by modern agriculture and poor knowledge of germplasm and of its scientific, social, cultural and economic importance, resulting in the loss of this treasure. In most of the crops including chickpea, only a fraction of the diversity of wild species is stored in the existing collections. In gene banks also, many accessions have been lost because of improper storage, poor seed viability following introduction and short storage viability even in good facilities. Further, much of this diversity is threatened by decades of underfunding and neglect as well as by wars and natural disasters. In genus *Cicer* six species, namely *C. atlanticum*, *C. echinospermum*, *C. floribundum*, *C. graecum*, *C. isauricum* and *C. reticulatum,* were categorized as rare (R) and were included in the 1997 World Conservation Union (International Union for Conservation of Nature, IUCN) List of Threatened Plants (Walter & Gillett, 1998). The tertiary gene pool species, *C. bijugum,* has been considered a priority for collection. Due to the introduction of high-yielding varieties, a number of landraces carrying vast amount of genetic diversity are lost from farmers' fields in many countries (Berger, Abbo, & Turner, 2003). In Georgia, where chickpea is one of the traditional crops, local varieties are rarely cultivated today (Akhalkatsi, Ekhvaia, & Asanidze, 2012). Dekaprelevich and Menabde (1929) reported that three subspecies and 24 varieties were available in western Georgia – Racha-Lechkhumi, Svaneti and Imereti up to the 1920s, but in the 1970s the same three subspecies – *C. arietinum* subsp. *mediterraneum* G. Pop., *C. arietinum* subsp. *eurasiaticum* G. Pop., *C. arietinum* subsp. *orientalis* G. Pop. – and only 6 of 24 varieties – *C. arietinum* subsp. *mediterraneum* var. *ochroleucum* A. Kob., *C. arietinum* subsp. *mediterraneum* var. *rozeum* G. Pop., *C. arietinum* subsp. *eurasiaticum* var. *aurantiacum* G. Pop., *C. arietinum* subsp. *orientalis* var. *fulvum* G. Pop., *C. arietinum* subsp. *orientalis* var. *rufescens* G. Pop. and *C. arietinum* subsp. *orientalis* var. *rufescens brunneopunctatus* A. Kob. – were in cultivation (Kobakhidze, 1974). In Svaneti also, chickpea was traditionally available, but by the 1970s only one farmer was sowing it in the Kala community village Khe (Zhizhizlashvili & Berishvili, 1980). The genetic erosion of chickpea has also been noticed in the Mianwali district of Punjab along the Indus (Ahmad et al., 1984). Several *Cicer* species are found in eastern Anatolian deciduous forests in the centre of Southwest Asia (Turkey, Iran and Afghanistan), but the high level of habitat

conversion and low level of protection in this region is posing a major threat to the chickpea genetic diversity and has warranted considerable conservation concerns in recent years (Stolton, Maxted, Ford-Lloyd, Kell, & Dudley, 2006).

4.4 Status of Germplasm Resources Conservation

Large-scale collection and conservation efforts have been initiated to protect the crop biodiversity, and *ex situ* gene banks have been established by the Food and Agriculture Organization (FAO) and the World Bank for the collection and conservation of plant genetic resources. Globally, about 7.4 million germplasm accessions of different crops have been collected and/or assembled and conserved in over 1750 gene banks (FAOSTAT, 2010). For chickpea, there are a large number of gene banks conserving over 98,000 germplasm accessions comprising of landraces, modern cultivars, genetic stocks, mutants and wild *Cicer* species (http://apps3.fao.org/wiews/germplasm_query.htm?i_l=EN). The major gene banks holding chickpea germplasm are given in Table 4.2. The RS Paroda gene bank at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has the largest collection: 19,959 accessions of cultivated chickpea and 308 accessions of 18 wild *Cicer* species from 60 countries. These accessions were obtained from donations as well as from collection missions in different countries. Other major gene banks holding chickpea germplasm include the National Bureau of Plant Genetic Resources (NBPGR) (16,881 accessions), New Delhi, India; the International Centre for Agricultural Research in Dry Areas (ICARDA) (13,818 accessions), Aleppo, Syria; Australian Temperate Field Crops Collection (ATFCC) (8655 accessions), Horsham, Victoria; and Western Regional Plant Introduction Station (WRPIS), United States Department of Agriculture - Agricultural Research Service (USDA-ARS) (6789 accessions), Pullman (Table 4.2). Besides conserving germplasm accessions in these gene banks, duplication agreements have been negotiated for safety between gene banks within and outside the Consultative Group on International Agricultural Research (CGIAR) system for a majority of crops. At the global level, the Svalbard Global Seed Vault will definitely contribute to combating the loss of biological diversity, reducing vulnerability to climate change and securing future food production.

4.5 Germplasm Evaluation and Maintenance

The characterization, evaluation and maintenance of germplasm are essential for their effective utilization in crop improvement programmes and for efficient management of genetic resources. At ICRISAT chickpea germplasm accessions have been characterized and evaluated for various morpho-agronomic traits following the Chickpea Descriptors (IBPGR, ICRISAT, & ICARDA, 1993) since 1974. A multidisciplinary approach is followed for the characterization and evaluation of chickpea germplasm for various biotic and abiotic stresses and for agronomic and nutrition-related traits. Besides, germplasm sets are also evaluated jointly with National Agricultural Research Systems (NARS) scientists in different countries and more intensively with the

Table 4.2 Major Holdings of Chickpea Germplasm in Different Gene Banks of the World

Country	Institute	Wild Accessions	Wild Species	Cultivated Accessions	Total
Australia	Australian Temperate Field Crops Collection (ATFCC), Horsham, Victoria	246	18	8409	8655
Ethiopia	Institute of Biodiversity Conservation (IBC), Addis Ababa			1173	1173
Hungary	Institute for Agrobotany (RCA), Tápiószele	9	5	1161	1170
India	Indian Agricultural Research Institute (IARI), New Delhi			2000	2000
	International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru	308	18	19,959	20,267
	National Bureau of Plant Genetic Resources (NBPGR), New Delhi	69	10	16,812	16,881
Iran	College of Agriculture, Tehran University, Karaj			1200	1200
	National Plant Gene Bank of Iran, Seed and Plant Improvement Institute (NPGBI-SPII), Karaj			5700	5700
Mexico	Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (IA-Iguala), Iguala			1600	1600
Pakistan	Plant Genetic Resources Institute (PGRP), Islamabad	89	3 (1)	2057	2146
Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR), St. Petersburg			2091	2091
Syria	International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo	270	11 (1)	13,548	13,818
Turkey	Plant Genetic Resources Department, Aegean Agricultural Research Institute (AARI), Izmir	21	4	2054	2075
Ukraine	Institute of Plant Production n.a. V.Y. Yurjev of UAAS, Kharkiv			1021	1021

(*Continued*)

Table 4.2 (Continued)

Country	Institute	Wild Accessions	Wild Species	Cultivated Accessions	Total
USA	Western Regional Plant Introduction Station, USDA-ARS, Pullman	205	22	6584	6789
Uzbekistan	Uzbek Research Institute of Plant Industry (UzRIPI), Botanica			1055	1055

Source: http://apps3.fao.org/wiews/germplasm_query.htm?i_l=EN.

NBPGR, New Delhi. About 99% of chickpea germplasm accessions have been characterized for agronomic and morphological traits at ICRISAT. Chickpea has orthodox seeds that can be dried to low seed moisture content (about 5–7%) for efficient conservation. For conservation of germplasm, a two-tier system is being followed in the ICRISAT gene bank. Seeds are dried in cool and dry conditions to reduce the moisture content to a desired level (5%±1%) and then stored as active collections in medium-term storage (at 4°C, 20–30% relative humidity) in aluminium cans and as base collection in long-term storage (at −20°C) after packing in vacuum-sealed aluminium foil pouches. The entire chickpea collection consisting of 20,267 accessions is stored as active and base collection in the ICRISAT gene bank. A recent monitoring of the health of seed conserved for 10–25 years under medium-term storage has indicated greater than 85% seed viability for the majority of the accessions. Regeneration is one of the most important gene-bank activities, which aims at seed multiplication by maintaining the genetic integrity of the original sample. Accessions with declining seed viability (less than 75% seed germination) and/or quantity (<100 g) have high priority for regeneration. Further, the regeneration of accessions that have low viability is given the highest priority over accessions with low seed quantity. Besides, special requirements for seed multiplication may arise for accessions requiring safety duplication and repatriation. Breeding behaviour of the crop and the sample size are the two key factors affecting efficient regeneration. Since chickpea is a self-pollinated crop, regeneration is carried out in field without any control on pollination by using at least 80 plants for regenerating an accession. Regeneration of cultivated types is carried out in solarized fields during the post-rainy season. Solarization is the process of heating soil by covering it with polyethylene sheets during hot summer to control soilborne diseases like *Fusarium* wilt that represent a major limitation on chickpea growth during regeneration. Solarization is conducted for at least 6 weeks during the hottest part of the year. However, critical accessions of wild *Cicer* species that need long day length and cool weather to grow and produce seeds are regenerated under controlled greenhouse conditions (Figure 4.1). Newly acquired germplasm of foreign origin is first grown in the post-entry quarantine isolation area under the supervision of the National Plant Quarantine Services. Recently, the management practices of different gene banks were reviewed to develop the best practices and procedures for chickpea germplasm management (Upadhyaya et al., 2009; http://cropgenebank.sgrp.cgiar.org/).

Figure 4.1 Regeneration of wild *Cicer* species under controlled environmental conditions in the greenhouse at ICRISAT, Patancheru, India.

4.6 Use of Germplasm in Crop Improvement

4.6.1 Status of Germplasm in Chickpea Improvement

Since 1974, the ICRISAT gene bank has distributed about 321,251 chickpea seed samples to researchers in 88 countries. The evaluation of chickpea germplasm by national programmes has led to the release of 17 accessions directly as varieties in 15 countries. Studies have shown scanty use of germplasm (<1%) in chickpea improvement programmes. India has one of the largest chickpea improvement programmes and has released 126 chickpea cultivars in the past four decades. Surprisingly, 41% of cultivars have Pb 7 as one of the parents, with IP 58, F 8, S 26 and Rabat being the most extensively used parents (Kumar, Gupta, Chandra, & Singh, 2004). However, ICRISAT, has the largest chickpea germplasm collections; our chickpea breeding programme has used 12,887 (586 unique) parents including only 91 germplasm lines to develop the 3,548 advanced breeding lines; L 550 and K 850 being the most frequently used cultivars (Upadhyaya, Gowda, Buhariwalla, & Crouch, 2006). This shows the breeders' preference for selecting parental genotypes from their working collections. Working collections usually exhibit good agronomic performance and provide a quick way for the breeders to make steady progress in the shortest possible time. Further, the chances of diluting the agronomic performance

become higher with the involvement of new germplasm lines (Kannenberg & Falk, 1995). Thus, the use of parental genotypes from working collections results in recirculation of the same germplasm, hence the narrow genetic base of the cultivars. This results in genetic vulnerability, which has already caused havoc in the past, such as the southern corn leaf blight epidemic in United States of America during 1969–1970, due to the large-scale use of genetically uniform male sterile lines.

4.6.2 Small Subsets for Enhancing the Utilization of Germplasm

Frankel and Brown (1984) suggested that greater use of germplasm in crop improvement is possible if a small collection representing the diversity of the entire large collection is made available to researchers for meaningful evaluation and utilization. Frankel (1984) coined the term "core collection" to sample representative variability from the entire collection. A core collection contains 10% of the accessions from the entire collection that capture most of the available diversity in the species (Brown, 1989a). Thus, a core collection has a reduced size containing a diverse set of germplasm and is representative of the entire collection. Such core collections can be evaluated extensively and the information derived could be used to guide the more efficient utilization of the entire collection (Brown, 1989b).

4.6.2.1 Core Collection

Using passport information and characterization and evaluation data generated over a period of time, a chickpea core collection consisting of 1956 accessions has been developed from the global collection of 16,991 accessions from 44 countries at ICRISAT (Upadhyaya, Bramel, & Singh, 2001). Similarly, a core collection of 505 accessions was developed from 3350 chickpea accessions by the scientists at the USDA in Pullman, Washington (Hannan, Kaiser, & Muehlbauer, 1994). A kabuli chickpea core collection consisting of 103 accessions has been developed at the Seed and Plant Improvement Institute (SPII), Karaj, Iran (Pouresmael, Akbari, Vaezi, & Shahmoradi, 2009). Recently, a core collection consisting of 158 germplasm accessions has been developed for the Ethiopian chickpea germplasm collection at ICRISAT (Kibret, 2011) (Table 4.3).

4.6.2.2 Mini-Core Collection

The germplasm collections at the International Agricultural Research Center (IARC) gene banks are very large in size such as the International Maize and Wheat Improvement Center (CIMMYT) gene bank holding more than 100,000 wheat accessions and the International Rice Research Institute (IRRI) gene bank with over 110,000 rice accessions; hence, the core collections with about 10,000 accessions could be unmanageably large and unwieldy, which would restrict its proper evaluation and use by crop breeders. Even at ICRISAT, the chickpea core collection of 1956 accessions is too large for its meaningful multilocation evaluation. This forced the scientists to develop a new strategy to further reduce the size of the core collection without losing the spectrum of diversity. Upadhyaya and Ortiz (2001) postulated

Table 4.3 Small-Sized Subsets for Chickpea Germplasm

Crop	Accessions	Subset Developed	Accessions in Subset	Reference
Chickpea	16,991	Core collection	1956	Upadhyaya et al. (2001)
	3350	Core collection	505	Hannan et al. (1994)
	1002	Core collection	158	Kibret (2011)
	N/A	Kabuli chickpea core collection	103	Pouresmael et al. (2009)
	1956	Mini-core collection	211	Upadhyaya and Ortiz (2001)
	482	Mini-core collection	39	Biabani et al. (2011)
	N/A	Composite collection	3000	Upadhyaya et al. (2006)
	3000	Reference set	300	Upadhyaya et al. (2008a)

the mini-core concept following a seminal two-stage strategy for sampling the entire and core collections to develop a mini-core collection, which consists of roughly 10% of the accessions of the core collection (about 1% of the entire collection) representing the diversity of the entire collection with minimum loss of diversity. They suggested using the core collection as a basis for developing a mini-core collection. The first stage in constituting a mini-core collection thus involves developing a representative core collection (about 10%) from the entire collection using the available information on origin, geographical distribution, characterization and evaluation data. The second stage involves evaluation of the core collection for various morphological, agronomic and grain quality traits, and selecting a further set of about 10% accessions from the core collection. At both the stages, standard clustering procedures are used to create groups of similar accessions and various statistical tests are used to evaluate and validate core and mini-core collections. Following this strategy, a mini-core collection was constituted in chickpea (Upadhyaya & Ortiz, 2001), which consists of 211 accessions representing the diversity of over 16,000 accessions (Table 4.3). Validation studies of this mini-core collection with the core collection and of the core collection with the entire collection revealed that the mini-core and core collections represented adequate diversity for most of the traits detected in the entire collection and will improve the efficiency of identifying valuable genes in the entire large collections for their effective utilization in chickpea improvement programmes. Another chickpea mini-core collection consisting of 39 accessions has been developed at the WRPIS at Pullman, Washington, USA (Biabani et al., 2011).

4.6.2.3 Composite Collection and Reference Set

Large collections of chickpea germplasm are maintained by ICRISAT, India and ICARDA, Syria (Table 4.2). As a part of the Generation Challenge Programme

Table 4.4 Composition of Global Composite Collections of Chickpea Germplasm

Germplasm/Traits	No. of Accessions
Accessions from ICRISAT	
Core collection	1956
Cultivars/breeding lines	39
Ascochyta blight	13
Botrytis gray mold	8
Stunt	8
Fusarium wilt	50
Collar rot	9
Black root rot	8
Dry root rot	6
Helicoverpa	16
Leaf miner	5
Nematode	8
Low temperature	12
High temperature	4
Drought	10
Salinity	4
Early maturity	25
High protein	10
Multiseeded pods	7
Seed size	18
High-input responsive	4
Twin pods	8
Nodulation	8
Morphological diversity	35
Accessions from ICARDA	
Based on characterization and evaluation data	599
Based on agro-climatological data	110
Cicer echinospermum	7 (1 from ICRISAT)
Cicer reticulatum	13 (2 from ICRISAT)

(GCP; http://www.generationcp.org), ICRISAT and ICARDA jointly developed a global composite collection of 3000 accessions to capture the global diversity available in these two gene banks and other materials such as released cultivars, sources of resistance/tolerance to various biotic/abiotic stresses including wild species (Tables 4.3 and 4.4) (Upadhyaya et al., 2006). The composite collection, which includes core and mini-core collections (Table 4.4), was molecularly profiled using 48 Simple Sequence Repeat (SSR) markers to study its genetic structure. A total of 1683 alleles were detected, of which 935 were rare, 720 common and 28 most frequent. The alleles per locus ranged from 14 to 67 and averaged 35; the polymorphic information content was from 0.467 to 0.974, averaging 0.854; and the gene diversity ranged from 0.533 to 0.974 with an average of 0.869. Kabuli chickpea as a group were genetically more diverse than other seed types. Desi and kabuli shared

436 alleles, while wild *Cicer* shared 17 and 16 alleles with desi and kabuli types, respectively. Desi chickpea contained a higher proportion of rare alleles (53%) than kabuli (46%), while wild *Cicer* accessions were devoid of rare alleles. Several group-specific unique alleles were also detected as 104 in kabuli, 297 in desi, and 69 in wild *Cicer*. Geographically, 114 unique alleles were found each in West Asia (WA) and Mediterranean, 117 in SSEA, and 10 in African accessions. The accessions from SSEA and WA shared 74 alleles, while those from Mediterranean shared 38 and 33 alleles with WA and SSEA, respectively (Upadhyaya et al., 2008a). The composite collection was also characterized for qualitative and quantitative traits at ICRISAT. A reference set consisting of the 300 genetically most diverse accessions was selected based on SSR markers, qualitative and quantitative traits, and their combinations. The reference set based on 48 SSR markers (78.1% alleles) was similar to the reference set based on seven qualitative traits (73.5%), whereas the reference set based on both captured 80.5% of the alleles of the composite collection (1683 alleles) (Upadhyaya et al., 2008b). This demonstrated that both SSR markers and qualitative traits were equally effective in sampling allelic diversity.

4.6.3 Trait-Specific Germplasm for Use in Chickpea Improvement

Evaluation of germplasm accessions, especially the small subsets, has resulted in the identification of new sources of resistance/tolerance to important biotic/abiotic stresses as well as promising accessions for important agronomic traits as follows.

4.6.3.1 Biotic Stresses

Resistance to Diseases

Evaluation of the chickpea mini-core collection resulted in the identification of three accessions (ICC 1915, ICC 6306 and ICC 11284) moderately resistant to *Ascochyta* blight, 55 accessions (ICC 1180, ICC 2990, ICC 4533, ICC 4841, ICC 4872 and others) to *Botrytis* gray mold, six accessions (ICC 1710, ICC 2242, ICC 2277, ICC 11764, ICC 12328 and ICC 13441) to dry root rot, 21 asymptomatic (ICC 637, ICC 1205, ICC 1356, ICC 1396, ICC 2065 and others) and 24 resistant (ICC 67, ICC 95, ICC 791, ICC 867, ICC 1164 and others) to *Fusarium* wilt (Pande, Kishore, Upadhyaya, & Rao, 2006). Combined resistance to *Ascochyta* blight and *Botrytis* gray mold was identified only in one accession, ICC 11284; for *Botrytis* gray mold and dry root rot in two accessions (ICC 11764 and ICC 12328); for *Botrytis* gray mold and *Fusarium* wilt in 11 accessions (ICC 2990, ICC 4533, ICC 6279, ICC 7554, ICC 7819 and others); and for dry root rot and *Fusarium* wilt in four accessions (ICC 1710, ICC 2242, ICC 2277 and ICC 13441) (Pande et al., 2006).

Resistance to Insect Pests

The chickpea mini-core collection was evaluated for pod borer (*Helicoverpa armigera* L.) resistance. Five accessions (ICC 5878, ICC 6877, ICC 11764, ICC 16903 and ICC 18983) had very low leaf-feeding score under detached leaf assay screening; five accessions (ICC 12537, ICC 9590, ICC 7819, ICC 2482 and ICC 4533)

had least larval survival rate and five accessions (ICC 16903, ICC 6877, ICC 3946, ICC 11746 and ICC 18983) were identified as the best accessions for lower larvae weight, when compared to resistant control cultivar ICC 506-EB (ICRISAT Archival Report, 2009). Similarly, evaluation of the chickpea reference set consisting of 300 accessions identified 13 accessions (ICC 1230, ICC 2263, ICC 3325, ICC 4567, ICC 5135, ICC 6874, ICC 10466, ICC 11198, ICC 12307, ICC 14831, ICC 15406, ICC 15606 and ICC 16524) with low *H. armigera* damage and plant mortality, which also exhibited high yield potential under unprotected conditions (ICRISAT Archival Report, 2010). Further, one mini-core accession, ICC 4969, has been identified as a resistant source for pulse beetle (*Callosobruchus maculatus* F.) in both free-choice and no-choice tests (Erler, Ceylan, Erdemir, & Toker, 2009).

4.6.3.2 Abiotic Stresses

Drought

Drought stress, especially terminal drought stress, is one of the major adverse factors affecting chickpea production. The importance of an extensive and deep root system is well recognized as a means to improve drought tolerance and hence crop productivity through enhanced water uptake. Evaluation of chickpea mini-core accessions for the root traits using a cylinder culture system revealed a large genetic variability among accessions and identified two accessions (ICC 8261 and ICC 10885) with high root length density (RLD), six accessions (ICC 13124, ICC 14506, ICCV 2, ICC 8261, ICC 15333, ICC 7315) with large shoot to root length density ratio (S/RLD) and several accessions having a deep root system in comparison to the then-known most drought-tolerant accession, ICC 4958. A kabuli type landrace ICC 8261, from Turkey, had the most prolific root system, the largest RLD, as well as larger biomass allocation into the root system, which could be of high importance under severe drought conditions (Kashiwagi et al., 2005). Similarly, evaluation of 50 large-seeded kabuli germplasm accessions with four control cultivars (KAK 2, JGK 1, ICCV 2 and ICC 4958) for drought-avoidance root traits identified one accession, ICC 17450 (EC 543583) with larger RLD than ICC 4958, which could be utilized for a larger-seeded kabuli chickpea improvement programme (Kashiwagi, Upadhyaya, Krishnamurthy, & Singh, 2007). Kashiwagi, Krishnamurthy, Upadhyaya, and Gaur (2008) also used canopy temperature as a simple screening method to screen for drought tolerance and identified ICC 14799 as having the highest relatively cool canopy temperature, followed by ICC 867, ICC 3325 and ICC 4958. Similarly, evaluation of 289 chickpea accessions for drought tolerance has identified several promising accessions (ICC 2580, ICC 7272, ICCV 92311, ICC 3362, ICCV 95311, ICC 506 and EC 583311) with high grain yield, high harvest index (HI) and/or pest resistance and was to be evaluated further in multilocation trails (Mulwa, Kimurto, & Towett, 2010). Following field screening techniques, the chickpea mini-core germplasm accession ICC 13124 had the highest drought tolerance efficiency, least drought susceptibility index, the highest HI and minimum reduction in seed yield under drought, and was identified as the most drought-tolerant accession for moisture stress conditions (Parameshwarappa & Salimath, 2008; Parameshwarappa et al., 2010). Similarly,

evaluation of the chickpea mini-core for drought tolerance index over 3 years identified five accessions (ICC 867, ICC 1923, ICC 9586, ICC 12947 and ICC 14778) as highly drought tolerant (Krishnamurthy, Kashiwagi, Gaur, Upadhyaya, & Vadez, 2010). Of these five accessions, ICC 867 and ICC 14778 have also been found to maintain the coolest canopy temperatures (Kashiwagi et al., 2008).

Water Use Efficiency

The soil plant analysis development chlorophyll meter reading (SCMR) has been recognized as a useful measure to estimate leaf chlorophyll content for the plant's nitrogen acquisition capability and is a surrogate trait for selecting genotypes with improved nitrogen status leading to improved yield. Kashiwagi, Krishnamurthy, Singh, and Upadhyaya (2006) evaluated the chickpea mini-core collection and identified two accessions, ICC 16374 and ICC 4958, with high and stable SCMR values. Similarly, based on transpiration efficiency (TE) and carbon isotope discrimination ($\delta^{13}C$), promising accessions were identified such as ICC 5337 and ICC 4958 are having high $\delta^{13}C$ under stress condition, and ICC 5337 having the highest TE under stress and well-watered conditions. Later, evaluation of the chickpea mini-core collection for SCMR identified ICC 4958 as having the best SCMR performance. The same genotype, ICC 4958, has also been identified to possess the most prolific and deep root systems as well as the largest relatively cool canopy temperature (Kashiwagi et al., 2008), which makes it a unique breeding material for improving the acquisition of both soil water and soil nitrogen. Additional accessions with high SCMR values, such as ICC 1422, ICC 10945, ICC 16374 and ICC 16903, were also identified (Kashiwagi, Upadhyaya, & Krishnamurthy, 2010).

Salinity

Two hundred and eleven chickpea mini-core germplasm accessions and 41 popular varieties and breeding lines were evaluated under saline conditions (100 mM NaCl; pot screening) and 10 highly tolerant accessions (ICC 10755, ICC 13124, ICC 13357, ICC 15406, ICC 15697 and others) were identified (Serraj, Krishnamurthy, & Upadhyaya, 2004). Similarly, 263 chickpea accessions comprising 211 mini-core accessions and some lines reported as tolerant to sodicity, popular cultivars and breeding lines, and one cultivar released by the Central Soil Salinity Research Institute (CSSRI) for salinity tolerance (CSG 8962) were evaluated under saline conditions (80 mM NaCl; pot screening) to identify salinity-tolerant chickpea genotypes based on their seed yield under salinity (Vadez et al., 2007). Sixteen salinity-tolerant accessions yielding more than the previously identified salt-tolerant genotype CSG 8962 were identified. Of these, three accessions, ICC 5003, ICC 15610 and ICC 1431, had about 20% higher yield than the tolerant control, CSG 8962. Vadez et al. (2007) also reported that the desi genotypes had more salinity tolerance than the kabuli genotypes. Recently, Krishnamurthy, Turner, et al. (2011) also evaluated chickpea germplasm accessions including 211 mini-core accessions for salinity tolerance and identified 12 accessions (ICC 9942, ICC 6279, ICC 11121, ICC 456, ICC 12155 and others), which were highly tolerant in both a Vertisol and an Alfisol soil. Of these, one accession, ICC 9942, had the highest and most consistent seed yield performance in both soil types.

Heat Tolerance

Evaluation of 35 chickpea germplasm accessions selected from the core collection along with a control cultivar, ICCV 92944, for tolerance to heat stress identified ICC 14346 as the most heat-tolerant germplasm accession, followed by ICC 5597, ICC 5829, ICC 6121, ICC 7410, ICC 111916, ICC 13124, ICC 14284, ICC 14368 and ICC 14653. These accessions were consistently high yielding (>1400 kg/ha) as compared with the control, ICCV 92944 (1333 kg/ha) (Upadhyaya, Dronavalli, Gowda, & Singh, 2011). Similarly, Krishnamurthy, Gaur, et al. (2011) evaluated the chickpea reference set collection for heat tolerance at two locations (Patancheru and Kanpur) in India and identified 18 stable heat-tolerant accessions (ICC 456, ICC 637, ICC 1205, ICC 3362, ICC 3761 and others).

4.6.3.3 Agronomic Traits

Early Maturity

Chickpea breeding programmes aim at developing early-maturing cultivars especially to increase crop adaptation by avoiding terminal drought and high temperature stress in the sub-tropics. Twenty-eight early-maturing chickpea germplasm accessions (ICC 16641, ICC 16644, IC 11040, ICC 11180, ICC 12424 and others), which were similar or earlier than control cultivars Harigantars and ICCV 2 and produced about 23% more seed yield as compared to the average of four control cultivars (ICCV 2, Harigantars, ICCV 96029 and Annigeri) have been identified (Upadhyaya, Dwivedi, Gowda, & Singh, 2007).

Large Seed Size

In chickpea, seed size and colour are important traits for trade purposes. Large-seeded kabuli cultivars with a 100-seed weight of >40 g have higher consumer preference and fetch about three times higher price in the market. Evaluation of 65 large-seeded kabuli germplasm lines in three sets and across environments identified the six best large-seeded kabuli chickpea genotypes in three sets having high stability. One accession, ICC 14190, a *Fusarium* wilt–resistant large-seeded (37.4 g 100-seed weight) landrace from India, ranked first with average yield of 1430 kg/ha and high productivity (13.64 kg/ha/day). Three accessions, ICC 14194, ICC 7344 and ICC 7345, were early-flowering, extra-large-seeded types (48.2–54.1 g 100-seed weight), with grain yields similar to the best control, L 550. The other two superior lines were ICC 17452 (54.0 g 100-seed weight) and ICC 19189 (50.7 g 100-seed weight), both early-flowering, extra-large-seeded types with grain yield similar to the control KAK 2. All these accessions exhibited high stability with regression value near unity and deviation near zero (Gowda, Upadhyaya, Dronavalli, & Singh, 2011). Kaul, Kumar, and Gurha (2007) evaluated 150 kabuli chickpea germplasm accessions belonging to diverse geographical regions for phenological and morpho-agronomic traits at Kanpur, India, and identified four large-seeded kabuli accessions, ICC 12033, ICC 14199, ICC 14197 and ICC 14203 (46.2–60.2 g 100-seed weight and originating from Mexico) having high yield potential of >18 q/ha. In a similar study, nine large-seeded accessions (ICC 7345, ICC 11883, ICC 17450, ICC 17452,

ICC 17456, ICC 17457, ICC 18591, ICC 19189 and ICC 19195) having 100-seed weight ranging from 50.0 to 61.6 g and high yield (1154.4–1708.3 kg/ha) comparable to the control cultivar, KAK 2 (35.4 g 100-seed weight and 1359.5 kg/ha yield) have been identified for their use in developing new large-seeded kabuli cultivars with a broad genetic base (Kashiwagi et al., 2007).

Yield and Component Traits

Evaluation of the chickpea core collection for 14 agronomic traits identified 39 accessions (19 desi, 15 kabuli and 5 intermediate) performing better for a combination of agronomic traits such as early maturity, seed size and grain yield (Upadhyaya et al., 2007). The most desirable accessions having high seed yield and greater 100-seed weight than controls are ICC 1836 among the desi type and ICC 5644, ICC 7200, ICC 8042, ICC 10783 and ICC 11904 among the kabuli type; for early maturity and greater 100-seed weight than controls are ICC 6122, ICC 8474 and ICC 12197 in desi, ICC 8155, ICC 12034, ICC 14190 and ICC 14203 among kabuli type, and ICC 4871 among intermediate type (Upadhyaya et al., 2007). These accessions represent new and diverse sources of germplasm for use in breeding programmes to develop new chickpea cultivars. Meena et al. (2010) identified six promising and diverse accessions, ICC 14778, ICC 6279, ICC 4567, ICC 4533, ICC 1397 and ICC 12328, for more than one trait for use in chickpea improvement. Further, evaluation of the chickpea mini-core collection under three environments identified one accession, ICC 13124, promising for earliness, large seed size, and high yield per plant in all the three environments, and concluded that this accession is best suited for cultivation under both rain-fed and irrigated conditions during the post-rainy season (Parameshwarappa, Salimath, Upadhyaya, Patil, & Kajjidoni, 2011).

4.7 Limitations in Germplasm Use

Although plant breeders recognize the limitations of working with collections and the importance of crop genetic resources, yet they are often reluctant to use these resources for several reasons. The main reason for the low utilization of germplasm in crop improvement programmes is the lack of information on the large number of accessions, particularly for traits of economic importance such as yield, stable resistance/tolerance to biotic/abiotic stresses and nutrition-related traits, which often show high genotype × environment interactions and require replicated multilocational evaluation. However, the large size of germplasm collections makes it a costly and resource-demanding task. Another major reason for the low use of germplasm is the apprehensions among breeders about poor adaptability of germplasm and a linkage load of many undesirable genes associated especially with utilizing exotic germplasm and wild relatives in crop improvement programmes. While using unknown and wild germplasm, comparatively more effort and time is needed to generate breeding materials. Further, inadequate linkages between gene banks and germplasm users, lack of an informative and user-friendly gene bank database management system, restricted access to germplasm collections due to limited seed availability and

regulations governing germplasm exchange are the important factors responsible for the low use of germplasm in chickpea improvement programmes.

4.8 Germplasm Enhancement Through Wide Crosses

The narrow genetic base of cultivated chickpea is one of the major limitations in improving chickpea production and productivity. Further, the global production is affected drastically by several biotic and abiotic constraints. Limited genetic variation present in the cultivated type of chickpea germplasm necessitates the utilization of wild *Cicer* species for germplasm enhancement. Wild *Cicer* species have been extensively screened and several of them have been reported to have very high levels of resistance/tolerance to many biotic and abiotic stresses, which includes resistance to *Ascochyta* blight (Collard, Ades, Pang, Brouwer, & Taylor, 2001; Croser, Ahmad, Clarke, & Siddique, 2003; Pande, Ramgopal, et al., 2006; Rao, Reddy, & Bramel, 2003; Singh, Hawtin, Nene, & Reddy, 1981; Singh & Reddy, 1993; Stamigna, Crino, & Saccardo, 2000), *Botrytis* gray mold (Pande, Ramgopal, et al., 2006; Rao et al., 2003; Stevenson & Haware, 1999), *Fusarium* wilt (Croser et al., 2003; Infantino, Porta-Puglia, & Singh, 1996; Rao et al., 2003), *Helicoverpa* pod borer (Sharma, Chen, & Muehlbauer, 2005), drought (Croser et al., 2003; Kashiwagi et al., 2005; Toker, Canci, & Yildirim, 2007), cold (Berger et al., 2012; Croser et al., 2003; Singh, Malhotra, & Saxena, 1990; Singh, Malhotra, & Saxena, 1995; Toker, 2005) and drought and heat (Canci and Toker, 2009). Besides resistant/tolerant sources, wild *Cicer* species harbour beneficial alleles/genes for high seed protein (Rao et al., 2003; Singh & Pundir, 1991) and improvement of agronomic traits in cultivated chickpea. Keeping in view the importance of wild *Cicer* species, most of the chickpea improvement programmes emphasize utilizing wild species to develop new cultivars with a broad genetic base. Of the eight annual wild *Cicer* species, only *C. reticulatum* is readily crossable with cultivated chickpea resulting in a fertile hybrid, whereas for exploitation of the remaining seven annual wild *Cicer* species for chickpea improvement, specialized techniques such as application of growth hormones, embryo rescue, ovule culture and other tissue culture techniques have been suggested by various researchers (Badami, Mallikarjuna, & Moss, 1997; Lulsdorf, Mallikarjuna, Clarke, & Tar'an, 2005; Mallikarjuna, 1999; Mallikarjuna & Jadhav, 2008). Utilization of the *C. reticulatum* accession ILWC 119 in a crossing programme has resulted in the development of two cyst–nematode-resistant chickpea germplasm lines: ILC 10765 and ILC 10766 (Malhotra, Singh, Vito, Greco, & Saxena, 2002). Promising high-yielding lines with good agronomic and seed traits, such as early flowering and high 100-seed weight, have also been obtained from crosses involving *C. reticulatum* and *C. echinospermum* with cultivated chickpea (Jaiswal, Singh, Singh, & Singh, 1986; Malhotra et al., 2003; Singh, Gumber, Joshi, & Singh, 2005; Singh, Jaiswal, Singh, & Singh, 1984; Singh & Ocampo, 1997; Upadhyaya, 2008). High-yielding cold-tolerant lines with high biomass have been obtained from *C. arietinum* × *C. echinospermum* crosses (ICARDA, 1995). Using various techniques, interspecific hybrids have been produced between *C. arietinum*

and *C. judaicum* (Singh, Singh, Asthana, & Singh, 1999; Verma, Ravi, & Sandhu, 1995; Verma, Sandhu, Rrar, & Brar, 1990), *C. arietinum* × *C. pinnatifidum* (Badami et al., 1997; Mallikarjuna, 1999; Mallikarjuna & Jadhav, 2008; Verma et al., 1990), *C. arietinum* × *C. cuneatum* (Singh & Singh, 1989), and *C. arietinum* × *C. bijugum* (Singh et al., 1999; Verma et al., 1990) to exploit the possibility of introgression of desirable alien genes from these wild *Cicer* species into the cultivated chickpea. These interspecific hybrids have contributed significantly towards the development of genomic resources for chickpea improvement. From *C. arietinum* × *C. judaicum* cross, a pre-breeding line IPC 71 having a high number of primary branches, more pods per plant and green seeds has been developed for use in chickpea improvement programmes (Chaturvedi & Nadarajan, 2010).

4.9 Chickpea Genomic Resources

Average chickpea productivity is less than $1\,t\,ha^{-1}$, which is much less than its potential, $6\,t\,ha^{-1}$ (Singh, 1985). Biotechnological tools can help to increase chickpea productivity by using the marker-assisted selection (MAS) approach in breeding programmes (Varshney, Graner, & Sorrells, 2005; Varshney, Nayak, May, & Jackson, 2009). Trait mapping provides the first step to employ MAS in breeding programmes. Recent developments in genomics technology have helped to explain the mechanism of complex traits controlling chickpea productivity and the genetic architecture of traits of economic importance to accelerate breeding programmes. A number of marker-trait associations have been identified in chickpea along with the dense genetic maps which have allowed MAS to become a routine in breeding programmes (Kulwal, Thudi, & Varshney, 2011; Varshney, Hoisington, & Tyagi, 2006). A huge amount of genomic and genetic resources developed by ICRISAT in collaboration with partners have regularly been used in accelerating the genomic and breeding application to increase chickpea productivity. Since 2005, ICRISAT has regularly been focussing on the development of molecular markers, construction of comprehensive genetic and consensus maps, identification of marker-trait associations and Quantitative Trait Loci (QTLs), and initiation of molecular breeding for various disease resistance and drought tolerance in chickpea.

4.9.1 Molecular Markers and Genotyping Platforms

A number of marker systems have been introduced recently, such as hybridization-based diversity arrays technology (DArT) and sequence-based single nucleotide polymorphism (SNP) markers. These marker systems can easily be automated and provide medium- to high-throughput genotyping. Still, microsatellite (SSR) markers are the marker of choice for geneticist and breeders. SSRs are highly polymorphic, multi-allelic and codominant in nature; therefore suitable for genotyping the germplasm with a narrow genetic base and for segregating populations (Gupta & Varshney, 2000). Development of SSRs was mainly dependent on the screening of size-selected genomic and cDNA libraries, but recently *in silico* approaches of

mining the expressed sequence tags (ESTs) and Bacterial Artificial Chromosome (BAC)-end sequences have also become popular for the identification of genic SSRs (Varshney, Glaszmann, Leung, & Ribaut, 2010). To supplement the chickpea genomics, more than 2000 SSR markers (Table 4.5) have been developed in the past few years using various approaches including genomic DNA libraries (Gaur et al., 2011; Nayak et al., 2010), cDNA libraries (Varshney, Hiremath, et al., 2009) and 454/FLX transcript reads (Garg, Patel, Tyagi, & Jain, 2011; Garg, Patel, Jhanwar, et al., 2011; Hiremath et al., 2011). On the other hand, a new set of 487 functional markers including EST-SSRs, Intron-targeted primers (ITP), expressed sequence tag polymorphisms and SNPs have been developed by the National Institute of Plant Genome Research (NIPGR), New Delhi, India (Choudhary, Gaur, Gupta, & Bhatia, 2012).

ICRISAT in collaboration with DArT Pty Ltd., Australia, has also developed another marker resource namely DArT arrays representing 15,360 features (Table 4.5) for chickpea (Varshney et al., 2010). This set has regularly been used for diversity studies and saturating linkage maps (Thudi et al., 2011). These arrays showed very little polymorphism when screened on the elite chickpea germplasm (Thudi et al., 2011), and the parental genotypes of mapping populations showed only 35% polymorphism when screened with these DArT arrays. This suggests that DArT arrays are not cost-effective to screen the cultivated chickpea germplasm. Another type of marker system, SNP, is gaining popularity in several crop species due to its genome-wide distribution, abundance, flexibility of automation and amenability to high throughput. For identification of SNP, three different approaches were used. First, RNA sequencing approach was used to sequence the parents of mapping population. Alignment of these short reads led to identification of thousands of SNPs. The second approach focussed on the allele-specific sequencing of parental genotypes using conserved orthologous sequence markers and led to identification of 768 SNPs (Table 4.5). In the third approach, 220 candidate genes were sequenced on 2–20 genotypes and 1893 SNP were identified based on allele-specific sequencing (Gujaria et al., 2011). In total, a large number of SNPs were identified and made available for use in chickpea improvement. To use these SNPs in breeding programmes and other applications, selection of an appropriate genotyping platform is very important. University of California – Davis in collaboration with its partners has developed Illumina GoldenGate assays for 768 SNPs. These GoldenGate assays are cost-effective only when dealing with large number of SNPs to genotype a large number of samples. However, where fewer markers are required for genotyping, another genotyping platform, BeadXpress based on VeraCode technology, suits well. Therefore, VeraCode assay for 96-plex SNP (Table 4.5) has been developed at ICRISAT to be used on Illumina's BeadXpress system (R. K. Varshney, unpublished data). Another SNP genotyping platform, KASPar, developed by KBiosciences (www.kbioscience.co.uk), provides a flexible and cost-effective assay for SNP genotyping. ICRISAT has developed 2068 KASPar assays (Table 4.5) in chickpea (Hiremath et al., 2012).

In recent years, next-generation sequencing (NGS) technologies have been adapted by researchers to produce a huge amount of sequencing data at very low cost and in less time. In chickpea, two NGS approaches 454 and Illumina were used for

Table 4.5 Summary of Genomic Resources in Chickpea Developed at ICRISAT, India

Resource	Number
SSRs	Approx. 2000
SNPs	9000
DArTs	15,360
GoldenGate assays	768 SNPs
KASPar assays	2068 SNPs
VeraCode assays	96 SNPs
Sanger ESTs	Approx. 30,000
454/FLX reads	435,018
TUSs	103,215
Illumina reads (million reads)	>108 (4 parents)

characterization of the chickpea transcriptome. Sanger sequencing was used to generate the EST from drought- and salinity-stress-challenged cDNA libraries. 454/FLX sequencing was undertaken to generate 435,018 transcript reads (Table 4.5), which were used along with the Sanger ESTs to improve the chickpea transcript assembly (Hiremath et al., 2011). In a similar study, National Institute of Plant Genome Research (NIPGR) generated a hybrid assembly with 34,760 tentative consensus sequences (Garg, Patel, Jhanwar, et al., 2011). Recently, a transcriptome of a wild chickpea, *C. reticulatum* (genotype PI 489777) with 37,265 *C. reticulatum* tentative consensus (CrTC) was reported using GS-FLX Roche 454 NGS technology (Jhanwar et al., 2012). Previously, the higher cost and need for time and expertise were the main constraints in whole-genome sequencing, but recent advancements in NGS technologies have allowed initiating genome sequencing at very low cost and less time. Very recently, ICRISAT in collaboration with Beijing Genomics Institute (BGI), Shenzhen, China and other international collaborators reported the draft whole-genome shotgun sequence of CDC Frontier, a kabuli chickpea variety (Varshney et al., 2013). Along with the genome sequence, resequencing of 90 cultivated and wild chickpea accessions has also been reported. An effort to sequence ICC 4958, a desi landrace, has also been initiated at NIPGR, New Delhi. These resources can be used for chickpea improvement through molecular breeding and to explain chickpea genome diversity and domestication events.

4.9.2 Genetic Maps and Trait Mapping

A first step in crop improvement using molecular breeding/genomics-assisted breeding is the discovery of marker-trait association between the trait of interest and a genetic marker. However, QTL analysis has suffered severely from the lack of saturated genetic maps. Large-scale genomic resources developed by ICRISAT and partners during the last 5 years have been used for the construction of comprehensive/consensus genetic maps in chickpea. An interspecific reference mapping population has been developed from a cross, ICC 4958×PI 489777 and used for generating genetic

maps (Upadhyaya, Thudi, et al., 2011). The first genetic map in chickpea was developed on this reference population (ICC 4958×PI 489777) using markers like Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and very few SSR markers. To saturate this map, a high-density chickpea genetic map with 1291 loci has been developed by Thudi et al. (2011). This map comprises a range of markers starting from BES-SSRs (157), genic molecular markers (145), DArT (621) and earlier published legacy markers (368), spanning a total of 845.56 cM across eight linkage groups (LG) with an average marker distance of 0.65 cM. The number of markers on each LG ranged from 68 (LG 8) to 219 (LG 3). Genetic maps constructed using the gene-based markers are referred to as *transcript maps*. In chickpea, a transcript map with 126 genic molecular markers, including 53 CAPS-SNPs, 55 EST-SSRs and 18 CISR loci has been developed (Gujaria et al., 2011). In another study using the same reference population, an advanced linkage map spanning 1497.7 cM with 406 loci including 177 gene-based markers and 126 genomic SSRs (gSSRs) has been developed (Choudhary et al., 2012). Recently, KASPar assays have been adopted for SNP genotyping and been used to develop a second-generation genetic map with 1328 loci including 625 Chickpea KASPar Assay Markers (CKAMs), 314 TOG-SNPs and 389 already published markers with an average inter-marker distance of 0.59 cM (Hiremath et al., 2012).

Besides interspecific mapping populations, several intraspecific mapping populations have also been developed to identify the markers associated with *Fusarium* wilt (Sharma, Winter, Kahl, & Muehlbauer, 2004; Sharma et al., 2005), *Ascochyta* blight (Anbessa, Taran, Warkentin, Tullu, & Vandenberg, 2009; Iruela et al., 2007) and drought. For drought tolerance in chickpea, ICRISAT has developed two intraspecific mapping populations (ICC 4958×ICC 1882 and ICC 283×ICC 8261) (Chamarthi et al., 2011). Both populations were used for the construction of SSR-based genetic maps comprising 240 and 170 loci, respectively. QTL analysis using the extensive phenotyping data revealed a genomic region that harbours QTLs for several root-related and other drought tolerance–related traits contributing approximately 35% of the phenotyping variation. Therefore, this genomic region has been targeted for introgression in elite chickpea lines to enhance drought tolerance using the marker-assisted backcross (MABC) approach.

4.9.3 Molecular Breeding

Once the QTLs for trait of interest are identified, the next step is to use this information in a crop improvement programme using genomic-assisted breeding for developing superior lines with better response to stress and high yield. With the recent development in NGS technology, it has become common practice to use molecular markers for phenotype prediction and selection of progenies for the next generation in breeding (Varshney et al., 2012). Several genomics-assisted breeding approaches, namely MABC, marker-assisted recurrent selection (MARS) and genomic selection have regularly been used in crop improvement programmes. MABC focusses on the introgression of the QTL and/or genomic region associated with the trait(s) of interest from a donor parent into an elite recurrent parent using molecular markers

(Hospital, 2005). This approach leads to the generation of near-isogenic lines (NILs) containing only the major gene/QTL from the donor parent, while retaining the whole genome of the recurrent parent (Gupta, Kumar, Mir, & Kumar, 2010). MABC can also be used for gene pyramiding, where different genes for the same trait or for different traits are accumulated in one background.

In chickpea, ICRISAT has been working on two MABC programmes. The first initiative, supported by the CGIAR GCP and the Bill & Melinda Gates Foundation, focusses on improved drought response in elite chickpea lines. Efforts have been made to introgress the genomic region harbouring QTLs for several drought-related traits into JG 11 genetic background from the germplasm accession, ICC 4958. BC_3F_4 lines have been generated and were evaluated under both rain-fed and irrigated conditions in India, Ethiopia and Kenya in the main crop season during 2011–2012. Results of the first-year field trial were very encouraging: the BC lines possessed the RLD of the donor parent with the seed quality and yield of the recipient parents. BC lines showed 6–11% higher yield in the rain-fed condition, while in the irrigated condition, the gains were up to 24%. The success story of JG 11 inspired several institutes, such as Indian Institute of Pulses Research (IIPR), Kanpur and Indian Agricultural Research Institute (IARI), New Delhi from India, Egerton University, Kenya, and Ethiopian Institute of Agricultural Research (EIAR), Ethiopia, to start MABC programmes for introgressing this genomic region from ICC 4958 into the leading varieties of different regions.

In an another initiative, sponsored by the Department of Biotechnology (DBT), Government of India, ICRISAT in collaboration with Jawaharlal Nehru Krishi Vishwavidyalaya (JNKVV) of Jabalpur, Mahatma Phule Krishi Vidyapeeth (MPKV) of Rahuri and ARS-Gulbarga has been working on gene pyramiding of resistance to two races (foc1 and foc3) for *Fusarium* wilt (FW) and two QTLs conferring resistance to *Ascochyta* blight (AB). Efforts have been initiated for introgression of resistance to FW from WR 315 and resistance to AB from ILC 3279 into elite chickpea cultivars (C 214, JG 74, Pusa 256, Phule G12 and Annigeri-1) from different agro-climatic zones through MABC. Presently, homozygous BC_3F_4 lines are available for preliminary evaluation for resistance to FW and AB.

4.10 Conclusions

The presence of enormous genetic variation and the means to exploit such variability is the key to success of crop improvement programmes. Large collections of chickpea germplasm comprising landraces and wild *Cicer* species have been conserved in various gene banks worldwide, representing a large spectrum of diversity in the genus *Cicer*. Development and evaluation of small subsets such as core and mini-core collections have resulted in the identification of trait-specific germplasm accessions for important abiotic and biotic stresses as well as for agronomic and nutrition-related traits, which results in the enhanced utilization of genetic resources for developing broad-based climate-resilient chickpea cultivars. Besides cultivated type germplasm, new sources of variability for traits of interest exists in wild *Cicer*

gene pools, which can be exploited using widespread hybridization techniques. Promising lines having resistance genes and good agronomic performance have been developed from crosses involving cultivated and wild *Cicer* species. Further, recent advances in plant biotechnology in combination with the traditional breeding approaches, coupled with genomics and transgenic technologies, provide new tools to exploit the genes locked up in cross-incompatible secondary and tertiary gene pools. The availability of genomic resources such as the development of molecular markers, genetic and physical maps and the generation of expressed sequenced tags (ESTs), genome sequencing and association studies revealing marker-trait associations has facilitated the identification of QTLs and discovery of genes associated with tolerance/resistance to abiotic and biotic stresses including agronomic traits. These advancements in chickpea genomic resources can assist in identifying and tracking allelic variants associated with beneficial traits and identifying desirable recombinant plants with the markers of interest, which will accelerate the chickpea improvement programmes.

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