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# Managing and Discovering Agronomically Beneficial Traits in Chickpea Germplasm Collections

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## Abstract

Access to crop biodiversity is foremost to address new challenges to agricultural production. The chickpea genetic resources maintained at ICRISAT genebank have been characterized for morpho-agronomic traits. The passport and characterization data were used to form representative subsets: core/mini core collections and genotyping data of composite collection was used to form reference set. The chickpea core and mini core collections, respectively, consist of 1956 and 211 accessions, while reference set 300 accessions. These subsets are ideal genetic resource to dissect population structure and diversity, identify new sources of variations, mine allelic variation, and conduct association genetics to identify QTLs associated with agronomic traits which upon validation may be used in applied breeding. Using mini core collection, a number of accessions with early maturity, high yield and large seed size, seed nutritional traits, and stress tolerance were identified, including some with high yield and multiple stress tolerance. A systematic program is underway to introgress wild *Cicer* gene(s) to enhance levels of resistance and to broaden the genetic base of cultigen gene pool.

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## 5.1 Introduction

Plant genetic resources are the basic raw materials and their judicious use in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for future and simultaneously increase agricultural production and food security. Key to successful crop improvement is a continued supply of genetic diversity including new or improved variability for target traits. International Crops Research Institute for the Semi-Arid

Tropics (ICRISAT) has global responsibility to collect, conserve, maintain, characterize, evaluate, document and distribute the wealth of genetic variation of its six mandate crops and five small millets. The germplasm collections maintained in genebanks are the source for detailed characterization of agronomically beneficial traits such as tolerance to abiotic and biotic stresses, yield, nutrition, and grain quality. In this chapter, we report the current status of chickpea germplasm of both cultivated and wild types preserved in RS Paroda genebank at Patancheru, India and the major activities carried out by the genebank scientists to promote use of germplasm in breeding and genomics of chickpea.

## 5.2 Global Chickpea Genetic Resources

Global collection of chickpea genetic resources consists of 99,877 accessions conserved across 120 national/international genebanks in 64 countries. Of these, 1476 accessions are of wild *Cicer* types. Table 5.1 represents the list of major genebanks holding chickpea collections greater than 1000 accessions. Together, they contain 87,341 accessions, 98.3% cultivated and 1.7% the wild *Cicer* types. ICRISAT genebank currently holds 308 wild *Cicer* accessions representing eight annuals and 10 perennial species (Table 5.2).

**Germplasm conservation at ICRISAT:** Currently ICRISAT genebank holds the largest chickpea germplasm collection (20,764 accessions) representing 59 countries of origin.

**New collection assembled:** Between 2004 and 2012, a total of 3332 accessions from USDA-USA (2098), ICARDA Syria (900), Nepal (265), Azerbaijan (44), and Bulgaria (25) were incorporated into the ICRISAT collection.

## 5.3 Conservation and Regeneration

**Conservation:** The base germplasm collection at ICRISAT is conserved under long-term storage conditions ( $-20^{\circ}\text{C}$ ), while the active collection is maintained under medium term storage at  $4^{\circ}\text{C}$

and 30% RH. The moisture content during seed processing for storage as active and base collections should be 10% and 6–7%, respectively. About 350 g of chickpea seed per accession is preserved in aluminum screw cap containers as the active collection. Vacuum-sealed standard aluminum foil pouches are used for conserving 200 g of seed per accession in the base collection (Upadhyaya and Gowda 2009). As a safety backup, seed samples of 16,996 chickpea accessions were deposited in Svalbard Global Seed Vault, Norway.

**Regeneration:** Chickpea is a cool-season grain legume crop grown in climates ranging from semi-arid tropics to temperate environments. Seed regeneration is an important aspect of management of genetic resources. Reproductive biology largely influences procedures used for regeneration. Chickpea being self-pollinated (unlike cross-pollinated species) can be easily regenerated under field conditions.

Chickpea accessions are regenerated when seed quantity in the active collection is less than 75 g or when viability falls below 85%; whereas accessions in the base collection are regenerated when seed viability falls below 90%. An adequate quantity of seeds should be used for regeneration to maintain as much of the original variation as possible within an accession. At ICRISAT, two four-meter rows containing approximately 80 plants are grown and harvested to provide regenerated seed of each accession. Accessions being regenerated are regularly monitored during the cropping season to detect and eliminate off-type plants. Data on discrete phenotypes such as growth habit, flower and seed color and seed shape is recorded during regeneration and compared to previously generated passport information to ensure integrity of each accession (Upadhyaya and Gowda 2009; <http://cropgenebank.sgrp.cgiar.org/index.php/crops-mainmenu-367/chickpea-mainmenu-360/regeneration-mainmenu-374>). Wild *Cicer* species are regenerated in a glasshouse under extended light (18 h) conditions. Seeds are scarified before planting to overcome dormancy due to hard seed coat.

**Table 5.1** List of genebanks holding more than 1000 chickpea germplasm accessions

Institute	Wild Cicer		Cultivated	Total
	Species	Accession		
Australian Temperate Field Crops Collection (ATFCC), Horsham Victoria, Australia	18	246	8409	8655
Institute of Biodiversity Conservation (IBC), Addis Ababa, Ethiopia			1173	1173
Institute for Agrobotany (RCA), Tápiószéle, Hungary	5	9	1161	1170
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India	19	308	20,456	20,764
National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India	10	69	14,635	14,704
National Plant Gene Bank of Iran, Seed and Plant Improvement Institute (NPGBI-SPII), Karaj, Iran			5700	5700
Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas (INIA-Iguala), Iguala, Mexico			1600	1600
Plant Genetic Resources Program (PGRP), Islamabad, Pakistan	3	89	2057	2146
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (VIR), St. Petersburg, Russian Federation			2767	2767
International Centre for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syrian Arab Republic	11	540	15,194	15,734
Plant Genetic Resources Department, Aegean Agricultural Research Institute (AARI), Izmir, Turkey	4	21	2054	2075
Institute of Plant Production n.a. V.Y. Yurjev of UAAS, Kharkiv, Ukraine			1760	1760
Western Regional Plant Introduction Station, USDA-ARS, Pullman, USA	21	194	7844	8038
Uzbek Research Institute of Plant Industry (UzRIPI), Botanica, Uzbekistan			1055	1055
Total		1476	85,865	87,341

([http://www.fao.org/wiews-archive/germplasm\\_query.htm?i\\_l=EN](http://www.fao.org/wiews-archive/germplasm_query.htm?i_l=EN))

## 5.4 Characterization and Evaluation

Characterization refers to recording easily-observed and highly heritable characters expressed in all environments; while evaluation of agronomically beneficial traits often requires more extensive screening tests and data recording. Accessions are usually grown in augmented block designs using standard checks at every 10 or 20 accessions and characterized following chickpea descriptors (IBPGR, ICRISAT, and ICARDA 1993). To date, about 98% of chickpea germplasm

has been characterized for morpho-agronomic traits, 63% for seed protein content, and 35% for biotic stresses, namely fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri* (Padwick) Snyd. & Hans.), ascochyta blight (*Ascochyta rabiei* [Pass.] Labr.), botrytis gray mold (*Botrytis cinerea* Pers. Ex Fr.) and colletotrichum blight (*Colletotrichum dematium* (Pers ex Fr) Grove). All the characterization and evaluation data can be accessed through <http://genebank.icrisat.org/>. Large range variations among entire collection accessions were noted for both quantitative and qualitative traits (Table 5.3, Figs. 5.1 and 5.2).

**Table 5.2** Status of wild *Cicer* species accessions conserved at ICRISAT genebank

Species	No. of accessions	Chromosome number ( $2n$ ) <sup>a</sup>	Country of origin
<b>Annual</b>			
<i>C. bijugum</i>	49	16	Iraq, Syria and Turkey
<i>C. chorassanicum</i>	4	16	Afghanistan
<i>C. cuneatum</i>	5	16	Ethiopia
<i>C. echinospermum</i>	18	16	Turkey
<i>C. judaicum</i>	70	16	Afghanistan Ethiopia, India, Israel, Jordan, Lebanon, Morocco, Syria, and Turkey
<i>C. pinnatifidum</i>	42	16	Ethiopia, Israel, Lebanon, Syria, and Turkey
<i>C. reticulatum</i>	36	16	Turkey
<i>C. yamashitae</i>	7	16	Afghanistan
<b>Perennial</b>			
<i>C. anatolicum</i>	3	14, 16	Turkey
<i>C. canariense</i>	1		Spain
<i>C. floribundum</i>	1		Turkey
<i>C. macracanthum</i>	5		Pakistan
<i>C. microphyllum</i>	52		India and Pakistan
<i>C. montbretii</i>	2	16, 24	Turkey
<i>C. multijugum</i>	1		Russian Federation
<i>C. nuristanicum</i>	2		Pakistan
<i>C. pungens</i>	9	14	Afghanistan
<i>C. rechingeri</i>	1		Afghanistan
Total	308		

<sup>a</sup>Saxena, M.C. & K.B. Singh (eds) 1987, pp 1–34. The Chickpea. C.A.B International, Wallingford, Oxon, UK

## 5.5 Documentation

Documentation of information on germplasm collections is critical for efficient genebank operations. Information on passport, characterization, inventory (season and location seeds produced, seed viability and date germination test conducted, date seed stored, moisture content, quantity and availability of seeds, and 100-seed weight) and distribution of each accession is maintained at ICRISAT using Genebank Information Management System (GIMS). GIMS is a standalone facility developed internally to meet the demands

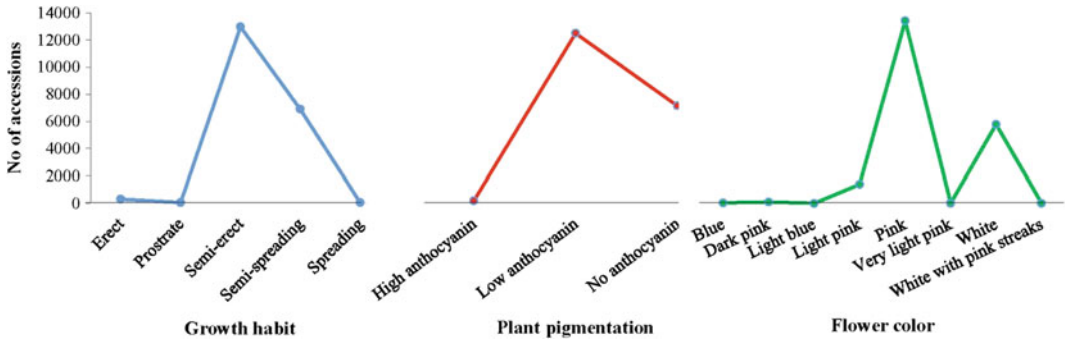
of genebank documentation activities at ICRISAT. Passport information regarding chickpea germplasm stored at ICRISAT genebank can be browsed through <http://genebank.icrisat.org/> and <http://www.genesys-pgr.org>.

## 5.6 Access to the Collection

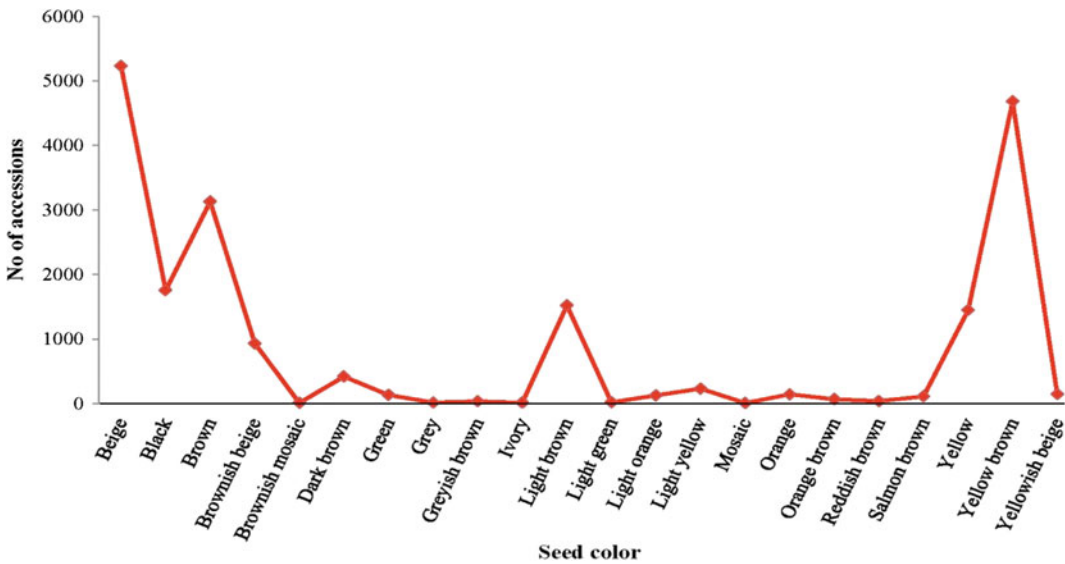
All the FAO-designated germplasm are available to researchers after signing with ICRISAT the Standard Material Transfer Agreement of International Treaty on Plant Genetic Resources for Food and Agriculture. To date, ICRISAT

**Table 5.3** Range and mean as observed for quantitative traits in entire collection as well in desi, Kabuli and pea-type chickpea accessions evaluated at Patancheru, India

Character	# accessions characterized	Entire		Desi		Kabuli		Pea	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
Plant height (cm)	20,219	14.0–105.7	39.2	14.0–80.8	36.7	15.0–96.6	45.9	19.0–105.7	41.3
Plant width (cm)	20,287	13.3–210.8	45.1	13.3–187.0	43.2	14.0–187.0	51.1	19.0–210.8	43.7
Basal primary branches number	20,284	0.2–15.7	2.8	0.2–15.7	2.8	0.7–11.2	2.8	1.0–6.0	2.7
Apical primary branches number	20,284	0.0–12.0	1.6	0.0–12.0	1.6	0.0–7.8	1.5	0.0–8.0	1.7
Basal secondary branches number	20,284	0.0–15.2	3.2	0.0–15.2	3.3	0.0–13.7	3.1	0.0–10.7	3.0
Apical secondary branches number	19,982	0.0–28.2	4.7	0.0–28.2	4.9	0.0–21.0	4.1	0.0–15.2	4.5
Tertiary branches number	20,283	0.0–30.8	1.3	0.0–30.8	1.2	0.0–24.8	1.6	0.0–13.0	1.1
Days to flowering	20,339	28–152	62	28–152	61	31–102	66	39–100	62
Flowering duration	18,319	11–104	33	13–104	34	11–99	33	14–93	36
Days to maturity	20,217	70–196	116	70–196	114	77–164	123	86–173	117
Pods per plant	20,210	2.0–263.0	42.0	2.0–263.0	46.7	2.0–202.0	32.4	5.0–155.0	37.7
Seeds per pod	20,151	1.0–3.2	1.2	1.0–3.0	1.3	1.0–2.9	1.1	1.0–3.2	1.2
100 Seed weight (g)	20,285	1.0–71.1	17.9	1.0–53.6	14.4	1.7–71.1	27.5	2.7–59.9	18.7
Seed yield (kg ha <sup>-1</sup> )	20,167	22–5130	1250	41–5130	1314	22–3464	1079	39–3840	1217



**Fig. 5.1** Variation in growth habit, plant pigmentation, and flower color as observed in entire collection of chickpea germplasm maintained at ICRIASAT Genebank



**Fig. 5.2** Variation in seed color as observed in entire collection of chickpea germplasm maintained at ICRIASAT Genebank

genebank has distributed 350,958 chickpea germplasm samples to researchers in 88 countries, with Indian NARS (27%) and ICRIASAT scientists (56%) being the largest beneficiary, and 87 other countries receiving the remaining 17% of seed samples distributed.

### 5.7 Global Crop Register for Chickpea

Germplasm register provides better knowledge on unique holdings in addition to passport information on collections, and an opportunity to improve

accuracy of the existing data on germplasm collections. Access to the crop-specific accession based information on germplasm collections would benefit the germplasm user community. The ‘Global Crop Register for Chickpea’ was developed by ICRIASAT for priority collections and collections maintained in common (GPG2 Activity 3.3) at different genebanks. This chickpea crop register is a searchable database via Web site through which the global chickpea data of ICRIASAT (20,602 accessions), ICARDA (13,818 accessions), and USDA-ARS (12,426 accessions) could be cross-referred through ICRIASAT

chickpea data set to identify unique chickpea collections maintained by each genebank.

## 5.8 Forming Representative Subsets for Trait Discovery and Utilization

**Representative subsets:** Both conventional core and mini core collections and genotype-based reference set have been formed to enhance greater utilization of chickpea genetic resources in breeding and genomics. The core collection consists of 1956 accessions (Upadhyaya et al. 2001), while mini core collection 211 accessions (Upadhyaya and Ortiz 2001). For forming genotype-based reference set, a global composite collection (3000 accessions) was formed composed of 80% landraces, 9% advanced breeding lines, 2% cultivars, 1% wild species, and 8%

accessions of unknown origin (Upadhyaya et al. 2006). This composite collection was then molecularly profiled using 50 SSR markers and the genotyping data on 48 SSRs was statistically analyzed to form a reference set comprised of 300 genetically most diverse accessions, capturing 78% allelic diversity of the 1683 composite collection alleles (Upadhyaya et al. 2008). Mini core collection is an ideal resource for allele mining, association genetics, mapping and cloning of genes, and applied breeding for the development of elite genetic materials/cultivars.

**Stress tolerance:** Researchers at ICRISAT and elsewhere have extensively evaluated mini core collection and reported a number of accessions with multiple stress tolerance (Table 5.4), for example, ICC 6874 and ICC 14402 resistant to drought, salinity, heat, fusarium wilt, and legume pod borer; ICC 12155 to drought, salinity, heat, fusarium wilt, and botrytis gray mold;

**Table 5.4** Sources of resistance to abiotic and biotic stress using mini core collection in chickpea

Stress	Summary of stress resistant germplasm	References
<b>Abiotic stress</b>		
Drought	ICC# 283, 456, 637, 708, 867, 1205, 1422, 1431, 1882, 2263, 2580, 3325, 4495, 4593, 4872, 5337, 5613, 5878, 6874, 7272, 7323, 7441, 8261, 8950, 10399, 10945, 11121, 11944, 12155, 12947, 13124, 14402, 14778, 14799, 14815, 15868, 16524, 16796	Reviewed in Upadhyaya et al. (2013)
	ICC 7571	Kashiwagi et al. (2013)
Salinity	ICC# 283, 456, 708, 867, 1431, 2263, 2580, 3325, 4495, 4593, 4872, 5613, 5878, 6279, 6874, 7272, 7441, 8261, 9942, 10399, 10945, 11121, 11944, 12155, 13124, 14402, 14778, 14799, 15868, 16524, 16796	Reviewed in Upadhyaya et al. (2013)
Heat	ICC# 283, 456, 637, 708, 1205, 1882, 2263, 4495, 5613, 5878, 6874, 7441, 10945, 11121, 11944, 12155, 13124, 14402, 14778, 14799, 14815, 15868	
Chilling stress	Reproductive stage: ICC 16348 and ICC 16349	Kumar et al. (2011)
<b>Biotic stress</b>		
<i>Fusarium</i> wilt	ICC# 1710, 1915, 2242, 2277, 2990, 3325, 4533, 5135, 6279, 6874, 7184, 7554, 7819, 9848, 12028, 12037, 12155, 13219, 13441, 13599, 13816, 14199, 14402, 14831, 15606, 15610	Reviewed in Upadhyaya et al. (2013)
Dry root rot	ICC# 1710, 2242, 2277, 11764, 12328, 13441	
<i>Ascochyta</i> blight	ICC# 1915, 7184, 11284	
<i>Botrytis</i> gray mold	ICC# 2990, 4533, 6279, 7554, 7819, 9848, 11284, 11764, 12028, 12037, 12155, 12328, 13219, 13599, 13816, 14199, 15406, 15606, 15610	
Legume pod borer	ICC# 3325, 5135, 6874, 14402, 14831, 15406, 15606	
Herbicide	ICC# 2242, 2580, 3325	

ICC 3325 to drought, salinity, fusarium wilt, and herbicide; ICC 6279 to salinity, fusarium wilt, and botrytis gray mold, and ICC 2580 to drought, salinity, and herbicide. More importantly, many of these accessions on average produced about 1.5 t seed yield ha<sup>-1</sup> (Upadhyaya et al. 2013), thereby agronomically comparable with controls.

The physiological basis of stress tolerance revealed that conservative water use (i.e., less during the vegetative growth stage could keep more soil water available during reproductive growth) in addition to drought avoidance root traits are associated with improved grain yield under drought stressed environments in chickpea (Zaman-Allah et al. 2011; Purushothaman et al. 2013; Kashiwagi et al. 2013). In addition, canopy temperature depression (CTD) is positively associated with grain yield and biomass, and thereby a cooler canopy temperature at mid reproductive stage can be used as selection criterion for drought tolerance (Purushothaman et al. 2015). For heat stress, researchers used heat tolerance index as measure of tolerance to identify heat stress tolerant chickpea germplasm (Krishnamurthy et al. 2011; Devasirvatham et al. 2015). Phenology is negatively correlated with grain yield at high temperature, while plant biomass, pod number, filled pods/seeds plant<sup>-1</sup> are positively correlated. Upadhyaya et al. (2011) evaluated 35 early maturing germplasm accessions for their tolerance to heat stress and identified tolerant lines. Cold tolerant chickpeas are less affected due to stress-related leaf injury but showed greater ascorbic acid and proline than cold-sensitive germplasm (Kumar et al. 2011). Further, they detected higher activity of enzymes related to carbohydrate metabolism such as  $\beta$ -amylase, invertase, and sucrose synthase in cold tolerant than cold susceptible germplasm.

**Early maturity:** Early maturity helps chickpea to avoid terminal drought and heat stress, and thereby diverse sources of early maturity germplasm are needed to enhance chickpea adaptation in the subtropics. Upadhyaya et al. (2007) detected substantial genetic variation for days to flowering and maturity among 28 early maturing

desi chickpea germplasm across five environments. ICC# 11040, 11180, 12424, 16641, and 16644 were earliest to mature, similar to or earlier than controls (Harigantars and ICCV 2), and produced about 23% more seed yield than the mean of the controls. ICC# 16641 and 16644 also showed higher 100-seed weight than controls (Annigeri and ICCV 2). Further analysis delineated this germplasm into three clusters, with maturity the main basis of delineation of the first cluster from others, while pod yield and its associated traits the basis for delineation of the second cluster from the others. Seed size is an important trait in Kabuli chickpea, and those with a 100-seed weight of >40 g fetch higher market price, largely because of consumer preference. When evaluated large-seeded Kabuli germplasm lines, Gowda et al. (2011) identified a few large-seeded high-yielding lines such as ICC 17109 and ICC 17452 (100-seed weight >50 g) with moderate stable seed yield across environments. Thus, these germplasm would be useful in breeding broad-based, early maturing and high-yielding cultivars.

**Seed nutritional traits:** Widespread micronutrient malnutrition results in an enormous negative socio-economic impact on the society, and crop biofortification is an ideal approach to minimize the adverse impact of micronutrient malnutrition. Similarly, protein is an important nutrient. Research to date suggests adequate genetic variation for seed iron (Fe), zinc (Zn), and protein among chickpea mini core accessions, i.e., from 54 to 76 ppm Fe and 31 to 59 ppm Zn and from 19.2 to 23.7% protein. Accessions with highest seed Fe, Zn and protein content could be used in breeding programs. Diapari et al. (2014) reported eight SNP loci associated with Fe and/or Zn concentrations in chickpea. One SNP on chromosome 1 is associated with both Fe and Zn. Three and two SNPs on chromosome 4, respectively, are associated with Zn and Fe. Two additional SNPs, one on chromosome 6 and the other on chromosome 7, were also found associated with high Fe and Zn concentrations, respectively. These SNPs after



validation could be used in marker-assisted breeding to enhance seed nutritional value (Fe and Zn) of chickpea.

### 5.9 Broadening Cultigen Genepool Using Wild *Cicer* Species

**Species description and trait discovery:** The genus *Cicer* comprises 43 wild species (35 perennials and 8 annuals) and a cultivated chickpea (*C. arietinum* L.), which were grouped into three gene pools based on crossability with chickpea. The primary gene pool consists of chickpea and the progenitor species, *C. reticulatum*, which is freely crossable with chickpea. The secondary genepool consists of *C. echinospermum*, a species that is crossable with chickpea, but with reduced fertility of the resulting hybrids and progenies. The remaining six annuals and 35 perennial species form the tertiary genepool, which require specialized techniques for gene transfer into the cultivated genetic background (reviewed in Sharma et al. 2013). *Cicer* species have shown a very high level of resistance to ascochyta blight, botrytis gray mold, fusarium wilt, and pod borer and tolerance to drought, cold, and heat stress (reviewed in Sharma et al. 2013). An accession from *C. echinospermum* showed reproductive tolerance at lower temperature (10 °C) under field conditions, which commenced podding earlier and yielded more than Rupali, the most productive chickpea. Further, when this accession was evaluated under controlled environment conditions, pollen germination, viability and frequency on the stigma surface, and pod set in relation to cultivated chickpea were unaffected by low post-anthesis temperatures (13/7 °C). *Cicer echinospermum* is therefore considered a good source of low temperature tolerance (Berger et al. 2012) and supports previous observations about freezing tolerance in *C. echinospermum* accessions (Saeed et al. 2010). *Cicer reticulatum* and *C. pinnatifidum* are also reportedly resistant to drought and heat stress (up to 41.8 °C) (Canci and Toker 2009).

**Pre-breeding:** It refers to the development of intermediate products with specific characteristics and minimum linkage drag that breeders can use in their breeding programs to develop productive cultivars. Ascochyta blight and botrytis gray mold are two devastating diseases in chickpea. Crosses involving cultivated and wild *Cicer* (*C. echinospermum*, *C. reticulatum*, and *C. pinnatifidum*) resulted in progenies with resistance to ascochyta blight and/or botrytis gray mold (Ramgopal et al. 2013; Kaur et al. 2013). Further, *C. reticulatum* contributed resistance to cyst nematode, *C. echinospermum* to cold tolerance, and *C. reticulatum* and *C. echinospermum* to early maturity and large seed weight in chickpea (reviewed in Sharma et al. 2013). Pod borer (*Helicoverpa armigera*) is one of the major constraints to chickpea production, with very low level of resistance in cultivated germplasm. *C. reticulatum* is reported resistant to pod borer. At ICRISAT, work is in progress to develop pre-breeding populations including resistance to biotic stresses such as botrytis gray mold, dry root rot, and pod borer using cultivated chickpea and *C. reticulatum*, and *C. echinospermum* accessions following simple and complex crosses (Shivali Sharma, ICRISAT, person. commun.).

### 5.10 Conclusion

Climate change and variability are likely to constrain chickpea production worldwide. Significant progress has been achieved since the formation of core and mini core subsets in identifying accessions with agronomically beneficial traits (early maturity, seed size, seed yield, seed nutritional traits, and stress tolerance) for use in chickpea breeding and genomics. Several sources of resistance to stress have been identified among wild *Cicer* accessions and efforts are on to develop pre-breeding populations (intermediate products) at ICRISAT and elsewhere to facilitate greater access and use of such lines in chickpea breeding. The chickpea researchers can access germplasm by signing material transfer agreement with ICRISAT.

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