Indian J. Genet., 76(4): 515-529 (2016)
DOI: 10.5958/0975-6906.2016.00070.5

Chickpea genetic resources and its utilization in India: Current status and future prospects

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(Received: October 2016; Revised: November 2016; Accepted: November 2016)

Abstract

Chickpea is recognized as most nutritious pulse crop and with respect to acreage, it ranks at the top among pulses in India. Realizing the significance of plant genetic resources, special efforts were made by the National Bureau of Plant Genetic Resources (NBPGR) to collect the chickpea germplasm from different states of India including certain useful introductions from other countries. A large number of germplasm accessions including wild species were characterized and evaluated for various agro-morphological traits using chickpea minimal descriptor.Thus, extensive germplasm collections now exist in various gene banks of the world including India. As far as germplasm maintenance is concerned, a core set developed by ICRISAT comprising of 1956 accessions and mini core set of 211 accessions representing diversity for seed yield and its component traits. Further, core set developed by NBPGR consisting of 1103 accessions extracted from 14651 accessions conserved in the Indian National Gene Bank revealed that 70% of materials belong to Indian origin. The characterization and evaluation experiments of chickpea conducted across the country led to the registration of some unique germplasm accessions for different trait of interest. However, using crop wild relatives, several interspecific crosses and advance pre-breeding lines were developed by the pulse research institutions in India. The trait of interest incorporated especially from *C. reticulatum*, *C. echinospermum* and *C. judaicum* species for widening the genetic base of cultivated gene pool. Some pre-breeding lines have been suggested as useful donors in national chickpea crossing programmes.

Key words: Chickpea, genetic resources, pre-breeding, utilization, documentation

Introduction

Chickpea (*Cicer arietinum* L.) is the most important grain legume crops in semi-arid tropics, especially, in the rainfed ecology of Indian sub-continent, Mediterranean regions, West Asian and North African areas, Eastern Africa and Latin America. It probably originated in South-Eastern Turkey and adjoining Syria, since most of the annual wild *Cicer* species are predominantly growing in these regions. Chickpea is a self-pollinating true diploid (2n=2x=16) annual species with a genome size of 740 Mbp (Arumuganathan and Earle, 1991). Domesticated chickpea (*Cicer arietinum* L.) has two distinct forms i.e., *desi* (small seeded, angular shape and coloured seeds with higher percentage of fibre) types and *kabuli* types (large seeded, owl's head shaped, beige coloured seeds with a low percentage of fibre). Chickpea seed contains protein, fiber, calcium, potassium, iron, phosphorus, magnesium, zinc, as well as substantial amount of selenium, sodium and copper, which make it nutritionally best composed edible dry legume for human consumption globally (Esha 2010). In Asia, it is the second most important grain legume after soybean, which contributes 86.73% of global pulse production from 89.89% area. The global area under chickpea is about 11.08 mha with a total production of 9.77 mt and an average productivity of 882 kg/ha (FAO, 2014). In India, chickpea is most important annual legume crop species in respect of both area and

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Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; http://epubs.icar.org.in/journal/index.php/IJGPB

production reaching to 9.93 mha and 9.53 mt, respectively with a productivity of 960kg/ha during 2013-2014. The major chickpea growing states are, Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Rajasthan, Maharashtra, Gujarat and Karnataka, which covers over 95% area. Unfortunately, in terms of production and productivity of chickpea in the country is still low. Genetic improvement of chickpea is constrained due to non-availability of appropriate germplasm and narrow genetic base of varieties developed so far (Kumar et al. 2004). Conventional breeding led to the development of crop varieties with narrow genetic base which is evident from plateau in yield gains (Singh et al. 2014). *Ascochyta* blight epidemics caused by *Ascochyta rabiei* in North India in 1980 and 1982 (Singh et al. 1982, 1984) resulted in to severe losses in chickpea production. During the course of evolution, chickpea like other crops was subjected to genetic bottlenecks and subsequent founder effect that resulted in narrow genetic base. Thus, the progress achieved through conventional breeding for developing genetically superior varieties is not in pace with the current need of the country, which is evident from the stagnant productivity of chickpea during the past two decades (Varshney et al. 2010). On the other hand, plant genetic resources including crop wild relatives (CWRs) are the reservoir of useful genes/alleles for an array of major biotic and abiotic stresses including desirable agro-morphological traits, that provide basic raw materials for further genetic enhancement (Bains et al. 2012). Therefore, systematic exploration and collection of chickpea germplasm including wild species from diversity rich areas, their characteri-zation and evaluation, maintenance, conservation and utilization into the elite genetic backgrounds are of considerable significance in view of improving the chickpea production in the country.

Origin, distribution, diversity and gene pool

Chickpea has originated in an area of present-day South-eastern Turkey and Syria, where three wild annual *Cicer* species, *C. bijugum*, *C. echinospermum* and *C. reticulatum*, closely related to chickpea, are found. From here, chickpea spread with human migration towards West and South via the Silk Route (Singh et al. 1997). Four centers 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).

As far as distribution is concerned, the *Cicer* species occurs from sea level (e.g. *C. arietinum, C. montbretii*) to over 5000 m (*C. microphyllum*) near glaciers in the Himalayas. The cultivated species, *C.arietinum* is found only in cultivation and can not colonize without human intervention. The wild species e.g., *C. reticulatum* and *C. bijugum* occur in weedy habitats (fallow or disturbed habitats, road sides, cultivated fields of wheat, and other places not touched by man or cattle), mountain slopes among rubble (e.g. *C. pungens*, *C. yamashitae*), on forest soils, in broad-leaf or pine forests (e.g. *C. montbretii*, *C. floribundum*), and also grown naturally in stony and desert areas of Himalayas in India (*C. microphyllum*) (Chandel 1984). The genus *Cicer* has 9 annual and 34 perennial species and is classified into three gene pools based on their compatibility with cultivated chickpea following (Harlan and de Wet 1971) the gene pool concept. The primary gene pool consists of domesticated chickpea, its landraces and the immediate progenitor species, *C. reticulatum*, the species which are easily 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 pollen fertility of the resulting hybrids and their advance progenies. The tertiary gene pool consists of remaining six annual and 34 perennial species which are not readily crossable with cultivated chickpea and require specialized techniques for gene transfer into cultivated backgrounds of chickpea.

Germplasm collection through exploration

The basic aim of collecting germplasm is to capture the substantial amount of genetic variability in the smallest sample size (Singh and Singh 1997), and the first exploration mission led by the Regional Pulse Improvement of US Department of Agriculture (USDA) was undertaken during 1970s in India collecting ~7000 chickpea accessions. Thereafter, the National Bureau of Plant Genetic Resources (NBPGR) took lead in conducting several exploration trips within the country in association with other national and international agencies. The area explored for the collection of chickpea germplasm comprised part of Rajasthan, Odhisa, Maharashtra, part of Gujarat, eastern parts of Arunachal Pradesh, Bihar, southern parts of Karnataka and Tamil Nadu (Singh and Singh 1997). Further, special efforts for exploration and collection of chickpea germplasm were made under the National Agricultural Technology Project (NATP) during 2000-2005. The bureau has also made explorations in collaboration with the International Crops Research

Institute for Semi-arid Tropics (ICRISAT) Patancheru, to collect the germplasm from arid parts of Rajasthan, Bundelkhand region of Uttar Pradesh, Madhya Pradesh, Gujarat, Maharashtra and Telangana. However, a lot of interest has been generated in the wild *Cicer* species with the realization that they are the sound wealth of genes/alleles not only to biotic and abiotic stresses, but also for elite agro-morphological traits (Van der Maesen and Pundir 1984). Many other explorations were also undertaken in the north-western Himalayan region to collect wild *Cicer* species. The bureau has collected some accessions of *Cicer microphyllum* from different ecological habitats, especially from Kukumseri, Triloki Nath, Throat and Mayar Valley in Lahaul and Lossar and Tabo in Spiti region of Himachal Pradesh.The International Crops Research Institute for Semi-Arid Tropics Patancheru has also made several collection missions in Afghanistan, Turkey, Syria and Pakistan and collected many samples of *C. microphyllum, C. nuristanicum* and *C. macrocanthum* species.

Germplasm introduction and conservation

Scientific activities on the introduction and conservation of genetic resources pertaining to agricultural crops for breeding purpose has been taken up after the classical work (Vavilov 1926) on the centres of origin and realization by plant breeders for its significance in genetic improvement. Chickpea, being an important pulse crop received due attention in the introduction and conservation of genetic resources in India. So far, 56,925 accessions including trial lines were introduced from 56 countries (Gautam et al. 2000).The International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria has been an important source of introduction from where about 17,880 accessions of chickpea were introduced for their use in the national breeding programmes. Some promising exotic accessions were also introduced from Syria, USA, Spain, Australia, Bangladesh, Israel, Afghanistan and Greece. A set of 105 accessions of global wild annual *Cicer* species was also introduced from the Biodiversity and Integrated Gene Management Unit (BIGM) of ICARDA (Singh et al. 2014). However, R. S. Paroda gene bank at ICRISAT holds about 20,267 accessions including wilds (GCDT, 2014).The major chickpea germplasm including wild species preserved in *ex-situ* collections in different gene banks around the world are presented in Table 1. Chickpea has orthodox seeds, which can be dried and stored for a long period with a minimum loss of seed viability. For conservation purpose, accessions are assigned a national identity number, dried to seed-moisture of around 5±2 per cent at 18^{o}C and 15 per cent relative humidity. The accessions meeting international standards for their conservation, seed viability should be more than 85 per cent and quantity of about 2,000 seeds, are transferred to long-term storage (LTS) in the gene bank for future use.

Germplasm characterization and evaluation

Adequate characterization and evaluation for agro-morphological traits is necessary to facilitate utilization of germplasm by breeders. To achieve this, a large number of germplasm accessions of chickpea have been characterized and evaluated in batches over the years in various institutions across India. The genetic variation ranges for some desirable traits namely, plant pigmentation (green to high pigmented), growth habit (erect, semi-erect, spreading, semi-spreading and prostrate), flower colour (blue, light blue, dark pink, pink, light pink, white and white-pink striped), seed coat colour (black, brown, light brown, dark brown, reddish brown, greyish brown, salmon brown, grey, brown beige, beige, yellow, light yellow, yellow brown, orange yellow, orange, yellow beige, ivory white, green, light green, variegated and black brown mosaic), plant height (14-105 cm), plant width (13-124 cm), days to flowering (33-107 days), flowering duration (13-75 days), days to maturity (84-169), number of pods/plant (2-238 pods), seeds/pod (1.0-3.2 seeds), seed weight (3.8-59 gm), seed shape (Angular, Owl's head, Pea shaped), seed testa texture (rough, smooth, tuberculated), seed yield (70-5130 kg/ha) and seed protein (12-29.6%) (www.icrisat. org; (Narayan and Macefield 1976; Singh and Tuwafe 1980; Pundir et al. 1985; Teshale 1987; Arora and Tripathi 1991; Jana and Singh 1993; Shukla 1998). First large scale evaluation of chickpea germplasm for various agro-morphological traits was taken up by Narayan and Macefield (1976). They evaluated 5,477 accessions for various yield attributing traits. This was followed by a no. of other relevant evaluations for resistance to major diseases. Elite genetic resources were identified for several biotic stresses *viz*., wilt (Satpute and Rao 1995; Halila and Strange 1997), *Ascochyta* blight (Muhammad et al. 1985; Wadud and Riaz 1988; Singh and Reddy 1993; Reddy et al. 1983), collar rot (Sugha et al. 1991), stunt (Shukla et al. 1985), root knot nematode (Gupta and Verma 1989), bruchids (Ahmad et al. 1995) and leaf miner (Singh and Weigand 1996). As far as maintenance of germplasm is concerned, ICRISAT has developed a core collection consisting of 1956 accessions and mini core set of 211

Table 1. Chickpea germplasm holdings in different gene banks of the world

Country/gene bank	Institution	No. of wild accessions	No. of cultivated accessions
Global gene bank	International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru Hyderabad, India	308	19959
Australia	Australian Temperate Field Crops Collection (ATFC)	241	8414
Bangladesh	Bangladesh Agricultural Resources Institute (BARI)	-	752
Brazil	Embrapa Hortalicas	-	775
Canada	Agriculture and Agri-Food Canada	2	507
Ethiopia	Institute of Biodiversity Conservation	-	1173
Germany	Leibniz Institute of Plant Genetics and Crop Plant Research	11	522
Greece	Fodder Crops and Pastures Institute	-	445
Hungary	Institute for Agro Botany	5	1165
India	National Bureau of Plant Genetic Resources	241	14651
India	Regional Station, Akola	-	813
Iran	Tehran University	-	1200
Iran	National Plant Gene Bank of Iran	-	5700
Japan	National Institute of Agrobiological Sciences	-	682
Mexico	Instituto Nacional de Investigaciones Agricolas	-	1600
Pakistan	Plant Genetic Resources Institute	24	2122
Philippines	University of the Philippines	-	407
Russian Federation	N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry	-	2091
Spain	Instituto Nacional de Investigacion y TecnologiaAgraria y Alimentaria, Centro de Recursos Fitogeneticos	-	644
Spain	Instituto Andaluz de Investigacion Agroalimentaria y Pesquera, Centro de Investigacion y Formacion Agroalimentaria Cordoba	-	608
Syrian Arab Republic	International Centre for Agricultural Research in Dry Areas	270	13,192
Turkey	Plant Genetic Resources Department	22	2054
Ukraine	Institute of Plant Production n.a. V.Y. Yurjev of UAAS	-	1021
USA	Western Regional Plant Introduction Station, USDA-ARS, Washington State University	202	6561
Uzbekistan	Uzbek Research Institute of Plant Industry	-	1055

Source: Global crop diversity trust (2014)

accessions (Upadhyaya et al. 2001). However, on the basis of allelic diversity data of global composite collection, a reference set of most diverse 300 accessions was also developed (Upadhyaya et al. 2008). Further, by using the core and mini core collections, various germplasm lines have been selected for agro-morphological traits, biotic and abiotic stresses at ICRISAT and other national chickpea improvement institutions the country. Furthermore, the NBPGR at New Delhi has also characterized 14651 accessions of chickpea and developed a core set of 1103 accessions using qualitative and quantitative data (Archak et al. 2016). Singh et al. (2014) characterized and evaluated a set of 88 wild annual *Cicer* accessions under two growing conditions of north-western India. The frequency distribution of seven annual *Cicer* species exhibited a wide range of intraspecific variation for some of the important morphological plant characteristics. Plant pigmentation showed variation in *C. reticulatum*, *C. judaicum*, and *C. pinnatifidum*

along with lightly pubescent leaves, except *C. yamashitae*, where it was densely pubescent. Number of leaflets leaf^{-1} also showed remarkable variation in the majority of *Cicer* species except in *C. yamashitae*. Likewise, in most of the *Cicer* species, seed shape was angular, with the exception of *C. arietinum* and *C. bijugum*, which were irregular, rounded and pea shaped, respectively. Testa texture was rough in *C. arietinum, C. reticulatum, C. judaicum, C. pinnatifidum, and C. yamashitae,* and in *C. echinospermum* and *C. bijugum*, it was tuberculated of pattern. Substantial variation in seed color was observed in *C. arietinum*, *C. reticulatum*, *C. judaicum*, and *C. pinnatifidum*. The seed colour is black in *C. echinospermum* and *C. yamashitae* and brown in *C. bijugum*. The summary of evaluation of *Cicer* species against important agro-morphological traits including major biotic and abiotic stresses is presented in Table 2 and Fig. 1.

Germplasm diversity and characterization

Molecular markers are very useful in elucidating the genetic diversity of germplasm and tagging genes of economic significance. Simon and Muehlbauer (1997) developed an integrated genetic linkage map of chickpea with 9 morphological, 27 isozyme, 10 RFLP and 45 RAPD markers covering the length of 550 cM. Ahmad (1999) and Sudupak et al. (2002) used Random Amplified Polymorphic DNA (RAPD) markers to study the genetic relationships among annual *Cicer* species and suggested that *C. arietinum, C. reticulatum* and *C. echinospermum* grouped in one cluster and *C. yamashitae* and *C. chorassanicum* in second cluster. However, *C. pinnatifidum, C. bijugum* and *C. judaicum* fell in third cluster and *C. cuneatum* is accommodated separately in fourth cluster.

Paucity of polymorphic molecular markers in chickpea has been a major limitation in the improvement of this important legume. Hence, in an attempt to develop sequence-tagged microsatellite sites (STMS) markers from chickpea, a microsatellite enriched library from the *C. arietinum* cv. Pusa 362 nuclear genome was constructed for the identification of CA/GT, and CT/GA, microsatellite motifs. A total of 92 new microsatellites were identified, of which 74 functional STMS primer pairs were developed by NIPGR, India. These markers were validated using 9 chickpea and one *C. reticulatum* accession. Further, cloning and sequencing of size variant alleles at two microsatellite loci revealed that the variable numbers of AG repeats in different alleles were the major source of polymorphism. Point mutations were found to occur both within and immediately upstream of the long tracts of perfect repeats, thereby bringing about a conversion of perfect motifs into imperfect or compound motifs. Such events possibly occurred in order to limit the expansion of microsatellites and also lead to the birth of new microsatellites. The microsatellite markers developed in this study will be useful for genetic diversity analysis, linkage map construction as well as for depicting intraspecific microsatellite evolution (Sethy et al. 2006).

At ICRISAT, SSR markers are being screened on a panel of 12 parental genotypes representing six intraspecific mapping populations derived from ICC 506EB × Vijay, ICC 3137 × IG 72953, ICC 3137 × IG 72933, ICC 283 × ICC 8261, ICC 4958 × ICC 1882, ICCV 2 × JG 62 crosses and one interspecific (*C. arietinum* × *C. reticulatum*) reference mapping population, ICC 4958 × PI 489777 (Varshney et al. 2007). A total of 20,162 (18,435 high quality) drought- and salinity- responsive ESTs were generated from ten different root tissue cDNA libraries of chickpea (Varshney et al. 2009). BLASTN analysis of unique sequences with ESTs of four legume species (*Medicago, Lotus*, soybean and groundnut) and three model plant species (rice, *Arabidopsis* and poplar) provided insights on conserved genes across legumes as well as novel transcripts for chickpea. Of 2,965 (46.3%) significant unigenes, only 2,071 (32.3%) unigenes could be functionally categorised according to Gene Ontology (GO) descriptions. A total of 2,029 sequences containing 3,728 simple sequence repeats (SSRs) were identified and 177 new EST-SSR markers were developed. Besides SSR markers, 21,405 high confidence single nucleotide polymorphisms (SNPs) in 742 contigs (with $\geq$ 5 ESTs) were also identified

Jain et al. (2013) generated a 520 Mb draft genome sequence of *C. arietinum* using next-generation sequencing (NGS) platforms, bacterial artificial chromosome (BAC) end sequences and a genetic map to facilitate genetic improvement of chickpea varieties. Subsequently, Varshney et al. (2013) reported ~738-Mb draft whole genome sequence of *C. arietinum* for trait improvement, whose advanced version was later published by Parween et al. (2015). Patil and Kamble (2014) provided comparative protein profiling of wild chickpea and its induced mutants to assess genetic variation among mutants and parental genotypes. Further, a study of Khatodia et al. (2014) produced transgenic chickpea plants expressing *cry1Aa3* gene, which showed resistance against *Helicoverpa armigera*, thus providing an opportunity

Table 2. Sources of useful traits identified in *Cicer* species for introgression of useful traits into elite genetic background of chickpea

Trait of interest	*Cicer* species	Reference
Biotic stress		
Ascochyta blight	*C. arietinum, C. judaicum, C. reticulatum, C. montbretii, C. bijugam, C. pinnnatifidum, C. cuneatum, C. echinospermum*	Vander Maesen and Pundir 1984; Singh and Reddy 1993; Singh et al.1994; Singh et al. 1998; Pande et 2010; Collard et al. 2001; Collard et al. 2003; Ahmad et al. 2013; Shah et al. 2005; Pande et al. 2005; Pande et al. 2006; Kaur et al. 2013; Singh et al. 2014; Benzohra et al. 2014
Fusarium wilt	*C. arietinum, C. reticulatum, C. bijugam, C. judaicum, C. pinnnatifidum, C. echinospermum, C. cuneatum*	Nene et al. 1980; Vander Maesen and Pundir 1984; Kaiser et al. 1994; Infantino et al. 1996; Nguyen et al. 2004; Singh et al. 1994; Singh et al. 2005; Ahmad et al. 2013
Bruchids	*C. reticulatum*	Singh et al. 2010
Botrytis grey mould	*C. judaicum, C. bijugam, C. pinnnatifidum, C. reticulatum*	Singh et al. 1982; Vander Maesen and Pundir 1984; Haware et al. 1992; Pande et al. 2006; Basandrai et al. 2006; Basandrai et al. 2008; Kaur et al. 2013; Singh et al. 2014
Cyst nematode resistance	*C. bijugam, C. pinnnatifidum, C. reticulatum,*	Greco Di Vito 1993; Singh et al. 1994; Di Vito et al. 1996; Ahmad et al. 2013; Singh et al. 2010
Rust resistance	*C. bijugam, C. reticulatum, C.echinospermum*	Sillero and Alias 2012
Root knot nematode	*C. bijugum, C. judaicum, C. pinnnatifidum, C. reticulatum, C. echinospermum*	Singh et al. 2014
Resistance to soil borne diseases	*C. bijugum, C. cuneatum,C. judaicum, C. pinnnatifidum*	Reddy et al. 1991
Phytophthora root rot resistance	*C. reticulatum, C. bijugum, C. pinnnatifidum, C. Echinospermum*	Knights et al. 2008
Root-lesion nematodes	*C. echinospermum, C. reticulatum,*	Thompson et al. 2011
Stem rot	*C. reticulatum, C. pinnatifidum, C. judaicum, C. yamashitae*	Singh et al. 2007; Kaur et al. 2008
Helicoverpa pod borer tolerance	*C. bijugum, C. reticulatum, C. echinospermum, C. cuneatum, C. pinnatifidum, C. Microphyllum*	Kaur et al. 1999; Sharma 2004; Sharma et al. 2006
Leaf miner	*C. reticulatum, C. judaicum, C. bijugam, C. cuneatum*	Singh and Weigand 1994; Singh et al. 1994; Singh et al. 2010
Seed beetle	*C. cuneatum, C. judaicum, C. reticulatum, C. echinospermum*	Gupta and Parihar 2015
Abiotic stress		
Cold tolerance	*C. echinospermum, C.reticulatum, C. bijugum, C. pinnnatifidum, C. judaicum*	Singh et al. 1990; Singh et al. 1994; Sandhu 2004; Toker 2005; Berger et al. 2005
Drought tolerance	*C. anatolicum, C. reticulatum C. microphyllum, C. oxydon, C. montbrettii, C. pinnnatifidium, C. songaricum, C. echinospermum*	Toker et al. 2007; Canci and Toker 2009
Yield attributes		
Yield attributes	*C. reticulatum, C. pinnatifidum*	Jaswal and Singh 1989; Singh and Ocampo 1997; Singh et al. 2005; Singh et al. 2012; Singh et al. 2014
High no. of seeds plant^{-1}	*C. cuneatum, C. montbretii*	Robertson et al. 1995; Robertson et al. 1997; Singh et al. 2014

Fig. 1. Phenotyping and identification of promising accessions from the global wild annual *Cicer* collection for various traits of interest

to reach field efficacy level. Although quantitative trait loci (QTLs) controlling agro-morphological traits in chickpea were identified, a genome-wide scanning of wild *Cicer* accessions was lacking until revealed by the study of Saxena et al. (2014) and Das et al. (2015). Upadhyaya et al. (2016) reported QTL analysis for delineation of candidate genes for marker-assisted selection. Moreover, studies of Saxena et al. (2014) on allelic diversity, genetic structure and linkage disequilibrium pattern in wild *Cicer* species and Gupta et al. (2015) on ESTs from *C. arietinum* that has provided expediency in genetics, genomics and breeding of chickpea germplasm. Khajuria et al. (2015), Kujur et al. (2015) and Bajaj et al. (2015) employed genome-wide polymorphic SSR and single nucleotide polymorphism (SNP) markers to extrapolate trait-specific genes, allelic diversity and domestication patterns in chickpea. Thereafter, Das et al. (2015) and Srivastava et al. (2016) made use of insertion-deletion (InDel) markers for improving chickpea yield by identifying QTLs for breeding purposes. Also, recent reports of Srivastava et al. (2016) and Gupta et al. (2016) on RNA-seq analysis of *C. microphyllum* and draft genome sequence of *C. reticulatum*, have opened new advances in agronomic trait improvement of chickpea.

Genome mapping in chickpea

International Chickpea Genome Sequence Consortium has completed genome sequencing of CDC Frontier, a kabuli variety (http://www.icrisat.org/gt-bt/ICGGC/GenomeSequencing.htm). On the other hand ICC 4958, a *desi* landrace has been targeted and sequenced at NIPGR, New Delhi. In recent years, STMS markers were indeed applied for the generation of almost all published genetic maps of chickpea developed employing populations from crosses between *C. arietinum* and *C. reticulatum*, molecular marker based diversity and structural analysis (Bharadwaj et al. 2011a; Thudi et al. 2011; Choudhary et al. 2012; Subodh et al. 2015; Shefali et al. 2015). Several intra-specific mapping populations have also been used to identify the markers associated with traits like resistance to *Fusarium* wilt. Though, STMS markers were applied for the generation of almost all published genetic maps of chickpea, most genomic regions harbouring genes for important traits are not yet sufficiently saturated with co-dominant markers to apply MAS in plant breeding programs.

Registration and documentation of germplasm

Germplasm registration is an essential component for systematic and effective utilization in crop improvement programmes. The NBPGR has been designated as a nodal institute for germplasm registration. The first catalogue on chickpea, containing information on 25 descriptors of about 15,000 accessions was published by Pundir et al. (1998). Various germplasm accessions of chickpea have been registered at NBPGR, for specific characters: (1) IC 296691 (CGS 88101) with INGR number 98008 for salinity tolerance (2) IC 296738 (K850 LM), INGR13008 (GL84100) and INGR13009 (GL87045) for *Ascochyta* blight resistance; INGR number 99016 for multi-pinnate leaf with shorter internodes, (3) IC 296887 (H96-99) INGR 02003 for compact and tall plant type, (4) IC 296886 (EIOY(m) INGR 96008 for erect and dwarf growth habit, (5) IC 296430 (H-82-2(M) INGR 03031 for fast early vigor, early flowering and maturity with long internodes, (6) IC 395465 (chickpea mutant) INGR 03061 for fascinated broadened stem mutant, (7) IC 395466 (JMG-4) INGR 03062 to broad leaflets, (8) IC 395467 (OCW-JGM-5) INGR 03063 for curved flower, (9) IC 395468 (cymose inflorescence) INGR 03064 for mutant with cymose inflorescence, (10) IC573446 INGR 09108 for high yield dwarf and bushy type and (11) IC486088 INGR 13058 for upright podding and peduncle breeding behaviour.

Pre-breeding and germplasm utilization

Chickpea has intrinsically narrow genetic base in India. That limits plant breeder's progress today. The existing variability among indigenous germplasm has been exploited to reach to a maximum level of productivity. Wild *Cicer* species and exotic germplasm lines hold a wealth of useful alleles that, if we find them, can help in breaking yield barriers and enhance tolerance to array of stresses for stability (Labdi et al. 1996; Tayyar et al. 1996; Ahmad and Slinkard, 2003; Ahmad et al. 2005). However, systematic screening of wild *Cicer* collections by ICRISAT, Patancheru, IIPR Kanpur, IARI, New Delhi and NBPGR, New Delhi including some State Agricultural Universities (PAU, Ludhiana and MPKV, Rahuri Maharashtra) have prompted to initiate genetic base broadening activities. About 300 wild annual *Cicer* accessions and landraces have been characterized and evaluated for various agro-morphological traits and biotic and abiotic stresses. Chickpea landraces and the wild species are the repertoire of the genes which provide tolerance to abiotic and biotic stresses. Thirty STMS primer pairs were used to dissect the genetic diversity and relationship of 14 wild and one cultivated accessions of chickpea by Shubha et al. (2011). The thirty five

STMS primer pairs generated on an average 3.433 amplicons per primer pair. Polymorphic Information content (PIC) ranged from 0.246 to 0.775 and genetic similarity between cultivars ranged from 0.10 to 0.77. Dendrogram constructed after STMS marker data showed four distinct clusters with a tendency of accessions of similar species clustering together. They inferred that the secondary gene pool is very diverse and could introduce wide variations if used in breeding. Bharadwaj et al. (2011) studying molecular diversity and phylogeny in a geographical collection of chickpea which included lines from ICRISAT and ICARDA gene banks inferred that the cultivated chickpea lines from ICRISAT which are of Indian origin were grouped together while the wild species and ICARDA lines which originated from West Asia and North Africa (WANA) formed a distinct group. They advocated that greater genetic gains could be achieved by crossing Indian lines with the landraces of WANA region. Studying the genetic diversity of the primary gene pool, Choudary et al. (2011) opined that most of the diversity existed in the wild species while the structure analysis revealed a low polymorphism for SSR markers in the cultivated lines. Tapan et al. (2015) inferred that chickpea incurs heavy yield losses due to terminal heat and drought as it is largely grown under rainfed restricted irrigated conditions on residual soil moisture. Exploring the extent of natural variation among cultivated chickpea for drought tolerance is important to develop pre-breeding and breeding strategies for chickpea. Thirty seven landraces representing seven countries and fourteen provinces obtained from ICARDA and three bold seeded *kabuli* varieties each from IIPR Kanpur and MPKVV, Rahuri were evaluated for their Relative Water Content (RWC) and Membrane Stability Index (MSI), the established physiological parameters for drought tolerance. The analysis into MSI has indicated wide variability in the landraces for drought tolerance. RWC followed a similar pattern to MSI. The genotypes that were having higher MSI also had higher RWC indicating the soundness of the traits. The genotypes *viz*., IG5844a, 5856, 5867, 5884, 5887, 5894, 5896, 5906 and 5908 were found tolerant to drought based on RWC and MSI values and were proposed to be donors for drought tolerance.

Wild *Cicer* species and landraces possess substantial amount of genetic diversity and resistance to *Fusarium* wilt in *C. reticulatum*, *C. echinospermum*, *C. pinnatifidum* and *C. judaicum* (Nene and Haware, 1980; Singh et al. 1994; Kaiser et al. 1994; Infantino et al. 1996; Stamigna et al. 1998; Singh et al. 1998; Singh et al. 2005); *Ascochyta* blight resistance in *C. reticulatum*, *C. echinospermum*, *C. bijugum* and *C. cuneatum* (Singh et al. 1982; Singh and Reddy, 1993; Collard et al. 2001; Singh et al. 2014). Likewise, Botrytis grey mould resistance in *C. bijugum* (Singh et al. 1994; Kaur et al. 2013; Singh et al. 2014) and *Cyst* nematode resistance in *C. reticulatum* and *C. bijugum* (Singh et al. 1989; Singh and Reddy, 1991). Bruchid (seed beetle) resistance in *C. pinnatifidum*, *C. echinospermum* and *C. reticulatum* (Singh et al. 1994; Singh et al. 1998). Among abiotic stresses, cold tolerance in *C. judaicum*, *C. bijugum* and *C. microphyllum* (Chandel 1984; Sillero and Alias; 2012; Singh et al. 2007) and drought tolerance in *C. microphyllum* (Toker et al. 2007; Canci and Toker 2009). Further, overall performance for most of the abiotic stress resistance has been reported in *C. bijugum*, *C. pinnatifidum* and *C. judaicum* (Singh et al. 1994 and Singh et al. 1998). As far as utilization of wild *Cicer* species is concerned, several interspecific crosses have been attempted between *Cicer arietinum* and its annual wild relatives. There has been no report of successful hybridization between a perennial *Cicer* species and *Cicer arietinum.* Thus, interspecific hybrids between *Cicer arietinum* x *Cicer reticulatum* and *Cicer arietinum* x *Cicer echinospermum* have been successfully attempted by several workers (Verma et al. 1990). Verma et al. (1990) made large number of crosses between cultivated as female parent and *Cicer reticulatum*, *C. echinospermum*, *C. judaicum*, *C. bijugum* and *C. pinnatifidum*as male parents. They suggested the overall success rate of seed set 1.69%. However, other interspecific crosses have also been made at Indian Institute of Pulses Research, Kanpur, Indian Agricultural Research Institute, New Delhi and National Bureau of Plant Genetic Resources, New Delhi; Punjab Agriculture University, Ludhiana and Mahatma Phule Krishi Vidya Peeth, Rahuri and incorporated useful trait of interest. Certain pre-breeding lines have been developed from interspecific crosses at IIPR, Kanpur and PAU, Ludhiana (Singh et al. 2012). Among them, IPC71 (*Cicer arietinum* x *Cicer judaicum*) has been used as donor parent under National Chickpea Crossing Program. Singh et al. (2015) also attempted interspecific crosses and the study revealed a high level of heterosis for number of podsplant^{-1} and seed yieldplant^{-1} in F_1 generation. Three cross-combinations of Pusa 1103 x ILWC 46, Pusa 256 x ILWC 46 and Pusa 256 x ILWC 239 exhibited substantially higher variability for important yield-related traits.

The International Crops Research Institute for

Semi-Arid Tropics, Patancheru has led to the release of more than 50 genotypes directly as cultivars in various countries like, Australia, Algeria, Bangladesh, Cyprus, Ethiopia, India, Italy, Myanmar, Nepal, Oman, Syria, Sudan, Turkey and USA (http://ICRISAT.org.). Long back local germplasm was directly used for the development of cultivars to meet the immediate requirement. The most important varieties are Chaffa, Dohad yellow, BDN 9-3, Annegeri-1, JG 315, JG 74, Pragati and BG 287 and these selections are still popular in many parts of India. Thereafter, variability created through hybridization between germplasm accessions having desired characters and the widely adapted genotypes has been utilized. Further, Kumar et al. (2004) suggested that out of 126 chickpea released cultivars in the past four decades, in which most frequently used parental lines were PB7, IP58, F8 and S26. Therefore, narrow genetic diversity among modern released cultivars rendering them more vulnerable to array of stresses and adaptation. About 16 chickpea cultivars were developed from the best use of exotic germplasm for desirable traits like, bold seed, wider adaptation, Ascochyta blight resistance, drought tolerance and early maturity. Where, Pusa 261 was developed by utilizing P827 from Morocco and P9847 from Russia in a two-way cross approach. Likewise, Pusa 244 and Pusa 267 were developed from three-way cross including two exotic parents with one indigenous cultivar. However, pedigree analysis of all chickpea released cultivars revealed the use of only 87 accessions indicating insignificant part of germplasm utilization in chickpea breeding in India.

Future perspectives

The productivity of chickpea is compounded by narrow genetic base, biotic and abiotic stresses. The evaluation and identification of wild *Cicer* species will greatly aid in trait discovery. With the use of modern molecular tools and pre-breeding activities some useful traits can be introgressed in to cultivars, which can be further used to augment the yield of chickpea. The earlier studies indicated the usefulness of exploiting existing variability for broadening the genetic base in chickpea both at phenotypic and molecular levels. Since, most of the desirable gene complexes are present in non-crossable secondary and tertiary pools, there is an urgent need to augment germplasm collections in the primary gene pool and landraces. An identification of areas is required for further exploration and targeted trait specific collections particularly from WANA regions. Collaborative efforts among national and international research institutions would help the evaluation of chickpea germplasm systematically at hot spot centers.

Declaration

The authors declare no conflict of interest.

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