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Genomic tools and germplasm diversity for chickpea improvement

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Abstract

Chickpea is third most important grain legume grown in the arid and semi-arid regions of the world. In spite of vast germplasm accessions available in different genebanks, there has been very limited use of these accessions in genetic enhancement of chickpea. However, in recent years specialized germplasm sub sets like global composite collection, core collection, mini core collection and reference set have been developed. In parallel, significant genomic resources like molecular markers including simple sequence repeats (SSRs), single nucleotide polymorphsims (SNPs), Diversity Arrays Technologies (DArT) and transcript sequences e.g. expressed sequence tags (ESTs), short transcript reads have been developed. By using SSR, SNP and DArT markers, integrated genetic maps have been developed. It is anticipated that use of genomic resources and specialized germplasm sub sets such as mini core collection and reference set will facilitate identification of trait-specific germplasm, trait mapping and allele mining for resistance to biotic and abiotic stresses and for agronomic traits. Recent advances in genomics and bioinformatics offer the possibility of undertaking large scale sequencing of germplasm accessions so that modern breeding approaches such as genomic selection and breeding by design can be realized in near future for chickpea improvement.

Key words: germplasm repositories, reference collection, genomic resources, allelic diversity

Introduction

Chickpea (*Cicer arietinum* L.) is one of the oldest (earlier than 9500 BC) and widely cultivated pulse crops in over 50 countries of the world. Chickpea is a member of the West Asian Leolithic crop assemblage, associated with the origin of agriculture in the Fertile Crescent some 10,000 years ago (Lev-Yadun *et al*., 2000; Zohary and Hopf, 2000). It most probably originated in south-eastern Turkey and adjoining Syria. *C. bijugum*, *C. echinospermum*, and *C. reticulatum*, the wild annual species of *Cicer*, closely related to chickpea are predominantly found in this region. South-west Asia and the Mediterranean are the two primary centres of origin, and Ethiopia the secondary centre of diversity (Vavilov, 1926; 1951). Wild annual *Cicer* originated mainly in the Mediterranean regions having a wide ecogeographic range, differing in habitat, topographic and climatic conditions (Abbo *et al*., 2003; Berger *et al*., 2003). The four evolutionary bottlenecks in chickpea reported are (i) scarcity and limited distribution of the wild progenitor, *C. reticulatum*, (ii) founder effect associated with domestication, (iii) shift, early in the crop's history, from winter to spring sowing, and the attendant change from using rainfall as it occurs to a reliance on residual soil moisture, and (iv) replacement of locally evolving landraces by the elite cultivars produced by modern plant breeding (Abbo *et al*., 2003).

Chickpea is a self-pollinated crop, with $2n = 2x = 16$ chromosomes and a genome size of 740 Mb (Arumuganathan and Earle, 1991). Two distinct forms of cultivated chickpeas are desi (small seeds, angular shape, and coloured seeds with a high percentage of fibre) and kabuli types (large seeds, owl-head shape, beige coloured seeds with a low percentage of fibre). A third type, designated as intermediate or pea-shaped, is characterized by medium to small size, and round/pea-

shaped seeds. Hair like structures on its stems leaves and pods secrete acids that provide the first line of defense against pests, reducing the need for chemical sprays (Yadav *et al*., 2007).

Chickpea seeds contain protein, fiber, calcium, potassium, phosphorus, iron, zinc and magnesium along with appreciable quantities of selenium, sodium and copper, which make it one of the nutritionally best composed edible dry legumes, for human consumption (Esha, 2010). Chickpea like most other beans is a good source of cholesterol lowering fiber (Pittaway *et al*., 2006). In addition to lowering cholesterol, the high fiber content prevents blood sugar levels from rising too rapidly after a meal, making chickpea a good choice for individuals with diabetes, insulin resistance or hypoglycemia (McIntosh and Miller, 2001). Chickpea does not contain any anti-nutritional factors except the raffinose type oligosaccharides which cause flatulence (Williams and Singh, 1987). However the oligosaccharides can be neutralized by boiling or mere soaking in water (Queiroz *et al*., 2002).

Chickpea is the most important legume in Asia, which contributes 86.73% of global production from 89.89% area. The world 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, 2009). India accounts for 67.68% of this area (7.50 Mha), and 66.91% (6.54 Mt) of production followed by Pakistan (with 9.75% of area: 1.08 Mha and 0.741 Mt). Chickpea is also an important crop in Iran (0.56 Mha), Turkey (0.45 Mha) Myanmar (0.20 Mha), Australia (0.36 Mha), Ethiopia (0.23 Mha), Canada (0.04 Mha), Mexico (0.11 Mha), Syria (0.07 Mha), USA (0.04 Mha), Spain (0.02 Mha), and Eritrea (0.02 Mha) (FAO, 2009).

Chickpea is traditionally grown extensively as a low input crop under receding soil moisture status with minimum management. Despite its high morphological variability, genetic variation is low (Udupa *et al.*, 1993), probably a consequence of its monophyletic decadence from its wild progenitor *C. reticulatum* in the Fertile Crescent (Ladizinsky and Adler, 1976; Lev-Yadun *et al*., 2000; Abbo *et al*., 2003). The major constraints to chickpea productivity are biotic stresses like *Helicoverpa* pod borer and fusarium wilt, abiotic stresses like drought, extreme temperatures and salinity, apart from its poor response to better management. The progress achieved through conventional breeding for improved varieties is not in pace with the current requirements, which is evident from the stagnant production of chickpea during past two decades (see Varshney *et al*., 2010b). With the exception of soybean, to various extents legume crops, including chickpea, have suffered from lack of genomic resources for genetic and genomic analysis — they have literally been 'orphans' from the genomics revolution (Varshney *et al*., 2009a). Recent years have seen tremendous progress in the development of large scale genomic resources such as DNA-based molecular markers, comprehensive genetic maps, whole-genome transcription profiling techniques to identify genomic regions and genes underlying plant stress responses (see Varshney *et al*., 2009a; Varshney *et al*., 2010b). These genomic tools will be useful to understand and access the diversity conserved in *ex situ* germplasm collections for chickpea improvement (Glaszmann *et al*., 2010). This article discusses the global status of germplasm collection, development of mini core and reference sets, identification of trait-specific germplasm, advances in development of genomic resources and the utility of genomic and germplasm resources for chickpea improvement.

Germplasm Assembly

The genus *Cicer* has 43 species out of which *C. arietinum* is the only cultivated species. The species *C. arietinum* has wide variability with thousands of landraces spread over 50 countries and a large number of traditional cultivars which were grown and maintained by farmers worldwide. However, after the introduction of modern high yielding genetically uniform varieties much of the species diversity has been lost due to replacement of traditional varieties and landraces over wide areas. In addition, change in dietary habits, natural calamities, land and crop conversion (deforestation, developmental activities such as hydroelectric projects, roads, and urbanization), introduction of exotic crops etc. have further aggravated the situation. The vulnerability of genetically uniform modern varieties, which are planted to large areas, to new pests, diseases, climatic conditions and changes in the market needs is widely acknowledged. The diverse landraces, exotics and wild relatives hold a wealth of alleles, which, if included in breeding programs can help raise the yield levels and enhance stress resistance level of agronomically superior cultivars. This emphasized the need for preservation of germplasm which led to assembling and maintaining a very large number of germplasm accessions (over 97,400) by many countries and conserving them in their genebanks (WIEWS-FAO, 2009).

The ICRISAT genebank has the largest collection of 20,267 accessions from 60 countries including 308 accessions of 18 (8 annual and 10 perennial) wild *Cicer* species. Of these 4153 accessions were obtained from 65 collection missions in 15 countries and the remaining were donations from 19 countries. Two hundred thirty three out of the 308 wild accessions were donations from six countries and the remaining (75) were collected from Afghanistan, Turkey, Syria and Pakistan.

Germplasm repositories

Although germplasm exchange and plant introduction have been in practice sporadically for centuries. However, purposeful efforts started only in the 1920s and genebanks have been established in different countries. The major chickpea germplasm repositories (*ex situ*) in the world are listed in Table 1.

Characterization and evaluation

The characterization, evaluation and documentation of the germplasm are essential for utilization in crop improvement and for efficient management. Therefore all the chickpea accessions have been characterizes and evaluated at ICRISAT research farm, Patancheru, India (18^{o}N, 78^{o}E, 545 m.a.s.l.) for 7 qualitative and 13 quantitative traits, following the chickpea descriptors (IBPGR, ICRISAT and ICARDA, 1993). A multi-disciplinary approach is followed for characterization and evaluation of chickpea germplasm for biotic and abiotic stresses, agronomic traits and for updating and maintenance of databases. These germplasm accessions contain very useful diversity for crop improvement. Evaluation of wild species had resulted in identification of genes for resistance to botrytis grey mold in *C. judaicum* and *C. pinnatifidum* (Singh *et al*., 1982); for ascochyta blight in *C. bijugum* , *C. pinnatifidum* and *C. yamashitae* (Shah *et al*., 2005); for fusarium wilt in *C. bijugum* (Infantino *et al*., 1996). Two wild species *C. echinospermum* and *C. reticulatum* are both cross compatible with the cultivated *C. arietinum* and are reported to be resistant to several pests (cyst nematodes, leaf minor and bruchids) and diseases (fusarium wilt, ascochyta blight and Phytophthera), apart from tolerance to cold (Dwivedi *et al*., 2005).

Geographic patterns of diversity

The primary center of diversity is the Fertile Crescent (Abbo *et al*., 2003), where the crop was domesticated and later spread to the secondary centers – the north-east Africa, the Mediterranean Europe and the Indian sub-continent and more recently to Mexico and Chile (van der Maesen, 1972). The distribution of old landraces and wild relatives of chickpea occurs in three main regions from 8^{o} to 52^{o} N latitude and 8^{o} W to 85^{o} E longitude covering the: i) western Mediterranean, Ethiopia, Crete and Greece, ii) Asia-minor, Iran and Caucasus, and iii) Central Asia, Afghanistan and the Himalayan region (van der Maesen, 1972). The ICRISAT's chickpea germplasm collection represents this entire area, showing wide range of variation for various morphological and agronomic traits. The level of diversity found among the traits indicate that West Asia region in which southwest Asia, one of the primary centers of diversity is located was adequately represented by 5564 (33.1%) accessions in the ICRISAT genebank. This was also demonstrated by the highest diversity for the morphological descriptors and agronomic traits observed in this region. The principal component analysis (PCA) based hierarchical cluster analysis resulted in two clusters. The accessions from Africa, South Asia and Southeast Asia grouped together as cluster-I and the accessions from rest of the countries (the Americas, Europe, West Asia, Mediterranean region and East Asia) formed cluster-II (Upadhyaya, 2003). The accessions in cluster-I were predominantly desi type, short statured, with low plant anthocyanin, pink flowers, angular shaped and rough, brown seeds of low seed weight where as most accessions in cluster-II were predominantly of kabuli type with no anthocyanin pigmentation, beige colored seeds with smooth seed surface and high 100-seed weight.

Morphological diversity

Large phenotypic diversity exists for morphological, reproductive, yield, nutrient content and biotic / abiotic stress tolerance related traits in the chickpea germplasm. The variability ranges for some valuable traits are : plant pigmentation (green to pigmented), growth habit (5 types), flower colors (seven colors), seed coat color (21 colors), plant height (14 -105 cm), plant width (13 -124 cm), days to flowering (31 -107 days), flowering duration (11 -75 days), days to maturity (84 -169 days), pod number per plant (2-263), seeds per pod (1 -3.2), seed weight (4 - 71 g), seed shape (3 types), seed testa texture (3 types), seed yield (70 - 5100 kg/ha) and seed protein (8 - 30 %) (http://icrtest:8080/what-we-do/crops/ChickPea/Project1/pfirst.asp).

Low use of genebank germplasm collection

ICRISAT has provided 313,976 (till Sept, 2010) chickpea seed samples to researchers in 86 countries The evaluation of the chickpea germplasm by national programs has led to the release of 17 accessions directly as cultivars in 15 countries (Table 2). A small proportion of chickpea germplasm at ICRISAT and other genebanks has been used in crop improvement programs. For example in the ICRISAT chickpea breeding program (1978-2004), only 91 were germplasm accessions among 12887 (586 unique) parents used in the development of 3548 advanced breeding lines (Upadhyaya *et al*., 2006b). Two most frequently used cultivars were L 550 and K 850. In India, out of 126 chickpea cultivars released in the past four decades, 41% of them had Pb 7 as one of the parents; IP 58, F 8, S 26 and Rabat were also the most extensively used parents (Kumar *et al*., 2004). Plant breeders frequently use parental lines from their working collections only, as they make reasonable and steady progress in most cases and broadening the

adapted genetic base generally will dilute agronomic performance (Kannenberg and Falk, 1995). Plant breeders consider elite inbred lines as the best genetic resources simply because each line contains a combination of genetic traits that satisfies the marketplace (Troyer, 1990). Yet new germplasm, if used in crop improvement programs can (1) raise the genetic ceiling on improvement, (2) decrease vulnerability to biotic and abiotic stresses, and (3) add new developmental pathways and ecological adaptations (Kannenberg and Falk, 1995).

Although plant breeders recognize the limitation of their working collections and the potential value of wild and landrace resources, they are often reluctant to use these resources for following reasons:

a) lack of reliable knowledge about stable donors for specific traits,

b) a linkage load of many undesirable genes,

c) lack of germplasm assessment for economic traits that show high genotype-environment interaction and require expensive, laborious and replicated multi environment evaluation,

d) assumed risks: while dealing with unknown and wild germplasm lines breeders are apprehensive about the possibility of complete program failures; timescales may be too long; or the value of the new varieties may never allow costs to be recouped. Additionally, there is the possibility of introducing toxic, allergenic, or pharmaceutically active plant products into food products, risks that are virtually absent in crossing elite, widely grown germplasm (Heslop-Harrison, 2002).

e) the need of plant breeders for genetically diverse, trait specific and agronomically desirable parents is not met by the information available in the genebank databases

f) the restricted access due to limited seed availability and regulations governing international exchange.

Advances in Development of Large-Scale Genomic Resources

Until recently, a very limited number of genomic resources such as few hundred molecular markers, some fragmentary genetic maps were available in chickpea. In the past five years, however, several national and international initiatives have tackled the challenge of dearth of genomic resources for genetics and breeding of chickpea (see Varshney *et al*., 2010b; Fig. 1). As a result various type of genomic resources like microsatellite or simple sequence repeat (SSR)/ sequence tagged microsatellite markers (STMS), expressed sequence tags (ESTs), single nucleotide polymorphism (SNP), cleaved amplified polymorphic sequences (CAPS), conserved intron spanning primers (CISP) and Diversity Arrays Technology (DArT) markers have been developed for chickpea.

SSR markers

SSR markers are considered the markers of choice for plant genetics and breeding applications (Gupta and Varshney, 2000). In case of chickpea, however, only few hundred SSR markers were available until recently (Table 3). It is also important to note that majority of these markers were developed from targeted SSRs for assaying variation in particular repeat motifs. Furthermore, low level of polymorphism especially in the cultivated germplasm of chickpea posed a need for development of large scale SSR marker. Hence in order to increase the molecular marker repertoire and to develop genome wide SSR markers, ICRISAT in collaboration with University of Frankfurt, Germany, developed 311 SSR markers from SSR-enriched libraries (Nayak *et al*.,

2010) and 1344 SSR markers from BAC-end sequence mining approaches in collaboration with University of California Davis, USA (unpublished data; Table 3). As EST sequences from various tissues and developmental stages of chickpea have also been reported (Boominathan *et al*., 2004; Romo *et al*., 2004; Buhariwalla *et al*., 2005; Coram and Pang, 2005; Varshney *et al*., 2009b, Choudhary *et al*., 2009), a few hundred SSR markers have been developed from ESTs (Buhariwalla *et al*., 2005, Varshney *et al*., 2009b, Choudhary *et al*., 2009). As a result of above mentioned efforts, >2000 SSR markers representing the entire chickpea genome are available at present.

Transcript sequences and SNP markers

Molecular marker technologies, however, are currently undergoing a transition from largely serial technologies based on separating DNA fragments according to their size (SSR, AFLP), to highly parallel, hybridization-based technologies that can simultaneously assay hundreds to tens of thousands of variations especially in genes. This transition has already taken place in several major crop species like rice (Nasu *et al*., 2002), maize (Yan *et al*., 2009), soybean (Wu *et al*., 2010), and common bean (Hyten *et al*., 2010). In case of chickpea, only few hundred ESTs and some reports on identification of SNPs were available until recently. Recent years have witnessed significant progress in development of comprehensive resource of transcripts by using Sanger sequencing as well as 'next generation sequencing' (NGS) technologies (see Varshney *et al*., 2009c) that are being deployed for understanding genome dynamics as well as development of SNP markers.

Sanger sequencing of a number of cDNA libraries constructed from drought- and salinity-challenged tissues has provided about 20,000 ESTs (expressed sequence tags) in chickpea (Varshney *et al*., 2009b). Two NGS technologies namely Roche 454/FLX and Illumina/Solexa have also been used to sequence the transcriptomes of reference genotype or parental genotypes of several mapping populations of chickpea to access the gene space and develop functional markers. For instance, ca. 500, 000 transcript reads have been generated after sequencing the pooled and normalized RNA isolated from >20 tissues from different developmental stages. Combined analysis of Sanger ESTs together with 454/FLX transcript reads provided 103,215 tentative unique sequences (TUSs) in chickpea. In parallel, RNA of four chickpea lines that represent parents of different mapping populations, have been sequenced by using Illumina/Solexa sequencing approach that has resulted ca. 118 million reads for chickpea. Alignment of these Illumina/Solexa reads of these genotypes with transcriptome assemblies of the respective species has provided a large number (tens of thousands) of SNPs. Selected set of SNPs are being used to develop large-scale SNP genotyping platform in chickpea that will augment recently developed GoldenGate assay platforms for 768 SNPs by University of California-Davis, USA, National Centre for Genome Resources (NCGR), USA and ICRISAT.

High-throughput genotyping DArT platform

DArT offers a rapid and DNA sequence-independent shortcut to medium-density genome scans of any plant species (Kilian *et al*., 2005). A single DArT assay simultaneously types hundreds to thousands of SNPs and insertion/deletion polymorphisms spread across the genome. Hence in collaboration with DArT Pty Ltd, Australia extended DArT arrays with 15,360 features for chickpea have been developed at ICRISAT (see Varshney *et al*., 2010a).

Genetic maps

Because of limited amount of genomic resources and a low polymorphism in cultivated germplasm, initial genetic mapping studies were restricted to inter-specific mapping populations. These mapping populations were derived from wide crosses between *C. arietinum* and *C. reticulatum* and between *C. arietinum* and *C. echinospermum* (Collard *et al*., 2003). While several research groups used the *C. arietinum* × *C. reticulatum* mapping population for developing genetic map by deploying a variety of molecular markers (Table 4), the mapping population based on *C. arietinum* × *C. echinospermum* cross has been used occasionally.

ICRISAT in collaboration with several partners like University of California- Davis, USA, and University of Frankfurt, Germany has recently developed a comprehensive genetic map of chickpea that is comprised of >1500 marker loci including 315 SSR and 420 SNP loci. Part of this map has already been published (Nayak *et al*., 2010). Recently a consensus map with 555 loci also has been developed by Millan *et al*. (2010) based on five crosses i.e. FLIP 84-92C(3) × PI599072, Hadas × Cr205, ICC 4958 × PI489777, ILC 72 × Cr5-10 and ICCL 81001 × Cr5-9.

For trait mapping, it is however important to develop genetic maps based on intra-specific mapping populations (Fig. 1). In past, several genetic maps were also developed by employing intra-specific (kabuli and desi) crosses and QTLs/markers associated with different agronomic traits have been identified (Table 4). At ICRISAT also, recently two intra-specific maps have been developed for ICC 4958 × ICC 1882 (253 SSR loci) and ICC 283 × ICC 8261 (191 SSR loci). These maps have been used to identify the hotspot containing QTLs for several drought tolerance related traits in chickpea genome (unpublished data). Recently a consensus intra-specific genetic

map of chickpea has been constructed by merging linkage maps from 10 different populations based on SSR markers as bridging markers (Millan *et al*., 2010).

Assessment of Allelic Diversity in Germplasm Collections

Crop breeders are reluctant to select parental lines from thousands of available germplasm lines without knowing their performance especially for quantitative traits which are highly environment sensitive. Selecting a few lines from these vast pools of germplasm is like searching for a needle in a hay stack. Obviously it is more appropriate and attractive to have a small sample of a few hundred germplasm lines, based on critical evaluation, representing the entire diversity of the species. Genomic tools such as molecular markers developed as mentioned above may be useful to select such a representative set of diversity that can be useful in breeding programme (Glaszmann *et al*., 2010).

Genetic diversity studies: Almost all kind of molecular markers have been used for analysis of genetic diversity in chickpea germplasm. Majority of these studies however employed RAPD and AFLP markers. Although a limited number of genotypes were used for diversity analyses in majority of these studies, the main outcome of these studies was availability of a low level of genetic diversity in cultivated germplasm as compared to wild species. Details on some of these studies have been provided in Table S1.

Some diversity studies have also provided a general consensus about the members of the first crossability group which contains *C. arietinum* along with *C. reticulatum* (Ahmad, 1999; Iruela *et al*., 2002; Rajesh *et al*., 2002; Sudupak *et al*., 2002, 2004; Javadi and Yamaguchi, 2004;

Nguyen *et al*., 2004), suggested to be the annual progenitor of chickpea (Ladizinsky and Adler, 1976), and *C. echinospermum*, suggested to have played a significant role in the evolution of cultivated chickpea (Tayyar and Waines, 1996). The second crossability group contained *C. bijugum*, *C. judaicum* and *C. pinnatifidum* (Ahmad, 1999; Sudupak *et al*., 2002, 2004; Sudupak, 2004; Nguyen *et al*., 2004). The last three species, *C. yamashitae, C. chorassanicum* and *C. cuneatum*, were either not included in many studies or were differentially positioned with respect to the cultivated germplasm.

Allelic diversity in the global chickpea composite collection: A composite collection of 3000 lines, representing a wide spectrum of genetic diversity captured from the entire collection of chickpea germplasm preserved in ICRISAT and ICARDA genebanks was developed by Upadhyaya *et al*. (2006a). The composite collection which includes core and mini core collections was genotyped using 48 SSR markers, and field evaluated for seven qualitative and 17 quantitative descriptors. A total of 1683 alleles were detected, 935 rare and 748 common alleles. Gene diversity varied from 0.533 to 0.974. Kabuli as a group were genetically more diverse than other seed types. Several group-specific unique alleles were detected: 104 in kabuli, 297 in desi, and 69 in wild *Cicer*; 114 each in West Asia and Mediterranean, 117 in South and South East Asia, and 10 in African region accessions.

Core, Mini Core Collections and Reference Set for Enhancing the Use of Germplasm in Breeding

Selecting a representative sample of all the diversity in the large collection would facilitate the enhanced use of germplasm in the breeding programs. Such samples would be cost effective and

easy to maintain by individual breeders. A core collection (Frankel, 1984) is a subset, consisting of ~10% of total accessions, which captures most of the available diversity in the entire collection (Brown, 1989). At ICRISAT a core collection consisting of 1,956 accessions was developed (Upadhyaya *et al*., 2001). However, the size of core was still large for practical use by breeders to identify trait specific accessions for use in crop improvement.

To overcome the above mentioned constraint, Upadhyaya and Oritz (2001) postulated the mini core collection concept, where ~ 10% of core collection (1% of entire collection) is selected without loosing any diversity of the core or entire collection. Following these procedures a mini core set of 211 accessions in chickpea was developed at ICRISAT (Upadhyaya and Ortiz, 2001). This mini core collection is an "International Public Good" (IPG) now and ICRISAT has supplied 28 sets of chickpea mini core collection to national partners in several countries. The mini core collection has been thoroughly evaluated at ICRISAT and by national partners in diverse and multiple environments at several locations in Canada, India, Japan, Mexico, Sweden, USA and diverse trait-specific germplasm lines identified for use in crop improvement. This approach has provided a point of entry to the world chickpea germplasm and as a working collection for scientists to tackle their region specific problems. The detailed information is available in Upadhyaya *et al*. (2009).

Furthermore, based on allelic diversity data of global composite collection of chickpea, a 'reference set' of most diverse 300 accessions was selected (Upadhyaya *et al*., 2008). Genotype-based reference set on 48 SSR markers captured 1,315 alleles where as the reference set based on seven qualitative traits captured 1,237 alleles (Upadhyaya, 2008). Mining allelic variation in the

mini core collection and reference set will facilitate identification of diverse germplasm with beneficial traits for enhancing the genetic potential of chickpea globally and broaden the genetic base of cultivars.

Identification of trait-specific germplasm for use in improvement program: The use of genetic resources in the breeding programs have been mainly as sources of resistance to pests and diseases (Knauft and Gorbet, 1989), or as sources of male sterility, short stature or any such character with simple inheritance. In fact there have been fewer efforts for identifying germplasm lines for increasing yield potential than for pest resistance and nutritional quality (Halward and Wynne, 1991). Using the core\mini core approach a number of germplasm lines have been identified at ICRISAT and national programs. This includes tolerance to abiotic and biotic stresses and for agronomic characters such as early maturity (28 accessions, Upadhyaya *et al*., 2007b); large-seeded kabuli (49 accessions, Gowda *et al*., 2010); high yield (39 accessions, Upadhyaya *et al*., 2007a); resistance/ tolerance to biotic stresses (Pande *et al*., 2006) : Ascochyta blight (3), botrytis grey mold (55), wilt (67), dry root rot (5), multiple resistance (31); *Helicoverpa* resistance related traits (15 accessions - 5 each for low leaf feeding score, low larval survival and low larval weight, Upadhyaya *et al*., 2010); drought avoidance root traits (18 accessions, Kashiwagi *et al*., 2005; 5 accessions, Krishnamurthy *et al*., 2003); salinity tolerance (29 accessions, Serraj *et al*., 2004; 16 accessions, Vadez *et al*., 2007); 10 accessions high SPAD meter reading (Kashiwagi *et al*., 2010); Water use efficiency (6 accessions, and cool canopy temperature, 1 accession, Kashiwagi *et al*., 2006 a, b); high temperature tolerance (10 accessions, Upadhyaya *et al*., 2010).

Towards genomics-based germplasm research for chickpea improvement

As mentioned above, specialized germplasm collections such as composite collection, core collection, mini core collection and reference set representing global diversity are available now. In parallel significant genomic resources have been developed that can be used for detection and utilization of allelic diversity. Availability of high-throughput genotyping platform such as GoldenGate or Infinium assay (SNP genotyping), capillary electrophoresis (SSR genotyping) and DArT arrays (DArT genotyping) on appropriate germplasm collections mentioned will facilitate use of association genetics approach for identification of genes/markers associated with traits of interest to breeders. Advent of next generation sequencing technology has also encouraged chickpea community for undertaking genome sequencing effort. For instance, the National Institute of Plant Genome Research (NIPGR), New Delhi (India) is using Roche/454 and Applied Biosystem SOLiD (AB SOLiD) sequencing technologies (http://www.nipgr.res.in/NGCPCG/ngcpcg.html). Once the reference genome of chickpea is available, low-cost and faster re-sequencing technologies such as Illumina/Solexa and AB SOLiD will offer the possibilities to generate the genome sequences for core and mini core collections and reference set or composite collection in short-term and for the entire germplasm collection in long term. However association of genomic sequences/ haplotypes with traits of interest to breeders would require multi-location and precise phenotyping data as well as appropriate analytical tools on high computing bioinformatics platform. Nevertheless, advances in high-throughput phenotyping as well as bioinformatics platform (e.g. cloud computing) and tools are expected to facilitate initiation of 'genomics-assisted breeding' (Varshney *et al*., 2005) or 'Breeding by design' approaches such as 'genomic selection' (Jannink, 2010) in chickpea breeding in coming future.

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Table 1. Major genebanks holding chickpea germplasm

Institutes/ Genebanks	No. of wild accessions	No. of cultivated accessions
Australian Temperate Field Crops Collection, Australia	241	8414
Plant Genetic Resources Centre, BARI, Bangladesh	-	752
Embrapa Hortaliças, Brazil	-	775
Agriculture and Agri-Food Canada, Canada	2	507
Institute of Biodiversity Conservation, Ethiopia	-	1173
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany	11	522
Fodder Crops and Pastures Institute, Greece	-	445
Institute for Agrobotany, Hungary	5	1165
Indian Agricultural Research Institute (IARI), India	-	2000
International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), India	308	20267
National Bureau of Plant Genetic Resources (NBPGR), India	241	14463
Regional Station, Akola, India	-	813
Tehran University, Iran	-	1200
National Plant Gene Bank of Iran, Iran	-	5700
National Institute of Agrobiological Sciences (NIAS), Japan	-	682
Instituto Nacional de Investigaciones Agrícolas (INIA), Mexico	-	1600
Nuclear Institute of Agricultural & Biology (NIAB), Pakistan	-	500
Plant Genetic Resources Institute (PGRI), Pakistan	24	2122
Pulses Research Institute, Pakistan	-	520
University of the Philippines, Philippines	-	407
N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry, Russian Federation	-	2091
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria. Centro de Recursos Fitogenéticos, Spain	-	644
Instituto Andaluz de Investigación Agroalimentaria y Pesquera. Centro de Investigación y Formación Agroalimentaria Córdoba, Spain	-	608
International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria	269	12950
Plant Genetic Resources Department, Turkey	22	2054
Institute of Plant Production n.a. V.Y. Yurjev of UAAS, Ukraine	-	1021
Western Regional Plant Introduction Station, USDA-ARS, Washington State University, United States of America	177	6018
Uzbek Research Institute of Plant Industry, Uzbekistan	-	1055

Table 2. Chickpea germplasm lines released as cultivars in different countries

Accession	*Country of origin*	*Country of release*	*Assigned name*	*Year of release*
ICC 552	India	Myanmar	Yezin 1	1986
ICC 4951	India	Myanmar	ICC 4951	-
ICC 6098	India	Nepal	Radha	1987
ICC 8521	Italy	USA	Aztec	1980
ICC 8649	Afghanistan	Sudan	Shendi	1987
ICC 11879	Turkey	Turkey	Guney Sarisi 482	1986
		Algeria	-	1988
		Morocco	-	1987
		Syria	Ghab 1	1982
ICC 13816	USSR (former)	Algeria	-	1984
		Italy	Sultano	1987
		Syria	Ghab 2	1986
		Cyprus	Yialousa	1984
ICC 14911	USSR (former)	Turkey	-	1986
		Morocco	-	1987
ICC 4923	India	India	Jyothi	1978
ICC 4998	India	Bangladesh	Bina Sola 2	1994
ICC 14880	India	Australia	Hira	1997
ICC 237	India	Oman	ICC 237	1988
ICC 14302	India	India	Anupam	1984
ICC 14559	Bangladesh	Bangladesh	Bari Chhola 5	1995
ICC 3274	Iran	Bangladesh	Bari Chhola 7	1999
ICC 4994	India	Myanmar	Keyhman	1986
ICC 14808	India	Ethiopia	Yelbey	2006

Note: - = information not available

Table 3. Genomic resources available for chickpea

Marker type	*Number of markers developed*	*References*
Genomic SSR	28	Hüettel *et al*., 1999
	174	Winter *et al*., 1999
	10	Sethy *et al*., 2003
	233	Lichtenzveig *et al*., 2005
	13	Choudhary *et al*., 2006
	85	Sethy *et al*., 2006a, b
	63	Qadir *et al*., 2007
	311	Nayak *et al*., 2010
	1344	ICRISAT and UC-Davis, USA (unpublished)
EST-derived SSR	60	Choudhary *et al*., 2009
	77	Varshney *et al*., 2009b
	106	Buhariwalla *et al*., 2005
CAPS	32	Rajesh and Muehlbauer, 2008; Rajesh *et al*., 2005
	5	Varshney *et al*., 2007
DArT	15,360	DArT Pty Ltd, Australia and ICRISAT (unpublished data)
SNP	Ca. 9,000 identified and 768 on GoldenGate assay	ICRISAT, UC-Davis, USA and NCGR, USA

*UC-Davis - University of California, Davis, USA
NCGR - National Center for Genome Resources, New Mexico, USA
ICRISAT - International Crop Research Institute for Semi-Arid Tropics, Hyderabad, India

Table 4. Molecular genetic maps developed for chickpea

Mapping population	Marker loci mapped	References
Inter-specific (*C. arietinum* × *C. reticulatum)*		
ICC 4958 × PI 489777	29	Gaur and Slinkard, 1990a, 1990b
	120	Winter *et al*., 1999
	354	Winter *et al*., 2000
	56	Tekeoglu *et al*., 2002
	296	Pfaff and Kahl, 2003
	521	Nayak *et al*., 2010
PI 360177 × PI 489777 and PI 360348 × PI 489777	28	Kazan *et al*., 1993
ICC 4958 × PI 489777, PI 360177 × PI 489777 and PI 360348 × PI 489777	91	Simon and Muehlbauer, 1997
FLIP 84-92C × PI 599072	144	Santra *et al*., 2000
JG 62 × CA-2156	117	Rajesh *et al*., 2002
Hadas × Cr205	93	Abbo *et al*., 2005
ILC 72 × Cr5-10	89	Cobos *et al*., 2006
Inter-specific (*C. arietinum* × *C. echinospermum)*		
Lasseter × PI 527930	83	Collard *et al*., 2003
Intra-specific (*C. arietinum* × *C. arietinum)*		
ICCV 2 × JG 62	103	Cho *et al*., 2002
ILC 1272 × ILC 3279	55	Udupa and Baum, 2003
ICC 12004 × Lasseter	69	Flandez-Galvez *et al*., 2003
CA 2139 × JG 62, CA 2156 × JG 62	138	Cobos *et al*., 2005
JG 62 × Vijay, Vijay × ICC 4958	273	Radhika *et al*., 2007
ICC 4991 × ICCV 04516	84	Kottapalli *et al*., 2009
WR 315 × C 104	102	Sharma *et al*., 2004
Consensus map		
Five narrow crosses (Desi × Kabuli)	229	Millan *et al*., 2010
Five wide crosses (*C. arietinum* × *C. reticulatum*)	555	Millan *et al*., 2010

Legend to figure:

Figure 1: A holistic approach to harness germplasm diversity through genomic tools. Modern genomics technologies such as next generation sequencing and high-throughput genotyping platform together with appropriate germplasm and their precise phenotyping can be used to identify the QTLs/ alleles for the trait of interest by using linkage or association mapping approaches. QTLs or desirable alleles, subsequently, can be deployed through molecular breeding approaches such as marker-assisted selection (MAS) for developing the superior lines for traits of interest to the breeders.

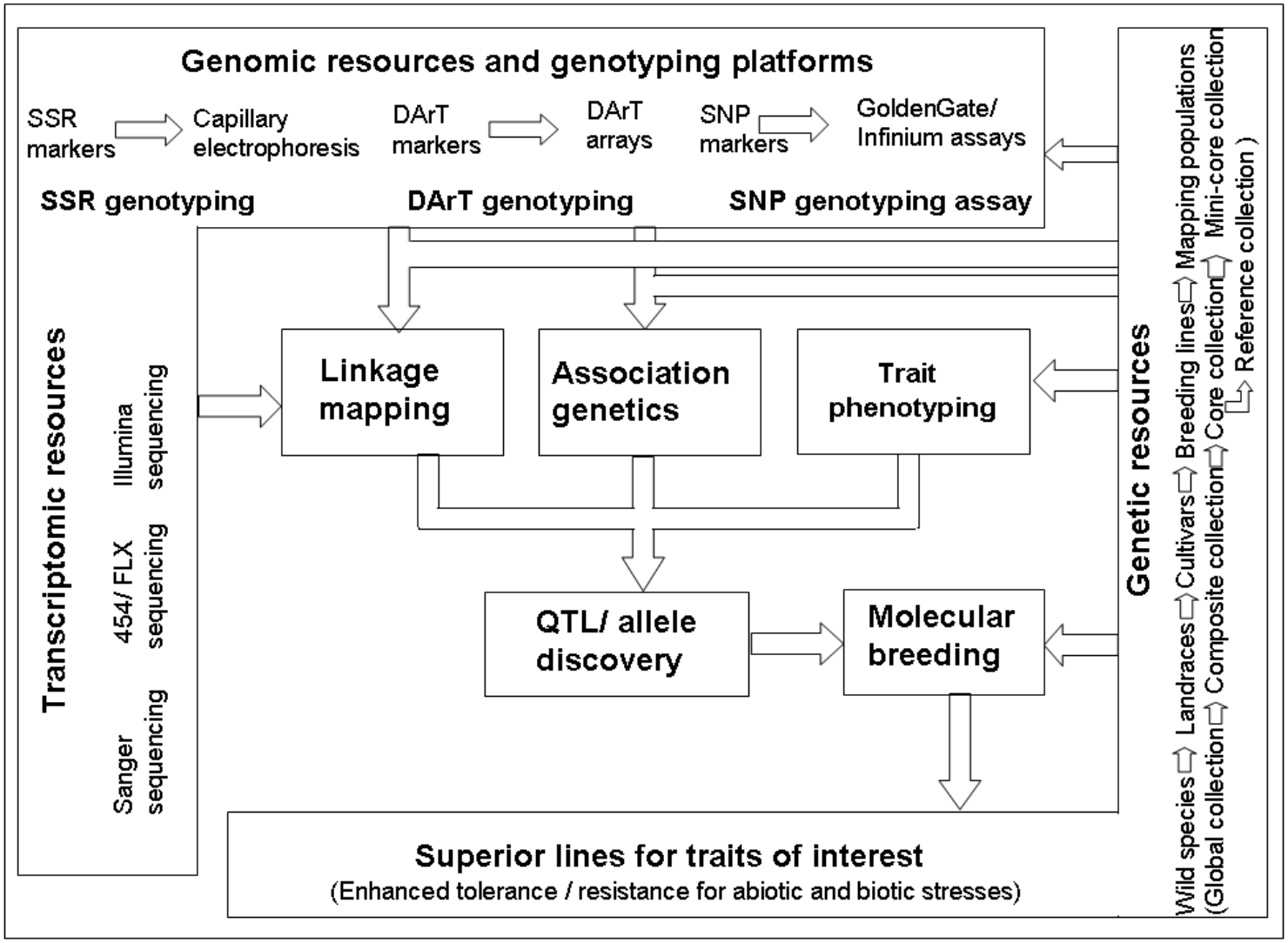
Genomic resources and genotyping platforms
SSR markers
Capillary electrophoresis
DArT markers
DArT arrays
SNP markers
GoldenGate/ Infinium assays
SSR genotyping
DArT genotyping
SNP genotyping assay
Transcriptomic resources
Illumina sequencing
454/ FLX sequencing
Sanger sequencing
Linkage mapping
Association genetics
Trait phenotyping
QTL/ allele discovery
Molecular breeding
Genetic resources
Wild species
Landraces
Cultivars
Breeding lines
Mapping populations
(Global collection
Composite collection
Core collection
Mini-core collection
Reference collection)
Superior lines for traits of interest
(Enhanced tolerance / resistance for abiotic and biotic stresses)

Figure 1

Table S1. Some genetic diversity studies in chickpea

Marker	Material	Outcome	Reference
RAPD			
75 RAPD	9 annual *Cicer* species (1 cultivated, 8 wild- *C. arietinum, C. reticulatum, C. echinospermum, C. bijugum, C. judaicum, C. pinnatifidum, C. chorassanicum, C. yamashitae, C. cuneatum*)	A total of 115 reproducibly scorable RAPD markers were generated, all except 1 polymorphic were utilized to deduce genetic relationships among the annual *Cicer* species. In addition to, species-diagnostic amplification four distinct clusters were observed.	Ahmad, 1999
7 RAPD primers	43 wild and cultivated accession representing ten species of *Cicer* (*C. montbretii, C. isauricum, C. anatolicum, C. incisum, C. pinnatifidum, C. judaicum, C. bijugum, C. echinospermum, C. reticulatum and C. arietinum*)	The dendrogram contained two main clusters, one of which comprised accessions of the four perennial species (*C. montbretii, C. isauricum, C. anatolicum and C. incisum*) together with the accessions of the three annual species (*C. pinnatifidum, C. judaicum and C. bijugum*), and the other cluster included the remaining three annual species (*C. echinospermum, C. reticulatum and C. arietinum*). Analysis of RAPD variation showed that *C. incisum* is the most similar perennial species to annuals, and *C. reticulatum* is the closest annual species to chickpea.	Sudupak *et al*., 2002
42 RAPD primers	19 wild *Cicer* accessions representing seven annual *Cicer* spp. (*C. echinospermum, C. reticulatum, C. pinnatifidum, C. judacium, C. cuneatum, C. yamashitae, C. arietinum*)	Diversity analysis provided three groups. The Group I included the cultivated species *C. arietinum*, *C. reticulatum* and *C. echinospermum*. Within this group, *C. reticulatum* accessions were clustered closest to the *C. arietinum, C. yamashitae*. The Group II was separated from the other	Talebi *et al*., 2009

		clusters. Group III (the annual tertiary group) included *C. judaicum, C. pinnatifidum* and *C. cuneatum.*	
16 RAPD	30 genotypes	No significant differences were observed between the mean percentage of the presence of RAPD markers between commercial cultivars and landraces.	Ahmad *et al*., 2010
ISSR			
15 ISSR markers	6 annual and 7 perennial wild species (*C. acanthophyllum, C. pungens, C. nuristanicum, C. anatolicum, C. microphyllum, C. oxyodon*)	The clustering pattern was in agreement with the data based on crossability, seed storage protein, isozyme, allozyme and RAPD marker analysis. 39% molecular variance was observed among annual and perennial groups. The results also suggested the monophyletic origin of wild annuals chickpea.	Rajesh *et al*., 2003
10 ISSR primers	12 chickpea genotypes (released cultivars and breeding lines)	In addition to the diversity analysis, one unique band was produced by the GGAGA primer in the BCP-15 genotype. This band may be linked to temperature tolerance phenotype.	Bhagyawant and Srivastava, 2008
AFLP			
AFLP(*Eco*RI and *Mse*I) 306 positions	47 accessions representing four perennial and six annual species	AFLP-based grouping of species revealed two clusters, Cluster I, includes three perennial species, *C. montbretii, C. isauricum* and *C. anatolicum*, while Cluster II consists of two subclusters, one including one perennial, *C. incisum*, along with three annuals from the second crossability group (*C. pinnatifidum*, *C. judaicum* and *C. bijugum*) and the other	Sudupak *et al*., 2004

		one comprising three annuals from the first crossability group (*C. echinospermum*, *C. reticulatum* and *C. arietinum*).	
214 AFLP marker loci	95 accessions that represented 17 species of *Cicer* (*C. arietinum, C. echinospermum, C. reticulatum C. bijugum, C. juidaicum C. pinnatifidum, C. anatolicum C. canariense, C. Cuneatum, C. flexuosum, C. macracanthum C. microphyllum, C. multijugum C. nuristanicum, C. oxyodon C. songaricum, C. yamashitae)*	Three main species groups were identified; Group I included the cultivated species *C. arietinum, C. reticulatum* and *C. echinospermum*. Within this group, *C. reticulatum* accessions were clustered closest to the *C. arietinum* cultivars 'Lasseter', 'Kaniva' and 'Bumper', supporting the hypothesis that *C. reticulatum* is the most probable progenitor of the cultivated species. Group II consists of *C. bijugum, C. judaicum* and *C. pinnatifidum.* While Group III contained all nine perennial species assessed and two annual species *C. yamashitae* and *C. cuneatum.* The genetic variation within a species was highest in *C. pinnatifidum* followed by *C. reticulatum* and lowest in *C. macracanthum.*	Nguyen *et al*., 2004
455AFLP	146 wild annual *Cicer* accessions (including two accessions of perennial *C. anatolicum* and six cultivars of chickpea)	Maximum genetic diversity of *C. reticulatum*, *C. echinospermum, C. bijugum* and *C. pinnatifidum* was found in southeastern Turkey, while Palestine was identified as the centre of maximum genetic variation for *C. judaicum*.	Shan *et al*., 2005

8 AFLP primer pairs	28 chickpea accessions from diverse origin	Greatest genetic diversity was found among accessions from Afghanistan, Iran and Lebanon.	Talebi *et al*., 2008b
SSR			
12 SSRs	78 genotypes (72 landraces, 4 cultivars, 2 wild species- *C. reticulatum* and *C. echinospermum*)	All the 76 accessions of cultivated chickpea could be readily distinguished with these markers. A significant positive correlation between the average number of repeats (size of the locus) and the amount of variation was observed.	Udupa *et al*., 1999
90 SSRs	40 accessions (39 annual, 1 perennial)	The degree of conservation of the primer sites varied between species depending on their known phylogenetic relationship to chickpea, ranging from 92.2% in *C. reticulatum*, chickpea's closest relative and potential ancestor, down to 50% for *C. cuneatum*	Choumane *et al*., 2000
11 SSR	29 accessions	Efficient marker transferability (97%) of the *C. reticulatum* STMS markers across other species of the genus was observed as compared to microsatellite markers from the cultivated species. Phylogenetic analysis clearly distinguished all the accessions	Sethy *et al*., 2006a
74 STMS	10 accessions (9 cultivated, 1 wild *C. reticulatum*)	The high levels of intra-specific genetic polymorphism in chickpea was clearly evident from dendrogram analysis. Sequence analysis of these amplicons suggested random point mutations followed by the subsequent expansion by replication slippage.	Sethy *et al*., 2006b

48 SSR markers	3000 accessions of composite collections	This was the most comprehensive genetic diversity studies in chickpea. In total, 1683 alleles were detected in 2915 accessions, of which, 935 were considered rare, 720 common and 28 most frequent. A number of group-specific alleles were detected: 104 in Kabuli, 297 in desi, and 69 in wild *Cicer*; 114 each in Mediterranean and West Asia (WA), 117 in South and South East Asia (SSEA), and 10 in African region accessions. Furthermore, based on comprehensive analysis, a 'reference set' was defined that includes broad-based elite breeding lines/cultivars with superior yield and enhanced adaptation to diverse environments. This is an ideal set of germplasm for allele mining, association genetics, mapping and cloning gene(s), and in applied breeding for the development of environments.	Upadhyaya *et al*., 2008
10 EST-SSR markers	58 accessions	Crossability-group-specific sequence variations were observed among *Cicer* species that were phylogenetically informative. The neighbor joining dendrogram clearly separated the chickpea cultivars from the wild *Cicer* and validated the proximity of *C. judaicum*	Choudhary *et al*., 2009
10 SSR markers	47 chickpea (*C. arietinum*) accessions including 21 induced mutation lines, 17 hybrid lines, 5 local cultigens, and 4 non-nodulating lines	UPGMA and ME (minimum evolution) trees classified the accessions into 6 groups and all but 6 accessions could be clearly separated. Grouping was mostly the same in the two phylogenetic trees, but the branching order	Khan *et al*., 2010

		differed greatly. Recent introgression among the parental lines is suggested for this reason.	
Miscellaneous			
*trn*T-F region in chloroplasts	29 accessions (representing 25 species *C. arietinum, C. bijugum C. cuneatum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum, C. yamashitae, C. chorassanicum, C. anatolicum, C. canariense, C. flexusoum, C. kermanense, C. microphyllum, C. montbretii, C. multijugum, C. nuristanicum, C. songaricum, C. spiroceras, C. subaphyllum, C. macracanthum, C. pungens, C. stapfianum, C. tragacanthoides, Lens ervoides, Pisum sativum*)	Phylogenetic analysis revealed three major clades in the genus *Cicer*. Inferred phylogenetic relationships supported multiple origins of annual species in the genus *Cicer*. Low variation within the most perennial species in the sequence regions suggests they	Javadi and Yamaguchi, 2004
Repeat unit length variation and internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA	76 accessions of 10 *Cicer* species (*C. reticulatum, C. echinospermum, C. bijugum, C. pinnatifidum, C. judaicum, C. chorassanicum, C. yamashitae, C. cuneatum, C. microphyllum)*	Cladistic analysis of ITS data revealed two major clades, clade I consisting of *C. arietinum*, *C. reticulatum* and *C. echinospermum*, and clade II comprised of *C. judaicum*, *C. chorassanicum*, *C. bijugum* and *C. cuneatum*. *C. microphyllum* grouped with the above four species. *C. pinnatifidum* was present as a separate branch. *C. yamashitae* emerged as the most distinct species.	Singh *et al*., 2008

12 RAPD, 8 ISSR	75 accessions belonging to 17 species of *Cicer* (*C. arietinum, C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum, C. yamashitae, C. cuneatum, C. anatolicum, C. multijugum, C. macracanthum, C. microphyllum, C. canariense, C. oxyodon*)	The dendrogram showed the variability between species was related to both growth habit and geographical origin	Iruela *et al*., 2002
17 random genomic and five heterologous probes in 65 probe-enzyme combinations	Five *desi* and five *kabuli* type chickpea cultivars	No polymorphism in chickpea varieties was detected with four RAPD markers studied. However, some degree of polymorphism between *C. arietinum* and its wild relative *C. reticulatum* was detected. The RFLP analysis of chloroplast and mitochondrial genomes showed no polymorphism.	Udupa *et al*., 2003 (check ref)
Microsatellite derived-RFLP	30 accessions	Greatest genetic diversity was observed in Pakistan, Iraq, Afghanistan, south-east Russia, Turkey and Lebanon. Lower genetic diversity was found in Iran, India, Syria, Jordan and Palestine	Serret *et al*., 2006

60 RAPD and 10 ISSR primers	19 chickpea cultivars and five accessions of its wild progenitor *C. reticulatum* Ladizinsky	The ISSR analysis clearly indicated that only six polymorphic markers are reliable for estimation of genetic diversity, while nearly 30 RAPD primers are required for the same. Genetic data produced through ISSR can be used to correlate with the relationship measures based on pedigree data and morphological traits to minimize the individual inaccuracies in chickpea.	Rao *et al*., 2007
33 RAPD and 9 morphological traits	36 genotypes	Correlation between the genetic distances was obtained with RAPD and morphological traits, indicating that there is a strong multi-locus association between molecular and morphological traits in these cultivars.	Talebi *et al*., 2008a
15 AFLP and 18 STMS primer pairs	21 cultivars of *C. arietinum*	The genetic similarity between cultivars varied from 0.30 to 0.85 for AFLP and 0.22 to 0.83 for STMS markers. Association of varietal type and flower colour was observed as cultivars E 100Ymu and Nabin (both Desi type and pink flower) clustered together in the dendrogram.	Singh *et al*., 2008