Chapter 3
Genetic Resources of Chickpea (*Cicer arietinum* L.) and Their Utilization

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Abstract Chickpea is a prominent grain legume crop providing cheap source of protein to the humankind. It originated in the Near East from the progenitor species *Cicer reticulatum* having a narrow distribution and genetic base. Moreover, during the course of domestication chickpea experienced various bottlenecks resulting in still narrow genetic variation in its two major forms 'Kabuli' and 'Desi'. Further genetic improvement would therefore depend on the exploration and introduction of useful genes from its wild relatives. The genus *Cicer* has 49 taxa including nine annual species. The genetic relationships among these and with the cultigen have been analyzed and elaborated by diverse methods including morphology, seed proteins, isozymes, karyotypes, FISH and various DNA markers. All these studies have resulted in demarcating primary, secondary and tertiary gene pools and show a very close relationship of the cultigen with two annual species *C. reticulatum* and *C. echinospermum* besides some perennial species. However, direct transfer of genes by hybridization has proved to be nearly impossible as the cultigen shows very poor or no crossability with any of the wild species except the progenitor species. This problem is being addressed by QTL mapping of mostly disease resistance loci from the RIL's produced from intra as well as interspecific crosses. Further efforts are being made to integrate genetic maps with physical maps. These methods provide a strong basis for genetic and genomic analysis of chickpea genome and facilitate further the use of molecular methods in breeding.

Keywords *Cicer arietinum* · Origin · Domestication · Interspecific relationships · Molecular maps

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3.1 Introduction

Chickpea (*Cicer arietinum* L.) is the only cultivated species of the genus *Cicer*, and as an important cool season grain legume it ranks second most important pulse crop being grown in about 50 countries on an area of 12 m ha with a total production of 11 m tons and productivity of 910 kg ha^{-1} (FAOSTAT 2012). With two third of the total world production occurring in India the other major producing countries are Pakistan, Myanmar, Iran, Turkey, Mexico, Canada and USA (FAOSTAT 2012). Chickpea provides a relatively cheap source of dietary protein and its seeds contain 20.3 % protein, approximately 40 % carbohydrates and 3–6 % oil (Gil et al. 1996). It is also rich in minerals and is a good source of calcium, magnesium, potassium, phosphorus, iron, zinc and manganese besides a number of vitamins (Ibricki et al. 2003; Wood and Grusak 2007). It also contains higher amount of carotenoids such as β-carotene than genetically engineered rice (Abbo et al. 2005). Moreover, in comparison to other legumes anti-nutritional factors are nearly absent (Muzquiz and Wood 2007). With all these nutritional components chickpea very well serves the purpose of a nutraceutical (Agharkar 1991; McIntosh and Topping 2000; Charles et al. 2002; Jukanti et al. 2012).

Two distinct types of chickpea are classified into, microsperma and macrosperma referring to the seed size (Cubero 1987). Commercially two distinct types are available 'Desi' with small angular dark brown seeds with rough surface, pink or purple flowers, anthocyanin pigments on the stems semi-erect or semi-spreading habit and 'Kabuli' with large ram-shaped seeds with smooth surface, white coloured flowers, lack of anthocyanin pigmentation and semi-spreading habit. These two types also have different centres of diversity as 'Kabuli' types with narrow genetic diversity mainly grow in Mediterranean region, central and West Asia, while 'Desi' with much wider genetic diversity in the Indian subcontinent and Ethiopia (van der Maesen 1972; Berger and Turner 2007). The 'Kabuli' types are generally considered to have evolved from 'Desi' types (Moreno and Cubero 1978; Hawtin and Singh 1980; Salimath et al. 1984; Gil and Cubero 1993) which is also supported by close similarity of seed coat texture between 'Desi' type of *C. arietinum* and *C. reticulatum* therefore implying a recent divergence of 'Kabuli' type from 'Desi' (Javadi and Yamaguchi 2004a). However, a white flower coloured mutation was isolated in M2 generation of some accession of *C. reticulatum*. This mutation also had cream coloured seeds as compared with dark coloured seeds in the parent *C. reticulatum* suggesting that this 'Kabuli' chickpea might have originated as a mutation of *C. reticulatum* (Toker 2009). Recently, the study on transcriptome sequencing of 'Kabuli' chickpea shows a higher similarity of transcripts between 'Kabuli' and 'Desi' as compared with these and the wild progenitor. It has been deduced that first cultivated chickpeas diverged from the wild progenitor and two cultivated types 'Kabuli' and 'Desi' diverged soon after that (Agarwal et al. 2012). However, the authors have not ruled out the possibility of both the cultivated types originating directly from the wild progenitor (Agarwal et al. 2012). The distinct genetic backgrounds of these two types has been shown by RAPD and

ISSR markers where they form two separate groups which, however, do not correlate with their geographical origin (Iruela et al. 2002). However, the analysis by STMS markers does not clearly demarcate 'Kabuli' and 'Desi' types (Rizvi et al. 2014). Now the differences between these two types are slowly merging due to plant breeding programmes requiring to combine the large seed size with local adaptation and vigour of 'Desi' types (Yadav et al. 2004). A third type named as intermediate type or 'pea shaped' has been identified with small to medium round pea-shaped seeds (Sharma et al. 2013).

3.2 Origin and Domestication

Chickpea is one of the founder grain crops having originated when the humans started domesticating the various plant species at the beginning of agriculture in the Fertile Crescent (Near East) 12,000–10,000 years ago with the cultivation of seven grain crops (*Triticum monococcum* L., *Triticum turgidum* L., *Hordeum vulgare* L., *Pisum sativum* L. *Lens culinaris*, *Cicer arietinum* L., *Vicia ervilia* (L.) Willd. and *Linum usitatissimum* L., a fiber crop) called founder crop package (Zohary and Hopf 2000; Lev-Yadun et al. 2000). Although many evidences have been recorded about chickpea cultivation, the earliest most authentic record is of 7260 B.C at Tell el-Kerkh, Syria where seed samples of both chickpea and progenitor *C. reticulatum* were clearly distinguishable (Tanno and Willcox 2006).

Chickpea has undergone many physiological and morphological changes since its evolution from the nearest parental species *C. reticulatum*. *C. reticulatum* has a very restricted distribution, currently reported from 18 locations in southeastern Turkey (Berger et al. 2003). The modern chickpea, therefore, has a narrow genetic base because of genetic bottlenecks it has experienced at various stages of domestication. According to Abbo et al. (2003) four such bottlenecks are the restricted distribution of the progenitor *C. reticulatum*, founder effects because of narrow genetic sampling in the initial stages, shift in the growing season from winter spring sowing to escape ascochyta blight, and finally replacement of locally existing land races by elite modern cultivars evolved by the breeders. However, despite the problems like disease susceptibility and manipulation of the growing season experienced by the early farmers the chickpea cultivation was necessitated by the advantages it provided in terms of nutritional superiority as some chickpea stocks analyzed were found to have high tryptophane levels (Karem et al. 2007; Abbo et al. 2005).

3.3 Taxonomy

The genus *Cicer* belongs to the family Fabaceae in the monogeneric tribe Cicerae Alef (Kupicha 1981). It consists of 49 taxa including nine annual species (van der Maesen et al. 2007; Donmez 2011; Ozturk et al. 2013). Traditionally, the genus

Cicer has been divided into two subgenera (*Pseudononis* and *Viciastrum*) and four sections *Cicer, Chamaecicer, Polycicer* and *Acanthocicer* based mainly on morphological characters and geographical distribution (Popov 1929; van der Maesen 1972, 1987). Recently, Davies et al. (2007), analyzed 104 characters in 35 recognized taxa of *Cicer* by multivariate statistics and proposed a new subgeneric classification with three subgenera, five sections and two series.

Genus Cicer
Subgenus Cicer
Section Cicer
Series Cicer

C. arietinum L.
C. echinospermum P.H. Davies
C. reticulatum Ladiz.

Series Pinnatifida

C. bijugum Rech.f
C. judaicum Boiss.
C. pinnatifidum Jaub. Sapach

Section Chamaecicer

C. atlanticum Coss. Ex Maire
C. incisum (Willd.) K. Maly

Sugenus Viciastrum
Section Annua

C. chorassanicum (Bunge) Popov
C. yamashitae Kitam

Section Polycicer

C. floribundum Fenzl.
C. graceum Orph.
C. heterophyllum Contandr. Pamuk C & Quezel
C. isauricum P.H. Davies
C. montbretii Jaub. & Spach.

Section Vicioides

C. acanthophyllum Borris
C. anatolicum Alef.
C. balcaricum Galushko
C. baldshuanicum (Popov) Lincz.
C. fedtschenkoi Lincz
C. flexuosum Lipsky
C. grande (Popov) Korotkova
C. incanum Korotkova

C. korshinsky Lincz.
C. laetum Rassulova & Sharipova
C. luteum Rassulova & Sharipova
C. macracanthum Popov
C. microphyllum Benth.
C. multijugum Maesen
C. nuristanicum Kitam
C. paucijugum (Popov) Nevski
C. pungens Boiss
C. rassulovie Lincz.
C. rechingeri Podlech
C. songaricum Steph. Ex DC
C. stapfianum Rech.f
C. subaphyllum Boiss
C. tragacanthoides Jaub & Spach

Subgenus Stenophylloma

C. canariense A.G. Guerra & G.P. Lewis
C. cuneatum Hochst. Ex A. Rich

Furthermore, a classification based on nuclear ITS and chloroplast trnK/matK and trns-trnG regions has been proposed (van der Maesen et al. 2007). This grouping of species on the basis of molecular data clearly brought out inadequacies of the earlier systems as section *Cicer* (subg. *Pseudononis*) and section *Acanthocicer* (subg. *Viciastrum*) have been shown to be polyphyletic and only section *Polycicer* (subg. *Viciastrum*) forms a well-supported monophyletic group. Furthermore, two African species *C. canariense* (section Polycicer) and *C. cuneatum* (section *Cicer*) form a highly supported basal clade in the phylogenetic tree (van der Maesen et al. 2007).

3.4 Phylogenetic Relationships Between Species

A proper assessment of the genetic variation present in various wild taxa and their phylogenetic relationship with each other and with the cultigen is of utmost importance to introduce wild characters of agronomic importance.

3.4.1 Morphological Characters

Morphological characters have been used to define relationships between different species of *Cicer*. A study on 228 accessions belonging to eight annual species and 20 'Kabuli' chickpea lines shows that the cultigen is more variable than wild

species and it also differs from the latter in terms of leaf area, growth habit, plant height, first pod height, pod dehiscence and 100 seed weight, the characters which changed during domestication. Further, it was found that *C. reticulatum, C. echinospermum, C. bijugum* were closest to the cultigen (Robertson 1997). Javadi and Yamaguchi (2004b) divided 17 species belonging to all the four sections into six plumule types. The type PI with spiral form of compound leaf and two adnate or separated stipular parts is characterized by *C. arietinum* and its two closest relatives *C. reticulatum* and *C. echinospermum*. The type PII with narrowly spiral form of compound leaf and two relatively close stipular parts included *C. yamashitae* and *C. chorassanicum* and the PIII included *C. pinnatifidum* and *C. judaicum* while the PIV included *C. cuneatum* and *C. canariense* and PV included mostly perennial species and PVI *C. bijugum* (Javadi and Yamaguchi 2004b).

Recently, Ozturk et al. (2013) investigated 17 *Cicer* taxa growing in Turkey by generating a data matrix prepared from 143 morphological, palynological and seed characters. The *Cicer* species were divided into two major groups with first group including *C. arietinum, C. reticulatum, C. echinospermum, C. bijugum, C. pinnatifidum* (section *Cicer*), *C. incisum* subsp. *incisum* and *C. incisum* subsp. *serpentinicola* (section Chamaecicer) and the second group comprised *C. heterophyllum* var. *heterophyllum, C. heterophyllum* var. *kassianum, C. uludereensis, C .isauricum, C. montbretii, C. floribundum* var. *floribundum, C. floribundum* var. *amanicola* (section Polycicer) and *C. anatolicum* (section *Vicioides*).

3.4.2 Karyotypes and Physical Mapping

Karyotypic comparison provides a basis for comparative study of gross structural changes taking place in the genome of different species within a genus. A number of studies have described minor variability in the karyotype of various accessions of both 'Kabuli' and 'Desi' *C. arietinum* (Kutarekar and Wanjari 1983; Mukherjee and Sharma 1987; Ohri and Pal 1991; Galasso and Pignone 1992; Venora et al. 1995; Akter and Alam 2005; Kordi et al. 2006). The karyotypes of the eight annual species have been described both by feulgen staining (Ohri and Pal 1991; Ocampo et al. 1992; Ahmad 2000) and by banding techniques (Tayyar et al. 1994; Galasso et al. 1996) while only four perennial species have been studied, e.g. C. *anatolicum* (Ahmad 1989; Hejazi 2011), *C. songaricum* (Ohri 1999), *C. oxyodon* (Hejazi 2011) and *C. canariense* (Pundir et al. 1993). All the species studied are diploid having $2n = 16$.

Karyotypes of five accessions of *C. arietinum* belonging to both 'Kabuli' and 'Desi' types studied by Ohri and Pal (1991) are more or less similar as the first and the longest chromosome which is median has a satellite on the longer arm and the rest of the chromosomes are median point, median, median submedian or submedian. The karyotypes of *C. reticulatum* and *C. echinospermum* are similar to that of *C. arietinum* but in case of *C. reticulatum* first two pairs which are median also have a satellite each on the longer arms. The karyotypes of these three species fall in

1b class of Stebbins (1958). The complement of *C. bijugum* has a satellite on the second pair the secondary constriction lies very near the centromere, and it also has a subterminal and a submedian pair. *C. pinnatifidum* has a satellite on the smallest pair and three submedian pairs, and both these species fall in 2a class of Stebbins (1958). In case of *C. cuneatum* the first pair has a satellite on the longer arm and it is peculiar in having three submedian pairs and therefore comes under 2b class. *C. judaicum* which has smallest complement of all the species, is also the most asymmetrical in having two subterminal pairs and a secondary constriction on the second pair, it falls in 3b class. In addition to these *C. yamashitae* studied by Ocampo et al. (1992) has mostly median and submedian chromosomes and the third pair is satellited. Tayyar et al. (1994) studied C-banding in all the nine species of *Cicer*. Mainly, centromeric C-bands were observed in addition to some intercalary bands which facilitated proper identification and pairing. The smallest haploid genome length was observed in *C. judaicum* and the longest in *C. arietinum*. There was no correlation between the amount of heterochromatin and the total haploid genome length as *C. chorassanicum* had the lowest (38.4 %) and *C. cuneatum* the highest (63.1 %) heterochromatin content. However, the C-banded karyotypes of *C. arietinum, C. reticulatum* and *C. echinospermum* were found to be quite similar. Tayyar et al. (1994) have classified two groups on the basis of heterochromatin content, i.e. *C. cuneatum* and *C. bijugum* with high heterochromatin content of 61.3 and 57.7 %, respectively and *C. pinnatifidum, C. judaicum, C. arietinum, C. reticulatum, C. yamashitae, C. echinospermum* and *C. chorassanicum* range from 38.4–46.0 %. Galasso et al. (1996) also studied C-banding in *C. arietinum, C. reticulatum* and *C. echinospermum* which showed similarity in the presence of mostly centromeric heterochromatic bands. The differences were found with regard to some intercalary bands as in case of chromosome B of *C. reticulatum* and the presence of satellites on the first two pairs in this species in contrast to other species which have a satellite only on the first pair. Fluorescent banding showed two pairs of chromosomes with CMA positive heterochromatin in all the three species. One site of this heterochromatin is located at the secondary constriction of a chromosome resembling chromosome A of *C. arietinum* and the other site in *C. echinospersmum* is present in subterminal position on the chromosome homoeologous to the chromosome B of *C. arietinum*, while in *C. reticulatum* this site is observed on the secondary constriction of second satellite chromosome. Karyotypes of three perennial species have been studied in some detail. In *C. anatolicum* secondary constriction is present on the longest chromosome and the rest of the chromosomes are either median or submedian, one smallest pair is median point. Though more asymmetrical, the kayotype of *C. anatolicum* resembles those of *C. arietinum, C reticulatum* and *C echinospermum* (Ahmad 1989). *C. songaricum* shows more symmetrical karyotype with three median point chromosomes while the others are either median or submedian and the third longest chromosome has a secondary constriction (Ohri 1999). In *C. oxyodon* satellite is present on the short arm of seventh pair and the remaining chromosomes are either median or submedian (Hejazi 2011).

Abbo et al. (1994) determined rDNA sites by fluorescent in situ hybridization (FISH). The cultigen which shows only one secondary constriction by feulgen staining, produces rDNA signals on three pairs of chromosomes. However, out of these only two pairs are regularly detected and the third is rarely observed perhaps due to low copy number. *C. reticulatum* as expected regularly has two pairs of hybridization sites corresponding to two pairs of satellite chromosomes as observed earlier (Ohri and Pal 1991; Ocampo et al. 1992; Tayyar et al. 1994). To account for three pairs of rDNA sites in the cultigens Abbo et al. (1994) have suggested a major translocation which removed one pair of satellite to another chromosome pair thus creating a major site, one of intermediate intensity and the remaining one with low intensity. Galasso et al. (1996), however, observed two pairs of hybridization sites each for the clone pTa71 (containing 18S-5.8S-25S rRNA genes) and clone pTa794 (containing 5S rRNA genes) in the three species, i.e. *C arietinum, C. reticulatum* and *C. echinospermum*. The presence of transcriptional activity by AgNOR staining revealed a major and a minor NOR pair in *C. reticulatum* but only one major active NOR pair in case of *C. arietinum* and *C. echinospermum* implying, therefore that the chromosome B showing CMA and pTa71 signaled an inactive remnant region which is active in *C. reticulatum*. This is further corroborated by the presence of four nucleoli (two large and two small) in interphase nuclei of *C. reticulatum* and only two large ones in *C. arietinum* and *C. echinospermum* (Galasso et al. 1996). However, the similarity in the size of 5S and 18-25S rRNA units of *C. arietinum* and *C. reticulatum* confirm their close relationship as between *C. bijugum, C. chorassanicum* and *C. echinospermum* with a smaller unit while *C. cuneatum* has the smallest 18-25S rRNA unit of all the *Cicer* species because of smallest intergenic spacer (Patil et al. 1995). It may be mentioned here that FISH has also been used on super stretched (extended 100 times) chromosomes of *C. arietinum* to increase the spatial resolution of neighbouring loci up to 70 kbp as compared to 5–10 mbp in case of metaphase chromosomes (Valarik et al. 2004).

Because of small size, the proper identification and pairing of the chromosomes of chickpea may be problematic even with banding techniques. This difficulty has been addressed by the physical mapping of molecular makers for specific chromosomes or arms. Gortner et al. (1998) used five simple sequence repeat oligonucleotides all of which produced hybridization signals with varying intensity and position, depending on the motif, on all the chromosomes. The metaphase chromosomes showed CA and GATA repeats mainly in the centromeric region while TA, A and AAC repeats occurring in dispersed manner. An *Arabidopsis* type of telomeric repeat (TTTAGGG)n produced a cluster of repeats on the short arm of chromosome B and a weaker signal on the short arm of chromosome A and very weak and inconsistent signals at the termini of other chromosomes (Gortner et al. 1998).

Similarly Staginnus et al. (1999) studied the physical mapping of four major repetitive families CaSat1, CaSat2, CaRep1 and CaRep2 on *Cicer arietinum* complement and their abundance and organization among eight other annual species. Major hybridization signals were observed with CaSat1 in the heterochromatin adjacent to secondary constriction of chromosome A and pericentric heterochromatin

block of chromosome B and in both cases the repetitive family hybridizes near rDNA site. On the other hand CaSat2 hybridizes to pericentric heterochromatin blocks of all 16 chromosomes. The retrotransposon like sequence CaRep1 and CaRep2 hybridize mostly on the DAPI-positive pericentric heterochromatic region of all chromosomes. The presence and organization of two satellite (CaSat1 and CaSat2) probes was observed by Southern hybridization on RsaI digested genomic DNA which form a ladder-like sequence on all annual species except *C. cuneatum* where no hybridization was seen. However, the variation in intensity of banding was observed in case of other species. For example, CaSat1 produced the strongest signals in *C. reticulatum, C. arietinum, C. echinospermum* and *C. chorassanicum*, somewhat weak signals in *C. yamashitae*, and very faint in *C. bijugum, C. pinnatifidum* and *C. judaicum*. It is interesting to note that CaSat2 produced similar pattern on DNA from the perennial *C. anatolicum* and annual species (Staginnus et al. 1999). Another family CaRep3 belonging to highly repetitive Ty3-gypsy like retrotransposon was also mapped and shown to be present in the intercalary heterochromatin of all the chromosome and on the distal parts of satellite chromosome A (Staginnus et al. 2010). The hybridization signals were particularly absent from centromeric region and secondary constriction of chromosome A. The restriction pattern of CaRep3 sequence and their relative abundance was similar in *C. reticulatum, C. echinospermum* and the cultigen, very different in *C. bijugum*, and either absent or divergent in *C. chorassanicum* and *C. yamashitae*. Staginnus et al. (2010) also detected two other, an LTR (CaTy) and a non-LTR (CaLin) retrotransposon family. Their physical location showed the presence of CaTy in the distal parts of intercalary heterochromatin and adjacent euchromatic regions, a pattern observed in all chromosomes. However, CaLin has a low presence and reveals considerable heterogeneity and signals were present only on the chromosome pairs A, B and D (Staginnus et al. 2010).

Successful flow sorting of individual chickpea (‘Kabuli’) chromosomes was done for the first time by Vlacilova et al. (2002) and subsequently, of the ‘Desi’ type by Zatloukalova et al. (2011). While in the former case (‘Kabuli’) 5 peaks A, B, C, G and H could be assigned to individual chromosomes leaving three tightly spaced peaks represented by chromosomes D, E and F, the ‘Desi’ types depicted four peaks represented by chromosomes A, B, E and H and two composite peaks representing chromosomes C and D, and F and G. This corroborates minor chromosomal variation in ‘Desi’ and ‘Kabuli’ types as observed by Ohri and Pal (1991). Out of the eight chromosomes of ‘Kabuli’ the largest chromosome A showing a nucleolar organizing region (NOR) with 45S rDNA locus, the second large chromosome B showing a hybridization signal of 5S rDNA locus and a large interstitial band of *Arabidopsis* type telomeric repeat and the second smallest chromosome G with a 5S rDNA locus could be definitely identified. However, one smallest chromosome H could be assigned to linkage group LG8 of Winter et al. (1999, 2000) by the sequence tagged microsatellite site (STMS) markers (Vlacilova et al. 2002). Zatloukalova et al. (2011) used some probes for major DNA repeats such as CaSat1, CaSat2, CaRep1 and CaRep2 which produced similar results as obtained by Staginnus et al. (1999). In addition to these 57 BAC clones carrying inserts of 20–100 kb were used as probes for FISH on flow sorted chromosomes. This

resulted in the identification of two clones localizing specifically to the chromosomes E and H which earlier lacked any cytological markers (Zatloukolova et al. 2011). Moreover, in this study STMS markers have been used to confirm LG8 to chromosome H, LG5 to chromosome A, LG4 to medium sized chromosome E and LG3 to the second largest chromosome B. However, the Chromosomes C and D were not flow sorted separately and jointly ascribed to LG6 and LG7 and likewise Chromosomes F and G to LG1 and LG2 (Zatloukalova et al. 2011).

3.4.3 Meiotic Associations in the Species

All the nine annual species including *C. arietinum* show normal formation of 8 bivalents at metaphase I and the chiasma frequency has been shown to be negatively correlated to genome length (Ahmad and Chen 2000). Likewise, a perennial species *C. canariense* also shows the normal meiosis with 8 bivalents (Pundir et al. 1993). In all the accessions of annual species studied by Ahmad and Chen (2000) only one chromosome pair was found to be associated with the nucleolus at pachytene and/or at diakinesis stage. This is an interesting observation especially in *C. reticulatum* where rRNA gene cluster has been mapped to two pairs of chromosomes (Abbo et al. 1994; Galasso et al. 1996). Additionally both rRNA gene sites are transcriptionally active albeit differentially and are thus capable of forming nuclei (Galasso et al. 1996). The association of only one pair with the nucleolus would thus indicate that perhaps the chromosome pair containing the lesser active of the rRNA site is not capable of associating with the nucleolus at pachytene/diakinesis since not a single PMC showed the expected association (Ahmad and Chen 2000).

3.4.4 Genome Size

Genome size has been determined for seven annual and one perennial species (Ohri and Pal 1991; Ohri 1999). The 2C DNA amounts range from 1.83 pg (*C. judaicum*) to 3.57 pg (*C. arietinum* ICC 5003). These seven species form three DNA groups whose means are separated by an interval of 0.8 pg. *C. judaicum* (1.83 pg) constitutes group I, *C. cuneatum* (2.50 pg), *C. bijugum* (2.54 pg), *C. pinnatifidum* (2.56 pg), *C. reticulatum* (2.65 pg) and *C. echinospermum* (2.56 pg) group II, while group III contains five cultivars of *C. arietinum* (3.30–3.57 pg) (Ohri and Pal 1991). *C. songaricum*, the only perennial species studied, shows a 2C DNA amount of 2.71 pg which is similar to the annual species included in group II (Ohri 1999). It is interesting to note that *C. reticulatum* has 22.3 % less DNA ($P < 0.01$) than *C. arietinum*. This has also been confirmed by Galasso et al. (1996) who reported 4C DNA amount of 5.30 and 5.22 pg for C. *reticulatum* and *C. echinospermum,* respectively and 6.57 pg for *C. arietinum*. These two studies while corroborating each other, however, do not agree with 2C DNA amount of 1.90 pg for *C. arietinum*

(Bennett and Smith 1976). Moreover, the genome size estimates by flow cytometry of 738 mb/1C (Arumuganathan and Earl 1991) and 2C of 1.74–1.80 pg (Ruperao et al. 2014) reported for different cultivars of *C. arietinum* and a kmer based estimate (~738 mb) of a Kabuli chickpea variety (Varshney et al. 2013) are significantly lower than reported by Ohri and Pal (1991) and Galasso et al. (1996).

3.4.5 Protein, Enzyme and DNA Markers

The electrophoretic data on seed proteins and allozyme/isozymes have been used by various studies to describe interspecific relationships. Ahmad and Slinkard (1992) analyzed both albumin and globulin fractions and found the profiles of *C. reticulatum* and *C. echinospermum* very similar to that of *C. arietinum,* and *C. judaicum* and *C. pinnatifidum* were established as different species to form a cluster with *C.bijugum* and *C. chorassanicum*, while *C. cuneatum* and *C. yamashitae* were placed in two separate groups.

Kazan and Muehlbauer (1991) studied isozyme variation at 30 loci and determined relationships between nine annual and one perennial species. A monophyletic origin of all annual species has been suggested because of the common presence of isozyme gene duplications. As expected a close allozyme similarity is observed in *C. reticulatum, C. echinospermum* and *C. arietinum* with *C. anatolicum* showing a close resemblance to this group. Similarly *C. bijugum, C. pinnatifidum* and *C. judaicum* form a second cluster and *C. yamashitae* and *C. chorassanicum* form the third group while *C. cuneatum* is distinctly separate from all other species. Other analyses by isozymes (Ahmad et al. 1992; Labdi et al. 1996; Tayyar and Waines 1996; Gargav and Gaur 2001) agree with this grouping except in case of some perennial species such as *C. microphyllum* (Gargav and Gaur 2001), and *C. anatolicum* and *C. songaricum* (Tayyar and Waines 1996) which cluster with *C. yamashitae* and *C. chorassanicum*. However, Sudupak and Kence (2004) placed *C. anatolicum* with two other perennial species *C. isauricum* and *C. montbretii* (section Polycicer) in a group separate from that containing six annual species including the cultigen and the progenitor species and also a perennial species *C. incisum* (section Chamaecicer) forming a cluster together.

The phylogenetic relationships of nine annual and some perennial species have also been studied using DNA-based molecular markers such as RAPD (Ahmad 1999; Sudupak et al. 2002; Iruela et al. 2002; Javadi and Yamaguchi 2004a; Talebi et al. 2009), ISSR (Rajesh et al. 2002; Iruela et al. 2002; Sudupak 2004; Amirmoradi et al. 2012; Ozturk et al. 2013), sequence tagged microsatellite sites (STMS) (Choumane et al. 2000; Sethy et al. 2006), AFLP (Sudupak et al. 2004; Nguyen et al. 2004; Shan et al. 2005), chloroplast sequence analysis (Javadi and Yamaguchi 2004c; Javadi et al. 2007), rDNA, RFLP and ITS sequences (Frediani and Caputo 2005; Singh et al. 2008; Javadi et al. 2007), start codon targeted (Scot) polymorphism and DAMD-PCR (Amirmoradi et al. 2012), EST markers (Buhariwala et al. 2005), iPBS retrotransposon markers (Andeden et al. 2013).

Some detailed studies have been done with larger taxon sampling comprising of different accessions of all the annual and some selected perennial species. Iruela et al. (2002) studied 75 accessions of 14 species including 8 annuals by RAPD. The dendrogram showed 4 groups and the first included all the perennial species of Asian origin i.e. *C. anatolicum, C. multijugum, C. macracanthum, C. microphyllum* and *C. oxyodon*, the second only *C. yamashitae*, the third *C. arietinum, C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum* and *C. bijugum* while the fourth had the African species *C. cuneatum and C. canariense*. Javadi and Yamaguchi (2004a) did RAPD analysis of 35 accessions of 6 perennial and 5 annual species. The different accessions of 'Kabuli' and 'Desi' types clustered together and showed close relationship with those of *C. reticulatum*. This group is related to *C. echinospermum* while *C. bijugum* shows a distant relationship with other annual species in the first cluster and in the other cluster comprising of mostly perennial species, with the exception of *C. chorassanicum* which forms a subgroup, the other subgroup consists of *C. spiroceras, C. macracanthum, C. oxyodon, C. anatolicum, C. tragacanthoides* while *C. canariense* is distantly related to all other species. Likewise, Sudupak et al. (2002) studied 43 accessions of wild and cultivated species and found two main clusters and in the first cluster one subcluster is formed by the accessions of *C. reticulatum and C. arietinum* in keeping with their close relationship, while the accessions of *C. echinospermum* form another subcluster. In the other main cluster *C. bijugum*, and *C. pinnatifidum* form separate clusters while *C. judaicum* is grouped outside these subclusters which are joined by *C. incisum* and the other perennial species *C. isauricum, C. anatolicum* and *C. montbretii* form the other main cluster. This study showed that *C. incisum* is closest to the annual species.

In a comprehensive AFLP analysis of 95 accessions of 17 species all the perennial species, i.e. *C. multijugum, C. nuristanicum, C. microphyllum, C. songaricum, C. flexuosum, C. macracanthum, C. anatolicum* and *C. oxyodon* grouped together along with one annual *C. yamashitae,* while *C. pinnatifidum, C. bijugum and C. judaicum* formed a group nearer to the perennial species and *C. arietinum, C. reticulatum, C. echinospermum* formed a distinct group with very low genetic distances while *C. cuneatum* and *C. canariense* were most distantly placed with respect to all other species (Nguyen et al. 2004). Similarly, an AFLP study of 47 accessions of four perennial and six annual species grouped all the perennial species together i.e. *C. montbretii, C. isauricum, C. anatolicum* while the other cluster had two subclusters one of which included one perennial *C. incisum* along with *C. pinnatifidum, C. judaicum and C. bijugum*, the other had *C. arietinum, C. reticulatum* and *C. echinospermum. C. incisum* was found to be closest to the annual species (Sudupak et al. 2004). Another AFLP study of 146 accessions of 8 annual and one perennial species showed similar results except that *C. yamashitae* grouped with the perennial *C. anatolicum* and *C. cuneatum* as expected was the most distant to all the other species (Shan et al. 2005). This study also brought out geographical patterns of variation as maximum genetic variation of *C. reticulatum, C. echinospermum, C. pinnatifidum, C. bijugum* occurs in southeastern Turkey while *C. judaicum* shows maximum variation in Palestine region (Shan et al. 2005).

The relationship of 30 species based on combined consensus tree based on two plastid sequences and ITS revealed three well-supported clades. The species clearly segregated into four geographical groups in three clades as *C. arietinum, C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum* and *C. incisum* form a monophyletic group in clade III as they all belong to Middle East. The African group forms a monophyletic clade I comprising of *C. cuneatum and C. canariense*. Clade II is divided into two subgroups and consists of west central Asian *C. anatolicum, C. macracanthum, C. flexuosum, C. rechingeri, C. spiroceras, C. stapfianum, C. subaphyllum, C. kermanense, C. tragacanthoides, C. multijugum* with two annuals *C. chorassanicum* and *C. yamashitae* forming a sister group to this subgroup. The other subgroup is formed by the species of Aegean-Mediterranean distribution i.e. *C. floribundum, C. graecum, C. isauricum, C. montbretii* (Javadi et al. 2007). Earlier Javadi and Yamaguchi (2004c) obtained similar results on 25 species based on trn T-F region of chloroplast DNA. This also shows *C. anatolicum* forming a monophyletic group with other perennial species rather than with annuals. Similarly, Frediani and Caputo (2005) did cladistic analysis of ITS1 and ITS2 of 20 species of *Cicer* and noted two clades, one of which included two African species *C. canariense* and *C. cuneatum* and in the other clade *C. arietinum, C. reticulatum* and *C. echinospermum* form a closed group while *C. bijugum, C. judaicum, C. pinnatifidum* form a separate group and annual species *C. yamashitae and C. chorassanicum* belong to perennial species, such as *C. pungens, C. flexuosum, C. multijugum, C. macracanthum, C. songaricum, C. anatolicum, C. oxyodon, C. graecum, C. montbretii* and *C. microphyllum* (Frediani and Caputo 2005).

The ISSR polymorphism was used to study six annual and seven perennial species (Rajesh et al. 2002). Out of the three main clusters formed the first was comprised of *C. acanthophyllum, C. macracanthum, C. pungens, C. nuristanicum, C. arietinum, C. reticulatum* and *C. echinospermum* in which as expected the latter three species form a closed group. The second cluster had *C. yamashitae, C. bijugum* and *C. judaicum* where the latter two species showed higher similarity and the third cluster had *C. anatolicum, C. microphyllum* and *C. oxyodon*. The clustering of the species shows that annual species are polyphyletic as the perennial species do not form a single cluster. In a similar ISSR study, Sudupak (2004) showed that the perennial *C. incisum* is closest to the annual species, i.e. *C. judaicum, C. pinnatifidum* and *C. bijugum* and the accessions of *C. arietinum* and *C. reticulatum* form a single subgroup which is joined by *C. echinospermum*. Remarkably, *C. anatolicum* is most distantly place in relation to all other species.

Singh et al. (2008) made a phylogenetic analysis of 76 accessions of 10 species using RFLP and ITS sequences of nuclear ribosomal DNA. The tree generated from RFLP of rDNA formed 5 clades with all the accessions of *C. arietinum, C. reticulatum* and *C. echinospermum* in clade I, *C. bijugum*, four accessions of *C. judaicum* and one accession of *C. yamashitae* in clade II, rest of the *C. judaicum* and *C. pinnatifidum* accessions and *C. chorassanicum* form parts of clade III and IV, *C. cuneatum* and *C. yamashitae* form clade V. *C. microphyllum* the only perennial species studied forms a separate branch in the tree. This study shows that *C. bijugum* is completely separate from *C. pinnatifidum* and *C. judaicum* and two

accessions of *C. yamashitae* are included in two different clades. Two clades were formed by ITS1 and ITS2 sequence analysis. One clade was constituted by *C. arietinum, C. reticulatum* and *C. echinospermum* the other clade consisted of *C. judaicum, C. chorassanicum, C. bijugum, C. cuneatum* and *C. microphyllum* where the latter two species are close together, while *C. pinnatifidum* and *C. yamashitae* constituted different branches in the tree. This study distinctly shows that *C. pinnatifidum* is distantly placed with respect to *C. bijugum* and *C. judaicum*.

Some other studies on smaller samples consisting of mostly annual species agree with the above reports. All the studies based on RAPD markers show a close relationship between *C. arietinum* and *C. reticulatum* with both of these along with *C. echinospermum* forming a group while *C. chorassanicum, C. yamashitae, C. pinnatifidum, C. judaicum, C. bijugum* cluster together and *C. cuneatum* shows a distant relationship with all other annual species (Ahmad 1999; Talebi et al. 2009). Choumane et al. (2000) and Sethy et al. (2006) analysed species relationships by STMSs and both the studies showed close genetic similarity between *C. arietinum, C. reticulatum* and *C. echinospermum*. This group was shown to be most closely related to the perennial species *C. anatolicum* by Choumane et al. (2000). Among the other species, *C. bijugum* and *C. pinnatifidum* are closely related as compared to *C. judaicum*, while *C. cuneatum* forms a distant group (Sethy et al. 2006). Similarly, Buhariwala et al. (2005) analysed EST-based markers to divide the species in three clusters, one cluster comprises of *C. arietinum, C. reticulatum* and *C. echinospermum*, the second *C. pinnatifidum, C. bijugum* and *C. judaicum*, and *C. yamashitae, C. chorassanicum* and *C. cuneatum* form the third cluster. Amirmoradi et al. (2012) obtained somewhat different grouping of 8 annual species, by using 3 marker types, i.e. start codon targeted (SCot) polymorphism, directed amplification of minisatellite DNA (DAMD-PCR) and ISSR. Five clusters were formed by ISSR where *C. arietinum* and *C. reticulatum* came together with *C. yamashitae* in the first, the second included *C. echinospermum*, the third *C. pinnatifidum*, fourth *C. cuneatum* and *C. bijugum* and the fifth *C. judaicum*. In Scot analysis, four clusters were formed. *C. arietinum* and *C. reticulatum* clustered with *C. yamashitae* and *C. pinnatifidum*, the second, third and fourth clusters were formed by *C. echinospermum, C. judaicum* and *C. bijugum* and *C. cuneatum,* respectively. Three clusters were observed in DAMD-PCR analysis and the first cluster had *C. arietinum* and *C. echinospermum*, the second *C. judaicum, C. bijugum* and *C. cuneatum* and the third *C. yamashitae* and *C. pinnatifidum*. In a recent study, Andeden et al. (2013) studied genetic diversity and relationships by iPBS-retrotransposons and ISSR markers, of 71 accessions of five annual species and the cultigen from its core area of origin and domestication. The combined ISSR and iPBS analysis divided the accessions in five groups in which *C. arietinum* and *C. reticulatum* form a single group. Another closely associated group belongs to *C. echinospermum* and the rest of the 3 groups are formed by *C. judaicum, C. pinnatifidum* and *C. bijugum*, respectively where *C. judaicum* and *C. bijugum* show greatest dissimilarity.

Genetic variation among 94 genotypes of eight annual species, including the cultigen, and one perennial species *C. microphyllum* has also been studied by single nucleotide polymorphism (SNP) and diversity array technology (DArT) by Roorkiwal et al. (2014). The UPGMA based on SNP markers formed two major groups, one consisting of cultivated genotypes and those of *C. reticulatum* and *C. echinospermum*, while in the other major group the genotypes of secondary gene pool and those of tertiary gene pools form different clusters. *C. reticulatum* shows particularly close relationship with its genotypes interspersed in the cultivated types. The other analysis (STRUCTURE) based on DArT data forms four clusters with a strong difference between cultivated and wild types, and the wild species form three clusters belonging to primary, secondary and tertiary gene pools. This study also brought out higher level of polymorphism among wild as compared to the cultivated genotypes. Moreover, *C. reticulatum* was found to be less diverse as compared to other wild species (Roorkiwal et al. 2014).

3.4.6 Interspecific Hybridization

It has already been mentioned that *C. arietinum* has a narrow genetic base, which crept in during its origin, as compared to its wild relatives (Abbo et al. 2003). This has been later confirmed by many studies using different DNA markers (Udupa et al. 1993; Choumane et al. 2000; Iruela et al. 2002; Nguyen et al. 2004; Choudhary et al. 2012a, b). This kind of situation makes it imperative to use the genetic variation present in wild relatives for further improvement with respect to the yield, nutritional quality and other characters providing resistance against various abiotic and biotic stresses. Many studies describe the extent of crossability of the cultigens with wild annual and some perennial species (Table 3.1).

Ladizinsky and Adler (1976a, b) studied crossability relationships between seven annual species (except *C. chorassanicum* and *C. yamashitae*) and meiotic behaviour of their hybrids. The cross between *C. arietinum* and *C. reticulatum* was most successful with fully viable F_1, regular meiosis and complete fertility. The F_1 was intermediate with respect to growth habit and seed structure and showed segregation in F_2 generation. The hybrid with one line of *C. arietinum*, however, showed a complex of four chromosomes and a bridge and a fragment at meiosis therefore indicating that the two parents differed by a translocation and an inversion (Ladizinsky and Adler 1976a). This again supports *C. reticulatum* as the wild progenitor of chickpea. *C. arietinum* and *C. echinospermum* show a low success rate, however, the F_1 which developed normally was highly sterile. Meiotic analysis showed 6II and a complex of four chromosomes as the two species differ by a translocation. Few seeds obtained from F_1 produced completely sterile F_2 progeny. *C. reticulatum* and *C. echinospermum* were very difficult to cross and only one F_1 showed normal development but was completely sterile. These two species also differed by a translocation as 6II and a complex of four chromosomes in seen at MI. Reciprocal crosses between *C. bijugum, C. pinnatifidum* and *C. judaicum* also

Table 3.1 Results of interspecific crosses involving *Cicer* species

	Author/s	Cross	F_1 status
1.	Ladizinsky and Adler (1976a, b)	*C. arietinum* × *C. reticulatum*	F_1 and F_2 fertile
		C. arietinum × *C. echinospermum*	F_1 Partially fertile
		C. reticulatum × *C. echinospermum*	F_1 fully sterile
		C. judaicum × *C pinnatifidum*	F_1 and F_2 partially fertile
		C. judaicum × *C. bijugum*	F_1 and F_2 partially fertile
		C. pinnatifidum × *C. bijugum*	F_1 and F_2 partially fertile
		C. arietinum × *C. cuneatum*	Failed
		C. judaicum × *C. cuneatum*	Failed
		C. pinnatifidum × *C. cuneatum*	Failed
2.	Mercy and Kakar (1975)	*C. arietinum* × *C. songaricum*	Failure of pollen germination and penetration of pollen tubes in style
3.	Pundir and Mangesha (1995)	*C. arietinum* × *C. echinospermum*	F_1 partially fertile
4.	Singh and Ocampo (1997)	*C. arietinum* × *C. reticulatum*	F_1 and F_2 Fertile
		C. arietinum × *C. echinospermum*	F_1 and F_2 partially fertile
5.	Badami et al. (1997)	*C. arietinum* × *C. pinnatifidum*	Albino plants obtained after embryo rescue
6.	Mallikarjuna (1999)	*C. arietinum* × *C. pinnatifidum*	Sterile F_1s after embryo rescue
7.	Stamigna et al. (2000)	*C. arietinum* × *C. judaicum*	Embryo abortion
		C. arietinum × *C. bijugum*	Embryo abortion
		C. arietinum × *C. pinnatifidum*	Embryo abortion
8.	Ahmad and Slinkard (2004)	*C. arietinum* × *C. echinospermum*	Viable embryo and seed formation
		C. echinospermum × *C. arietinum*	Embryo abortion
		C. arietinum × *C. pinnatfidum*	Embryo abortion
		C. pinnatifidum × *C. arietinum*	Embryo abortion
		C. arietinum × *C. judaicum*	Embryo abortion
		C. judaicum × *C. arietinum*	Embryo abortion
		C. arietinum × *C. chorassanicum*	Embryo abortion
		C. chorassanicum × *C. arietinum*	Embryo abortion
		C. arietinum × *C. yamashitae*	Embryo abortion
		C. yamashitae × *C. arietinum*	Embryo abortion
		C. arietinum × *C. cuneatum*	Embryo abortion
		C. cuneatum × *C. arietinum*	Embryo abortion
		C. arietinum × *C. bijugum*	Embryo abortion
9.	Clarke et al. (2006)	*C. arietinum* × *C. bijugum*	F_1 breakdown during embryogenesis
		C. arietinum × C. pinnatifidum	

(continued)

Table 3.1 (continued)

	Author/s	Cross	F_1 status
10.	Mallikarjuna et al. (2007)	C. arietinum × C. bijugum	Green F_1 plants selected after embryo rescue
11.	Kumari et al. (2011)	*C. arietinum* × *C. pinnatifidum*	Albino, partially green and green plantlets obtained after embryo rescue
		C. arietinum × *C. judaicum*	
12.	Clarke et al. (2011)	*C. arietinum* × *C. judaicum*	Albino, pale, green F_1 plants after embryo rescue
		C. arietinum × C. pinnatifidum	
13.	Abbo et al. (2011)	*C. judaicum* × *C. bijugum*	F_1 pollen stainability 50 %, F_2 breakdown
		C. judaicum × *C. pinnatifidum*	F_1 with protruding pistils & 30 % pollen stainability, F_2 breakdown
		C. cuneatum × *C. canariense*	50 % pollen stainability, F_2 breakdown
14.	Singh and Singh (2012)	*C. arietinum* × *C. judaicum*	F_1 partially sterile, 54 % pollen stainability
		C. judaicum × *C. arietinum*	

resulted in F_1s with intermediate morphology. Meiosis showed bivalents with variable univalents on the basis of which *C. pinnatifidum* was shown to be closer to *C. bijugum* than to *C. judaicum*. The hybrids though showed 30–50 % pollen fertility, did not result in any seed formation due to elongation of style (prezygotic barrier) at anthesis. However, hand pollination resulted in reasonably good seed production and F_2 progeny showing a close relationship between these three species. *C. cuneatum* which was crossed with *C. arietinum, C. judaicum* and *C. pinnatifidum* did not result in any viable seed though some empty pods were developed showing post zygotic barriers (Ladizinsky and Adler 1976a, b). Similar results were obtained by Abbo et al. (2011) in crosses involving *C. judaicum, C. bijugum* and *C. pinnatifidum*. The cross between *C. judaicum* and *C. bijugum* resulted in partially fertile F_1s which showed further breakdown in the F_2 and between *C. judaicum* and *C. pinnatifidum* resulted in F_1s with protruding styles which were backcrossed with *C. pinnatifidum* producing highly sterile BC_1F_1 plants. Interestingly, *C. cuneatum* × *C. canariense* cross succeeded resulting in F_1s with normal meiotic pairing and more than 50 % pollen stainability therefore supporting close relationship between these two species (Abbo et al. 2011; van der Maesen et al. 2007). Embryo rescue was used to obtain F_1 plants between *C. arietinum* and *C. judaicum* which showed intermediate characters and normal meiotic behaviour with 54 % pollen stainability (Singh and Singh 2012). There is no report of a successful cross between *C. arietinum* and any perennial species as with *C. songaricum* no hybrid seed was obtained despite large number of crosses (Mercy and Kakkar 1975). Hybridization of *C. arietinum* with *C. canariense* was possible as the pollen tubes germinated and the embryos grew up to globular stage and no plants were obtained (Mallikarjuna 2001).

3.4.7 Barriers to Hybridization

Barriers to interspecific crossability among the *Cicer* species occur at post zygotic level. The hybrid breakdown can occur due to various reasons such as embryo abortion (Ahmad et al. 1988; Bassiri et al. 1987; Badami et al. 1997; Mallikarjuna 1999; Stamigna et al. 2000; Ahmad and Slinkard 2004; Clarke et al. 2006; Mallikarjurna et al. 2011), albinism (Mallikarjuna and Jadhav 2008; Kumari et al. 2011; Clarke et al. 2011) or pollen sterility due to reduced chromosome pairing (Ladizinsky and Adler 1976a, b; Abbo et al. 2011). Another mechanism which may lead to failure of fertilization due to abnormal flower development causing protrusion of stigma in F_1 or F_2 progeny of certain crosses (Ladizinsky and Adler 1976b; Abbo et al. 2011). Mallikarjuna et al. (2011) have also described crosses between some annual and perennial species which resulted in various percentages of pod set but in no case a viable seedling was obtained.

3.5 Gene Pools of Chickpea

Redden and Berger (2007) included *C. reticulatum* along with various landraces and cultivars of *C. arietinum* in the primary gene pool, *C. echinospermum* in the secondary genepool, and rest of the annual and perennial species which are genetically highly differentiated from the cultigens comprised the tertiary gene pool. This demarcation has been altered a little by placing *C. reticulatum* in the secondary gene pool (Table 3.2, Mallikarjuna et al. 2011). This is quite appropriate considering the differential crossability success of *C. reticulatum* with various cultivars of

Table 3.2 Gene pools of *Cicer arietinum*

Primary	Secondary	Tertiary
Land races and cultivars of *C. arietinum*	*C. reticulatum*, *C. echinospermum*	*C. bijugum, C. judaicum, C. pinnatifidum, C. chorassanicum, C. yamashitae, C. cuneatum, C. atlanticum, C. incisum, C. incisum ssp. serpentinica, C. floribundum, C. floribundum var. amanicola, C. graecum, C. heterophyllum, C. heterophyllum* var. *kassianum, C. uludereensis, C. isauricum, C. montbretii, C. acanthophyllum, C. anatolicum, C. balcaricum, C. baldshuanicum, C. fedtschenkoi, C. flexuosum, C. grande, C. incanum, C. korshinskyi, C. laetum, C. luteum, C. macracanthum, C. microphyllum, C. multijugum, C. nuristanicum, C. paucijugum, C. pungens, C. rassuloviae, C. rechingeri, C. songaricum, C. stapfianum, C. subaphyllum, C. tragacanthoides, C. kermanense, C. mogoltavicum, C. oxyodon, C. spiroceras, C. canariense*

C. arietinum (used as female parent) and occurrence of inversions in crosses between *C. reticulatum* and some of the cultivars of *C. arietinum* (Ladizinsky and Adler 1976a) as well as by the differences in karyotypes and genome size (Ohri and Pal 1991; Galasso et al. 1996). Similar differences in crossability success have been shown between *C. echinospermum* and different lines of *C. arietinum* (Singh and Ocampo 1997; Collard et al. 2003; Mallikarjuna et al. 2011).

Many of the accessions of wild species belonging to secondary and tertiary gene pools have been identified for showing resistance to various abiotic and biotic stresses such as drought, suboptimal temperature, nutrient imbalance, salinity, ascochyta blight, fusarium wilt, botrytis grey mould, collar rot, leaf blight, pod borer, leaf minor, seed beetles, nematods, etc. (Toker et al. 2014). Nevertheless, it has already been mentioned that except in case of species belonging to the secondary gene pool the crosses of the cultigens with the species in tertiary gene pool invariably fail due to strong post zygotic barriers. In some cases even the plants obtained as a result of embryo rescue result in complete sterility (Mallikarjuna et al. 2011).

3.6 Molecular Maps

It has already been pointed out that the productivity of chickpea is adversely affected by some fungal diseases such as ascochyta blight and fusarium wilt in addition to some agronomic traits like flowering time, time to maturity, podding habit, etc. Efforts have been going on to map the genes of interest which may facilitate marker assisted selection and map based cloning of useful genes. However, the genetic variation within chickpea is minimal because of the bottlenecks it experienced during the course of domestication. Therefore, interspecific crosses have been attempted to maximize polymorphism for linkage analysis, though intraspecific crosses have also been used in some cases. To achieve this objective two types of mapping populations have been utilized to generate linkage maps, the F_2 population and recombinant inbred lines (RILs).

An integrated map has been prepared using 130 RILs from a wide cross between a *C. arietinum* cultivar resistant to fusarium wilt and *C. reticulatum*. A total of 354 markers including 118 STMSs, 96 DAFs, 70 AFLPs, 37 ISSRs, 17 RAPDs, 2 SCARs, 3 loci conferring resistance to various races of *Fusarium*, 8 isozymes and 3 cDNAs covered a distance of 2077.9 cM. Eight large and eight small linkage groups were identified with the average distance of 6.8 cM between the markers (Winter et al. 2000).

Another consensus map has been prepared by merging linkage maps from 10 different populations derived from five wide cross *C. arietinum* × *C. reticulatum* and five narrow ‘Desi’ × ‘Kabuli’ cross using STMS markers. The integrated map from wide crosses comprised of 555 loci including 135 STMSs and 33 cross genome markers distributed on eight linkage groups covering 652.67 cM. The map from narrow crosses involved 99 STMSs, 3 SCARs, 1 ASAP, *Fusarium* resistance

gene, five morphological markers and RAPD and ISSR markers distributed on eight linkage groups covering 426.99 cM (Millan et al. 2010).

Similarly a high density map has been developed, based on RIL population between *C. arietinum* and *C. reticulatum*, with the help of SSR markers from bacterial artificial chromosome (BAC)-end sequences (BESs) and diversity array technology (DArT) markers. The map comprised of 1291 markers on eight linkage groups spanning 845.56 cM. The number of markers per linkage group ranged from 68 (LG8) to 218 (LG3) with an average inter marker distance of 0.65 cM (Thudi et al. 2011).

Choudhary et al. (2012a, b) developed different types of 487 novel EST-derived functional markers such as EST-SSRs, ITP, ESTPs, and SNPs to maximize the detection of polymorphisms in a mapping population of 129 RILs derived from *C. arietinum* (*Fusarium* resistant drought tolerant) × *C. reticulatum* (*Fusarium* wilt susceptible) cross. These markers were integrated with previously published STM markers to produce an advanced linkage map containing 406 loci distributed on eight linkage groups covering 1497.7 cM with the average marker density of 3.68 cM.

Santra et al. (2000) produced a map of nine linkage groups from an RIL population of *C. arietinum* and *C. reticulatum* cross. A total of 116 markers (isozymes, RAPDs, ISSRs) covered a map distance of 981.6 cM with an average distance of 8.4 cM between markers. Two quantitative trait loci (QTL-1 and QTL-2) conferring resistance to ascochyta blight have been tagged with different markers. Same RIL population was used by Takeoglu et al. (2002) to integrate 50 sequence tagged microsatellite (STMS) markers and a resistant gene analogue (RGA) locus to prepare a map covering 1174.5 cM with an average distance of 7.0 cM between markers on nine linkage groups. Six STMS markers were integrated into map region where 2 QTLs reported by Santra et al. (2000) were located. Also 2 DAFs were shown to be tightly linked to QTL-1 in the same RIL population (Rakshit et al. 2003). Cobos et al. (2006) used RILs from a cross of *C. arietinum* (resistant parent) and *C. reticulatum* (susceptible parent) to prepare a linkage map covering a distance of 601.2 cM in 10 linkage groups. However, the QTL for resistance to ascochyta blight was shown to be different as compared to previous studies as it was located on linkage group2 (LG2). Aryamanesh et al. (2010) studied interspecific F_2 population to identify 3 QTLs explaining 49 % of variation for ascochyta blight resistance on LG3 and LG4.

A composite linkage map was prepared using two RIL populations from *C. arietinum* and *C. reticulatum* cross showing segregation for resistance to ascochyta blight, fusarium and rust diseases. It was possible to map loci conferring resistance to ascochyta blight and fusarium wilt by RGA markers. Association was detected between RGAs and genes that controlled resistance to fusarium wilt caused by races 0 and 5 (Palomino et al. 2009).

Collard et al. (2003) prepared a linkage map from F_2 population from *C. arietinum* (susceptible to ascochyta blight) and *C. echinospermum* (resistant to ascochyta blight). Map covered a distance of 570 cM and at least two QTLs for seedling resistance were located on LG4.

With an objective of studying nutritional characters the F_2 population from a cross between *C. arietinum* and *C. reticulatum* was studied with 91 STMS and 2 CytP450 markers to generate a linkage map consisting of nine linkage groups and covering 344.6 cM. Four QTLs for beta-carotene concentration, 1 QTL for lutein concentration and 3 QTLs for seed weight were identified (Abbo et al. 2005).

Cho et al. (2004) used F7 derived RILs from intraspecific cross of susceptible and a resistant accession to prepare a linkage map and identified regions associated with blight resistance, a major QTL for resistance to pathotype II of *Ascochyta rabiei* and two QTLs for resistance to pathotype I. Flandez-Galvez et al. (2003) prepared a linkage map from F_2 population of chickpea cultivars showing contrasting disease reaction to *A. rabiei*. Fifty one STMS, 3 ISSR, and 12 RGA markers mapped on eight linkage groups. The map covered a distance of 534.5 cM with an average of 8.1 cM between markers. Chickpea derived STMS markers were distributed throughout the genome, but RGA markers clustered with ISSR markers on the linkage groups LGI, II and III. With an objective to map genetic loci associated with QTLs for ascochyta blight resistance, Taran et al. (2007) developed an F_2 population of 186 plants derived from a cross between a 'Kabuli' and a 'Desi' cultivars. A total of 144 SSR markers and I morphological marker were assigned to eight linkage groups in a map spanning 1285 cM. One QTL each for ascochyta blight resistance was found on linkage groups LG3, LG4, LG6. Madrid et al. (2008) analyzed an RIL population from *C. arietinum* and *C. reticulatum* cross and identified a QTL for chickpea rust resistance on LG7. Two STMS markers were identified flanking this resistance gene.

Radhika et al. (2007) developed a composite intraspecific map from two RIL populations with one common parent. Three yield related traits were analyzed with different markers to prepare a map covering a 739.6 cM. The characters of double podding and seeds per pod were tagged by different markers and 8 QTLs were found to influence seed weight.

In order to analyze the complex drought related traits two intraspecific mapping populations were studied for segregation of drought tolerance related root traits. This resulted in a consensus map consisting of 352 loci and identification of 9 QTL clusters containing QTLs for drought tolerance traits which can be targeted for molecular breeding (Varshney et al. 2014).

3.7 Conclusions

Chickpea holds a prominent position among grain legumes providing relatively cheap source of protein to the humankind. It originated in Near East from its progenitor species *C. reticulatum* which has a very restricted distribution. During the process of origin various bottlenecks have resulted in a very narrow genetic base in the cultigens. Today chickpea is available as two main types 'Kabuli' and 'Desi' which are considered to have diverged after originating from *C. reticulatum*. The genus *Cicer* comprises 49 taxa including nine annual species. The phylogenetic

relationships of these species have been discussed on the basis of morphology, cytology, hybridization and molecular studies. These studies have resulted in the demarcation of primary secondary and tertiary gene pools. While crosses between taxa belonging to primary and secondary gene pools are feasible and result in hybrid progeny which is vegetatively and sexually viable, those involving tertiary gene pool are completely unsuccessful. Even the plants obtained by embryo rescue do not survive beyond a certain stage and are highly sterile. This produces a big constraint on the introduction of genes conferring resistance to various biotic and abiotic stresses and nutritional and yield components from the wild species to the cultigens. This problem is being addressed by QTL mapping of mostly disease resistance loci from the RIL's produced from intra as well as interspecific crosses. Further efforts are being made to integrate genetic maps with physical maps. These methods provide a strong basis for genetic and genomic analysis of chickpea genome and facilitate further the use of molecular methods in breeding.

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