Biotechnology and Genetic Engineering Reviews - Vol. 25, 267-314 (2008)

Chickpea Improvement: Role of Wild Species and Genetic Markers

RAKESH SINGH[1], PANKAJ SHARMA[2], RAJEEV K. VARSHNEY[3], S. K. SHARMA[1], N. K. SINGH[4]*

[1]National Research Centre on DNA Fingerprinting, National Bureau of Plant Genetic Resources, New Delhi-1100 12, INDIA, [2]Bayer BioScience Pvt. Ltd., Tollichowki, Hyderabad- 500 008, Andhra Pradesh, India, [3]International Crops Research Institute for Semi-Arid Tropic Crops (ICRISAT), Patancheru-502324, Andhra Pradesh, India and [4]National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi-110 012, India

Abstract

Chickpea is an important grain legume of the semi arid tropics and warm temperate zones, and forms one of the major components of human diet. However, a narrow genetic base of cultivated chickpea (*Cicer arietinum* L.) has hindered the progress in realizing high yield gains in breeding programs. Furthermore, various abiotic and biotic stresses are the major bottlenecks for increasing chickpea productivity. Systematic collection and evaluation of wild species for useful traits has revealed presence of a diverse gene pool for tolerance to the biotic and abiotic stresses. Relationships among the species of genus *Cicer* are presented based on crossability, karyotype and molecular markers. The reproductive barriers encountered during interspecific hybridization are also examined. Recent information on genetic linkage maps, comparison of isozymes

*To whom correspondence may be addressed (nksingh@nrcpb.org)

Abbreviations: AB-QTL, Advanced Backcross-Quantitative Trait Loci; AFLP, Amplified Fragment Length Polymorphism; ASAP, Allele Specific Associated Primers; BAC, Bacterial Artificial Chromosome; Dart, Diversity Array technology; ESTs, Expressed Sequence Tags; ICARDA, International Centre for Arid and Dry Land Agriculture; ICRISAT, International Crop Research Institute for the Semi Arid Tropics; ISSR, Inter Simple Sequence Repeats; MAS, Molecular Assisted Selection; PCR, Polymerase Chain Reaction; QTL, Quantitative Trait Loci; RAPD, Randomly Amplified Polymorphic DNA; RFLP, Restriction Fragment Length Polymorphism; RILs, Recombinant Inbred Lines; SAGE, Serial Analysis of Gene Expression; SCAR, Sequence Characterised Amplified Region; SNPs, Single Nucleotide Polymorphisms; SSR, Simple Sequence Repeats; STMS, Sequence Tagged Microsatellite Sequence.

and different DNA marker systems used for diversity analysis in chickpea germplasm, tagging of genes/QTLs for qualitative and quantitative traits and progress in application of marker assisted selection and genomics in chickpea are presented.

Introduction

Chickpea (*Cicer arietinum* L.), is the third most important cool season food legume in the world after dry beans and peas (FAOSTAT, 2004). It has been cultivated mainly in the Indian subcontinent, West Asia, and North Africa, but recently large acreages have been introduced in the Americas and Australia. Chickpea is a diploid with 2n = 2x = 16 (Arumuganathan and Earle, 1991) having a genome size of approximately 931 Mbp (http;//www.rbgkew.org.uk/cval/, *Table 1*). It is a highly self-pollinated crop with an outcrossing rate of less than 1%. It serves as an important source of protein in human diet and plays an important role in the enrichment of soil fertility. Two main types of chickpea cultivars are grown globally– *kabuli* and *desi,* representing two diverse gene pools. The *kabuli* types are generally grown in the Mediterranean region including Southern Europe, Western Asia and Northern Africa and the *desi* types are grown mainly in Ethiopia and Indian subcontinent. *Desi* chickpeas are characterized by angular seed shape, dark seed coat, pink flowers, anthocyanin pigmentation of stem, rough seed surface and either semi-erect or semi-spreading growth habit, whereas *kabuli* types generally have owl shaped seeds, beige coloured seeds, white flowers, smooth seed surface, lack of anthocyanin pigmentation and semi-spreading growth habit (Pundir *et al.*, 1985).

Table 1. General information on chickpea.

Features	*Specifications in chickpea*	*References*
Common name	Gram, *Chana* (Hindi)	
Botanical genera	*Cicer*	
Cultivated species	*arietinum*	
Related wild species	*Cicer reticulatum*	
Ploidy level and Chromosome number	*Diploid (2n= 2x= 16)*	Millan *et al*., 2006
Genome size	*931 Mbp*	http://www.rbgkew.org.uk
BAC libraries	3.8×	Rajesh *et al.*, 2004
	2.5×	Lichtenzveig *et al*., 2005
Binary BAC library	4.5×	Lichtenzveig *et al*., 2005
ESTs	~3000	Buhariwalla *et al*., 2005 http://www.icrisat.org/gt1/cpest/home.asp Coram and Pang, 2005a
SSR markers	~700	Winter *et al*., 1999 Huttel *et al*., 1999 Sethy *et al*., 2006a and 2006b Lichtenzveig *et al*., 2005 Choudhary *et al*., 2005 Varshney *et al*., unpub. Bhatia *et al*., unpub.
Gene arrays	768 feature microarray SAGE gene chips	Coram and Pang, 2005b P. Winter, Pers. comm.

The genus *Cicer*

The *Cicer* genus belongs to family Leguminoseae, sub-family Papilionaceae and tribe Cicereae. It encompasses 9 annual and 34 perennial wild species. Most of these species are found in West Asia and North Africa covering Turkey in the north to Ethiopia in the south, and Pakistan in the east to Morocco in the west (*Figure 1*). Of the 9 annual species, chickpea (*Cicer arietinum* L.) is the only cultivated species. The eight other annual species of chickpea are wild and include: *C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum, C. cuneatum, C. chorassanicum* and *C. yamashitae*. Van der Maesen (1987) classified the *Cicer* species into four sections based on their morphological characteristics, life cycle and geographical distribution. Eight annual species namely *C. arietinum, C. reticulatum, C. echinospermum, C. pinnatifidum, C. bijugum, C. judaicum, C. yamashitae* and *C. cuneatum* were placed in Section Monocicer, *C. chorassanicum* and *C. incisum* (perennial species) in section Chamaecicer, 23 perennial species in section Polycicer and seven woody perennial species in section Acanthocicer.

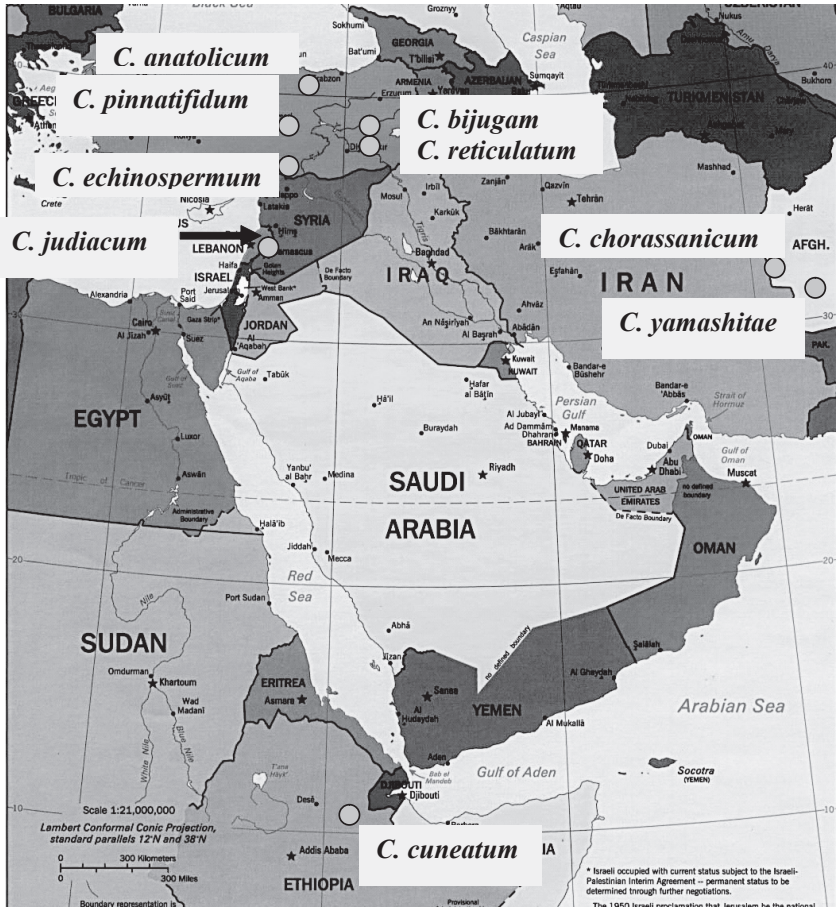

Figure 1. Geographical distribution of different *Cicer* species.

Genetic relationships among *Cicer* species

The knowledge of genetic relationships between the cultivated chickpea and its wild relatives is a prerequisite to track the evolution of cultivated species and also to determine the close relatives which can be exploited for introgression of useful traits into the cultigen in plant breeding programmes. Hawkes (1977) emphasized the need to understand crossability relationships, chemotaxonomic relationships and cytogenetical affinities between wild species and cultigen before making use of wild forms. Interspecific hybridization, seed storage protein profiles, isozymes, karyotype and molecular markers have been used as different criteria to investigate species relationships in the *Cicer* genus.

INTERSPECIFIC HYBRIDIZATION

Based on the studies on crossability and fertility of hybrids in interspecific crosses among seven annual species of chickpea, Ladizinsky and Adler (1976a) have placed seven annual *Cicer* species (except *C. chorassanicum* and *C. yamashitae*) into the following three crossability groups. Interspecies hybridization is possible within the group but not between the groups.

Group I: It is the most important group among the annual *Cicer* species because it includes the cultivated species *C. arietinum* along with its close wild relatives, *C. reticulatum* and *C. echinospermum*. The regular formation of 8 bivalents during meiosis, completely fertile F_1 hybrids and normal segregation of F_2 progenies in *C. arietinum* x *C. reticulatum* crosses clearly shows high cross compatibility between the two species (Pundir and van der Maesen, 1983; van der Maesen, 1980). The high crossability and thereby free gene exchange between the two species compared to other annual species supports the idea of *C. reticulatum* being the possible wild progenitor of cultivated chickpea.

Interspecific hybrids between *C. arietinum* and *C. echinospermum* develop normally but show high degree of sterility. The difference in pollen fertility among reciprocal crosses between *C. arietinum* x *C. echinospermum* indicated presence of maternal effects (Pundir and Mengesha, 1995; Singh and Ocampo, 1993), and suggests that the cultivated species should be always used as the female parent for interspecific hybridization. The low success rate of crosses has been observed when *C. echinospermum* was used as the female parent. Formation of a quadrivalent during meiosis in some crosses of *C. arietinum* x *C. echinospermum* indicated presence of a reciprocal translocation while paracentric inversions was seen in some other crosses leading to formation of unbalanced gametes (Ladizinsky and Adler, 1976b). Pundir and Mengesha (1995) observed regular meiosis with 8 bivalents at Metaphase I in 96% of pollen mother cells and lagging behind of one chromosome at Anaphase I in 6% of cells. The F_2 progenies of these two species, despite their similar chromosome number and almost regular meiosis in F_1, exhibited varying degrees of sterility (0–100%) (Pundir and Mengesha, 1995) and low pod set (Verma *et al.*, 1990). The sterility of the hybrid may be due to reciprocal translocation or cryptic structural changes or the involvement of genetic factors (Ladizinsky and Adler, 1976b).

Group II: It includes annual species *C. bijugum, C. judaicum* and *C. pinnatifidum*. The three species show close homology of chromosomes but no seeds were produced

in any of the F_1 hybrid combinations because of the unique post zygotic reproductive barrier. Despite high levels of pollen fertility in their F_1 hybrids, disharmony in the growth rate of the stigma and anthers at the time of anthesis of the F_1 interspecific hybrid leads to failure of seed set. However, fully fertile interspecific hybrids among the three species were produced by hand pollination. Pundir and van der Maesen (1983) reported partial sterility in F_1 hybrids between the three species and suggested the need of hand pollination for seed set. Crosses between the three annual wild species and *C. arietinum* yielded no viable seeds. By attempting multiple numbers of pollinations, interspecific hybrids have been produced between *C. arietinum* and group II species *C. judaicum, C. pinnatifidum* and *C. bijugum*. The success in producing interspecific hybrids depends upon the number of pollinations attempted and the specific cultivar-wild species combination (Verma *et al.*, 1990). The accession GLG 84038 of cultivated species exhibited the highest level of crossability with wild species, which indicates that the genetic variability within the cultivated species plays an important role in crossability with the wild species (Verma *et al.*, 1990 and 1995). Crosses between *Cicer arietinum* and *C. bijugum or C. judaicum* usually abort within 7 days after pollination. Use of *C. arietinum* as female parent and application of a combination of growth regulators to the pollinated pistils had a major effect on hybridization success. KAK-2, ICCV-2, Sonali or Macarena were better female parents with respect to number and the size of hybrid ovules/embryos obtained. Some successful hybrids between *C. arietinum* and *C. bijugum* have also been reported and their hybridity confirmed by molecular analysis (Lulsdorf *et al.*, 2005). A very low level of seed set has been reported in the cross *C. arietinum* x *C. pinnatifidum* (Badami *et al.*, 1997). However, the pod formation in this case required application of growth regulators to the pollinated pistils immediately after pollination. Ahmad *et al.* (1987) reported interspecific hybrids between *C. judaicum* x *C. chorassanicum*, which were highly sterile with a low pollen fertility of 4.8%. The stigma and style grew out of the keel petals at the time of anthesis, while the anthers remained inside in all hybrid flowers. Ahmad *et al.* (1988) also reported an interspecific hybrid between *C. chorassanicum* x *C. pinnatifidum,* and placed *C. chorassanicum* along with group II species and *C. yamashitae* in a separate group.

Group III: This includes only one annual species *C. cuneatum*, originated in Ethiopia. It exhibits cross incompatibility with group I and group II species and thus is considered as the most distant relative of the cultivated species. *C. cuneatum* is the only annual *Cicer* species having climbing growth habit.

KARYOTYPE STUDIES

All the annual species of *Cicer* have the same chromosome number 2n = 16 (Ladizinsky, 1976a). *C. arietinum* has three submetacentric and five metacentric chromosomes. The karyotypic studies showed significant difference in the nuclear DNA content of the annual species of chickpea ranging from 1.83 pg (*C. judaicum*) to 3.57 pg (*C. arietinum*). Based on the nuclear DNA content, the annual species of *Cicer* were classified into three groups (Ohri and Pal, 1992). Group 1: *C. judaicum*, Group 2: *C. cuneatum, C. bijugum, C. pinnatifidum, C. reticulatum* and *C. echinospermum* and Group 3: *C. arietinum. C. reticulatum*, the closest wild relative of chickpea and probably the wild progenitor of the cultivated species, differ from *C. arietinum* in

having one extra satellite pair and 22.3 per cent less 2C DNA content. However, no significant difference in DNA content was observed between *desi* and *kabuli* cultivars of chickpea despite their geographical divergence and clear phenotypic differences. The nuclear DNA content of *C. reticulatum* and *C. echinospermum* is similar and lower than *C. arietinum* (Galasso *et al.*, 1996).

Significant differences in the haploid genome content and C-heterochromatin content within and among the annual *Cicer* species have been observed (Tayyar *et al*., 1994). The mean haploid genome length ranged from 20 um in *C. judaicum* to 28.7 um in *C. arietinum*, which shows a positive correlation between chromosome length and total DNA content. Within accession variability for haploid genome content was observed for *C. bijugum*, *C. judaicum* and *C. reticulatum* and it is particularly high in *C. judaicum* (38%). *C. chorassanicum* has the lowest (38.4%) C-heterochromatin content whereas *C. cuneatum* has the highest (61.3%). Based on the total C-heterochromatin content, the nine annual *Cicer* species have been divided into two groups: *C. cuneatum* (61.3%) and *C. bijugum* (57.7%) in one group, and the rest of the species in another group (range 38.4 - 46.0%) (Tayyar *et al*., 1994). *C. cuneatum* and *C. bijugum* have quite similar karyotypes though they belong to different crossability groups and having distinct morphological characteristics, isozyme and seed storage protein profiles. The two species *C. judaicum* and *C. pinnatifidum* belonging to the same crossability group have small chromosomes and similar C-banding patterns. The three closely related species *C. arietinum* (43%), *C. reticulatum* (42%) and *C. echinospermum* (39%) exhibited almost similar amounts of C-banded heterochromatin (Galasso *et al.*, 1996) and also quite similar chromosome banding patterns (Tayyar *et al*., 1994).

Karyotype analyses have shown that only one chromosome pair of chickpea genome is associated with the nucleolar organizing regions (Ahmad and Hymowitz, 1993; Ocampo *et al.*, 1992). The satellite is present on the longest first chromosome in *C. arietinum, C. reticulatum* and *C. echinospermum* and an extra satellite in case of *C. reticulatum* is present on second chromosome. Based on the satellite information and karyotype asymmetry, *C. arietinum, C. reticulatum* and *C. echinospermum* were placed in one group and the rest of the species in other group (Ocampo *et al*., 1992). *C. anatolicum*, one of the 34 wild perennial relatives of chickpea, also has the same diploid chromosome number and exhibits karyotypic similarity with group I species (Ahmad, 1989). *C. anatolicum* also carries the satellite on the first chromosome. The highest degree of karyotype asymmetry was observed in *C. arietinum, C. reticulatum* and *C. echinospermum* which are considered as highly evolved since asymmetric karyotype is positively correlated with specialization and is more favoured during evolution. The karyotype of *C. arientinum* has been described as an asymmetrical type compared to the other pulse crops (Lavania *et al*., 1983).

SEED STORAGE PROTEIN PROFILING

Seed storage protein profiles have been used as a tool for assessing species relationships in many crop plants. Ladizinsky and Adler (1975) observed that protein electrophoretic profiles of *C. reticulatum* resemble closely to that of *C. arietinum*. Globulin fraction of seed storage proteins exhibited major differences in polypeptide composition among the members of group I species (Vairinhos and Murray, 1983). Distribution of legumin-like polypeptides in *C. arietinum* resemble more closely to *C. reticulatum* than *C.*

echinospermum. The accessions of cultivated species exhibited similar seed storage protein patterns (Ahmad and Slinkard, 1992), which show a very low genetic diversity in cultivated chickpea. However, distinct species-specific profiles were observed for each of the nine *Cicer* species. The studies have placed *C. arietinum*, *C. reticulatum* and *C. echinospermum* in group I, *C. bijugum*, *C. pinnatifidum*, *C. judaicum* and *C. chorassanicum* in group II, *C. yamashitae* in group III and *C. cuneatum* in group IV, which is consistent with the grouping of species based on crossability studies.

ISOZYME STUDIES

Isozymes have provided a valuable tool for the study of genetic variation in crop populations and to investigate species relationships. Kazan and Muehlbauer (1990) analysed 9 annual and one perennial species of chickpea for 19 enzymes representing 30 putative gene loci and classified the species into four groups based on their isozyme profiles: Group I: *C. arietinum*, *C. reticulatum*, *C. echinospermum* and *C. anatolicum*, Group II: *C. judaicum*, *C. bijugum* and *C. pinnatifidum*, Group III: *C. chorassanicum* and *C. yamashitae*, Group IV: *C. cuneatum.* Further studies on the annual species of chickpea using isozymes have also grouped the species in a similar pattern (Ahmad *et al.*, 1992; Labdi *et al*., 1996; Tayyar and Waines, 1996). However, there was a discrepancy in placement of *C. chorassanicum* with *C. yamashitae* in the same group based on isozyme studies. But *C. chorassanicum* and *C. yamashitae* have been assigned to different groups based on interspecific hybridization studies (Ahmad, 1988) and seed storage protein profiles (Ahmad and Slinkard, 1992). The species relationships based on randomly amplified polymorphic DNA (RAPD) markers also placed both the species in the same cluster as that determined by isozyme studies (Ahmad, 1999). Though *C. chorassanicum* is reproductively isolated from *C. yamashitae*, evolution of reproductive barrier(s) does not necessarily follow the divergence of isozyme loci (Ahmad *et al*., 1992). The perennial species *C. anatolicum* and *C. soongaricum* have been placed in one separate cluster along with *C. chorassanicum* and *C .yamashitae* (Tayyar and Waines, 1996).

MOLECULAR STUDIES

DNA markers have been used widely for fingerprinting of plant genomes, genetic diversity analysis and to understand the evolutionary relationships among crop species. Ahmad (1999) and Sudupak *et al*. (2002) used RAPD markers to investigate genetic relationships among the annual *Cicer* species. The RAPD analysis placed *C. arietinum, C. reticulatum* and *C. echinospermum* in a single cluster, *C. yamashitae* and *C. chorassanicum* in the next cluster, *C. pinnatifidum, C. bijugum* and *C. judaicum* in third cluster and *C. cuneatum* in the fourth cluster. AFLP markers have also been used to study the genetic relationships in the nine annual species (Sudupak *et al*., 2004) and the grouping was similar as found with the RAPD makers (Sudupak, *et al*, 2002; Shan *et. al.*, 2005). The grouping of annual *Cicer* species based on these RAPD analysis disagree with the previous crossability and isozyme based grouping in placing *C. chorassanicum* and *C. yamashitae* in a cluster next to *C. arietinum* (Tayyar and Waines, 1996). Successful interspecific hybrids of *C. arietinum* with

either *C. chorassanicum* or *C. yamashitae* have not been produced; instead they have been placed more closely to *C. arietinum* than group II species. However, the ability to interbreed should not be treated as the sole criteria in determining the degree of relatedness between species. Closely related species commonly loose the ability to interbreed and become genetically isolated due to small number of chromosomal structural mutations (Tayyar and Waines, 1996).

The sequences flanking microsatellite sites are generally conserved within species and also often in closely related species (Gupta and Varshney 2000). The flanking sequences of microsatellite loci of cultivated chickpea were found to be conserved in related annual species also (Choumane *et al*., 2000; Winter *et al.*, 1999). The highest degree of conservation was observed in *C. reticulatum* (92 per cent) and *C. echinospermum* (83 per cent), whereas *C. cuneatum* showed the lowest (50 per cent) (Choumane *et al.*, 2000), which further supports the crossability, isozyme and RAPD studies based grouping of species. *C. anatolicum*, a wild perennial relative of chickpea, showed 72 per cent conservation, which strongly supports the karyotypic studies (Ahmad, 1989). *Cicer* species present in group I are more closely related to the perennial relative *C. anatolicum* than to the other annual wild species. However, studies on crossability of *C. anatolicum* with the cultigen need to be done since *C. anatolicum* carries genes for resistance to Aschochyta blight (Muehlbauer *et. al.*, 1994b). Similarities in the length of the 5S and 18-25S rRNA units between *C. arietinum* and *C. reticulatum* have also been observed (Patil *et al*., 1995).

C. reticulatum is the only annual wild species that exhibits high cross-compatibility with the cultivated *Cicer* species and thereby open the possibility for unhindered gene transfer *via* hybridization. Molecular studies have also clearly indicated a close relationship between *C. arietinum*, *C. reticulatum* and *C. echinospermum*, which strongly support the idea of *C. reticulatum* as the possible wild progenitor of cultivated chickpea. In addition, morphological studies also indicated that the three annual species *C. reticulatum*, *C. echinospermum* and *C. bijugum* are closest to *C. arietinum* (Robertson *et al*., 1997). The distinct morphological characteristics, seed storage protein, isozyme and RAPD profiles of *C. cuneatum* show its distant relationship with the cultivated species (*Figure 2*). Based on the genetic relationships, Ladizinsky and Adler (1976a) have placed *C. reticulatum* in primary gene pool, *C. echinospermum* in secondary and the other annual wild species in tertiary gene pool following the classification of Harlan and De Wet (1971). Presence of duplication in gene loci encoding isozymes in all the annual *Cicer* species suggested monophyletic origin of the *Cicer* species (Kazan and Muehlbauer, 1990). The possibility of perennial species *C. anatolicum* being the progenitor of three group I species has yet to be studied. The species in secondary and tertiary gene pools could be effectively exploited for genetic enhancement of chickpea by overcoming pre-and post-fertilization barriers or through genetic transformation route.

Role of wild species in chickpea improvement

World chickpea germplasm lacks the desirable agronomic traits needed for ready utilization in varietal improvement programmes. Use of wild species and primitive cultivars provides a wider genetic base to the crop species and furthermore, they are the potential source of resistance genes for various biotic and abiotic stresses

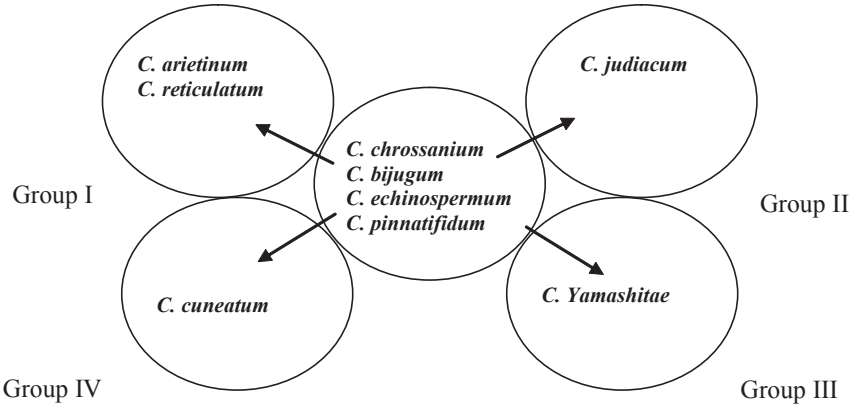

Figure 2. Schematic diagram depicting relationships between nine annual *Cicer species* based on different methods used to establish the relationships. Controversial species are placed in centre and arrow indicates the group in which they can be placed.

(Hawkes, 1977; Van der Maesen and Pundir, 1984). The genetic advance for yield in chickpea is low because of limited genetic variation present in the germplasm and therefore chickpea is classified as a recalcitrant crop (Van Rheenen *et al*., 1993). Lack of desired genetic variation in available germplasm of cultivated species necessitates the exploitation of related annual species for genetic improvement. Van Rheenen *et al*. (1993) emphasized use of mutation breeding and interspecific hybridization to broaden the genetic base of cultivated species. Interspecific or wide hybridization has been identified as a potential means of increasing the genetic variation and introduction of resistance genes in cultivated species from wild species (*Table 2*).

Table 2. Wild *Cicer* species with resistance to various biotic and abiotic stresses

Trait	Source of resistance	Reference (s)
Ascochyta blight	*C. echinospermum, C. pinnatifidum, C. bijugum, C. judaicum and C. montbretii*	Singh *et al.*, 1981,1998
Fusarium wilt	*C. bijugum, C. judaicum, C. pinnatifidum, C.reticulatum, C. echinospermum and C. cuneatum*	Nene and Haware, 1980 and Singh *et al.*, 1998
Leaf miner	*C. chorassanicum, C. cuneatum and C. judaicum*	Singh and Weigand, 1994 and Singh *et al*., 1998
Bruchids	*C. echinospermum, C. bijugum and C. judaicum*	Singh and Weigand, 1994 and Singh *et al*., 1998
Cyst nematode	*C. pinnatifidum, C. bijugum and C. reticulatum*	Singh *et al*., 1998
Gray mold	*C. judaicum, C. pinnatifidum*	Singh *et al*., 1982
Phytophthora root rot	*C. echinospermum*	Singh *et al*., 1994
Cold	*C. bijugum, C. reticulatum, C.echinospermum and C. pinnatifidum*	Singh *et al*., 1990 and 1995

YIELD IMPROVEMENT

Interspecific hybridization has played an important role in genetic enhancement of yield in many crops by facilitating transfer of useful traits from wild, weedy forms. Two QTLs for increased yield have been identified and transferred into cultivated rice (*Oryza sativa* L.) from its wild relative *Oryza rufipogon* through Advanced-Backcross QTL (AB-QTL) breeding method (Xiao *et al*., 1996 and 1998; Septiningsih *et al.* 2003). Similarly in tomato, the interspecific crosses have been used to identify and transfer QTLs for yield and soluble solid content (brix) from wild species to cultivated species through AB-QTL breeding method (Eshad and Zamir, 1995; Tanksley *et al*., 1996; Grandillo and Tanksley, 2005). Interspecific hybridization in chickpea, involving wild species of the primary gene pool, have demonstrated the possibility of increasing seed yield. High variability for yield per plant in F_2 generation in *C. arietinum* x *C. reticulatum* cross was observed, and some higher yielding F_3 progenies compared to the cultigen were isolated (Jaiswal *et al*., 1986). Transgressive segregants with early maturity were also isolated from interspecific crosses (Singh *et al*., 1984). High degree of heterosis for seed yield in the F_1s was observed in interspecific crosses *C. arietinum* x *C. echinospermum* (153%) and in *C. arietinum* x *C. reticulatum* (138.8%) (Singh and Ocampo, 1993). Occurrence of varying levels of sterility in the F_2 progenies in *C. arietinum* x *C. echinospermum* crosses can be effectively eliminated by exercising selection for completely fertile plants. Singh and Ocampo (1997) identified several transgressive segregants for agronomic traits in F_2 population of *C. arietinum* x *C. reticulatum* interspecific crosses. Twelve F_7 recombinant inbred lines produced higher seed yield than the cultigen and all were phenotypically uniform and free from undesirable traits of the wild species. The F_2 recombinants with very large number of secondary branches, high pod number and higher yield were isolated from a cross between *C. arietinum* x *C. judaicum* (Verma *et al*., 1995). Occurrence of high yielding transgressive segregants in the F_2 lines from different interspecific crosses may be due to formation of favourable gene complexes because of recombination and genetic reshuffling (Singh and Ocampo, 1993).

ABIOTIC STRESS TOLERANCE

Cold tolerance

Chickpea is traditionally grown during the spring season in the Mediterranean region including North Africa, West Asia and South Europe. Higher seed yields can be achieved by planting chickpea in winter season (Singh and Hawtin, 1979). The higher yields in winter sowing may result from better moisture availability and long growing season. However, occurrence of freezing temperatures during winter season may cause considerable damage to the crop at the time of flowering leading to flower and pod losses. Insulating chickpea from cold temperature damage by introducing genes for cold tolerance is necessary to realize higher yields during winter season. Cold tolerance is dominant over susceptibility and is controlled by at least five of genes. Both additive and non-additive gene effects play important role in the inheritance of cold tolerance with preponderance of additive effects (Malhotra and Singh, 1990). Involvement of epistatic interactions in addition to additive and

dominance gene effects suggests that selection after few generations of selfing would be more effective for cold tolerance (Malhotra and Singh, 1991). Singh *et al.* (1989b) developed a simple, reliable 1-9 scale field screening technique to evaluate chickpea germplasm and breeding material for cold tolerance for low to medium elevations in the Mediterranean environments. The genotypes with cold tolerance rating of 1-3 are highly desirable for breeding programmes and screening for cold tolerance must be carried out only after susceptible checks have suffered 100% mortality. Chickpea is a predominantly winter crop in Indian subcontinent and is highly susceptible to temperatures below 15° C.

Since the crop is highly susceptible to cold injury at late vegetative stage, screening germplasm lines and breeding lines at this stage would permit efficient selection of genotypes with cold tolerance (Singh *et al.*, 1995). Singh *et al.* (1989b) identified 15 germplasm lines and 6 breeding lines exhibiting cold tolerance of rating of 3 after evaluating 3,276 lines. Therefore, rigorous screening of germplasm of wild annual species and cultivated species must be done to identify sources of cold tolerance for use in the breeding programmes. Systematic evaluation of accessions of eight annual wild *Cicer* species revealed significant level of cold tolerance in *C. bijugum, C. reticulatum, C. echinospermum* and *C. pinnatifidum* (Singh *et al*., 1995; Singh *et al*, 1990). Some accessions of *C. bijugum* and *C. reticulatum* showed high degree of cold tolerance with ratings of 1 and 2. All lines of *C. chorassanicum, C. cuneatum* and *C. yamashitae* were susceptible to cold. None of the 5,515 accessions of cultivated species had a rating of 2, which shows lack of diversity for cold tolerance in the cultivated species. Singh *et al.* (1998) observed that only the accessions from *C. bijugum* and *C. reticulatum* recorded a rating of 2 and 3, and therefore these two species should be exploited for breeding cold tolerant chickpea genotypes. In cultivated species, two *kabuli* germplasm lines and one *kabuli* breeding line were tolerant to cold with a rating of 3 (Singh *et al*., 1995). Though cold tolerance was observed in cultivated species, the degree of tolerance was low as compared to the wild annual species. Moreover, the winter sown chickpea is affected severely by ascochyta blight. Therefore, cold tolerant genotypes coupled with resistance to ascochyta blight are needed for winter season crop to increase chickpea productivity and for this *C. bijugum* and *C. reticulatum* are the best sources.

Drought

Drought is the second most important abiotic factor limiting chickpea production. The exposure of chickpea plants to terminal drought is one of the major constraints to increasing productivity. Therefore, development of early maturing cultivars coupled with early growth vigour may help the varieties utilize the available soil moisture efficiently and produce relatively higher yields. The variability found in *C. judaicum* for early flowering (Robertson *et al*., 1997) needs to be exploited to breed early maturing genotypes. The involvement of a single recessive locus in controlling early time of flowering and dominant delayed flowering has been reported (Kumar and van Rheenen, 2000). Genotypes with the recessive allele in homozygous condition escape drought at the end of season by nearly 3 weeks in receding soil moisture situations and are known to stabilise chickpea seed yields in such environments. This recessive allele has much significance for breeding for early maturity and molecular mapping

of this locus will help chickpea breeders transfer the locus to locally adapted varieties through backcross method more efficiency.

BIOTIC STRESS TOLERANCE

Fusarium wilt

Fusarium wilt, caused by *Fusarium oxysporum f. sp. Ciceris* is an economically important disease of chickpea. Seven distinct physiological races of *Fusarium oxysporum* have been identified so far, of which 1, 2, 3 and 4 are prevalent in India (Haware and Nene, 1982) and the remaining races (0, 5 and 6) are reported from Spain (Jimenez –Diaz *et. al*., 1989). Race 1 is common in central India, whereas race 2 is common in northern India. Race 3 and 4 appear to be location specific in Punjab and Haryana states of India. The pathogen can survive in soil for more than six years even in the absence of chickpea and causes considerable yield losses. Breeding varieties resistant to fusarium wilt is an eco-friendly, economically viable strategy. Breeding programmes at national and international centres have resulted in the release of several chickpea cultivars resistant to fusarium wilt. But these cultivars do not show resistance across locations (Infantino *et al*., 1996) due to the prevalence of location specific races of the wilt pathogen.

Resistance to the different races of *Fusarium* wilt fungus in chickpea and pea is controlled by single genes (Muehlbauer and Kaiser, 1994). The genotypes of chickpea differ for their time to develop initial symptoms of wilt that indicates different degrees of resistance. The difference in time taken for the development of initial symptoms appears to be controlled by segregation of a single gene with early wilting partially dominant over late wilting (Upadhyaya *et al*., 1983). Resistance to fusarium wilt race 0 is governed by one or two independent genes (Tekeoglu *et al*., 2000; Rubio *et al*., 2003). Involvement of two independent loci in the inheritance of resistance to race 1 has also been observed (Singh *et al*., 1987; Upadhyaya *et al*., 1983). Presence of either of these genes in homozygous recessive form increases time to develop wilt symptoms (late wilting), but both the genes must be in homozygous recessive form to confer resistance. Late wilting chickpea genotypes would be of great value in reducing the yield losses by delaying the wilt development. These late wilting lines may carry useful resistance genes that can confer complete resistance when complemented with other resistance genes. Gumber *et al.* (1995) reported that resistance to race 2 of fusarium wilt is controlled by two genes, one gene must be present in the homozygous recessive form, and the second gene must have its dominant allele (whether in homozygous or heterozygous condition) for complete resistance. The resistance to race 4 of *Fusarium oxysporum* is controlled by a single recessive gene (Tullu *et al*., 1998). For race 5, only one resistance gene has been described and mapped so far (Tekeoglu *et al*., 2000; Winter *et al.*, 2000). The genetics of resistance to various other diseases of chickpea has been discussed in detail by Singh and Reddy (1991).

Nene and Haware (1980) evaluated 102 accessions representing six annual wild *Cicer* species for resistance to fusarium wilt. All accessions of *C. bijugum* and some accessions of *C. judaicum*, *C. reticulatum*, *C. echinospermum* and *C. pinnatifidum* showed resistance to fusarium wilt, whereas all the accessions of *C. yamashitae* were highly susceptible. Some accessions of *C. bijugum*, *C. judaicum* and *C. reticulatum*

were completely free from wilt damage, and are therefore potential source of genes for Fusarium wilt resistance that could be transferred into the cultivated species. Seventeen lines resistant to race 0 and only one line resistant to race 5 were identified from 1,904 lines of *kabuli* chickpea tested, showing the paucity of resistance in the cultivated chickpea accessions (Jimnez-Diaz *et al.*, 1991). Race 1 of Fusarium is the most widespread in India, to which 160 resistant lines, including both *desi* and *kabuli*, have been identified. Fifty-two accessions representing 11 wild *Cicer* species were evaluated for resistance to races 0 and 5 but promising source could not be identified in the accessions of the primary gene pool. Resistance to highly virulent race 5 was identified in the accessions of *C. bijugum*, *C. cuneatum* and *C. judaicum*, and resistance to race 0 was observed in accessions of *C. bijugum*, *C. chorassanicum*, *C. cuneatum*, *C. judaicum* and *C. pinnatifidum*. Resistance to race 0 was also observed in a perennial species *C. canariense*. Some accessions of *C. bijugum*, *C. judaicum* and *C. pinnatifidum* with combined resistance to races 0 and 5 of fusarium wilt and ascochyta blight have also been identified. Different accessions of *C. bijugum*, *C. judaicum*, *C. reticulatum*, *C. pinnatifidum, C. echinospermum* and *C. cuneatum* were found to be resistant to fusarium wilt in other studies (Singh *et al*., 1998).

Ascochyta blight

Ascochyta blight, caused by *Ascochyta rabiei*, is the most important foliar disease of chickpea. The disease is particularly severe on winter sown chickpea, favoured by cool and wet weather conditions and attacks the crop at both vegetative and podding stages. The yield losses of chickpea due to blight range from 10 to 100 per cent under severe natural epidemics (Nene and Reddy, 1987; Singh, 1990). Most of the resistances to blight identified so far is under multigenic control (Muehlbauer and Kaiser, 1994). The chickpea lines exhibiting resistance to 3-5 races of *Ascochyta rabiei* were identified after evaluation of 1,069 germplasm lines (Singh, 1990). Reddy and Singh (1983) observed resistance to ascochyta blight in 0.29 and 0.06% of *kabuli* and *desi* accessions, respectively. Total 19,343 *Cicer* germplasm accessions which includes both *kabuli* and *desi* types were screened for resistance to six races of *A. rabiei* and 14 lines (9 *desi* and 5 *kabuli* accessions) of durable resistance at both vegetative and podding stage were identified by ICARDA, Syria (Singh and Reddy, 1993a). More concerted efforts at ICARDA, Syria led to the development of 92 lines resistant to all the six physiological races of *A. rabiei*, which have registered 33% more seed yield than the original resistant sources. Planting of these highly resistant lines in winter season increases the prospects of achieving higher yields in the Mediterranean region (Singh and Reddy, 1996). The ascochyta blight resistant breeding lines showed no association with late maturity and plant height, as a result 28 resistant lines coupled with early maturity have been developed. These lines are highly suitable for regions with short growing seasons.

The pathogen *Ascochyta rabiei* is inherently unstable and continuously evolves new races in complex interaction with host, which makes resistance ephemeral and limits the effective life of resistant lines under production. The evolution of new races has made the plant breeders to change their strategy from oligogenic resistance to pyramiding of genes to provide durable resistance. It is also equally important to understand the race distribution of *A. rabiei* in different chickpea growing regions, which will help in

selective deployment of varieties to provide effective resistance and to save resistance genes from being defeated by the races of the pathogen. The development of DNA markers for different races of the pathogen help in rapid diagnosis of the pathogens and also to conduct pathogen surveys more frequently and systematically compared to the analysis of pathogens based on differential sets. Resistance to ascochyta blight was observed in the accessions belong to *C. pinnatifidum*, *C. judaicum* and *C. montbretii* (Singh *et al.*, 1981). The wild species *C. echinospermum*, *C. pinnatifidum*, *C. bijugum* and *C. judacium* possessed high degree of resistance to ascochyta blight (Singh and Reddy 1993b; Singh *et al*., 1998).

Cyst nematode

Cyst nematode (*Heterodera ciceri*) is another major biotic stress to chickpea particularly in Syria. Identification of source of resistance in the cultivated and or annual wild species is a prerequisite to breed varieties with high degree of resistance. Di Vito *et al*. (1988) evaluated 2001 lines of *C. arietinum* and 20 lines of wild *Cicer* species for resistance to cyst nematode on 0-5 scale. Only 1% of the lines of cultivated species showed resistance with a rating of 2, which clearly indicates lack of adequate resistance to cyst nematode in the cultivated species. The accessions from *C. bijugum* registered a rating of 2 and 3. 91% of accessions from *C. bijugum* recorded a rating of 2 and the lines of other annual *Cicer* species were highly susceptible (Singh *et al.*, 1989). Di Vito *et al*. (1996) tested 7,258 lines of *C. arietinum* and 102 lines of eight annual species and observed resistance in the accessions from *C. bijugum*, *C. pinnatifidum* and *C. reticulatum*. All the lines of cultivated species and accessions from other annual species were susceptible. Several resistant plants were identified in F_2 and F_3 generations from crosses between cultigen and a resistant accession of *C. reticulatum*, showing the importance of wild species in chickpea improvement. High degree of resistance in the accessions of *C. pinnatifidum, C. bijugum* and *C. reticulatum* has also been reported (Singh *et al*., 1998). Some accessions of *C. yamashitae* were moderately resistant with a rating of 3.

Phytophthora root rot

Phytophthora root rot, caused by *Phytophthora megasperma*, is a major disease in north-eastern Australia. Many of the cultivated varieties have inadequate resistance to phytophthora root rot and yield substantially less than winter cereals. A chickpea cultivar Barwon with increased phytophthora resistance was released in 1992 (Singh *et al*., 1994). It is necessary to search for other annual wild *Cicer* species for additional sources of resistance. Among the two closely related annual species of chickpea, *C. echinospermum* accessions have shown superior phytophthora resistance (Singh *et al*., 1994).

Gray mold

Gray mold, *Botrytis cinerea*, is second most important foliar disease of chickpea and is prevalent in sub-mountainous region of northern India, as well as in Bangladesh

and Nepal. The presence of resistance to gray mold was observed in the accessions of *C. pinnatifidum* and *C. judaicum* (Singh *et al.*, 1982).

Leaf miner and Bruchids

Leaf miner (*Liriomyza Cicerina*) causes considerable damage to chickpea in West Asia, North Africa and Southern Europe. 7,000 germplasm accessions of cultivated species and 200 accessions of eight wild annual species have been evaluated by Singh and Weigand (1994) to find source(s) of resistance to leaf miner. The germplasm of cultivated species lack adequate degree of resistance and only ten lines with moderate levels of resistance have been identified. In case of wild species, two accessions of *C. cuneatum* and ten of *C. judaicum* have exhibited resistance with a rating of 2 (highly resistant) and some accessions of *C. judaicum*, *C. pinnatifidum* and *C. reticulatum* were identified with a rating of 3 (resistant). Bruchids (*Callosobruchus chinensis*) are the important stresses on chickpea in the Mediterranean region. Singh *et al*. (1998) evaluated the germplasm accessions of cultivated and annual wild species for resistance to leaf miner and bruchids. All the accessions of *C. chorassanicum, C. cuneatum* and some accessions from *C. judaicum, C. bijugum, C. pinnatifidum* and *C. echinospermum* showed resistance to leaf miner. All the accessions of *C. echinospermum*, and some accessions of *C. bijugum*, *C. judacium*, *C. pinnatifidum*, *C. reticulatum* and *C. cuneatum* have shown resistance to bruchids.

Pod borer

Among numerous pests attacking chickpea plants and seeds, pod borer (*Helicoverpa armigera*) is the most devastating one. An integrated breeding approach has been adopted for helicoverpa control in chickpea involving both non-preference/antibiosis and avoidance. Several accessions resistant to helicoverpa have been identified at ICRISAT. The chickpea cultivars and wild *Cicer* species have been found to differ significantly in their ability to inhibit *Helicoverpa armigera* gut proteinases (Patankar *et al*., 1999). But none of the species offered complete protection against pod borer by inhibiting gut proteinases. *C. bijugum* exhibited highest inhibition (36%) followed by *C. echinospermum* and *C. arietinum* (cv Vijay) (33%). The chickpea cultivars showed differential inhibition activity of gut proteinases, which indicate that *H. armigera* is adapted to a wide range of host protein inhibitors. The lack of effective resistance in *Cicer* species necessitates genetic transformation of chickpea with heterologous proteins to provide resistance against pod borer. The annual and perennial species possessing resistance to various biotic and abiotic stresses are summarized in *Table 2*.

REPRODUCTIVE BARRIERS

Among the different annual wild *Cicer* species, successful hybridization of *C. arietinum* has been achieved only with *C. reticulatum* and *C. echinospermum*. Occurrence of partial sterility in the crosses of *C. arietinum* x *C. echinospermum* can easily be overcome by practicing selection on the progenies. Since different accessions of *C. reticulatum* and *C. echinospermum* harbour resistance or tolerance to fusarium wilt,

ascochyta blight, cyst nematode, bruchids, phytophthora root rot and cold (*Table 2*), these two species could be effectively exploited for introgression of useful traits into the cultivated species. The species with high degree of resistance to multiple stresses are present only in the tertiary gene pool, but these exhibits cross incompatibility with *C. arietinum* and therefore have little scope for utilization.

The failure of the interspecific crosses may be due to the operation of either pre- or post-fertilization barriers or both. The pre-fertilization barriers include failure of pollen to germinate, bursting of pollen tubes within the style and inability of the pollen tubes to reach the embryo sac, whereas post-fertilization barriers mainly relate to somatoplastic sterility (collapse of the zygotes due to imbalance in embryo-endosperm relationship). Strong pre-fertilization barriers like failure of pollen grain germination and lack of penetration of pollen tubes in the stylar tissues were observed in *C. arietinum* x *C. soongaricum* cross (Mercy and Kakar, 1975). No inhibition of growth of pollen tubes in interspecific crosses was observed among the annual *Cicer* species, which suggests that the failure of seed formation may be due to slow growth of pollen tube or collapse of fertilized ovules (Bassiri *et al*., 1987). But entry of the pollen tube into the ovule and occurrence of fertilization in all interspecific cross combinations has been reported (Ahmad *et al*., 1988). Large differences were observed in time taken from pollination to fertilization in normal and reciprocal interspecific crosses. For instance, fertilization took place in 49.2 ± 18.4 h when *C. cuneatum* was used as a female parent in a cross with *C. arietinum*, whereas fertilization was completed in 25.6 ± 5.8 hrs in a reciprocal cross. Therefore, choosing the right species as a female parent is an important factor in the success of interspecific crosses but deleterious maternal effects also have to be considered at the same time.

Shrivelled crossed seeds collected 30 days after pollination from *C. arietinum* x *C. pinnatifidum* cross produced albino seedlings. Hence, ovules collected from the pods 18 days after pollination was cultured for 10 days, embryos were taken out from the ovules, which also have produced albino seedlings upon *in vitro* culturing. The albino plants produced multiple shoots, developed green pigmentation upon subculturing for 3-4 weeks and exhibited poor rooting ability. The plants developed wilting symptoms and did not survive beyond 12 days after transferring to soil. The hybrid shoots with poor rooting system grew for more than one month upon grafting to the female parental stocks and subsequently withered after reaching a height of 12 cm. The strong post-zygotic reproductive barriers between *C. arietinum* x *C. pinnatifidum* reflected in poor seed set, high hybrid embryo necrosis, albinism and poor adaptability to form roots (Badami *et al*., 1997).

The occurrence of embryo abortion in all crosses except with *C. reticulatum* clearly demonstrates presence of post-fertilization crossability barriers. It may be possible to transfer desirable traits from these species to the cultigen by overcoming the post fertilization barriers through tissue culture techniques by excising the fertilized ovules and growing them on an artificial medium (Ahmad *et al*., 1988; Bassiri *et al.*, 1987; Singh and Ocampo, 1993; 1997). Alternatively, somatic hybridization by protoplast fusion has also been used to transfer genes between sexually incompatible plant species. For example, somatic hybrids with improved resistance to bacterial soft rot were produced in brassica by protoplast fusion between *Brassica rapa* and *Brassica oleracea* (Ren *et al*., 2000). The technique can be useful to circumvent reproductive barriers that operate between the *Cicer* species (Singh *et al*., 1984), provided that

regeneration and differentiation from protoplasts of the crop is possible. A hybrid plant between *C. arietinum* and *C. cuneatum* was successfully produced through embryo rescue technique but it did not flower (Singh and Singh, 1989). However, more detailed studies on wide hybridization and standardization of tissue culture techniques needs to be carried out for rapid exploitation of wild species from the tertiary gene pool.

Genetic markers

The genetic markers include morphological, biochemical and DNA variants tightly linked with the traits of agronomic importance so that they can be employed in indirect selection. Morphological markers can be scored quickly without specialized lab equipment but their expression at specific growth stages and the possibility of analysing only a limited number of markers in a single population limit their use in breeding programmes. Many protein variants of an enzyme, referred to as isozymes, can be resolved by electrophoresis and are very useful genetic markers. The alleles of most isozyme markers segregate in a codominant manner and rarely show epistatic interactions, which allows accumulation of many polymorphic isozyme loci in a single F_2 population and increases the efficiency of gene mapping. Once the map location of isozyme genes are known, they can be used efficiently as biochemical markers to map other genes for morphological, physiological and phytopathological traits (Tanksley and Rick, 1980). However, only a relatively small number of protein variants may exist between the two parents and this limits the total number of protein loci that can actually be scored in a given mapping population (Young, 1994). DNA markers on the other hand are not influenced by environment and do not exhibit epistatic interactions. The limitless availability of DNA markers permits construction of linkage maps with hundreds or even thousands of markers using a single mapping population. DNA markers have been widely used to: a) identify and discriminate closely related cultivars, b) assess taxonomic and phylogenetic relationships of a crop species, c) construction of high density genetic linkage maps, d) study genome organization and e) tagging of loci affecting qualitative or quantitative traits (Azhaguvel *et al*., 2006). The major DNA markers include restriction fragment length polymorphisms (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites or Simple sequence repeats (SSRs), diversity array technology (DArt) and single nucleotide polymorphisms (SNPs). The PCR based markers have been widely used because of their technical simplicity, ease of screening large number of samples in a short period of time and can be carried out in a moderately equipped laboratory.

RFLPs exhibit variation as length polymorphisms due loss or gain of restriction sites in the hybridizing DNA fragments during Southern hybridization and have been used for the first time to construct genetic linkage maps in humans (Botstein *et al*., 1980). Majority of the RFLP markers are codominant in nature with a segregation ratio of 1:2:1 in F_2 population. RAPDs are generated by PCR amplification of genomic DNA with 10-mer oligonucleotides of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al*., 1990). Most, but not all, of the RAPD markers inherit in a dominant fashion and are scored as presence/absence polymorphisms. RAPD polymorphisms can reflect point mutations in the primer annealing sites or in length differences between primer annealing sites due to insertion or deletion mutations (Williams *et a*l., 1990).

Use of dominant RAPD markers can be enhanced with identification of coupling and repulsion phase markers linked to the gene of interest. The coupling and repulsion markers can be used together as a codominant pair and will be equally informative as codominant markers in detecting heterozygotes in segregating populations (Haley *et al.*, 1994; Johnson *et al*., 1995). AFLP combines principles of both RFLP and RAPD and involves four steps: a) restriction digestion of genomic DNA and ligation of oligonucleotide adapters, b) pre-selective amplification of the restricted fragments with primers specific to adapters, c) selective amplification of a set of restriction fragments, and d) gel electrophoretic separation of the amplified fragments (Vos *et al*., 1995). The AFLP polymorphisms occur as presence or absence of the amplified fragments and thus behave as dominant markers. AFLP based polymorphism has been used in chickpea for the development of genetic linkage map (Winter *et al*., 2000) and to assess genetic diversity within and between different chickpea species (Ngyen *et al*., 2004). The highest genetic variation was observed in *C. pinnatifidum* and the lowest in *C. macracanthum*. The cultivated species *C. arietinum* was closet to the wild species *C. reticulatum*, its most probable progenitor (Ngyen *et al*., 2004)**.**

Simple sequence repeats (SSRs) are tandem arrays of two to six nucleotide base pair repeats that occur ubiquitously throughout the genome (Tautz, 1989). The unique sequences flanking the SSR (also known as microsatellite) are generally conserved within a species. The flanking sequences are utilized to design forward and reverse primers to amplify the corresponding SSR loci, which are also referred to as sequence tagged microsatellite sites (STMS) markers (Beckmann and Soller, 1990). The SSR markers have several advantages: a) highly abundant, b) codominant in nature, c) rapidly typed via PCR, d) hypervariable, e) highly reproducible. Replication slippage within the SSR stretches leads to a change in the length of the SSR, which is reflected as length polymorphism between individuals of a population (Schlotterer and Tautz, 1992). Akkayya *et al*. (1992) demonstrated that length polymorphism of SSRs between lines occur due to variation in the number of repeat units. Another approach using SSRs for the detection of polymorphism, called inter simple sequence repeat (ISSR), involves PCR amplification of genomic DNA between two microsatellite loci using a single primer composed of a microsatellite sequence anchored at the 3' or 5' end with one or few arbitrary, often degenerate nucleotides (Rajesh *et al*., 2002). These primers target simple sequence repeats that are abundant throughout the eukaryotic genome and do not require prior knowledge of DNA sequence for primer design. The ISSR markers are generally inherited as dominant markers (Ratnaparkhe *et al*., 1998a; Tsumura *et al*., 1996). The codominant markers such as isozymes, RFLPs and SSR markers have distinct advantages over dominant markers: a) they allow plant breeders to identify individuals homozygous for the desired alleles in the F_2 generation of a cross, without need for F_3 progeny testing (Yu *et al.*, 1991), and b) codominant nature permits easy transfer of markers between genetic maps of different crosses (Thomas and Scott, 1993). RFLP markers have been used in development of genetic linkage map of chickpea using mapping population from an inter-specific cross (Simon and Muehlbauer, 1997), whereas SSRs are the marker of choice for the development of genetic maps and tagging of genes (Winter *et al*., 1999; Huttel *et al*., 1999), inter and intra-specific variabibity (Choudhary *et al*., 2005; Sethy *et al*., 2006b) and phylogenetic study (Sethy *et al.*, 2006a).

Single nucleotide polymorphisms (SNPs), the new generation molecular markers, are the most abundant of all marker systems known so far (Gupta *et al*., 2001). On an

average an SNP occurs every 100-300 bp in the genome. SNPs may be found within a gene or may be found in its close proximity. When present in a gene, it may or may not be responsible for the mutant phenotype, but in either case it can be used as marker for tagging and positional cloning of the genes of interest. Several SNPs have been reported to be associated with genes of economic value in other crops e.g. SNP in waxy gene (*Wx*) controlling amylose content (Ayers *et al*., 1997), dwarfing gene in rice (Graland *et al.*, 2000), male sterility gene in onion (Alcala *et al.*, 1997) and for soybean cyst nematode resistance gene (Young, 1999). It is expected that similarly in chickpea, a high number of SNPs will be genetic determinants, therefore associations among SNPs and the traits of economic value will be of major interest to the chickpea breeders. However, so far no SNP work has been carried out in chickpea.

For a molecular marker to be useful to plant breeders, it must be assayed readily and reproducibly in a range of laboratories (King, 1994). Though RAPD markers are cost effective and easy to operate, lack of reproducibility and dominant inheritance limit their use in plant breeding programmes. However, the problem can be overcome by converting RAPD markers to more robust SCAR markers (Paran and Michelmore, 1993). Lin *et al.* (1996) compared three different DNA mapping techniques- RFLP, RAPD and AFLP for their ability to detect polymorphisms and demonstrated that AFLP is the most efficient one. The comparison of RFLP, RAPD, AFLP and SSRs for their utility in germplasm analysis of soybean indicated that SSRs have the highest polymorphism information cotent (the ability of a marker to distinguish between genotypes), whereas AFLPs have the highest effective multiplex ratio (the number of loci simultaneously analysed per experiment) (Powell *et al*., 1996). The SSRs are highly reproducible across laboratories as compared to AFLP and RAPD markers (Jones *et al*., 1997). As a result, SSR markers have been developed in almost all plant species in large number (Gupta and Varshney 2000).

In recent years, due to availability of large numbers of expressed sequence tags (ESTs) in several plant species, SSRs have been developed from ESTs. Such SSRs are referred as EST-SSRs or genic SSRs and development of such SSR markers involves small cost and less time as compared to conventional method of SSR development (Varshney *et al*., 2005).

GENETIC DIVERSITY STUDIES

Knowledge of genetic diversity and relatedness in the germplasm of both cultivated and wild species is a prerequisite for their better utilization in crop improvement programmes. Genetic markers have been widely used to detect genetic variation at individual gene loci (alleles of a gene), among several loci or gene combinations, between individual plants within plant populations, or between populations. In chickpea, isozymes, RFLP, RAPD, synthetic oligonucleotide probes and SSRs have been used to assess genetic diversity.

Isozymes

As early as in 1988, Tuwafe and coworkers identified four polymorphic isozyme loci showing monogenic inheritance in 1,392 chickpea accessions representing 25 countries and reported greatest genetic diversity in the germplasm accessions originating from

Ethiopia, India, the Soviet Union, and Middle Eastern countries such as Turkey and Afghanistan. The Indian germplasm exhibited high average number of alleles per locus and heterozygosity. The *desi* and *kabuli* accessions of chickpea, though geographically different, shared common alleles for most of the allozymes (Kazan and Muehlbauer, 1990). A very low level of isozyme polymorphism was reported in the cultivated chickpea species (Ahmad *et al*., 1992; Gaur and Slinkard, 1990b; Kusmenoglu *et al*., 1992; Labdi *et al*., 1996; Oram *et al*., 1987; Tayyar and Waines, 1996; Van Rheenen, 1992). For instance, only 3 loci out of 23 isozyme loci tested showed polymorphism in the cultivated species (Tayyar and Waines, 1996). The isozyme markers have also been used to assess diversity within and between *Cicer* species. The *Cicer* species *C. reticulatum*, *C. pinnatifidum*, *C. bijugum* and *C. cuneatum* exhibited within species polymorphism (Ahmad *et al*., 1992). The proportion of polymorphic loci is highest in *C. reticulatum* and least in *C. arietinum* (Ahmad *et al*., 1992; Labdi *et al*., 1996; Tayyar and Waines, 1996). *C. reticulatum* also exhibits highest morphological variability among the annual wild species (Robertson *et al*., 1997), which shows a correlation between morphological and isozyme derived data. Isozymes reveal only genetic changes in coding regions of the genome that have resulted in changed amino acid sequences. Though isozyme analysis is comparatively simple, their use is often limited by the less number of enzymes and loci that can be resolved and often subject to post-translational modifications. Moreover, some enzymatic assays are possible only at certain growth stages and in specific tissues. Since most isozyme loci are monomorphic in cultivated chickpea, the paucity of isozyme polymorphism restricts their application in germplasm characterization and tagging of gene(s) of interest, but they can be effectively exploited in case of interspecific crosses (Kusmenoglu *et al*., 1992).

RFLP and RAPD markers

The RFLP and RAPD markers also show very low level of genetic variation between chickpea cultivars (Udupa *et al*., 1993; Van Rheenen, 1992). The low polymorphism may have been caused by narrow ancestry and self-pollinated nature of the crop (Van Rheenen, 1992). Sant *et al.* (1999) employed RAPD and oligonucleotide probes to assess genetic diversity between 29 elite Indian chickpea cultivars. Out of 35 RAPD primers tested, only 10 primers (29%) generated polymorphic patterns between the cultivars, which indicate narrow genetic base of the cultivated species. The genetic distance values ranged from 0.09 to 0.27 based on RAPD analysis, whereas genetic distance ranged from 0.42 to 0.61 with oligonucleotide probes. The experimental findings in our laboratory also supported the findings of Sant *et al.* (1999). Out of 78 RAPD primers tested, only 20 primers revealed polymorphisms between the chickpea cultivars (Singh *et al*. 2003). Most of the RAPD polymorphisms appeared as single major band polymorphisms (Singh *et al*. 2002). The low degree of genetic variation detected by RFLP and RAPD markers in cultivated chickpea limit mapping of large number of these markers in a given cross.

Oligonucleotide fingerprinting/ in-gel hybridization

Oligonucleotide fingerprinting involves use of synthetic oligonucleotide probes complementary to microsatellites or simple sequence repeats (SSRs) for in-gel or

Southern hybridization with genomic DNA digested with individual restriction enzymes and electrophoresed on agarose gel. The probes can be designed without any sequencing efforts. Since repetitive DNA is present ubiquitously in all eukaryotic genomes (Tautz, 1989), a large number of hybridizing fragments with varying sizes are generally obtained. These multi-locus probes may be useful for genetic characterization of cultivars and breeding lines, estimation of degree of relatedness and tagging of gene(s) of interest. If linkage with a gene is detected, sequence tagged microsatellites can be developed by sequencing the representative fragment, so that unique flanking sequences can be used as locus specific starting points for chromosome walking experiments (Weising *et al.,* 1992). Oligonucleotide probes representing microsatellites present as polymorphic repeats in the chickpea genome and detected intra as well as inter-specific variation in chickpea (Serret *et al*., 1997; Weising *et al*., 1992). The number of hybridizing fragments varied between different probes and from species to species and the fingerprints are somatically stable.

The di-, tri- and tetra-nucleotide repeat motifs like $(CAA)_5$ and $(GATA)_4$ revealed a considerable degree of intra as well as inter-accessional polymorphism in chickpea. The tetra-nucleotide motif $(GCCT)_4$ detected only inter-accessional polymorphism and these accession specific probes may serve as an invaluable tool for the identification of accessions and cultivars (Sharma *et al.*, 1995). Oligonucleotide probes revealed a wide range of variability in accessions from Pakistan, Iraq, Afghanistan, south-east Russia, Turkey and Lebanon, whereas low genetic diversity was found in accessions from India, Iran, Syria, Jordan and Palestine. Three centres of diversity have been proposed for chickpea: a) Pakistan and Afghanistan, b) Iraq, Turkey and Lebanon, and c) India. Future explorations for germplasm collection can be carried out in these centres of rich diversity (Serret *et al*., 1997).

The genetic diversity among chickpea cultivars revealed by oligonucleotide fingerprinting is much higher as compared to RAPD markers (Sant *et al*., 1999). Since these probes yield more polymorphisms, few probes will be sufficient for thorough coverage of the genome, and show high reproducibility as compared to RAPDs. Therefore, these oligonucleotide probes may be useful for developing molecular markers and for gene introgression in a crop like chickpea with a narrow genetic base. However, the approach is limited with the following disadvantages: a) Extensive clustering of simple sequence repeats, b) high mutation rates leading to unexpected fragments (non parental bands) in the progeny, c) dominant inheritance. The occurrence of non-parental bands during linkage analysis may be minimized by the inclusion of parental and F_1 DNAs in the segregation analysis and the preferred use of accession rather than individual specific probes (Sharma *et al*., 1995).

SSR markers

The majority of the SSRs amplify single loci with multiple alleles, and thus serve as viable alternative to synthetic oligonucleotide probes. The relatively simple interpretation and genetic analysis of single locus markers make them superior to multi-locus DNA markers, especially for map construction and DNA fingerprinting (Thomas and Scott, 1993). The ability of SSRs in detecting intra-specific variation in chickpea has been demonstrated by Huttel *et al.* (1999). Sixteen SSR loci detected 2-4 alleles at intra-specific level out of 22 loci tested. Two SSR loci, CaSTMS10 and

CaSTMS15 detected 25 and 16 alleles, respectively, upon testing on a large number of chickpea accessions. Sethy *et al.* (2006a) used 25 polymorphic SSR markers to analyse intra-specific genetic diversity within 36 geographically diverse chickpea accessions. Based on cloning and sequencing of size variant alleles at two microsatellite loci revealed that the variable numbers of AG repeats in different alleles were the major source of polymorphism. Recently Udupa *et al*. (2004) studied dynamics of microsatellite evolution in chickpea and for this they selected di ant tri nucleotide repeat (TA)n and (TAA)n, respectively, and based on polymorphism they observed that the two loci do not evolve in complete independence. Below a threshold level they evolve independently and above that threshold level if one allele increases in size the other closely linked locus decreases in its size and *vice versa*, without change in the overall ratio. Sethy *et al.* (2006b) cloned and sequenced microsatellite sequences from *C. reticulatum* and developed 11 SSR markers to analyse 29 accessions representing all nine annual *Cicer species*. Efficient marker transferability (97%) of the *C. reticulatum* was observed as compared to microsatellite markers developed from cultivated species. Based on cluster analysis all the accessions (except two *C. judaicum* accessions) distinguished from one another and revealed intra and inter-species variability. An annual *Cicer* phylogeny was depicted which established higher similarity between *C. arietinum* and *C. reticulatum*. In the study placement of C. *pinnatifidum* in the second crossability group and its closeness to *C. bijugum* was supported. Two species *C. yamashitae* and *C. chorassanicum* were grouped distinctly and seemed to be genetically diverse from members of first crossability group. The above studies suggest that microsatellite markers are highly suitable for genome analysis in chickpea because of their rich allelic diversity and informativeness compared to other DNA markers.

GENETIC LINKAGE MAPS

Development of high density integrated genetic linkage maps based on molecular markers is a prerequisite for use in marker assisted selection (MAS) and positional cloning of agronomically important genes in a crop species (Azhaguvel *et al.*, 2006). In chickpea, generally two types of mapping populations, F_2 and recombinant inbred lines (RILs) derived from interspecific as well as intra-specific crosses have been used to construct linkage maps. The F_2 populations are easy to develop, but these are transient in nature as the seeds derived from selfing of F_2 will not breed true. The RILs are created by single seed descent method from F_2 plants through at least 5 or 6 generations of selfing until their genomes could be virtually homozygous. RILs are highly preferred for genome mapping as compared to F_2 populations because of their distinct advantages: a) The RILs constitute an immortal population because true breeding seed can be generated upon selfing of these lines, b) The true breeding material can be exchanged between laboratories, which would facilitate mapping of several traits using a single population by different workers at the same time, c) Since these lines undergo several rounds of meiosis during the inbreeding process, they provide more opportunity for recombination and segregation of genomic segments. Spurious QTL-marker associations due to linkage errors are less likely with RILs than with F_2 or backcross populations (Mansur *et al*., 1996). However, the process of generating RILs is time consuming, and some regions of the genome may tend to stay heterozygous longer than expected from theory (Young, 1994).

The genetic linkage maps developed using morphological, isozyme and different DNA markers are presented in *Table 3*. Since intra-species polymorphism in cultivated chickpea is extremely low, interspecific crosses (*C. arietinum* x *C. reticulatum*) have been exploited to saturate genetic linkage maps with more number of markers (Gaur and Slinkard 1990b; Kazan *et.al*. 1993). Simon and Muehlbauer (1997) developed an integrated genetic linkage map of chickpea with morphological, isozyme and DNA markers covering the length of 550 cM. Since RFLPs and RAPDs detected low genetic polymorphism in cultivated species (Sant *et al.*, 1999; Udupa *et al.*, 1993), genetic maps based on highly polymorphic marker systems like SSR and AFLP would be of great value in marker assisted selection for trait(s) of interest. A total of 174 microsatellite loci have been reported by Winter et al. (1999) and 112 STMS loci distributed over 11 linkage groups covering a length of 613 cM were mapped. The information provided on the number of alleles detected by each SSR loci will help the breeder or geneticist to choose markers with the greatest probability of detecting polymorphism.

The integrated map developed by Santra *et al.* (2000) cover a length of 981.6 cM with a density of one marker per 8.4 cM. With the integration of different kinds of genetic markers, Winter *et al*. (2000) developed a more comprehensive linkage map of chickpea with 303 markers with an average distance of 6.8 cM between two consecutive markers, covering a map length of 2077.9 cM. The genetic map shows clustering of all marker types at specific genomic regions. The restriction enzymes used to generate AFLP markers influence their genomic distribution (Young *et al*., 1999). The distribution of EcoR1 and Pst1 enzyme derived AFLP markers has been studied in great detail, and found that 34% of the Eco-AFLP markers segregated into clusters, whereas Pst-AFLP markers mapped to the lower marker density regions. The clustering of markers may be due to location of markers in the regions of reduced recombination, most likely near the centromeres. In general, recombination is inhibited near centromeres, in heterochromatin, and in regions introgressed from wild relatives (Young, 1994). Only 10.9% of the Pst-AFLP markers were observed within the Eco-AFLP cluster regions, which clearly indicate that the genomic regions marked by the clusters are highly methylated. In case of chickpea, all the 70 mapped AFLP markers are Eco-AFLP type, and hence have the possibility of forming clusters in central regions. Ahmad and Hymowitz (1993) observed that all the chickpea chromosomes have heterochromatic regions, proximal to and on either side of the centromere.

Most of the SSR loci are also clustered at central positions flanked by regions of low marker density (Winter *et al*., 1999, 2000). Akkayya *et al.* (1995) observed random distribution of the SSR loci in soybean genome by placing 40 SSR loci on soybean genetic map. However, with the mapping of 606 SSR loci in soybean, Cregan *et al.* (1999) observed clustering of SSR markers. But in rice, microsatellite markers are distributed relatively uniformly throughout the genome even after mapping of 312 microsatellites (Temnykh *et al*., 2000), which shows differential genome organization of the crop species. Simple sequence repeats are predominantly located in extensively methylated regions of the chickpea genome (Sharma *et al*., 1995). Therefore, the distribution of microsatellites in high methylated regions near centromeric region may result in the formation of clusters. These clusters in the central regions may mark the heterochromatic regions surrounding the centromeres. Since majority of the structural genes reside in hypo methylated euchromatic regions, the use of Pst-AFLP derived

Table 3. Progress in the development of genetic linkage maps of chickpea

Mapping population	Marker Type	No. of markers	Linkage groups	Genome Coverage (cM)	References
F_2 population					
C.arietinum x *C.reticulatum*	Morphological and isozyme	29	7	200	Gaur and Slinkard, 1990a, 1990b
C.arietinum x *C.reticulatum* and C.arietinum x C.echinospermum	Morphological and isozyme	28	8	257	Kazan *et al*., 1993
C.arietinum x *C.reticulatum* and *C.arietinum* x *C.echinospermum*	Morphological, isozyme, RFLP and RAPD	91	10	550	Simon and Muehlbauer, 1997
C. arietinum x *C. echinospermum*	SSR, RAPD, ISSR and RGA	83	8	570	Collard *et al*., 2003
RIL population					
C.arietinum x *C.reticulatum*	STMS	120	11	613	Winter *et al*., 1999
C.arietinum x *C.reticulatum*	SSR, DAF, AFLP, ISSR, RAPD, isozyme, cDNA, SCAR and morphological	354	16	2077.9	Winter *et al*., 2000
C.arietinum x *C.reticulatum*	RAPD, ISSR, isozyme, and morphological	116	9	982	Santra *et al*., 2000
C.arietinum x *C.reticulatum*	RAPD, ISSR and morphological		23		Hajj- Moussa *et al*., 2001
C.arietinum x *C.reticulatum*	Addition of RGA Potkin 1-2 171 to linkage group 5 of Santra *et al*. (2000)	117	9	-	Rajesh *et al*., 2002
C.arietinum x *C.reticulatum*	Addition of SSR and RGA marker to map of Santra *et al*., 2000	51	9	1,175	Tekeoglu *et al*., 2002
C.arietinum x *C.reticulatum*	Addition of 47 defense response gene markers to the map of Winter *et al*., 2000	296	12	2500	Pfaff and Kahl., 2003
C.arietinum x *C.reticulatum*	SSR and Cyt P450	93	9	344.6	Abbo *et al*., 2005
Intraspecific (F_2)					
C.arietinum (ICC1 × Lasseter)	SSR, ISSR and RGA	66	8	534.5	Flandez-Galvez *et al*., 2003b

Table 3. Contd.

Mapping population	Marker Type	No. of markers	Linkage groups	Genome Coverage (cM)	References
Intraspecific (RIL)					
C.arietinum (ILC1272 × ILC3279)	SSR and Ascochyta blight resistance loci	55	8	-	Udupa and Baum, 2003
C.arietinum (ICCV2 × JG62)	SSR, RAPD, ISSR and morphological	111	14	297	Cho *et al*., 2002
C.arietinum (PI 359075 × FLIP 84-92C)	SSR	53	11	318.2	Cho *et al*., 2004
C.arietinum (Kabuli × Desi)	RAPD, ISSR, SSR and morpho-logical	160	10	427.9	Cobos *et al.*, 2005

markers may be useful to saturate the regions with low marker density (Young *et al.*, 1999), which would increase the likelihood of identifying tightly, linked markers to the gene(s) of interest.

Identification of more microsatellite loci and integration with morphological, biochemical and other DNA markers would help in thorough coverage of the genome and enhance the process of tagging of simply inherited as well as quantitatively inherited traits. Genetic linkage maps based on molecular markers allow comparative mapping of related crops to study syntenic relationships and conserved linkages. Chickpea genome exhibits more similarity with the pea genome as compared to lentil (Simon and Muehlbauer, 1997). Pea, lentil and chickpea have several common linkage groups consisting of homologous genes (Gaur and Slinkard, 1990a). The presence of conserved linkage groups in these closely related genera will help to predict the location of non-mapped genes in one genus based on their location in the other genus (Gaur and Slinkard, 1991). The genetic linkage maps of chickpea that have been developed based on interspecific crosses are particularly useful for gene introgression from wild relatives (Mohan *et al*., 1997).

GENE TAGGING AND MARKER ASSISTED SELECTION

Molecular marker assisted selection (MAS) is based on the principle that if a gene (or block of genes) is tightly linked to an easily identifiable genetic marker it may be more efficient to select in a breeding programme for the marker than for the trait itself. The effectiveness of MAS depends on the strength of linkage between the marker and the gene locus controlling the trait of interest, and genetic control of the trait (Hayward *et al*., 1994). In case of single major gene, MAS can be the most effective means of transfer of the desired gene by backcross breeding. Isozymes and DNA markers have been widely used to tag gene(s) conferring resistance to various fungal, bacterial and viral diseases in different crop species and provide an opportunity to

pyramid genes in various combinations. The molecular markers and their applications in plant breeding programmes have been reviewed extensively (Gupta *et al.*, 1999; Melchinger, 1990; Mohan *et al*., 1997). Various factors, including fungal inoculum levels and environmental conditions such as soil temperature and/or moisture level often influence disease development in field experiments. Molecular markers permit genotyping of individuals at seedling stage itself for the trait of interest in a disease free environment. In chickpea, several gene(s) conferring resistance to biotic stresses as well as agronomic traits have been tagged using molecular markers (*Table 4*).

Agronomic traits

Cold and drought are the major constraints to chickpea productivity worldwide but they are quantitative traits where gene tagging has been difficult. Litchtenzveig *et al.* (2006) used an intraspecific cross of *kabuli* and *desi* cultivars and mapped two QTLs responsible for days to flowering. This work may help in identifying genes responsible for early flowering which may help in developing varieties which may escape terminal drought. QTLs for flower colour, double podding, seed coat thickness, seed weight and carotenoids have been also reported (Cobos *et al.*, 2005; Abbo *et al*., 2005; Rajesh *et. al*., 2002) using interspecific as well as intraspecific mapping populations, but none of these QTLs have shown very tight linkage with the markers needed for MAS, Furthermore, the results of none QTL mapping experiment have rarely been validated in different crosses to check their robustness (*Table 4*).

Fusarium wilt resistance

Resistance to race 1 of fusarium wilt is controlled by a single recessive locus and RAPD markers linked to the resistance gene were identified (Mayer *et al*., 1997). The two RAPD markers UBC-170_{550} and CS-27_{700} are linked with the fusarium wilt resistance and susceptibility alleles, respectively, and are located on the same side of the locus. The two marker loci are 7 cM away from the locus that controls resistance to fusarium wilt race 1. Some resistant lines possessing the marker CS-27_{700} were identified, due to recombination between marker and the resistance gene loci. Allele-specific associated primers (ASAPs) developed for the marker UBC-170_{550} amplified DNA from both resistant and susceptible plants, and thus exhibited locus specificity instead of allele-specificity. However, there could be sequence differences between the two amplified fragments of same size. Amplification of similar size fragments from both resistant and susceptible plants with allele-specific primers has been previously reported (Horejsi *et al*., 1999; Paran and Michelmore, 1993). The loss of polymorphism during conversion of RAPD marker to an allele-specific SCAR marker shows that the RAPD polymorphism is caused by mutations at the primer binding site. The longer allele-specific primers can tolerate mismatches in the nucleotide sequence, and anneal to regions with common sequence in both genotypes resulting in loss of polymorphism.

Paran and Michelmore (1993) suggested different approaches to solve this problem: a) performing the polymerase chain reaction at higher annealing temperatures to eliminate weak bands, b) restriction digestion of the fragments from both the

Table 4. List of mapped genes/QTLs for agronomic traits and disease resistance in chickpea

Trait	Mapping Population	QTL/ gene	Reference
beta-Carotene concentration	F_2, interspecific (*C.arietinum* x *C.reticulatum)*	4 QTLs	Abbo *et.al*., 2005
Seed Weight	F_2, interspecific (*C.arietinum* x *C.reticulatum)*	3 QTLs	Abbo *et.al*., 2005
Flower colour	Intraspecific (*C. arietinum*) Kabuli × Desi	Tagged with maker GAA47	Cobos *et. al.*, 2005
Single pod/double pod	Intraspecific (*C. arietinum*) Kabuli × Desi	Tagged with maker TA80	Rajesh *et al*., 2002; Cobos *et. al.*, 2005
Time to flowering	Intraspecific (*C. arietinum*) Kabuli × Desi	SSR makers	Lichtenzveig *et al.*, 2006
Fusarium wilt resistance for race 1	Interspecific (*C.arietinum* x *C.reticulatum*)	ASAP	Simon and Muehlbauer, 1997
Fusarium wilt resistance for race 3	BAC library		Rajesh *et al*., 2004
Fusarium wilt resistance for race 4 and 5	RIL, interspecific (*C.arietinum* x *C.reticulatum)*	RAPD and ISSR	Winter *et. al*. 2000
Fusarium wilt resistance, race 0	RILs, Intraspecific (*C. arietinum*)	RAPD	Rubio *et al*., 2003
Fusarium wilt resistance, race 0	RILs, Intraspecific (*C. arietinum*)	RAPD and STMS	Cobos *et. al.*, 2005
Aschochyta blight	RIL, interspecific (*C.arietinum* x *C.reticulatum*)	2 QTL	Tekeoglu *et al*., 2002
Aschochyta blight Pathotype I & II	Intraspecific (*C. arietinum*)	3 QTLs	Cho *et. al.,* 2004
Aschochyta blight	F_2, interspecific (*C.arietinum* x *C.reticulatum*)	2 QTLs	Collard *et. al.*, 2003
Aschochyta blight	Intraspecific (*C. arietinum*)	3 QTLs	Flandez-Galvez *et. al.*, 2003a and 2003b
Aschochyta blight		2 QTLs	Millan *et al*., 2003
Aschochyta blight	RIL, interspecific (*C.arietinum* x *C.reticulatum*)	1 QTL	Rakshit *et. al*., 2003
Aschochyta blight Pathotype I & II	*C.arietinum* (ILC1272 × ILC3279)	3 QTLs	Udupa and Baum, 2003
Aschochyta blight	*C.arietinum* (Kabuli × Desi)	2 QTLs	Irula *et al*., 2006

genotypes to reveal polymorphism, which are generally referred as cleaved amplified polymorphic sequences (CAPS), c) sequencing of both the fragments and designing allele-specific primers based on the difference(s) in the nucleotide sequence. No

polymorphism was detected upon restriction digestion of 550bp fragment from the resistant and susceptible genotypes. Therefore, construction of specific primers by utilising the differences in the nucleotide composition of the fragments from both the genotypes may help in distinguishing resistant and susceptible individuals. The two RAPD markers CS-27_{700} and UBC-170_{550} are also linked to a recessive gene conferring resistance to race 4 of fusarium wilt (Tullu *et al*., 1998), suggesting that some of the Fusarium wilt resistance genes may be in clusters. Only two RAPD markers associated with resistance or/ susceptibility were found upon testing of 370 (Mayer *et al*., 1997) and 420 (Tullu *et al*., 1998) random primers, exhibiting low level of genetic variation in cultivated chickpea germplasm. Both the RAPD markers are located on the same side of the resistance gene with a genetic distance of 9 map units from the resistance locus. The two RAPD markers are mapped to linkage group VI of the chickpea and the genes for resistance to race 1 and 4 are only 5 cM apart.

Ratnaparkhe *et al*. (1998a) reported an ISSR marker linked to a gene conferring resistance to race 4 of fusarium wilt using recombinant inbred lines. The ISSR marker UBC-855_{500} is linked in repulsion phase with the wilt resistance gene at a genetic distance of 5.2 cM, and is highly suitable for practical use. The ISSR marker UBC-855_{500} was also mapped to linkage group VI. It co-segregated with CS-27_{700}, and was located 0.6 cM from the CS-27_{700}, indicating that genes for resistance to race 1 and 4 are linked. The ISSR marker UBC-855_{500} was amplified using simple sequence repeat $(AC)_8$YT as a primer in a PCR reaction. A new approach has been used by Ratnaparkhe *et al*. (1998b) for marker enrichment in the desired genomic regions, It is possible to amplify different sequences containing AC repeats by changing the nucleotide residues at 3' and 5' anchor positions. The ISSR primers UBC 825 and UBC 857, with a composition of $(AC)_8$T and $(AC)_8$YG respectively, amplified marker fragments of 1200 bp and 800 bp respectively and the two markers are linked to the fusarium wilt resistance gene. The ISSR marker UBC 825_{1200} is located 5.0 cM from *Foc4*, a gene conferring resistance to fusarium wilt race 4, and is closer to the gene than the two markers UBC 855_{500} and CS 27_{700} reported earlier. The markers located at a genetic distance of less than 5 cM would be of practical use (Tanksley, 1983). A resistance gene for fusarium wilt race 5, *Foc5* was also mapped to the same linkage group as *Foc4*. The abundance of di-, tri- and tetra-nucleotide repeats near fusarium wilt resistance gene clusters facilitated the generation of more tightly linked markers by one or two nucleotide changes at the 3' and 5' anchor positions. Winter *et al.* (2000) showed that the $CS27_{700}$ ASAP is linked to *Foc4* and *Foc5* genes for resistance to fusarium wilt races 4 and 5 at a distance of 3.7 and 18 cM, respectively. Sharma *et al*. (2004) used intraspecific cross to map the resistance to fusarium wilt race 3 and mapped *foc-3* gene 0.6 cM from SSR markers TA96 and T27 and STS maker CS27A. Linkage between *foc-3* and other chickpea wilt wilt resistance gene *foc-1* and *foc-4* was established. Cobos *et. al*. (2005) used intraspecific cross to map the resistance to fusarium wilt race 0 and found that on linkage group III, a resistance gene *Foc-0* is flaked by $OPJ20_{600}$ and SSR maker TR59. It appears that the genes conferring resistance to different races of fusarium wilt are functionally related and are organized as complex gene families on a particular chromosome in the genome of chickpea. In a recent review, Milan et al. (2006) has summarized all the studies related with molecular mapping of *Foc* genes and showed a cluster of several *Foc* genes on linkage group II. Detailed analysis of more than two hundred diseases resistance genes on rice chromosome 11

and 12 has revealed evolution of these genes by tandem duplication and divergence under the selective pressure of fast evolving rice pathogens (Rice chromosome 11 and 12 sequencing consortium, BMC Biology 2006).

Ascochyta blight

Resistance to many fungal diseases inherit quantitatively, and such resistance is controlled by multiple loci, known as quantitative trait loci (Young, 1996). Since a quantitative character is governed by the joint action of several genes and their interactive effects, it is very difficult to manipulate these traits in breeding programmes. However, the complex traits can be dealt effectively as single gene traits, if they could be resolved into their individual genetic components. The high density linkage maps based on molecular markers provide an opportunity to identify, map and measure the effects of genes underlying quantitative traits (Tanksley *et al*., 1989).

The inheritance studies have indicated involvement of more than one gene in controlling resistance to ascochyta blight (Vir *et al*., 1975; Singh and Reddy 1983; Tewari and Pandey 1986). Involvement of two major complementary recessive genes in controlling resistance to ascochyta blight has been reported (Santra *et al.*, 2000 and Tekeoglu *et al.*, 2000). Molecular markers linked to the two major QTLs and one minor QTL conferring resistance to ascochyta blight were identified using recombinant inbred lines derived from interspecific cross *C.arietinum* x *C.reticulatum* (Tekeoglu *et al.*, 2000). Two major QTLs, QTL-1 and QTL-2 accounted for 50.3% and 45.0% of the total estimated phenotypic variation and were mapped to linkage groups 6 and 1, respectively. The reported DNA markers are linked to the three QTLs for resistance in coupling phase. The QTL-1 is flanked by two RAPD markers (UBC $733b_{850}$ and UBC $181a_{1850}$), which are 10.7 cM apart. The second major QTL is flanked by an ISSR marker (UBC $836b_{700}$) and an isozyme marker Dia 4 and are separated by 5.9 cM distance. A minor QTL, QTL-3 was mapped to linkage group 4 and is flanked by two ISSR markers (UBC $681a_{1750}$ and UBC $858b_{1400}$) spaced 11.7 cM apart. Later Tekeoglu *et al*. (2002) added sequence tagged microsatellite site (STMS) makers to the same linkage map and confirmed the two QTLs for the blight resistance identified by Santra *et. al*. (2000). The linked molecular markers permit rapid and precise transfer of QTLs into superior crop varieties. The size of the population and thorough coverage of the genome with molecular markers play an important role in identification of more tightly linked markers and also additional minor QTLs. Flandez-Galvez *et. al*. (2003a; 2003b) used an intraspecific cross for linkage map construction and identified six QTLs for resistance to ascochyta blight in the three genomic regions; the major QTLs were located on linkage group III. Iruela *et. al.* (2006) identified two QTLs associated with resistance to ascochyta blight using RILs derived from the cross of Kabuli x Desi. The ascochyta blight resistance genes have been also searched in the wild relatives using an interspecific cross between *C. arietinum* and *C. echinospermum* that identified two QTLs associated seedling resistance (Collard *et. al*. 2003).

As the ability of microsatellites in detecting intraspecific variation has already been demonstrated in chickpea they must be employed along with AFLP markers to identify tightly linked markers to the gene(s) of interest and to saturate the genomic region(s) with more number of markers, which could provide the basis to start with chromosome walking for the isolation of genes. Once developed, the primer sequences

can be easily shared across laboratories. Moreover, undesirable and deleterious genes (linkage drag) linked with gene(s) of interest are often transferred from wild species during backcrossing (Stam and Zeven, 1981). In some cases, mutation breeding has been used to break undesirable linkages (Knott, 1980), but it is a tedious process and often unreliable. It is also not possible to recognize the plants with reduced linkage drag by phenotypic selection. Use of RFLP based selection in selecting individuals that have experienced recombination near the gene of interest has been suggested by Tanksley *et al*. (1989). Molecular markers bracketing the gene(s) of interest can reduce the unwanted chromosomal segments surrounding the target gene to less than 2 cM within two backcross generations, which otherwise would require more than 10 backcrosses. However, the ability to detect recombination events in a region of interest depends upon how tightly the markers are linked to the gene of interest and the number of plants assayed in each generation. Therefore it may be anticipated that molecular markers will accelerate the process of transferring genes, particularly recessive genes, to desirable adapted cultivars through backcross method. Udupa and Baum (2003) attempted to elucidate the genetics of pathotype specific blight resistance. Although they identified genomic regions for pathotype-specific blight resistance in chickpea but genetic information was not sufficient to clarify the mechanism. Later Cho *et. al*. (2004) using RIL population constructed a mapped resistance to pathotypes I and II using SSR marker and identified single and two QTLs governing resistance for pathotype II and I, respectively.

MOLECULAR MARKERS IN PATHOGEN FINGERPRINTING

The pathogens of chickpea biotic stresses are generally soil or seed-borne and survive in soil up to 6 years even in the absence of the host plant (Haware *et al*., 1996). The effectiveness of host resistance to the fungal pathogens is threatened by appearance of new pathogenic races that overcome resistance genes (Kaiser *et al*., 1994). Therefore, it is highly desirable to determine the genetic variability existing within the pathogen population in order to breed chickpea cultivars with durable resistance.

Fusarium wilt pathogen

A total of seven distinct races, viz. 0, 1, 2, 3, 4, 5 and 6 of *Fusarium oxysporum f. sp. Ciceri,* have been reported so far by their differential reaction with chickpea lines (Kelly et al., 1994). Differentiation of physiological races using differential hosts is very tedious and takes at least 40 days to complete. The identification of pathotypes with molecular markers is more rapid, cheaper and less labour-intensive. Molecular markers can be used to differentiate species, formae speciales, races and even isolates of fungal pathogens. Race 0 isolates cause the yellowing syndrome, whereas the other races cause the typical fusarium wilt and necrosis. RAPD analysis of these isolates placed the yellowing and wilts inducing races into two separate groups. Two fragments of approximately 1.6 kb were observed with primer KS in wilt-inducing isolates (races 1, 2, 3, 4, 5 and 6), whereas only one fragment was amplified in the yellow-inducing isolates.

Oligonucleotide fingerprinting has been used to distinguish four races viz., 1, 2, 3 and 4 prevalent in India (Barve *et al.*, 2001). All the four Indian races were distinguishable

based on oligonucleotide fingerprints generated by the probes $(AGT)_5$, $(ATC)_5$, and $(GATA)_4$. The races 1 and 4 are closely related at a similarity index value of 76.6%, whereas race 3 is very distinct from others at a similarity value of 26.7%. Race-specific markers, which would be of great use in continuous monitoring of racial distribution in different regions, could not to be identified. The regional distribution of different races of pathogens explains why some varieties that are resistant in one region become susceptible in other.

Ascochyta blight pathogen

Oligonucleotide fingerprinting has been used to distinguish isolates of *Ascochyta rabiei* collected from a single field (Morjane *et al.,* 1994). Based on the fingerprint patterns derived from probes $(CA)_8$, $(CAA)_5$, $(CAT)_5$ and $(GATA)_4$, a total of twelve different fungal haplotypes were observed at various frequencies within a single field. The field populations of *Ascochyta rabiei* are genetically heterogeneous and this heterogeneity at molecular level can be determined precisely and reliably. RAPD analysis of *Ascochyta rabiei* isolates collected from India, Syria, America and Pakistan showed a correlation between genetic distances with geographical distribution. The isolates of *Ascochyta rabiei* within the state of Punjab are distant from the isolates of other north western states of India. A RAPD marker $UBC756_{1600}$ specific to the Indian isolates was identified (Santra *et al*., 2001). Conversion of specific RAPD marker fragment(s) to single locus SCAR (sequence characterised amplified regions) markers increases the reproducibility and makes the analysis simpler.

Chickpea genomics

High throughput tools of functional genomics have revolutionized biological research and are predicted to have great impact on plant breeding which will gradually evolve in to genomics-assisted molecular breeding for crop improvement (Varshney *et al*., 2005). New technologies promise to resolve constraints in defining unambiguous associations between specific gene sequences and phenotype. As a result, the concept of expression markers has been developed, that capitalizes on the establishment of so called expression fingerprints, i.e. specific patterns of all genes responding to a stress. In contrast to the random molecular markers, that detect discriminatory mutations in different individuals, expression fingerprints are more likely to reflect the resistance potential of a plant against a stress, and hence a source for candidate genes underlying a particular trait. Such expression markers or functional markers will certainly be the markers of choice (Anderson and Lübberstedt, 2003). Thus, functional genomics will drive the development and application of new types of precision markers for complex traits (Morgante and Salaimini, 2003).

A resource of about 2000 ESTs associated with chickpea drought was generated at ICRISAT (Buhariwalla *et al.,* 2005). Similarly by using the Super SAGE methodology, over 220,000 Super-SAGE tags describing the differential transcription profiles of chickpea roots and nodules have already been sequenced at University of Frankfurt (G. Kahl and P. Winter, *personal communications*). A custom made gene chip for identifying genes expressed during abiotic and biotic stresses has been developed in Australia (Coram and Pang, 2005b). These resources, together with advances in automation will

accelerate the functional genomics or gene expression studies in near future. Further, by analyzing the expression levels of genes or clusters of genes within a segregating population, it is possible to map the inheritance of expression pattern in an approach called 'genetical genomics' (Jansen and Nap, 2001) or 'expression genetics' (Varshney *et al.*, 2005). The QTLs, identified using expression data in quantitative fashion together with the phenotyping data, are commonly called eQTLs. The eQTLs can be classified as *cis* or *trans* acting based on location of transcript compared to that of the eQTL influencing expression of that transcript (de Koning and Haley, 2005). Because of this feature, eQTL analysis makes it possible to identify factors influencing the level of mRNA expression. The regulatory factor (second order effect) is of specific interest because more than one QTL can be putatively connected to a *trans*-acting factor (Schadt *et al*., 2003). Thus, the mapping of eQTLs allows multi-factorial dissection of the expression profile of a given mRNA or cDNA, protein or metabolite into its underlying genetic components as well as localization of these components on the genetic map (Jansen and Nap, 2001). Subsequently, the eQTL analysis for each gene or gene product analyzed in the segregating population can identify the regions of the genome influencing its expression. The co-localization of candidate genes with QTLs controlling a particular phenotype supports the use of the candidate gene a potential source for developing "perfect marker(s)" for selecting the phenotype in marker-assisted breeding and the procedure is termed as 'genomics-assisted breeding' (Varshney *et al*., 2005).

Development of SSR markers has accelerated chickpea genomics and offered great help to study the complex agronomic trait. In chickpea, several hundreds of SSR markers have been developed and mapped on intra- and inter-specific mapping populations (Winter *et al*., 1999; Huttel *et al.*, 1999; Lichitenzveiz *et al*., 2005, Choudhary *et al.*, 2005 and Sethy *et al.*, 2006a; 2006b). The linkage maps developed by using SSR markers in an intra-specific mapping populations in the cultivated gene pool is more important to breeding programme, because of uneven recombinations and segregation distortion in inter-specific mapping populations (Cho *et al.*, 2002; Flandez-Galvez *et al.*, 2003a). At present SSR markers are available for the complete chickpea genome. The National Institute for Plant Genomic Research, New Delhi (India) and International Crop Research Institute for Semi Arid Tropics (ICRISAT), Patencheru, Hyderabad have made significant progress in the development and utilization of microsatellite markers (Choudhary *et al*., 2005; Sethy *et al*., 2006a and 2006b, Varshney *et al.*, unpublished). More than 100 SSR markers have been developed and many more are under development at NIPGR. These SSR markers have been tested for their cross-species transferability, for intra-specific variability analysis within *C. arietinum* and phylogenetic analysis of all the nine annual *Cicer* species (Sethy *et al*., 2006a; 2006b). At ICRISAT more than 200 SSRs have been identified for development of the markers. The SSR markers developed so far will saturate the intra- and inter-specific genetic maps (Lichtenzveig *et al*., 2005; Choudhary *et al*., 2005; Sethy *et al*., 2006a; 2006b, Varshney *et al*., unpublished and Bhatia *et al.*, *personal communication*). These saturated maps will further help in physical mapping and isolation of disease resistance genes by map based cloning. Rajesh *et al*., (2004) has constructed a Bacterial Artifical Chromosome (BAC) library of Chickpea germplasm line, FLIP 84-92C to facilitate positional cloning of disease resistance gene and physical mapping of the genome. The BAC library was screened with a SSR maker Ta96 which was tightly linked to gene (*foc-3*) for resistance to fusarium wilt. The BAC library may contain other genes also hence; this is the first step in the positional gene cloning in chickpea.

The functional markers generated from genic sequence (e.g. ESTs) have definite advantage over the markers generated from anonymous random regions of the genome, because they are completely linked to desired trait alleles (Anderson and Lubberstedt, 2003; Varshney *et al.*, 2005; Buhariwala *et al*., 2005). At ICRISAT, Buhariwala *et al.* (2005) have developed an EST library from two very closely related chickpea genotypes (*Cicer arietinum*). A total of 106 EST-based markers were designed from 477 sequences with functional annotations and tested on *C. arietinum*. Forty-four EST markers were polymorphic when screened across nine *Cicer* species (including the cultivated species) and were used to study the species relationship. Most of the species clustered as reported in the previous studies, and hence it further supported the classification of primary (*C. arietinum*, *C. echinospermum* and *C. reticulatum*), secondary (*C. pinnatifidum*, *C bijugum* and *C. judaicum*), and tertiary (*C. yamashitae*, *C. chrossanicum* and *C. cuneatum*) gene-pools. A large proportion of EST alleles (45%) were only present in one or two of the accessions tested whilst the others were represented in up to twelve of the accessions tested.

Conclusions

The chickpea productivity is often limited due to various biotic and abiotic stresses. Lack of requisite genetic variation in the cultivated species has necessitated systematic collection, documentation and evaluation of wild *Cicer* species for use in chickpea variety improvement programmes. Various studies have clearly indicated the possibility of transferring resistance genes and desirable co-adapted gene complexes from unexploited wild annual species to the cultivated species. At this juncture, the valuable genetic resources present in the primary gene pool can be successfully utilized in plant breeding programmes for genetic enhancement of chickpea. However, most of the wild species possessing high degree of resistance to multiple stresses are present in the secondary and tertiary gene pools where hybridization with the cultivated species is often limited by reproductive barriers. Gene introgression from the wild species can be monitored effectively with the use of molecular markers, which will expedite the transfer of agronomically important traits into desirable genetic backgrounds for the development of varieties with durable resistance through marker assisted selection. Molecular markers such as SSR and SNP are useful for construction of high density genetic maps of chickpea. These maps will be useful in identification of genes/QTLs associated with stress resistance as well as quality traits for undertaking extensive molecular breeding in chickpea. Systematic pathogen surveys to identify new virulence and to get an insight into their regional distribution will help in devising appropriate management strategies for controlling important fungal diseases. Novel biotechnological approaches are required for introgression of useful traits from the wild *Cicer* species, a resource that is yet to be harnessed effectively.

References

Abbo, S., Molina, C., Jungmann, R., Grusak, A., Berkovitch, Z., Reifen, R., Kahl, G., Winter, P. And Reifen, R. (2005). Quantitative trait loci governing

carotenoid concentration and weight in seeds of chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics.* **111**,185-95.

Ahmad, F., Slinkard, A.E. And Scoles Gj. (1987). The cytogenetic relationship between *Cicer judaicum* Boiss. and *Cicer chorassanicum* (Bge.) M. Pop. *Genome* **29**, 883-86.

Ahmad, F., Slinkard, A.E. And Scoles, G.J. (1988). Investigations into the barrier(s) to interspecific hybridization between *Cicer arietinum* L. and eight other annual *Cicer* species. *Plant Breeding* **100**,193-98.

Ahmad, F. (1988). Interspecific hybridization and genetic relationships among the annual *Cicer* L. species. Ph.D. thesis, University of Saskatchewan, Saskatoon, Canada.

Ahmad, F. (1989). The chromosomal architecture of *Cicer anatolicum* Alef., a wild perennial relative of chickpea. *Cytologia* **54**, 753-57.

Ahmad, F., Gaur, P.M. And Slinkard, A.E. (1992). Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L. *Theoretical and Applied Genetics* **83**, 620-27.

Ahmad, F. And Slinkard A.E. (1992). Genetic relationships in the genus *Cicer* L. as revealed by polyacrylamide gel electrophoresis of seed storage proteins. *Theoretical and Applied Genetics* **84**, 688-92.

Ahmad, F. And Hymowitz, T. (1993). The fine structure of chickpea (*Cicer arietinum* L.) chromosomes as revealed by pachytene analysis. *Theoretical and Applied Genetics* **86**, 637-41.

Ahmad, F. (1999). Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. *Theoretical and Applied Genetics* **98**, 657-63.

Akkayya, M.A., Bhagwat, A.A. And Cregan, P.B. (1992). Length polymorphisms of simple-sequence repeat DNA in soybean. *Genetics* **132**, 1131-39.

Akkayya, M.A., Shoemaker, R.C., Specht, J.E., Bhagwat A.A. And Cregan, P.B. (1995). Integration of simple sequence repeat DNA markers into a soybean linkage map. *Crop Science* **35**, 1439-45.

Alcala, J., Giovannoni, J.J., Pike, L.M. And Reddy, A.S. (1997). Application of Genetic Bit Analysis (GBA™) for allelic selection in plant breeding. *Molecular Breeding* **3**, 495–2.

Anderson, J.R. And Lübberstedt, T. (2003). Functional markers in Plants. *Trends in Plant Science* **8**, 554-60.

Arumuganathan, K. And Earle, E.D. (1991). Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter* **9**, 208-218.

Ayers, N.M., Mcclug, A.M., Larkin, P.D., Bligh, H.F.J., Jones, C.A. And Park, W.D. (1997). Microsatellites and a single nucleotide polymorphism differentiate apparent amylase classes in an extended pedigree of US rice germplasm. *Theoretical and Applied Genetics* **94**, 773-81.

Azhaguvel, P., Vidya, S.D., Sharma, A. And Varshney, R.K. (2006). Methodological advancement in molecular markers to delimit the gene(s) for crop improvement.

In: Floriculture, Ornamental and Plant Biotechnology Advances and Tropical Issues. Eds. Texiera da Silva, J., pp 460-99. Global Science Books, London.

Badami, P.S., Mallikarjuna, M. And Moss, J.P. (1997). Interspecific hybridization between *Cicer arietinum* and *C. pinnatifidum*. *Plant Breeding* **116**, 393-95.

Barve, M.P., Haware, M.P., Sainani, M.N., Ranjekar, P.K. And Gupta, V.S. (2001). Potential of microsatellites to distinguish four races of *Fusarium oxysporum f.sp. ciceri* prevalent in India. *Theoretical and Applied Genetics* **102**, 138-47.

Bassiri, A., Ahmad, F. And Slinkard, A.E. (1987). Pollen grain germination and pollen tube growth following in vivo and in vitro self and interspecific pollinations in annual *Cicer* species. *Euphytica* **36**, 667-75.

Beckmann, J.S. And Soller, M. (1990). Towards a unified approach to genetic mapping of eukaryotes based on sequence-tagged microsatellite sites. *Bio/Technology* **8**, 930-32.

Botstein, D., White, R.L., Skolnick, M. And Davis, R.L. (1980). Construction of a genetic linkage map in man using restriction fragment polymorphism. *American Journal of Human Genetics* **32**, 314-31.

Buhariwalla, H.K., Jayashree, B., Eshwar, K., Crouch, J.H. (2005). ESTs from chickpea roots with putative roles in drought tolerance. *BMC Plant Biology* **5**, 16.

Cho, S., Kumar, J., Shultz, J.F., Anupama, K., Tefera, F. And Muehlbauer, F.J. (2002). Mapping genes for double podding and other morphological trait in chickpea. *Euphytica* **125**, 285-92.

Cho, S., Chen, W. And Muehlbauer, F.J. (2004). Pathotype-specific factors in chickpea (*Cicer arietinum* L.)for quantitative resistance to ascochyta blight. *Theoretical and Applied Genetics* **109**,733-39.

Choudhary, S., Sethy, N.K., Shokeen, B. And Bhatia, S. (2006). Development of sequence tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). *Molecular Ecology Notes* **6**, 93-95.

Choumane, W., Winter, P., Weigand, F. And Kahl, G. (2000). Conservation and variability of sequence tagged microsatellite sites (STMSs) from chickpea (*Cicer arietinum* L.) within the genus Cicer. *Theoretical and Applied Genetics* **101**, 269-78.

Cobos, M.J., Fernandez, M.J., Rubio, J., Kharrat, M., Moreno, M.T., Gil, J. And Millan T. (2005). A linkage map of chickpea (Cicer arietinum L.) based on populations from Kabuli x Desi cross; location of genes for resistance to fusarium wilt race 0. *Theoretical and Applied Genetics* **110**, 1347-53.

Collard, B.C.Y., Pang, E.C.K., Ades, P.K. And Taylor P.W.J. (2003). Prelimnary investigation of QTLs associated with seedling resistance to ascochyta blight from *Cicer echinospermum*, a wild relative of chickpea. *Theoretical and Applied Genetics* **107**, 719-29.

Coram, T.E., Pang, E.C.K. (2005*a*). Isolation and analysis of candidate ascochyta blight defence genes in chickpea. Part I. Generation and analysis of an expressed sequence tag (EST) library. *Physiological and Molecular Plant Pathology* **66**, 192–200.

Coram, T.E., Pang, E.C.K. (2005b). Isolation and analysis of candidate ascochyta blight defence genes in chickpea. Part II. Microarray expression analysis of putative defence-related ESTs. *Physiological and Molecular Plant Pathology* **66**, 201–210.

Cregan, P.B., Jarvik, K.T., Bush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.L., Kaya, N., Van Toai, T.T., Lohnes, D.G., Chung, J. And Specht, J.E. (1999). An integrated genetic linkage map of the soybean genome. *Crop Science* **39**, 1461-90.

De Koning, D.J. And Haley, C.S. (2005). Genetical genomics in humans and model organisms. *Trends in Genetics* **21**, 377-81.

Di Vito, M., Greco, N., Singh, K.B. And Saxena, M.C. (1988). Response of chickpea germplasm lines to *Heterodera ciceri* attack. *Nematologia Mediterranea.* **16**, 17-18.

Di Vito, M., Singh, K.B., Greco, N. And Saxena, M.C. (1996). Sources of resistance to cyst nematode in cultivated and wild *Cicer* species. *Genetic Resources and Crop Evolution* **43**, 103-7.

Eshed, Y. And Zamir, D. (1995). An introgression line population of Lycopersicon pennelli in the cultivated tomato enables the identification and fine mapping of yield-associated QTLs. *Genetics* **143**, 1807-17.

Faostat-Agriculture (2004). Database. http://www.fao.org/waicent/portal/statiistic.es.asp.

Flandez-Galvez, H., Ford, R., Pang, E.C.K. And Taylor, P.W.J. (2003a). An intra-specific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged based microsatellite site and resistance gene along markers. *Theoretical and Applied Genetics* **106**, 1447-53.

Flandez-Galvez, H., Ades, P.K., Ford, R., Pang, E.C.K., Taylor, P.W.J. (2003b). QTL analysis for ascochyta blight resistance in an Intraspecific population of chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* **107**, 1257-65.

Galasso, I., Frediani, M., Maggiani, M., Cremonini, R. And Pignone, D. (1996). Chromatin characterization by banding techniques, in situ hybridization, and nuclear DNA content in Cicer L. (Leguminosae). *Genome* **39**, 258-65.

Gaur, P.M. And Slinkard, A.E. (1990a). Genetic control and linkage relations of additional isozyme markers in chickpea. *Theoretical and Applied Genetics* **80**, 648-56.

Gaur, P.M. And Slinkard, A.E. (1990b). Inheritance and linkage of isozyme coding genes in chickpea. *Journal of Heredity* **81**, 455-61.

Gaur, P.M. And Slinkard, A.E. (1991). An isozyme gene map of chickpea. *International Chickpea Newsletter* **24**, 25-28.

Graland, S. H., Lewin, L., Blakeney, A. And Henry, R. (2000). In: Plant and Animal Genome VIII Conference, 9–12 January, San Diego, USA (http://www.intl-pag.org/pag/8/abstracts/).

Grandillo, S. And Tanksley, S.D. (2005). Advanced Backcross QTL Analysis;

results and perspectives. In: The wake of Double Helix; from the Green Revolution to the Gene Revolution. Eds. Tuberosa, R., Phillips, R.L. and Gale, M., pp115-32. Edizioni Avenue Media, Bologna.

Gumber, R.K., Kumar, J. And Haware, M.P. (1995). Inheritance of resistance to fusarium wilt in chickpea. *Plant Breeding* **114**, 277-79.

Gupta, P.K., Varshney, R.K., Sharma, P.C. And Ramesh B. (1999). Molecular markers and their applications in wheat breeding. *Plant Breeding* **118**, 369-90.

Gupta, P.K. And Varshney, R.K. (2000). The development and use of microsatellite markers for genetic analysis and Plant Breeding with emphasis on bread wheat. *Euphytica* **113**, 163-85.

Gupta, P.K., Roy, J.K. And Prasad, M. (2001). Single nucleotide polymorphism; a new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Current Science* **80 (4)**, 524-35.

Haley, S.D., Afanador, L. And Kelly, J.D. (1994). Selection for monogenic pest resistance traits with coupling- and repulsion phase RAPD markers. *Crop Science* **34**, 1061-66.

Harlan, J.R. And De Wet, J.M.J. (1971). Toward a rational classification of cultivated plants. *Taxon* **20**, 509-17.

Haware, M.P. And Nene, Y.L. (1982). Races of *Fusarium oxysporum f.sp.ciceri*. *Plant Diseases* **66**, 809-10.

Haware, M.P., Nene, Y.L. And Natarajan, M. (1996). Survival of *Fusarium oxysporum f. sp. ciceri* in soil in the absence of chickpea. *Phytopathologie Mediterranea* **35**, 9-12.

hawkes, j.g. (1977). The importance of wild germplasm in Plant Breeding. *Euphytica* **26**, 615-21.

Hayward, M.D., Mcadam, N.J., Jones, J.G., Evans, C., Evans, G.M., Forster, J.W., Ustin, A., Hossain, K.G., Quader, B., Stammers, M. And Will J.K. (1994). Genetic markers and the selection of quantitative traits in forage grasses. *Euphytica* **77**, 269-75.

Horejsi, T., Box, J.M. And Staub, J.E. (1999). Efficiency of randomly amplified polymorphic DNA to sequence characterized amplified region marker conversion and their comparative polymerase chain reaction sensitivity in cucumber. *Journal of the American Society for Horticultural Science* **124**, 128-35.

Huttel, B., Winter, P., Weising, K., Choumane, W., Weigand, F. And Kahl, G. (1999). Sequence-tagged microsatellite markers for chickpea (*Cicer arietinum* L.). *Genome* **42**, 210-17.

Infantino, A., Porta-Pugalia, A. And Singh, K.B. (1996). Screening wild *Cicer* species for resistance to fusarium wilt. *Plant Diseases* **80**, 42-44.

Irula, M., Rubio, J., Barro, F., Cubero, J.I., Millan, T. And Gil, J. (2006). Detection of two QTL for resistance to Ascochyta Blight in an intraspecific cross of chickpea (*Cicer arietinum* L.): Development of SCAR markers associated

to resistance. *Theoretical and Applied Genetics* **112**, 278-87.

Jaiswal, H.K., Singh, B.D., Singh, A.K. And Singh, R.M. (1986). Introgression of genes for yield and yield traits from *C. reticulatum* into *C. arietinum*. *International Chickpea Newsletter* **14**, 5-8.

Jansen, R.C. And Nap, J.P. (2001). Genetical genomics; the added value for segregation. *Trends in Genetics* **17**, 388-91.

Jimenez-Diaz, R.M., Trapero-Crass, A., Carbera De, L.A. And Coina, J. (1989). Races of Fusarium oxysporum f.sp. ciceri infecting chickpeas in Southern Spain. In: Vascular wilt diseases of plants. Eds. Tjamos, E.C. and Beckman, C.H., pp 515-520. NATO ASI series H.

Jimenez-Diaz, R.M., Singh, K.B., Trapero-Casas, A. And Trapero-Casas, J.L. (1991). Resistance in kabuli chickpeas to Fusarium wilt. *Plant Diseases* **75**, 914-18.

Johnson, E., Miklas, P.N., Stavely, J.R. And Martinez-Crusado, J.C. (1995). Coupling-and repulsion phase RAPDs for marker-assisted selection of PI 181996 rust resistance in common bean. *Theoretical and Applied Genetics* **90**, 659-64.

Jones, C.J., Edwards, K.J., Castaglione, S., Winfield, M.O., Sala, F., Van De Wiel, C., Bredemeijer, G., Vosman, B., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Malcevschi, A., Marmiroli, N., Aert, R., Volckaert, G., Rueda, J., Lincareo, R., Vazquez, A. And Karp, A. (1997). Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding* **3**, 381-90.

Jones, N., Ougham, H. And Thomas, H. (1997). Markers and mapping; we are all geneticists now. *New Phytologist* **137**, 165-77.

kaiser, w.j., alcala-jimenez, a.r., hervas-vargas, a., trapero-casas, j.l. and jimenez-diaz, r.m. (1994). Screening of wild cicer species for resistance to races 0 and 5 of *Fusarium oxysporum f.sp. ciceris*. *Plant Diseases* **78**, 962-67.

Kazan, K. And Muehlbauer, F.J. (1990). Allozyme variation and phylogeny in annual species of Cicer (Leguminosae). *Plant Systematics and Evolution* **175**, 11-21.

Kazan, K., Muehlbauer, F.J., Weeden, N.F. And Ladizinsky, G. (1993). Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* **86**, 417-26.

Kelly, A., Alcala-Jimenez, A.R., Bainbridge, B.W., Heale, J.B., Perez-Artes, E. And Jimenez-Diaz, R.M. (1994). Use of genetic fingerprinting and random amplified polymorphic DNA to characterise pathotypes of *Fusarium oxysporum f.sp. ciceris* infecting chickpea. *Phytopathology* **84**, 1293-98.

King, G.J. (1994). Progress in mapping agronomic genes in apple (The European Apple Genome Mapping Project). *Euphytica* **77**, 65-69.

Knott, D.R. (1980). Mutation of a gene for yellow pigment linked to Lr 19 in wheat. *Canadian Journal Genetics Cytology* **22**, 651-54.

Kumar, J. And Van Rheenen, H.A. (2000). A major gene for time of flowering in

chickpea. *Journal of Heredity* **91**, 67-68.

Kusmenoglu, I., Muehlbauer, F.J. And Kazan, K. (1992). Inheritance of isozyme variation in ascochyta blight-resistant chickpea lines. *Crop Science* **32**, 121-27.

Labdi, M., Robertson, L.D., Singh, K.B. And Charrier, A. (1996). Genetic diversity and phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism. *Euphytica* **88**, 181-88.

Ladizinsky, G. And Adler, A. (1975). The origin of chickpea as indicated by seed protein electrophoresis. *Israel Journal Botany* **24**, 183-89.

Ladizinsky, G. And Adler, A. (1976a). Genetic relationships among the annual species of *Cicer* L. *Theoretical and Applied Genetics* **48**, 197-3.

Ladizinsky, G. And Adler, A. (1976b). The origin of chickpea *Cicer arietinum* L. *Euphytica* **25**, 211-17.

Lavania, U.C. And Lavania, S. (1983). Karyotype studies in Indian Pulses. *Genetica Agraria.* **37**, 299-08.

Lichitenzveiz, J., Scheuring, C., Dodge, J., Abbo, S. And Zhang, H.B. (2005). Construction of BAC and BIBAC libraries and their application for generation of SSR markers for genome analysis of chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* **110**, 492-10.

Lichitenzveiz, J., Bonfil, D.J., Zhang, H.B., C., Shtienberg, D., And Abbo, S. (2006). Mapping quantitative trait loci in chickpea associated with time to flowering and resistance to *Didymella rabiei* the causal agent of Ascohyta blight. *Theoretical and Applied Genetics* **113**, 1357-69.

Lin, J.J., Kuo, J., Ma, J., Saunders, J.A., Beard, H.S., Macdonald, M.H., Kenworthy, W., Ude, G.N. And Matthews, B.F. (1996). Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Molecular Biology Reporter* **14**, 156-69.

Lulsdorf, M., Mallikarjuna, N., Clarke, H. And Tar'an, B. (2005). Finding solutions for interspecific hybridisation problems in chickpea (*Cicer arietinum* L.). In: 4th International Food legumes Research Conference, Oct. 18-22, pp 44. New Delhi, India.

Malhotra, R.S. And Singh, K.B. (1990). The inheritance of cold tolerance in chickpea. *Journal of Genetics and Breeding* **44**, 227-30.

Malhotra, R.S. And Singh, K.B. (1991). Gene action for cold tolerance in chickpea. *Theoretical and Applied Genetics* **82**, 598-1.

Mansur, L.M., Orf, J.H., Chase, K., Jarvik, T., Cregan, P.B. And Lark, K.G. (1996). Genetic mapping of agronomic traits using recombinant inbred lines of soybean. *Crop Science* **36**, 1327-36.

Mayer, M.S., Tullu, A., Simon, C.J., Kumar, J., Kaiser, W.J., Kraft, J.M., Muehlbauer, F.J. (1997). Development of a DNA marker for fusarium wilt resistance in chickpea. *Crop Science* **37**, 1625-29.

melchinger, a.e. (1990). Use of molecular markers in breeding for oligogenic disease resistance. *Plant Breeding* **104**, 1-19.

Mercy, S.T. And Kakar, S.N. (1975). Barrier to interspecific crosses in *Cicer*. *Proceedings of Indian National Science Academy* **41**, 78-82.

Millan, T., Rubio, J., Irula, M., Daly, K., Cubero, J.I. And Gil, J. (2003). Marker associated with Ascochyta blight resistance in chickpea and their potential in marker assisted selection. *Field Crop Research* **84**, 373-84.

Millan, T., Clarke, H.J., Siddique, K.H.M., Buhariwalla, H.K., Gaur, P.M., Kumar, J., Gil, J., Kahl, G. And Winter, P. (2006)Chickpea molecular breeding-New tools and concepts. *Euphytica* **147**, 81-3.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T.G., Yano, M., Bhatia, C.R. And Sasaki, T. (1997). Genome mapping, molecular markers and marker assisted selection in crop plants. *Molecular Breeding* **3**, 87-3.

Morgante, M. And Salaimini, F. (2003). From plant genomics to breeding practice. *Current Opinion in Biotechnology* **14**, 214-19.

Morjane, H., Geistlinger, J., Harrabi, M., Weising, K. And Kahl G. (1994). Oligonucleotide fingerprinting detects genetic diversity among *Ascochyta rabiei* isolates from a single chickpea field in Tunisia. *Current Genetics* **26**,191-97.

Muehlbauer, F.J. And Kaiser, W.J. (1994). Using host plant resistance to manage biotic stresses in cool season food legumes. *Euphytica* **73**, 1-10.

Muehlbauer, F.J., Kaiser, W.J. And Simon, C.J. (1994). Potential for wild species in cool season food legume breeding. *Euphytica* **73**, 109-14.

Nene, Y.L. And Haware, M.P. (1980). Screening chickpea for resistance to wilt. *Plant Diseases* **64**, 379-80.

Nene, Y.L. And Reddy, M.V. (1987). Chickpea diseases and their control. In: The chickpea. Eds. Saxena, M.C. and Singh, K.B., pp 233-70. CAB International/ Oxon, U.K.

Nguyen, T.T., Taylor, P.W.J., Redden, R.J. And Ford, R. (2004). Genetic diversity estimates in Cicer using AFLP analysis. *Plant Breeding* **123**, 173-79.

Ocampo, B., Venora, V., Errico, A., Singh, K.B. And Saccardo, F. (1992). Karyotype analysis in the genus Cicer. *Journal of Genetics and Breeding* **46**, 229-40.

Ohri, D. And Pal, M. (1992). The origin of chickpea (*Cicer arietinum* L.); karyotype and nuclear DNA amount. *Heredity* **66**, 367-72.

Oram, R.N., Shaikh, M.A.Q., Zaman, K.M.S. And Brown, A.H.D. (1987). Isozyme similarity and genetic differences in morphology between Hyprosola, A high yielding, high protein mutant of chickpea (*Cicer arietinum* L.) and its parental cultivar. *Environmental and Experimental Botany* **27**, 455-62.

Paran, I. And Michelmore, R.W. (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics* **85**, 985-93.

Patankar, A.G., Harsulkar, A.M., Giri, A.P., Gupta, V.S., Sainani, M.N., Ranjekar, P.K. And Deshpande, V.V. (1999). Diversity in inhibitors of trypsin and *Helicoverpa armigera* gut proteinases in chickpea (*Cicer arietinum*) and its wild relatives. *Theoretical and Applied Genetics* **99**, 719-26.

Patil, P.B., Vrinten, P.L., Scoles, G.J. And Slinkard, A.E. (1995). Variation in the ribosomal RNA units of the genera Lens and Cicer. *Euphytica* **83**, 33-42.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S. And Rafalski, A. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* **2**, 225-38.

Pundir, R.P.S. And Van Der Maesen, L.J.G. (1983). Interspecific hybridization in Cicer. *International Chickpea Newsletter* **8**, 4-5.

Pundir, R.P.S., Rao, N.K. And Van Der Maesen, L.J.G. (1985) Distribution of qualitative traits in the world germplasm of chickpea (*Cicer arietinum* L.). *Euphytica* **34**, 697-3.

Pundir, R.P.S. And Mengesha, M.H. (1995). Cross compatibility between chickpea and its wild relative, *Cicer echinospermum* Davis. *Euphytica* **83**, 241-45.

Rajesh, P.N., Tullu, A., Gil, J., Gupta, V.S., Ranjekar, P.K. And Muehlbauer, F.J. (2002). Identification of an STMS marker for double-podding gene in chickpea. *Theoretical and Applied Genetics* **105**, 604-7.

Rajesh, P. N., Coyne, C., Meksem, K., Sharma, K.D., Gupta, V. And Muehlbauer, F. J. (2004). Construction of a HindIII Bacterial Artificial Chromosome library and its use in identification of clones associated with disease resistance in chickpea. *Theoretical and Applied Genetics* **108**, 663-9.

Rakshit, S., Winter, P., Tekeoglu, M., Munoz, J.J., Paff, T. And Benko-Iseppon, A.M. (2003). DAF maker tightly linked to a major locus for Ascochyta blight resistance in chickpea (*Cicer arietinum* L.). *Euphytica* **32**, 23-30.

Ratnaparkhe, M.B., Santra, D.K., Tullu, A. And Muehlbauer, F.J. (1998a). Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. *Theoretical and Applied Genetics* **96**, 348-53.

Ratnaparkhe, M.B., Tekeoglu, M. And Muehlbauer, F.J. (1998b). Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. *Theoretical and Applied Genetics* **97**, 515-19.

Reddy, M.V. And Singh, K.B. (1984). Evaluation of a world collection of chickpea germplasm accessions for resistance to ascochyta blight. *Plant Diseases* **68**, 900-1.

Ren, J.P., Dickson, M.H. And Earle, E.D. (2000). Improved resistance to bacterial soft rot by protoplast fusion between *Brassica rapa* and B. *oleracea*. *Theoretical and Applied Genetics* **100**, 810-19.

Robertson, L.D., Ocampo, B. And Singh, K.B. (1997). Morphological variation in wild annual Cicer species in comparison with the cultigen. *Euphytica* **95**, 309-19.

Rubio, J., Moussa, E., Kharrat, M., Moreno, M.T., Milla´, N.T. And Gil, J. (2003). Two genes and linked RAPD markers involved in resistance to Fusarium oxysporum f. sp. ciceris race 0 in chickpea. *Plant Breeding* **122**,188–91.

Sant, V.J., Patankar, A.G., Sarode, N.D., Mhase, L.B., Sainani, M.N., Deshmukh, R.B., Ranjekar, P.K. And Gupta, V.S. (1999). Potential of DNA markers in detecting divergence and in analysing heterosis in Indian elite chickpea cultivars. *Theoretical and Applied Genetics* **98**, 1217-25.

Santra, D.K., Tekeoglu, M., Ratnaparkhe, M., Kaiser, W.J., Muehlbauer, F.J. (2000). Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. *Crop Science* **40**, 1606-12.

Santra, D.K., Singh, G., Kaiser, W.J., Gupta, V.S., Ranjekar, P.K. And Muehlbauer, F.J. (2001). Molecular analysis of *Ascochyta rabiei* (Pass.) Labr., the pathogen of ascochyta blight in chickpea. *Theoretical and Applied Genetics* **102**, 676-82.

Schadt, E.E., Monks, S.A., Drake, T.A., Lusis, A.J., Che, N., Colinayo, V., Ruff, T.G., Milligan, S.B., Lamb, J.R., Cavet, G., Linsley, P.S., Mao, M., Stoughton, R.B. And Friend, S.H. (2003). Genetics of gene expression surveyed in maize, mouse and man. *Nature* **422**, 297-1.

Schlotterer, C. And Tautz, D. (1992). Slippage synthesis of simple sequence DNA. *Nucleic Acids Research* **20**, 211-15.

Septiningsih, E.M., Trijamiko, K.R., Moeljopawiro, S. And Mccouch, S.R. (2003). Identification of quantitative trait loci for grain quality in an advanced backcross population derived from the Oryza sativa variety IR64 and the wild relative O. rufipogon. *Theoretical and Applied Genetics* **107**, 1433-41.

Serret, M.D., Udupa, S.M. And Weigand, F. (1997). Assessment of genetic diversity of cultivated chickpea using microsatellite-derived RFLP markers; Implications for origin. *Plant Breeding* **116**, 573-78.

Sethy, N.K., Choudhary, S., Shokeen, B. And Bhatia, S. (2006a). Identification of microsatellite markers from *Cicer reticulatum*; molecular variation and phylogenetic analysis. *Theoretical and Applied Genetics* **112**, 347-57.

Sethy, N.K., Shokeen, B., Edwards, K.J. And Bhatia, S. (2006b) Development of microsatellite markers and analysis of intraspecific genetic variability in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* **112**, 1416-28.

Shan, F., Clarke, H.C., Plummer, J.A., Yan, G., Siddique, K.H.M. (2005). Geographical patterns of genetic variation in the world collections of wild annual *Cicer* characterized by amplified fragment length polymorphisms. *Theoretical and Applied Genetics* **110**,381-91.

Sharma, P.C., Winter, P., Bunger, T., Huttel, B., Weigand, F., Weising, K. And Kahl, G. (1995). Abundance and polymorphism of di-, tri- and tetra-nucleotide tandem repeats in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* **90**, 90-96.

Sarma, K.D., Winter, P., Kahl, G. And Muehlbauer, F.J. (2004). Molecular mapping of *Fusarium oxysporum f. sp. ciceris* race 3 resistance gene in chickpea. *Theoretical and Applied Genetics* **108**, 1243-48.

Simon, C.J. And Muehlbauer, F.J. (1997). Construction of a chickpea linkage

map and its comparison with maps of pea and lentil. *Journal of Heredity* **88**, 115-19.

Singh, B.D., Jaiswal, H.K., Singh, R.M. And Singh Ak. (1984). Isolation of early-flowering recombinants from the interspecific cross between *Cicer arietinum* and *C. reticulatum*. *International Chickpea Newsletter* **11**, 14-16.

Singh, G., Kapoor, S. And Singh, K. (1982). Screening chickpea for gray mold resistance. *International Chickpea Newsletter* **7**, 13-14.

Singh, H., Kumar, J., Smithson, J.B. And Haware, M.P. (1987). Complementation between genes for resistance to race 1 of *Fusarium oxysporum f.sp.ciceri* in chickpea. *Plant Pathology* **36**, 539-43.

Singh, K.B. And Hawtin, G.C. (1979). Winter planting. *International Chickpea Newsletter* **1**, 4.

Singh, K.B., Hawtin, G.C., Nene, Y.L. And Reddy, M.V. (1981). Resistance in chickpeas to *Ascochyta rabiei*. *Plant Diseases* **65**, 586-87.

Singh, K.B. And Reddy, M.V. (1983). Inheritance of resistance to ascochyta blight in chickpea (*Cicer arietinum* L.). *Crop Science* **23**, 9-10.

Singh, K.B., Di Vito, M., Greco, N. And Saxena, M.C. (1989a). Reaction of wild *Cicer* spp. lines to *Heterodera ciceri*. *Nematologia Mediterranea* **17**, 113-14.

Singh, K.B., Malhotra, R.S. And Saxena, M.C. (1989b). Chickpea evaluation for cold tolerance under field conditions. *Crop Science* **29**, 282-85.

Singh, K.B. (1990). Patterns of resistance and susceptibility to races of *Ascochyta rabiei* among germplasm accessions and breeding lines of chickpea. *Plant Diseases* **74**, 127-29.

Singh, K.B., Malhotra, R.S. And Saxena, M.C. (1990). Sources for tolerance to cold in *Cicer* species. *Crop Science* **30**, 1136-38.

Singh, K.B. And Reddy, M.V. (1991). Advances in disease resistance breeding in chickpea. *Advances in Agronomy* **45**, 191-22.

Singh, K.B. And Ocampo, B. (1993). Interspecific hybridization in annual *Cicer* species. *Journal of Genetics and Breeding* **47**, 199-4.

Singh, K.B. And Reddy, M.V. (1993a). Resistance to six races of *Ascochyta rabiei* in the world germplasm collection of chickpea. *Crop Science* **33**, 186-89.

Singh, K.B. And Reddy, M.V. (1993b). Sources of resistance to ascochyta blight in wild *Cicer* species. *Netherland Journal Plant Pathology* **99**, 163-67.

Singh, K.B. And Weigand, S. (1994). Identification of resistant sources in *Cicer* species to *Liriomyza cicerina*. *Genetic Resources and Crop Evolution* **41**, 75-79.

Singh, K.B., Malhotra, R.S., Halila, M.H., Knights, E.J. And Verma, M.M. (1994). Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. *Euphytica* **73**, 137-49.

Singh, K.B., Malhotra, R.S. And Saxena, M.C. (1995). Additional sources of tolerance to cold in cultivated and wild *Cicer* species. *Crop Science* **35**, 1491-97.

Singh, K.B. And Reddy, M.V. (1996). Improving chickpea yield by incorporating

resistance to ascochyta blight. *Theoretical and Applied Genetics*. **92**, 509-15.

Singh, K.B. And Ocampo, B. (1997). Exploitation of wild *Cicer* species for yield improvement in chickpea. *Theoretical and Applied Genetics* **95**, 418-23.

Singh, K.B., Ocampo, B. And Robertson, L.D. (1998). Diversity for abiotic and biotic stress resistance in the wild annual *Cicer* species. *Genetic Resources and Crop Evolution* **45**, 9-17.

Singh, R., Durga Prasad, C., Singhal, V. And Randhawa, G.J. (2002). Analysis of Genetic diversity in *Cicer arietinum* L. using Random Amplified Polymorphic DNA Markers. *Journal Plant Biochemistry and Biotechnology* **11,** 109-12.

Singh, R., Durga Prasad, C., Singhal, V. And Randhawa, G.J. (2003). Assessment of genetic diversity in chickpea cultivars using RAPD, AFLP and STMS markers. *Journal of Genetics and Breeding* **57**, 165-74.

Singh, R.P. And Singh, B.D. (1989). Recovery of rare interspecific hybrids of gram *Cicer arietinum* x *C. cuneatum* L. through tissue culture. *Current Science* **58**, 874-76.

Stam, P. And Zeven, A.C. (1981). The theoretical proportion of the donor genome in near-isogenic lines of self fertilizers bred by backcrossing. *Euphytica* **30**, 227-38.

Sudupak, M.A., Akkaya, M.S. And Kence, A. (2002). Analysis of genetic relationships among perennial and annual *Cicer* species growing in Turkey using RAPD markers. *Theoretical and Applied Genetics* **105**, 1220-28.

Sudupak, M.A., Akkaya, M.S. And Kence, A. (2004). Genetic relationships among perennial and annual *Cicer* species growing in Turkey assessed by AFLP fingerprinting. *Theoretical and Applied Genetics* **108**, 937-44.

Tanksley, S.D. And Rick, C.M. (1980). Isozymic gene linkage map of the tomato; Applications in genetics and breeding. *Theoretical and Applied Genetics* **57**, 161-70.

Tanksley Sd. (1983). Molecular markers in Plant Breeding. *Plant Molecular Biology Reporter* **1**, 3-8.

Tanksley, S.D., Young, N.D., Paterson, A.H. And Bonierbale, M.W. (1989). RFLP mapping in Plant Breeding; New tools for an old science. *Bio/Technology* **7**, 257-64.

Tanksley, S.D., Grandillo, S., Fulton, T.M., Zamir, D., Eshed, Y., Petiard, V., Lopez, J. And Beck-Bunn, T. (1996). Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. *Theoretical and Applied Genetics* **92**, 213-24.

Tautz D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research* **17**, 6463-71.

Tayyar, R.I., Lukaszewski, A.J. And Waines, J.G. (1994). Chromosome banding patterns in the annual species of *Cicer*. *Genome* **37**, 656-63.

Tayyar, R.I. And Waines, J.G. (1996). Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation. *Theoretical and Applied Genetics* **92**, 245-54.

Tekeoglu, M., Santra, D.K., Kaiser, W.J. And Muehlbauer, F.J. (2000). Ascochyta blight resistance in three chickpea recombinant inbred line populations. *Crop Science* 40, 1251-56.

Tekeoglu, M., Rajesh, P.N., Muehlbauer, F.J. (2002). Integration of sequence tagged microsatellite sites to chickpea genetic map. *Theoretical and Applied Genetics* **105**, 847-54.

Temnykh, S., Park, W.D., Ayers, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y.G., Ishii, T. And Mccouch, S.R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **100**, 697-12.

Tewari, S.K., Pandey, M.P. (1986). Genetics of resistance to ascochyta blight in chickpea (*Cicer arietinum* L.). *Euphytica* **35**, 211-15.

Thomas, M.R. And Scott, N.S. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theoretical and Applied Genetics* **86**, 985-90.

Tsumura, Y., Ohba, K. And Strauss, S.H. (1996). Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and Sugi (*Cryptomeria japonica*). *Theoretical and Applied Genetics* **92**, 40-45.

Tullu, A., Muehlbauer, F.J., Simon, C.J., Mayer, M.S., Kumar, J., Kaiser, W.J. And Kraft, J.M. (1998). Inheritance and linkage of a gene for resistance to race 4 of fusarium wilt and RAPD markers in chickpea. *Euphytica* **102**, 227-32.

Tuwafe, S., Kahler, A.L., Boe And A., Ferguson, M. (1993). Inheritance and geographical distribution of allozyme polymorphisms in chickpea. *Journal of Heredity* **79**, 170-74.

Udupa, S.M., Sharma, A., Sharma, R.P. And Pai, R.A. (1993). Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. *Journal of Plant Biochemistry and Biotechnology* **2**, 83-86.

Udupa, S.M. And Baum, M. (2003). Genetic dissection of pathotype-specific resistance to ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using microsatellite markers. *Theoretical and Applied Genetics* **106**, 1196-2.

Udupa, S.M., Malhotra, R.S. And Baum, M. (2004). Tightly linked di- and tri-nucleotide microsatellites do not evolve in complete independence: evidence from linked (TA)n and (TAA)n microsatellites of chickpea (Cicer arietinum L.). *Theoretical and Applied Genetics* **108**, 550-57.

Upadhyaya, H.D., Haware, M.P., Kumar, J. And Smithson, J.B.(1983). Resistance to wilt in chickpea. I. Inheritance of late-wilting in response to race 1. *Euphytica* **32**, 447-52.

Vairinhos, F., Murray, D.R. (1983). The seed proteins of chickpea; Comparative studies of *Cicer arietinum*, *C. reticulatum* and *C. echinospermum* (Leguminosae). *Plant Systematics and Evolution* **142**, 11-22.

Van Der Maesen, L.J.G. (1980). Growing wild chickpeas. *International Chickpea Newsletter* **2**, 3-4.

Van Der Maesen, L.J.G And Pundir, R.P.S. (1984). Availability and use of wild *Cicer* germplasm. *Plant Geaet. Newsletters* **57**, 19-24.

Van Der Maesen, L.J.G. (1987). Origin, history and taxonomy of chickpea. In: The Chickpea. Eds. Saxena, MJ and Singh KB pp 11-34. CAB International, Cambridge.

Van Rheenen, H.A. (1992). Biotechnology and chickpea breeding. *International Chickpea Newsletter* **26**, 14-17.

Van Rheenen, H.A., Pundir, R.P.S. And Miranda, J.H. (1993). How to accelerate the genetic improvement of a recalcitrant crop species such as chickpea. *Current Science* **65**, 414-17.

Varshney, R.K., Graner, A. And Sorrells, M.E. (2005). Genomic-assisted breeding for crop improvement. *Trends in Plant Science* **10**, 621-30.

Verma, M.M., Sandhu, J.S., Brar, H.S. And Brar, J.S. (1990). Crossability studies in different species of *Cicer* (L.). *Crop Improvement* **17**, 179-81.

Verma, M.M., Ravi And Sandhu, J.S. (1995). Characterization of the interspecific cross *Cicer arietinum* L. x *Cicer judaicum* (Boiss). *Plant Breeding* **114**, 549-51.

Vir, S., Grewal, J.S. And Gupta, V.P. (1975). Inheritance of resistance to *Ascochyta rabiei* in chickpea. *Euphytica* **24**, 209-11.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van Der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kulper, M. And Zabeau, M. (1995). AFLP; a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-14.

Weising, M., Kaemmer, D., Weigand, F., Epplen, J.T. And Kahl, G. (1992). Oligonucleotide fingerprinting reveals various probe-dependent levels of informativeness in chickpea (*Cicer arietinum*). *Genome* **35**, 436-42.

Welsh, J. And Mcclelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**, 7213-18.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski And J.A., Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531-35.

Winter, P., Pfaff, T., Udupa, S.M., Huttel, B., Sharma, P.C., Sahi, S., Arreguin-Espinoza, R., Weigand, F., Muehlbauer, F.J. And Kahl, G. (1999). Characterization and mapping of sequence tagged microsatellite sites in the chickpea genome. *Molecular and General Genetics* **262**, 90-1.

Winter, P., Benko-Iseppon, A.M., Huttel, B., Ratnaparkhe, M., Tullu, A., Sonnante, G., Pfaff, T., Tekeoglu, M., Santra, D., Sant, V.J., Rajesh, P.N., Kahl, G. And Muehlbauer, F.J. (2000). A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* x *C. reticulatum* cross; localization of resistance genes for fusarium wilt races 4 and 5. *Theoretical and Applied Genetics* **101**, 1155-63.

Xiao, J., Li, J., Grandillo, S., Ahn, S.N., Yuan, L., Mccouch, S.R. And Tanksley, S.D. (1996). Genes from wild rice improve yield. *Nature* **384**, 223-24.

Xiao, J., Li, J., Grandillo, S., Ahn, S.N., Yuan, L., Tanksley, S.D. And Mccouch

S.R. (1998). Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon. Genetics* **150**, 899-9.

YOUNG, N.D. (1994). Plant genome mapping. In: Encyclopedia of Agricultural Sciences.

YOUNG, N.D. (1996). QTL mapping and quantitative disease resistance in plants. *Annual Review Phytopathology* **34**, 479-1.

YOUNG, W.P., SCHUPP, J.M. AND KEIM, P. (1999). DNA methylation and AFLP marker distribution. *Theoretical and Applied Genetics* **99**, 785-90.

YU, Z.H., MACKILL, D.J., BONMAN, J.M. AND TANKSLEY, S.D. (1991). Tagging genes for blast resistance in rice via linkage to RFLP markers. *Theoretical and Applied Genetics* **81**, 471-76.