Chapter 4
Cicer

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4.1 Introduction

Chickpea (*Cicer arietinum* L.) is one of the earliest grain crops cultivated by man and has been found in Middle Eastern archeological sites dated at 7500–6800 BC (Zohary and Hopf 2000). Chickpea is grown in about 50 countries with an estimated 95% of the cultivated area in the developing countries. Chickpea production is particularly important in the countries of South Asia and accounts for about 71% of global area devoted to the crop. Chickpea can fix up to 140 kg nitrogen ha^{-1} and meet up to 80% of its nitrogen requirement from symbiotic nitrogen fixation (Saraf et al. 1998). Substantial amounts of nitrogen remain in the soil following the chickpea crop, which is beneficial to subsequent crops. Chickpea crop residues add much needed organic matter for the maintenance of soil health, long-term fertility, and sustainability of the ecosystems. Chickpea is an important source of protein for millions of people in developing countries. Chickpea has the highest nutritional compositions of any dry edible grain legume and does not contain any anti-nutritional factors. In addition to having high protein content (20–22%), chickpea is rich in fiber and minerals (phosphorus, calcium, magnesium, iron, and zinc), and its lipid fraction is high in unsaturated fatty acids (Williams and Singh 1987). Chickpea contains higher amounts of carotenoids such as β-carotene than genetically engineered "golden rice" (Abbo et al. 2005).

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4.2 Origin and Taxonomy

Chickpea is a Neolithic crop that had its origin in the fertile crescent some 10,000 years ago (Lev-Yadun et al. 2000; Zohary and Hopf 2000). Evidence suggests the region of southeastern Turkey and adjoining Syria (van der Maesen1987) as the center of origin. The progenitor species of chickpea, *Cicer reticulatum*, grows there even today. Further evidence of its origin comes from seeds that date back to 5450 BC that have been unearthed from excavations at Hac near Burdur in Turkey (Helbaek 1970). From Turkey, chickpea diverged in two directions: one into the Western Province, where it is grown in spring and summer, and the other into the eastern and southern parts of the region, where it is grown in the cool dry season.

Various studies have shown that chickpea rests on a narrow genetic base (Millan et al. 2006) and this is the reason for the crop being susceptible to a range of diseases and pests. According to Abbo et al. (2003), a series of four evolutionary bottlenecks are responsible for the narrow genetic base of the crop. Unlike pea, barley, lentil, and wheat, the progenitor species of chickpea, *C. reticulatum* (Ladizinsky and Adler 1976), is narrowly distributed in southeastern Turkey and harbors limited adaptive variation compared to those of wheat or barley (Berger et al. 2003). Based on both genetic and archeological evidence (Zohary 1999; Lev-Yadun et al. 2000), the limited founding species is called the domestic bottleneck. Chickpea is considered to be the victim of a series of bottlenecks. The crop underwent a bottleneck when it was shifted from being an autumn to a spring-sown crop, probably to avoid Ascochyta blight that is less severe in spring-sown crops. Spring-sown chickpea had to be vernalization insensitive, thus further narrowing the genetic

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base. Selection to suit post-rainy season, cropping further reduced the genetic diversity. Replacement of landraces by elite cultivars produced by modern breeding caused yet another bottleneck. With the change in climatic conditions and the evolution of several pathogens, selection will be rigorous for chickpea germplasm to withstand abiotic and biotic stresses. This process of selection will further narrow the genetic base and contribute an additional bottle neck to the chickpea crop.

4.3 Gene Pools of Chickpea

Chickpea is endowed with rich germplasm in the form of wild species. The genus *Cicer* is classified into three gene pools based on its crossability with cultigen. Based on their crossability with cultivated species, wild species, both annual and perennial, have been grouped. Using the classification proposed by Harlan and de Wet (1971), a modification of the classification is proposed. Although the modification does not deviate much from the previously proposed gene pools for chickpea, the secondary gene pool is strengthened by the placement of *C. reticulatum*. The proposed classification is similar to the recent classification proposed by van der Maesen et al. (2007). The primary gene pool consists of cultivated species and landraces. The secondary gene pool consists of the progenitor species, *C. reticulatum* and *C. echinospermum*, a species that is crossable with *C. arietinum* but with reduced fertility of the resulting hybrids and progenies; nevertheless, both are cross-compatible with the cultigen and do not need in vitro interventions to produce hybrids. The tertiary gene pool consists of all the annual and perennial *Cicer* that are not crossable with cultivated *C. arietinum*. All the perennial *Cicer* species are considered to be in the tertiary gene pool as none of the species of this group are known to cross readily with the cultivated species and produce mature seeds (Mallikarjuna and Muehlbauer unpublished results). In general, the transfer of desirable traits from wild species may accompany tightly linked undesirable genes/traits commonly referred to as "linkage drag." Utilization of molecular markers can be used effectively for transfer of genes of interest from wild to cultivated species.

4.3.1 Primary Gene Pool

Cultivated chickpea has vast collections of landraces and improved cultivars that are maintained at the international centers, ICRISAT and ICARDA, and at numerous national gene repositories including USDA, USA. ICRISAT maintains 17,258 accessions (135 wild, 17,123 cultivated). Although a large number of cultivated accessions are conserved in the gene bank, there is fatigue in the utilization of the germplasm, as the sheer numbers are intimidating. ICRISAT chickpea breeders used just 83 germplasm lines, in contrast to their use of 480 breeding lines for the development of 3,430 advanced varieties (ICCV numbers) during the same period (1978–2004) (Upadhyaya et al. 2008). India has released 126 cultivars between 1967 and 2003. Pedigree analysis of 86 cultivars developed from crosses has revealed that although 95 progenitors were involved, just ten of these contributed 35% of the genetic base (Shivkumar et al. 2004). The five most frequently used ancestors were Pb7, IP 58, F 8, Rabat, and S 26. Furthermore, about 41% of the cultivars developed through crossing have Pb7 as an ancestor. This suggests that many cultivars share a narrow genetic base. Further, a global composite collection was developed that comprised 3,000 accessions. The composite collection is made up of 80% landraces, 9% advanced breeding lines, 2% cultivars, 1% wild species, and 8% whose precise status is unknown (Upadhyaya et al. 2008). It is envisaged that such collections would enhance the utilization of germplasm in improvement of chickpea. This is an ideal set of germplasm for allele mining, association genetics, mapping and cloning gene(s), and applied breeding. Nevertheless, due to narrow ancestry and numerous bottlenecks, chickpea has limited genetic variation in the primary gene pool. In spite of the above-mentioned constraints, extensive international breeding efforts have led to the development of over 300 improved varieties (Gowda and Gaur 2004). Potential yield of chickpea is estimated to be 5.0 ton ha^{-1}, but the average yield is around 0.8 ton ha^{-1} as various biotic and abiotic stresses reduce yield. Drought is a serious abiotic stress, which has led to the development of cultivars that can escape terminal drought through early maturity. ICRISAT has invested in the development of short-duration cultivars such as ICCV 2, ICCV 3, and KAK 2.

Chickpea is highly self-pollinating with an outcrossing rate of less than 1%. Two main types of chickpea cultivars are grown globally, representing two diverse subgene pools: Kabuli and Desi. The Kabuli types are generally grown in the Mediterranean region, southern Europe, western Asia, and northern Africa and the Desi types are grown mainly in Ethiopia and the Indian subcontinent.

4.3.2 Secondary Gene Pool

C. reticulatum and *C. echinospermum* are the two wild species in the secondary gene pool. *C. reticulatum* crosses with *C. arietinum*, resulting in completely fertile hybrids and progenies. *C. reticulatum* is considered as the wild progenitor of *C. arietinum* based on morphological and cytological similarities as well as crossability (Ladizinsky and Adler 1976), molecular analysis, and their cohabitation at the center of origin of the crop (Zohary and Hopf 1988). Many accessions of *C. reticulatum* have been identified with useful genetic variation and there are various reports (see below) on its utilization to introgress useful traits into the cultivated species (Ascochyta blight resistance: Collard et al. 2003; botrytis gray mold resistance: Mallikarjuna unpublished data; *Helicoverpa armigera* resistance: Mallikarjuna et al. 2007a; nematode resistance: Malhotra et al. 2002; high yield: Singh and Ocampo 1997). Hence, *C. reticulatum* represents a potential source of genes for broadening the genetic base of cultivated chickpea.

Another potential source of increased genetic variation is *C. echinospermum*. Natural hybrids between chickpea and *C. reticulatum* and between *C. echinospermum* and *C. reticulatum* have been reported by ICARDA (Irula et al. 2002), which strongly suggests compatibility among these three species and the reason for compatible pollinations. Mature seed set is observed, when these species are crossed with each other. Although *C. echinospermum* readily crosses with cultivated chickpea, cytological studies have shown meiotic abnormalities leading to certain degrees of pollen sterility (Pundir and Mengesha 1995). Molecular studies have shown that *C. echinospermum* is not as closely related to cultivated chickpea as *C. reticulatum* (Choumane et al. 2000).

Ladizinsky and Adler (1976) reported that *C. reticulatum* and *C. echinospermum* differed from each other by a major reciprocal translocation, and their hybrid was completely sterile. They also stated that *C. echinospermum* also differed from the cultigen by the same translocation and their hybrids were also sterile. However, with some accessions of *C. echinospermum*, it was possible to obtain completely fertile selections (Singh and Ocampo 1997). Collard et al. (2003) used an accession of *C. echinopsermum* (PI 527930) and developed an interspecific population and identified quantitative trait loci (QTL) associated with seedling resistance to Ascochyta blight. The same accession of *C. echinospermum* crossed with a different cultivar of chickpea set mature seeds, but the F_1 hybrid did not have fertile pollen grains. Meiosis was normal until the tetrad stage after which, instead of tetrads, polyads were observed. It was not possible to obtain F_2 seeds, but backcrossing the F_1 hybrid with female parent, chickpea cultivar KAK 2 gave rise to mature seeds (Mallikarjuna unpublished results). This shows that genotype of the female parent is important while attempting crosses with wild *Cicer* species and that crosses with *C. echinospermum* often have disturbed meiosis (Pundir and Mengesha 1995). The rationale of placing both the species in the secondary gene pool is their crossability with the cultigen.

4.3.3 Tertiary Gene Pool Species

Annual *Cicer*: Many of the species in this group harbor important traits/genes necessary for the improvement of chickpea, such as *H. armigera* resistance in *C. judaicum*, *C. pinnatifidum*, and *C. bijugum* (Sharma et al. 2005a); Ascochyta blight resistance in *C. judaicum*, *C. bijugum*, and *C. pinnatifidum* (Pande et al. 2006); botrytis gray mold resistance in *C. judaicum* (Pande et al. 2006), and drought resistance in *C. pinnatifidum* (Bhattarai and Fettig 2005). Various studies have shown that wild species in the tertiary gene pool are distantly related to cultivated chickpea; however, crossing techniques are being improvized for making wide crosses in chickpea using tertiary gene pool species (Mallikarjuna 1999; Mallikarjuna et al. 2007c; Mallikarjuna and Jadhav 2008). Many of the incompatible *Cicer* species in the tertiary gene pool that do not cross easily with cultivated chickpea have been used in crosses followed by hormone applications, embryo rescue, and attempts to root hybrid shoots/plants in vitro. Although hybrid

shoots/plants have been obtained, hybrids have proven to be fragile and have not withstood transfer to glasshouse/field and hence are not yet available for chickpea improvement.

4.3.3.1 Barriers to Interspecific Crosses

It is now known that the barriers to hybridization are post-zygotic (Ahmad et al. 1988; Mallikarjuna 1999) meaning that pollinations take place but the zygote, which is a few celled, begins to abort by 3–5 days after pollination. Badami et al. (1997) were able to postpone the abscission of pollinated pistils to 15–18 days by the application of growth regulators. This facilitated the growth of the hybrid embryo to early cotyledonary stage of development and being 0.5–1.0 mm in size (Mallikarjuna 1999).

Embryos of size 0.5 mm or less did not grow directly on culture medium while 0.3–0.4 mm size embryos responded well to specific growth hormones when cultured as in-ovulo embryo culture. Embryo response was maximum when Zeatin was used in combination with indole acetic acid in in-ovulo embryo culture medium. Hybrid embryos emerged out of the ovules after 3–4 weeks of culture (Mallikarjuna 1999). Similar response was not obtained when zeatin was replaced with other cytokinins, which reduced the number of responding embryos (Mallikarjuna unpublished). In peanut ovule culture, emergence of the developing embryo is observed as seen in chickpea (Mallikarjuna and Sastri 1985), but in pigeonpea ovule culture, the developing embryo never emerges out of the ovule; hence, in such cases, it is important to dissect the developing embryo out of the ovule.

The best time to save the aborting seeds/ovules was when the hybrid embryo had reached its maximum growth and development, being at the cotyledonary stage of development, which was 15–18 days after pollination. If left longer on the plant, the pods turn yellow, indicating abortion of the hybrid seed. It was possible to save aborting hybrid embryos from the cross *C. arietinum* × *C. pinnatifidum* by in-ovulo embryo culture. Some of the hybrid shoots were pale yellow in color and scanning electron microscopy (SEM) studies showed that the chloroplasts were abnormal. Use of a cytokinin in culture medium in combination with light helped the conversion of leucoplastids to chloroplasts (Badami et al. 1997). Overall, the hybrids between *C. arietinum* and *C. pinnatifidum* were fragile with the leaves resembling those of *C. pinnatifidum*. The color of the flower was pale violet resembling the violet color of the male parent and the pollen was 100% non-viable (Mallikarjuna 1999).

In an attempt to check if *C. reticulatum* and *C. echinospermum* could be considered bridge species, as a means of transferring of genes from species of the tertiary gene pool to the cultigen, crosses were carried out between *C. reticulatum* and *C. pinnatifidum* and between *C. echinopsermum* and *C. pinnatifidum*. In both the crosses, embryos from crossings aborted 15–20 days after pollination. Rescuing hybrid embryos in vitro gave rise to albino plants (Mallikarjuna and Jadhav 2008). Although mature seeds were not obtained in the cross between *C. reticulatum* and *C. bijugum*, hybrid shoots were green (Mallikarjuna et al. 2007a). It is suggested that a larger number of *C. reticulatum* accessions should be used to identify compatible combinations to obtain viable and green hybrid plants between *C. reticulatum*/*C. echinospermum* and *C. pinnatifidum*.

Crosses between incompatible annual *Cicer* species as the female parent (*C. pinnatifidum* and *C. bijugum*) and cultivated chickpea as the pollen donor produced mature seeds consistently. These presumed hybrid seeds germinated well and all the plants resembled the female parent. Molecular profiling and high pollen fertility of the presumed hybrids indicated that the plants were identical to the female parent. In the crosses with cultivated chickpea and *C. cuneatum*, a similar situation was observed (Gaur personal communication). Although further investigations are necessary, the results may indicate apomictic seed production. This type of seed production is most prevalent when *C. pinnatifidum* or *C. bijugum* were used in crosses with cultivated chickpea as the pollen donor (Mallikarjuna 2003).

Development of haploid plants from chickpea anther culture and microspore culture is now possible (Grewal et al. 2009). Mallikarjuna et al. (2005) demonstrated that multicellular microspores can also be obtained through wide cross between chickpea and *C. pinnatifidum*. The hybrid gave rise to multicellular microspores in large numbers with divisions in all the microspores in some plants. Culturing such microspores may give rise to haploid plants that could be

further utilized, through chromosome doubling, for gene transfer to *C. arietinum*.

4.3.3.2 Perennial *Cicer*

There are 34 perennial wild *Cicer* species, which require very specific soil and environmental conditions for growth and reproduction. Traits of interest such as resistance to Ascochyta blight (Muehlbauer et al. 1994), *H. armigera* (Sharma et al. 2006), Fusarium wilt (Kaiser et al. 1994), and drought resistance (Toker et al. 2007) are present in this gene pool. Perennial *Cicer* species survived the severe frost conditions and resumed their vegetative growth with the onset of summer in the USDA-ARS nursery located at the Washington State University, Pullman, USA. All the perennial *Cicer* species have larger plant morphology compared to the annual *Cicer* species, with robust vegetative growth (Fig. 4.1a, b). The flowers are larger and produce multi-seeded fruits/pods (Fig. 4.1c, d). Some of the perennial *Cicer* species have morphological modifications, such as spines and tendrils. Some of the characters that chickpea would benefit from perennial *Cicer* are large and robust vegetative growth, large pods with multiple seeds (Fig. 4.1c, d), drought and cold tolerance, Ascochyta blight, and insect resistance.

Application of growth regulators was mandatory to obtain pod set in the crosses involving perennial *Cicer* (Table 4.1). Pollinations were growth regulator (gibberellic acid 75 mg/L + naphthalene acetic acid 10 mg/L + kinetine 10 mg/L)-aided. Without the application of growth regulators, immature pods aborted by 6 days after pollination. Growth regulators were able to retain the pods from cross pollinations for 15 days or more. Pollinated pistils were observed under a microscope for pollen response. Pollen grains germinated on the stigma (Fig. 4.1e) and post-fertilization changes were observed (Fig. 4.1g, h).

Microscopic techniques were used to determine the barriers to successful hybridization between *C. arietinum* and *C. anatolicum*. Fluorescence microscopy of pollen tube growth and development in these hybrid crosses showed that pollen was able to germinate, and the resulting pollen tubes penetrated the stigma, style, ovary, and ovule tissues. Traditional light microscopy was used to examine *C. arietinum* and *C. anatolicum* hybrid embryo and endosperm development. When *C. arietinum* (cultivar Myles) and *C. anatolicum* (PI 561078) were self-fertilized resulting in the formation of a zygote and endosperm nucleus (Figs. 4.2 and 4.3, respectively). The zygote developed into an embryo that passed through the globular and heart stages and developed distinct cotyledons by 10 days after pollination. The endosperm nucleus gave rise to the rapidly dividing endosperm tissue. In *C. arietinum* × *C. anatolicum* hybrids, embryo and endosperm growth was arrested after several cell divisions around 4–5 days after pollination and the embryo and endosperm subsequently broke down 6–8 days after pollination (Fig. 4.4). Because the endosperm was non-viable, nutrients available for its development were instead used by the nucellus, which became overgrown. In *C. anatolicum* × *C. arietinum* hybrids, fertilization occurred, but the resulting zygote and endosperm nucleus failed to begin cell division to produce an embryo and endosperm (Fig. 4.5). The post-zygotic failure of this cross is most likely due to a lack of cooperation between the diverse genomes or slow growth of the pollen tube to deliver the gametes before the abscission of the flower, to form and maintain a viable embryo and endosperm.

Crosses were carried out between chickpea cultivar Myles and perennial *Cicer* species *C. oxyodon*, *C. songaricum*, and *C. microphyllum*. Pod set was observed in all the three cross combinations (Fig. 4.1g, h); many of the pods had immature seeds, but none of them matured. Pod set between *C. reticulatum* and *C. oxyodon* was 15%. In other cross combinations using *C. anatolicum*, *C. nuristanicum*, *C. multijugum*, and *C. microphyllum*, pods were observed, but if seeds developed, they were immature and non-viable (Fig. 4.1k). Crosses with *C. echinospermum* and *C. oxyodon* yielded 1% pod set and crosses with *C. songaricum*, *C. microphyllum*, *C. microcanthum*, *C. nuristanicum*, and *C. multijugum* did not set any pods. Crosses between *C. pinnatifudum* and *C. oxyodon* had 10% pod set, and there was no pod set when crosses were made with *C. microcanthum*. Pod set was not observed when *C. judaicum* was crossed with *C. nuristanicum*, *C. macrocanthum*, and *C. oxyodon*. Crosses with *C. bijugum* and *C. oxyodon* yielded 15% pod set, and 30% pod set when crossed with *C. anatolicum*. It was observed that *C. bijugum* was a better female parent with respect to pod set when crossed with perennial *Cicer* species (Table 4.1). But this may not be significant as *C. bijugum* cannot be used as bridge species between

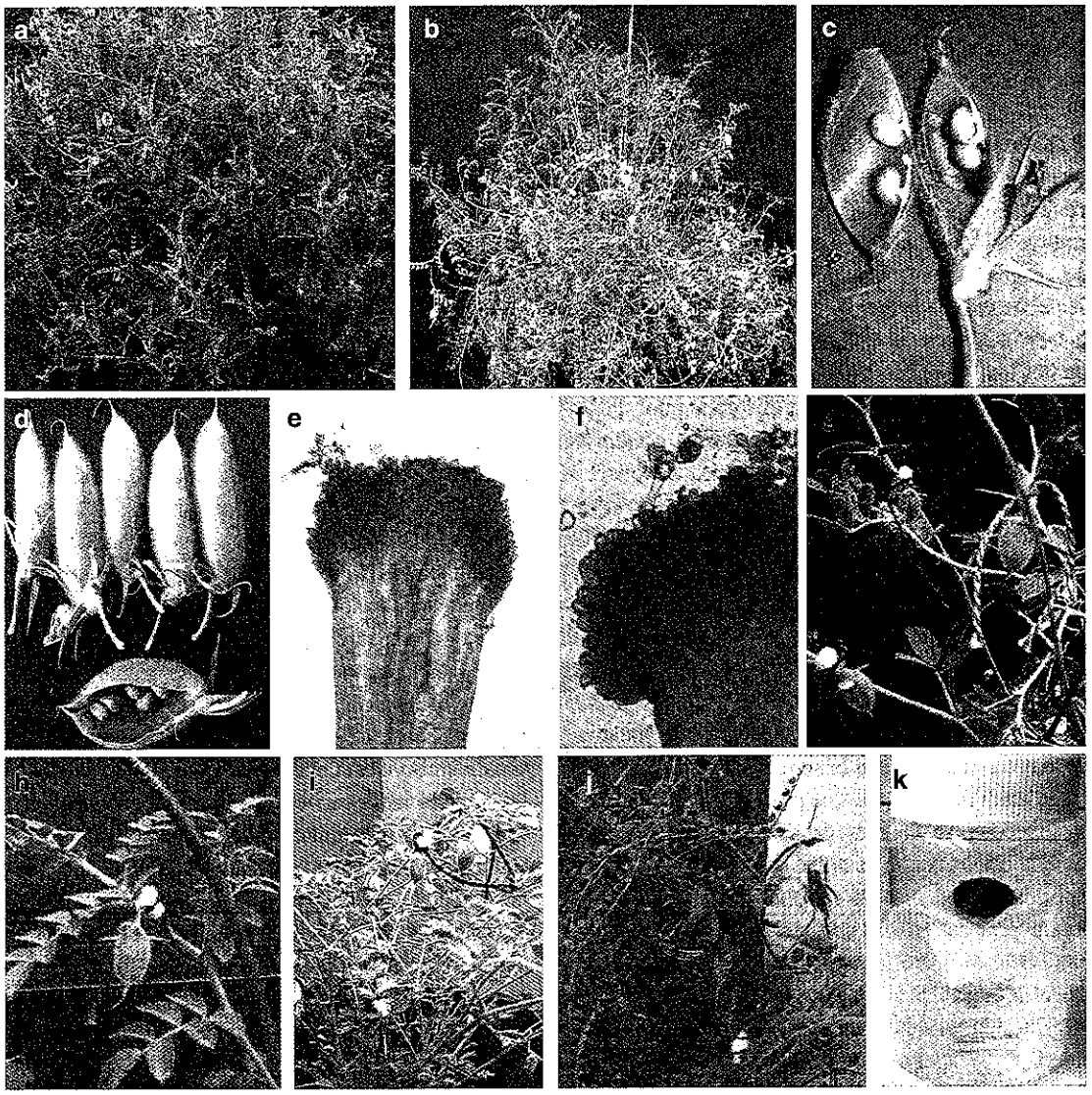

Fig. 4.1 Interspecific hybridization using perennial *Cicer* species. (**a**) *Cicer multijugum* plant. (**b**) Annual *Cicer*. (**c**) *Cicer microphyllum* pod with multiple seeds. (**d**) *C. oxyodon* pod. (**e**) *C. oxyodon* pollinated with *C. reticulatum* pollen. (**f**) *C. multijugum* with *C. oxyodon* pollen. (**g**) Cultivated chickpea with *C. songaricum* pollen. (**h**) Cultivated chickpea with *C. oxyodon* pollen. (**i**) *C. reticulatum* with *C. microphyllum* pollen. (**j**) Perennial *Cicer* with *C. reticulatum* pollen. (**k**) Immature seed from the cross *C. bijugum* × *C. oxyodon*

cultivated chickpea and perennial *Cicer* species as it does not set mature seeds when crossed with cultivated chickpea. Reciprocal crosses using *C. oxyodon* as the female parent and *C. reticulatum* as the pollen donor set a large number of pods (24%; Fig. 4.1j). *C. nuristanicum* had 9% pod set when crossed with *C. reticulatum*. It was possible to retain pods from crossing with perennial *Cicer* species for 15–17 days by application of growth regulators, thereby encouraging the growth of hybrid seeds. But it was not possible to rescue hybrid embryos by culturing them in vitro (Fig. 4.1k).

Hybrid seed size was not more than 2.5–3.0 mm, except for the immature seed from the cross *C. bijugum* × *C. oxyodon* (Fig. 4.1k). Such seeds were collected for in-ovulo embryo culture. Immature seeds showed initial swelling, but no further response even after 60 days of culture. Ovule culture techniques were similar to that used for incompatible crosses involving

annual *Cicer* species (Mallikarjuna 1999). Ovules remained green in culture and enlarged. None of the embryos grew to form a seedling.

4.4 International Transfer of Germplasm

Thus far none of the perennial *Cicer* species have been grown successfully in tropical or subtropical environments where the annual *Cicer* species grow. This is a major impediment for the growth and utilization of perennial species. If the pollen of the perennial *Cicer* species can be preserved for utilization in the regions of the world where these species do not grow, the bottleneck in the utilization of these species to develop crossability techniques can be overcome. This difficulty in the use of perennial *Cicer* germplasm might be overcome through transshipment of viable pollen. Pollen from cultivated and wild species can be collected and preserved for 14 days, at 4–6°C in a desiccator, beyond which the viability of the pollen decreases. Pollinations are successful if the pollen is utilized within 14 days of collection and preservation (Mallikarjuna et al. 2007b).

Table 4.1 Pod set in the crosses between annual and perennial *Cicer* species[a]

Female parent	Male parent	Pod set (%)
Chickpea cv Myles	*C. oxyodon*	18.0
Annual *Cicer*	Perennial *Cicer*	
C. reticulatum	*C. oxyodon*	15.00
C. echinospermum	*C. oxyodon*	1.00
C. pinnatifidum	*C. oxyodon*	10.00
C. bijugum	*C. oxyodon*	15.00
C. bijugum	*C. anatolicum*	30.00
C. bijugum	*C. nuristanicum*	20.00
C. bijugum	*C. Microphyllum*	5.00
Perennial *Cicer*	Annual *Cicer*	
C. microphyllum	*C. reticulatum*	35.0
C. oxyodon	*C. reticulatum*	24.0
C. microphyllum	*C. echinopsermum*	22.00

[a]Crosses were carried out in 2006 at WSU, USA

4.5 Genetic Diversity in *Cicer* Species

A genetic diversity analysis among cultivated and wild chickpea is important for detecting variation in traits of interest. Various biochemical markers can be used; however, only molecular markers are discussed in this review. Narrow ancestry, recent domestication, and high percentage of self-pollination are reflected in the apparent minimal amount of molecular polymorphism between chickpea cultivars (van Rheenen 1992; Udupa et al. 1993). Several molecular markers have detected minimal polymorphism. For example, only 29% of random amplified polymorphic DNA (RAPD) markers were polymorphic and identified narrow genetic distance of 0.09–0.27 within 29 Indian cultivars. Compared to RAPD markers, restriction fragment length polymorphisms (RFLPs) and microsatellite-based markers, such as sequence tagged microsatellite site (STMS) and intersimple sequence repeat (ISSR), detected greater polymorphism in several studies (Serret et al. 1997a, b; Sant et al. 1999;

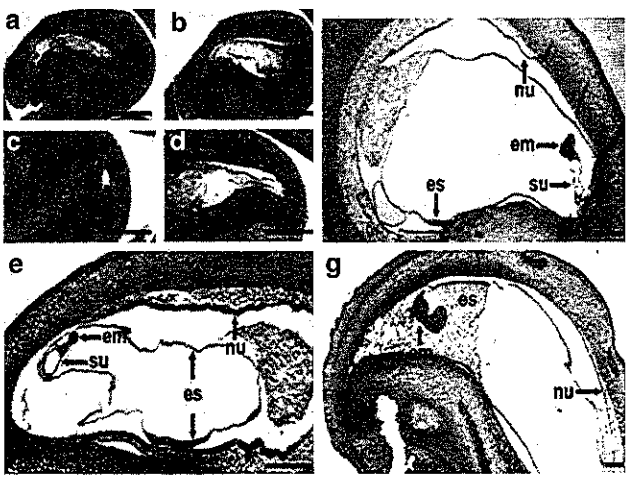

Fig. 4.2 Embryo and endosperm development in self-pollinated *C. arietinum* ovules (**a**, **b**) 0 days after pollination (DAP), (**c**, **d**) 2 DAP, (**e**) 7 DAP, (**f**) 9 DAP, and (**g**) 10 DAP. Scale bars represent 200 μm. *cc* central cell, *eg* egg, *em* embryo, *en* endosperm nucleus, *es* endosperm, *nu* nucellus, *su* suspensor, *zy* zygote

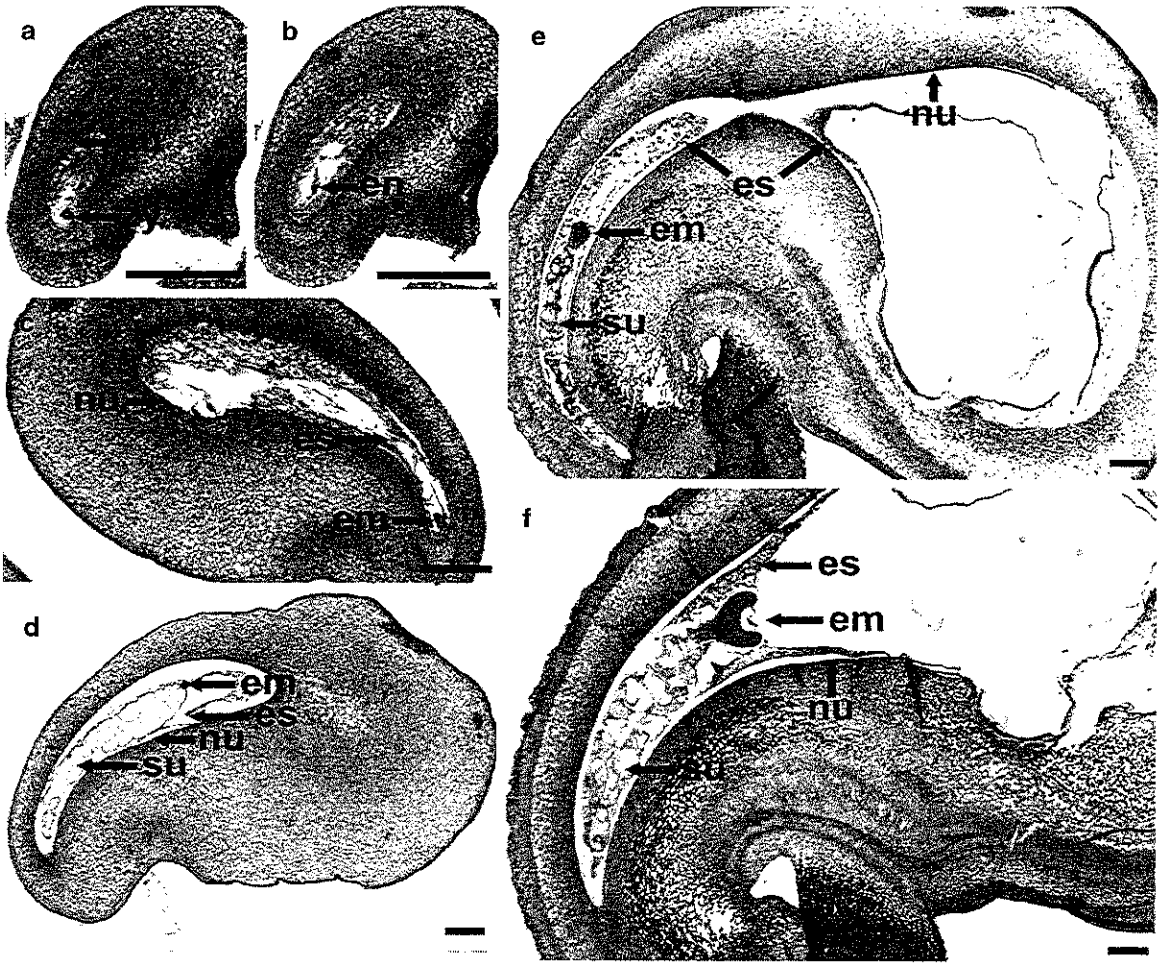

Fig. 4.3 Embryo and endosperm development in self-pollinated *C. anatolicum* ovules (**a**, **b**) 1 day after pollination (DAP), (**c**) 4 DAP, (**d**) 6 DAP, (**e**) 8 DAP, and (**f**) 10 DAP. Scale bars represent 200 μm. *em* embryo, *en* endosperm nucleus, *es* endosperm, *nu* nucellus, *su* suspensor, *zy* zygote

Choumane et al. 2000; Rajesh et al. 2003; Sethy et al. 2006). When oligonucleotide probes were employed, the genetic distance ranged from 0.42 to 0.61 (Sant et al. 1999). Oligonucleotide markers revealed intra- and interaccessional polymorphism in chickpea. Wide range of variability in accessions from Pakistan, Iraq, Afghanistan, Russia, Turkey, and Lebanon were detected. Accessions from India, Jordan, Palestine, Syria, and Iran showed low levels of polymorphism (Sharma et al. 1995; Serret et al. 1997a, b). Sethy et al. (2006) used simple sequence repeat (SSR) markers developed from *C. reticulatum* to study diversity between nine annual *Cicer* species. The study showed greater similarity between cultivated chickpea and *C. reticulatum*. *C. pinnatifidum* was closer to *C. bijugum*, but the two species *C. yamashitae* and *C. chorassanicum* were distinct from all the other species.

Choumane et al. (2000) used STMS markers to study the relationship between the *Cicer* species. Their study showed a close relationship between *C. arietinum*, *C. reticulatum*, *C. echinospermm*, and a perennial *Cicer* species *C. anatolicum*. SSR markers (Staginnus et al. 1999) showed a close relationship between *C. anatolicum* and annual wild *Cicer* species, opening up avenues to consider *C. anatolicum* as the progenitor species of the annual wild *Cicer* species. Using ISSR markers, Rajesh et al. (2002) concluded that annuals are more recent than perennials and might have evolved from the perennial *Cicer* species. Many of the perennial *Cicer* species showed a closer relationship

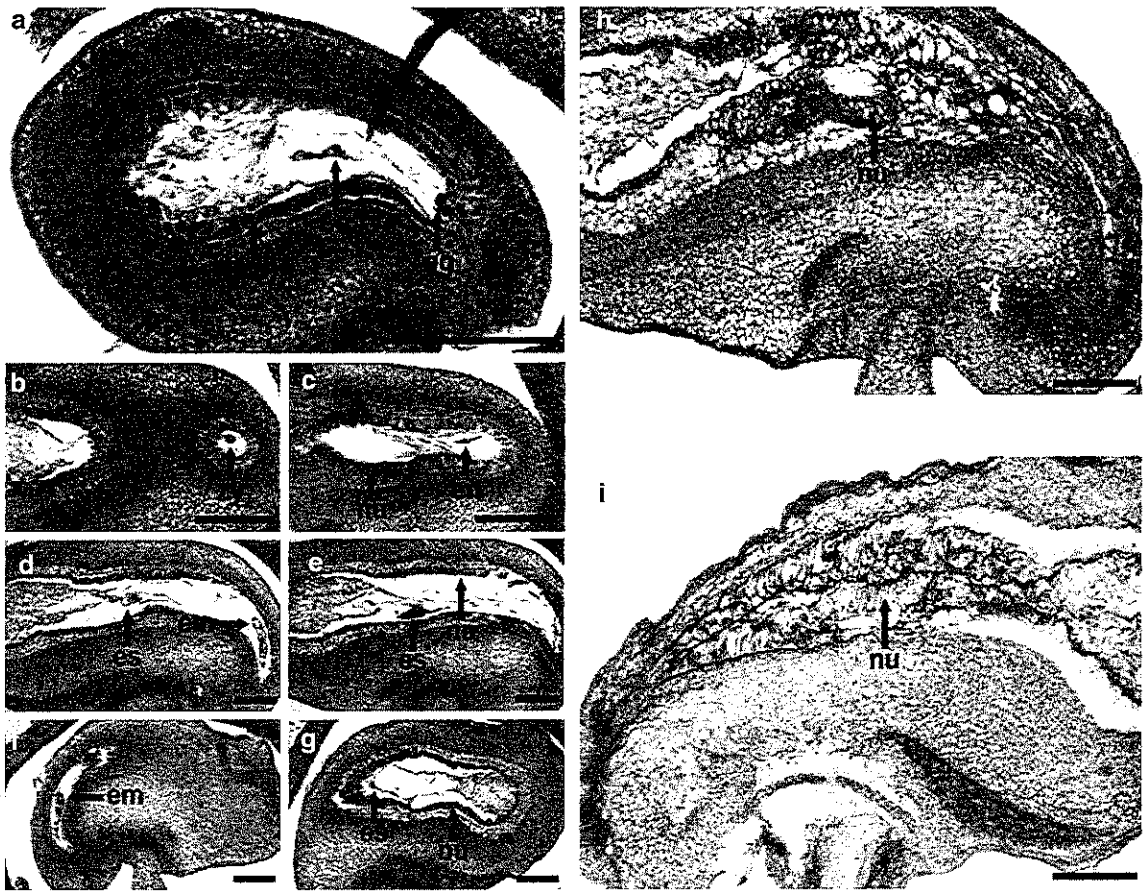

Fig. 4.4 Embryo and endosperm development resulting from the interspecific cross *C. arietinum* × *C. anatolicum* (**a**) 0 days after pollination (DAP), (**b**, **c**) 2 DAP, (**d**, **e**) 4 DAP, (**f**, **g**) 5 DAP, (**h**) 8 DAP, and (**i**) 10 DAP. Scale bars represent 200 μm. *cc* central cell, *eg* egg, *em* embryo, *en* endosperm nucleus, *es* endosperm, *nu* nucellus, *zy* zygote

with cultivated chickpea, *C. reticulatum*, and *C. echinospermum* than with other annual wild *Cicer* species. Here, *C. anatolicum* was more closely related to other perennial *Cicer* species, such as *C. oxyodon* and *C. microphyllum* than to cultivated chickpea or to *C. reticulatum*.

In conclusion, a more comprehensive analysis including a greater number of accessions and a more effective molecular marker system is required to establish a clearer understanding of the phylogenetic relationships among annual and perennial *Cicer* species. It is clear from crossability experiments that *C. reticulatum* is more closely related to cultivated chickpea than any other annual or perennial *Cicer* species. Crossability experiments do not show close relationship between cultivated chickpea and *C. anatolicum*.

4.6 Broadening the Genetic Base by Introducing Useful Genetic Variation from Wild *Cicer* Species

Chickpea is prone to be susceptible to 47 diseases (Nene and Reddy 1987) and 54 insect pests (Reed et al. 1987) attack chickpea. Of these, Fusarium wilt (*Fusarium oxysporum f.* sp. *ciceri*), Ascochyta blight (*Ascochyta rabiei*), root rot (*Rhizoctonia bataticola*), botrytis gray mold (*Botrytis cinerea*), and nematodes (*Meloidogyne incognita*, *M. javanica*, *Heterodera ciceri*) are considered to be globally important. Among the insects, pod borer (*H. armigera*) is the most important pest. Among abiotic stresses, drought

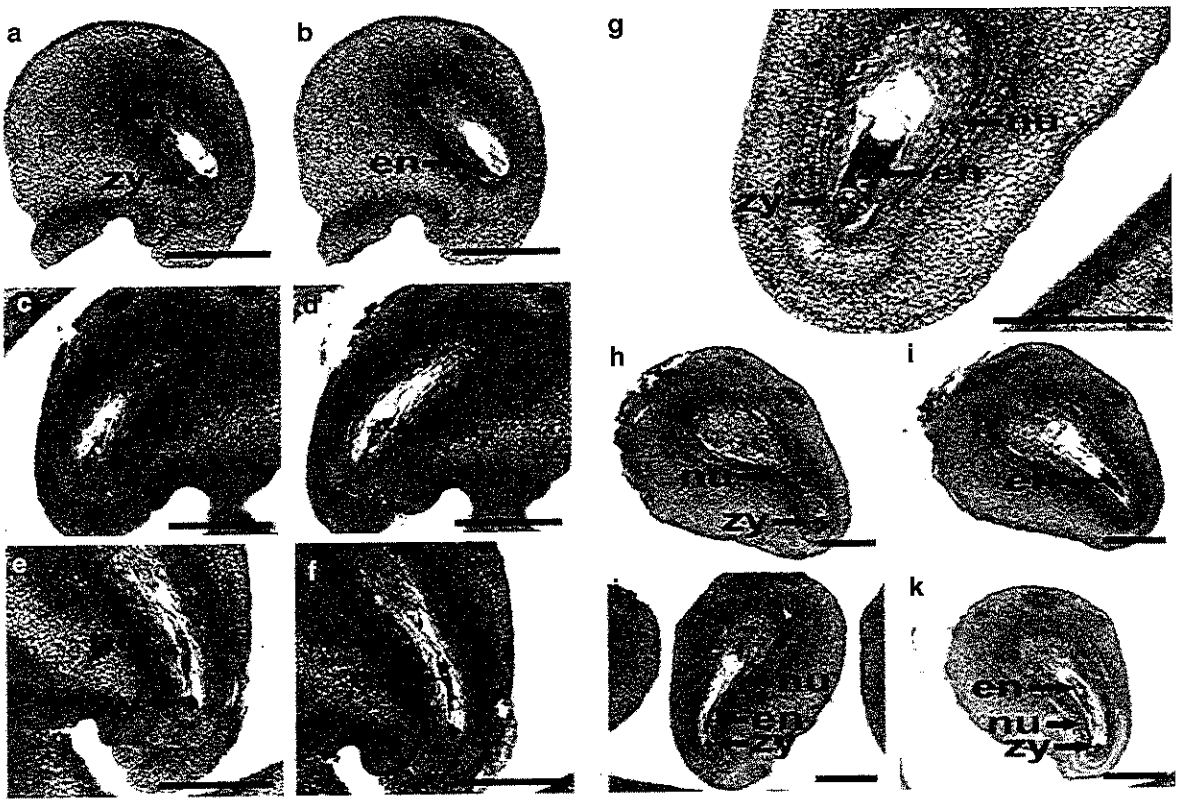

Fig. 4.5 Embryo and endosperm development resulting from the interspecific cross *C. anatolicum* × *C. arietinum* (**a**, **b**) 1 day after pollination (DAP), (**c**, **d**) 3 DAP, (**e**, **f**) 4 DAP, (**g**) 5 DAP, (**h**, **i**) 6 DAP, (**j**) 8 DAP, and (**k**) 10 DAP. Scale bars represent 200 μm. *en* endosperm nucleus, *nu* nucellus, *zy* zygote

is very important. Because of these constraints, world mean yield of chickpea is about 0.8 ton ha^{-1}, which is a serious reduction of yield from an estimated potential of 5 ton ha^{-1}. However, this high estimate is made under very favorable growing conditions not often available for chickpea production. Grain yield is limited by several biotic and abiotic stresses including diseases, such as Fusarium wilt and Ascochyta blight, and abiotic stresses, such as heat and drought. Unavailability of adequate resistance sources to important stresses within the crop species and narrow genetic base are the limitations to its productivity (van Rheenen 1991). Hence, wild relatives with broader diversity can be utilized in breeding chickpea for disease and pest resistance (Lenne and Wood 1991). The resistance to diseases and pests available in germplasm of the primary gene pool is often minimal with a limited number of sources. Nevertheless, resistance to a number of diseases has been incorporated into the elite genotypes. Selection pressure on pathogen populations due to the widespread use of homogeneous host plant resistance may result in more virulent strains, which may overcome the resistance. Hence, discovery and use of alien genes for resistance from wild species provide the way for sustaining crop improvement through pre-emptive breeding. Available resistance in wild *Cicer* species and their utilization to overcome various biotic and abiotic stresses and other useful traits are described below.

4.6.1 Ascochyta Blight

Among the foliar fungal diseases, Ascochyta blight, caused by *A. rabiei*, is the most devastating disease of chickpea. It is reported to occur in as many as 30 countries (Singh and Reddy 1991). It causes serious yield losses in India, Pakistan, and the countries around the Mediterranean region, where cool and humid climates prevail. Crop loss due to the disease can be 100% (Nene 1982). Under favorable weather conditions, the disease takes epidemic proportions. Such epidemics

have occurred in India, Pakistan, the US, Pacific northwest, Australia, and Syria (Malhotra et al. 2003). More than 60 years of efforts in managing the disease through cultural practices, chemical control, and exploitation of host plant resistance available in the cultivar have not resulted in satisfactory control of the disease. The reason for the failure of the elite resistant genotypes developed from intraspecific crosses within chickpea is attributed to the low levels of resistance caused by the development of more virulent pathotypes in different chickpea-growing regions, for example, Italy (Stamigna et al. 2000), Syria, Lebanon (Reddy and Kabbabeh 1985), and Pakistan (Jamil et al. 2000).

Several wild species are identified to be resistance sources for Ascochyta blight in chickpea, such as *C. reticulatum* and *C. echinospermum* (Collard et al. 2001). A linkage map was constructed based on an interspecific F_2 population derived from the cross between cultivated chickpea and *C. echinopsermum* (PI 527930; Collard et al. 2003). Santra et al. (2000) reported two quantitative trait loci (QTLs), QTL1 and QTL2, that confer resistance to Ascochyta blight in US. These QTLs accounted for an estimated 34.4% and 14.6% of the total phenotypic variance (Santra et al. 2000; Tekeoglu et al. 2000, 2002). Two QTLs were identified for seedling resistance and five markers were associated with stem resistance, four of which were also associated with seedling resistance in Australia (Collard et al. 2003). Udupa and Baum (2003) identified a single major gene conferring resistance to the pathotype 1 and two QTLs for pathotype II. Rakshit et al. (2003) mapped OPS06, a DNA amplification fingerprinting (DAF) marker, between the flanking markers at QTL1 of Santra et al. (2000) along with several other DAF markers.

The environmental effect of Ascochyta blight QTL1 and QTL2 was analyzed at Eskisehir, Turkey (Tekeoglu et al. 2004), using CRIL-7 (Santra et al. 2000) developed in the USA from the interspecific cross FLIP84-92C × PI 599072. The same two QTLs were identified at both the locations indicating their robust nature, with some differences. The effect of QTL1 was greater than QTL2 at Pullman, Washington, USA, whereas the effect of QTL2 was greater than QTL1 at Eskisehir, Turkey, indicating possible differences in pathogen population and environmental interactions (Tekeoglu et al. 2004). Irula et al. (2006) used an intraspecific population developed from the cross ILC3279 × WR315 and identified two QTLs that were the same as that of Santra et al. (2000). More recently, Pande et al. (2006) identified moderate levels of resistance in accessions belonging to *C. cuneatum*, *C. pinnatifidum*, *C. judaicum*, and *C. bijugum*, and accessions of *C. judaicum* and *C. bijugum* showed higher levels of resistance. Some accessions of *C. echinospermum* have also shown resistance to Ascochyta blight (Pande et al. 2006).

4.6.2 Botrytis Gray Mold

Botrytis gray mold (BGM) is prevalent in 14 countries, including the three main chickpea-growing countries, India, Pakistan, and Turkey. The disease occurs regularly but increased damage occurs when there are rains and high humidity during flower/pod formation. Screening of chickpea germplasm and breeding lines in India and Nepal has failed to identify high levels of resistance (Singh and Reddy 1991). Among the wild species, resistance has been identified in *C. bijugum* (Haware et al. 1992), *C. pinnatifidum*, and *C. judaicum* (van der Maesen and Pundir 1984). More recently, Pande et al. (2006) identified BGM resistance in *C. echinospermum* accessions, apart from *C. bijugum*, *C. pinnatifidum*, and *C. judaicum* accessions. It was possible to transfer BGM resistance from *C. echinospermum* to the cultigens, and the nature of resistance was found to be monogenic and recessive (Mallikarjuna unpublished results). Stevenson and Haware (1999) attributed resistance in *C. bijugum* to BGM with high concentrations of chemical maackiain (200–300 $\mu g/g^{-1}$) compared to low concentrations of maackiain (70 $\mu g/g^{-1}$) in susceptible wild and cultivated species.

4.6.3 Fusarium Wilt

Fusarium wilt (*F. oxysporum f.* sp. *Ciceri*) is a major constraint to chickpea production and yield losses due to wilt have been estimated at 10–90% (Jimenez-Diaz et al. 1989; Singh and Reddy 1991). The pathogen persists in soil year after year even in the absence of the host, which renders its control difficult (Haware et al. 1996). Vertical resistance to wilt is available in

cultivated chickpea (Sharma et al. 2005). There are eight distinct physiological races of wilt namely 0, 1A, 1B/C, 2, 3, 4, 5, and 6 (Haware and Nene 1982; Jimenez-Diaz et al. 1993; Kelly et al. 1994). The pathogen exhibits variation with respect to occurrence, regional specificity, and disease symptoms (Sharma et al. 2009). Races 0 and 1B/C cause yellowing syndrome, whereas 1A, 2, 3, 4, 5, and 6 lead to wilting syndrome. Genetics of resistance to two races (1B/C and 6) is yet to be determined; however, for other races, resistance is governed either by monogenes or by oligogenes. The individual genes of the oligogenic resistance mechanism delay the onset of disease symptoms, a phenomenon called as late wilting. Slow wilting, i.e., slow development of disease after onset of disease symptoms also occurs in reaction to pathogen; however, its genetics is not known.

STMS markers have revealed significant interspecific and intraspecific polymorphism in chickpea. Markers linked to six genes that govern resistance to six races (0, 1A, 2, 3, 4, and 5) of the pathogen have been identified and their position on chickpea linkage maps elucidated. These genes lie in two separate clusters on two different chickpea linkage groups. While the gene for resistance to race 0 is situated on linkage group (LG 5) of Winter et al. (2000), those governing resistance to races 1A, 2, 3, 4, and 5 spanned a region of 8.2 cm on LG 2. The cluster of five resistance genes was further subdivided into two subclusters of 2.8 cm and 2.0 cm, respectively.

Wild relative *C. judaicum* roots have three isoflav-3-enes, together with two pterocarpan glycosides. Initial experiments have shown that these chemical compounds may confer resistance to Fusarium wilt fungi (Stevenson and Veitch 1996). Later, these compounds were isolated in many annual and perennial *Cicer* species (Stevenson and Veitch 1998).

4.6.4 H. armigera *(Pod Borer)*

The legume pod borer [*H. armigera* (Hubner)] is an important pest of chickpea globally and brings down the yield of the crop. Losses due to *H. armigera* are estimated at US$927 million on chickpea and pigeonpea worldwide (Gowda 2005). It is polyphagous insect and attacks more than 182 plant species. The levels of resistance to pod borer in cultivated chickpea is not up to the desired level and other management options such as biological and chemical control have their own limitations. Therefore, development of host plant resistance is the best option as this can be coupled with management options.

Resistance to pod borers has been reported in wild *Cicer* species, namely, in accessions of *C. bijugum*, *C. pinnatifidum*, *C. judaicum*, *C. reticulatum*, and *C. echinospermum* (Sharma et al. 2005a). Mallikarjuna et al. (2007a) utilized *C. reticulatum* and *C. echinospermum* and obtained progeny that consistently showed low field damage (10% or less) due to pod borers. Laboratory bioassay using third instar larvae fed on the pods of resistant plants showed reduced larval weight, delayed pupation, failure to pupate or death before pupation, and in some cases, abnormal adults. This shows that antibiosis mechanism of resistance exists in these wild *Cicer* species, which can be transferred and exploited in a breeding program to develop cultivars with resistance against the insect.

Simmonds and Stevenson (2001) identified for the first time four isoflavonoids, namely, judaicin 7-0-gluoside, 2-methoxy judaicin, maackiain, and judaicin, which deterred larval feeding by *H. armigera* at 100 ppm concentration. Flavonoids judaicin and maakiain retained their antifeedant activity at 50 and 10 ppm, respectively. Additionally, chlorogenic acid increased their antifeedant potency. These flavonoids may be the substances responsible for antibiosis mechanism of resistance observed by Mallikarjuna et al. (2007a). Antibiosis mechanism of resistance to *Spodoptera litura* was observed in interspecific progeny derived from wild species *Arachis kempff-mercadoi* with the presence of chlorogenic acid, quercetin, and rutin (Mallikarjuna et al. 2004). Flavonoids chlorogenic acid, quercetin, and rutin were present in larger quantities in *A. kempff-mercadoi* (Stevenson et al. 1993) than in the susceptible cultivated groundnut, and these substances were responsible for conferring resistance to *S. litura*.

4.6.5 *Bruchids* (Callosobruchus chinensis)

Many storage insects, specifically bruchids, are a serious pest of stored chickpea (Southgate 1978). Chickpeas stored as "dhal" harbor fewer bruchids than when

stored as whole grains. Bruchids lower seed viability. For control of bruchids, dusting with BHC, DDT, derris, lindane, or pyrethrum or fumigation with methyl bromide has been recommended (Duke 1981). Resistance to seed beetles is not available in chickpea genotypes (Di Vito et al. 1988). At ICARDA, 127 accessions of eight wild *Cicer* species were screened for resistance to seed beetles (Singh et al. 1994, 1998). Three accessions of *C. echinospermum*, six accessions of *C. judaicum*, and nine accessions of *C. bijugum* were free from pest damage. It is possible that some of the flavonoids present in wild *Cicer* species (Simmonds and Stevenson 2001) may be responsible for bruchid resistance. There is no report of successfully transferring bruchid resistance from wild *Cicer* species to cultivated forms.

4.6.6 Cyst Nematode (H. ciceri Vovlas, Greco, and Divito)

Cyst nematode is an important pest in West Asia and North Africa and causes heavy losses to chickpea production (Greco et al. 1988). Resistance to cyst nematode has been identified in accessions of *C. bijugum*, *C. pinnatifidum* and in one accession of *C. reticulatum* ILWC 119. Chickpea germplasm lines, ILC 10765 and ILC 10766, resistant to cyst nematode were derived from crosses utilizing *C. reticulatum* ILWC 119 (Malhotra et al. 2002).

4.6.7 High Protein Content and High Yield

Significant variation in seed protein content has been observed in wild *Cicer* species, with some of the species showing higher content compared with cultivated chickpea (Singh and Pundir 1991). Ocampo et al. (1998) are of the opinion that this may be due to the methodology used in the estimation. Utilization of wild relatives in sorghum (Cox et al. 1984), soybean (Li et al. 2008), and rice (McCouch et al. 2007) has shown that wide crosses could produce positive transgressive segregants with high yield.

Although the present-day chickpea cultivars have been developed to produce more than the traditional varieties, there is ample scope to increase the yield, as there is a gap between potential yield and the actual yield obtained. In order to introduce yield genes into chickpea cultivars, chickpea cultivars were crossed with *C. reticulatum* and *C. echinospermum* on the premise that recombination could result in progenies with high yield. Heterosis was visually recorded in F_1 plants and promising and uniform progenies were bulked in F_5. Lines with higher yield (39%) than the controls were observed in F_7 (Singh and Ocampo 1997).

4.6.8 Cold Tolerance

Cold conditions result in flower drop in chickpea, culminating in significant yield loss in the semi-arid tropics (Malhotra et al. 1997). In the Mediterranean region, winter sowing is more productive compared to traditional sowing in spring, and cold tolerance is an important prerequisite for winter sowing (Singh and Hawtin 1979). Many accessions of C. *bijugum*, one accession of *C. echinospermum*, 13 accessions of *C. reticulatum*, and one accession of *C. pinnatifidum* showed higher levels of cold tolerance than the cultivated species (Singh et al. 1990). There are no reports on transfer of cold tolerance from wild *Cicer* species to the cultigen.

4.6.9 Drought Tolerance

Chickpea is sensitive to water stress during the early pod development (Khanna-Chopra and Sinha 1987). As the crop is mostly grown under residual moisture situation, scanty and early cessation of rains can cause significant yield losses in chickpea. Recently, seven annual wild *Cicer* species were investigated for their root traits along with chickpea genotypes (Krishnamurthy et al. 2003). The root and shoot growth of annual wild *Cicer* species was relatively poor compared to cultivated chickpea genotypes. Among the annual wild *Cicer* species, *C. reticulatum* showed growth rates closer to cultivated genotypes. In a study, Canci and Toker (2009) reported a few accessions of *C. reticulatum* and *C. pinnatifidum* to perform better under drought conditions and those lines could be considered as the best available drought-resistant

sources for breeding purposes. Utilizing *C. reticulatum* in place of cultivated chickpea would bring in much desired genetic variation in the resultant population. Toker et al. (2007) also found drought-resistant accessions of *C. pinnatifidum* and *C. reticulatum* on par with currently available resistant sources. Some of the perennial *Cicer* species not only recovered after wilting and drying out above the ground level but also tolerated temperatures above 41.8°C. A dehydrin gene (*cpdhn1*) related to drought resistance was isolated from a cDNA bank prepared from ripening seeds of *C. pinnatifidum* (Bhattarai and Fettig 2005). Two perennial species, *C. microphyllum* and *C. montbretii*, have been reported to be drought tolerant, inferred from their distribution in alpine regions (Chandel 1984). Additionally, *C. stapfianum*, *C. subaphyllum*, and *C. pungens* have been found to be drought resistant based on their growing region (van der Maesen, personal communication). Most perennial *Cicer* species are known to have a long woody tap root, which can often penetrate to 2 m depth. Long root system is marked as an important trait for drought tolerance. This trait may be useful in chickpea, which is often grown under receding moisture conditions. Although systematic screening of perennial wild *Cicer* species to quantify the variation in root growth is required for their exploitation in crop improvement programs, it can be concluded on the basis of the available information that perennial *Cicer* species are good sources of drought tolerance/resistance.

4.7 Genomics Resources

The chickpea genome is considered homogeneous, based on the minimal polymorphism detected by molecular markers. Limited polymorphism may be due to the self-pollinating nature. Although RAPDs were used initially, the development of highly variable polymorphic SSR markers has replaced them. Linkage maps were developed using amplified fragment length polymorphism (AFLPs), RAPDs, ISSRs, RGAs (resistance gene analogs), and STMS markers (Santra et al. 2000; Winter et al. 2000; Cho et al. 2002, 2004; Collard et al. 2003; Flandez-Galvez et al. 2003). Development of numerous SSR markers has accelerated chickpea genomics and the study of important traits. In chickpea, several hundred SSR markers have been developed and mapped on both intra- and interspecific mapping populations (Huttel et al. 1999; Winter et al. 1999; Lichtenzveig et al. 2005; Sethy et al. 2006). Functional markers generated from genic sequence, expressed tag sequences (ESTs), are advantageous as they are linked to traits of interest. Buhariwalla et al. (2005) developed 106 EST markers, of which 14 contained SSR motifs, and these were the first chickpea EST-SSR markers. More recently, Choudhary et al. (2009) generated 822 ESTs from immature seeds as well as 1,309 ESTs from chickpea database. From these, 246 SSR motifs were identified and 60 were validated as functional markers. These markers showed low levels of intraspecies polymorphism and high level of interspecies polymorphism.

The complete plastid genome of chickpea was sequenced by Jansen et al. (2008), and it was found to be 125,319 bp in size. The genome encodes 108 genes, including four rRNAs, 29 tRNAs, and 75 proteins. The sequence provides valuable information on the intergeneric spacer regions among legumes and endogenous regulatory sequences for plastid genetic engineering.

Jayashree et al. (2005) reported the development of a chickpea root-specific EST database comprising of over 2,800 EST sequences. This was constructed from using substractive suppressive hybridization (SSH) of root tissues from two closely related chickpea genotypes possessing different sources of drought avoidance and tolerance. The database provides researchers in chickpea genomics with a major resource for data mining association with root traits and drought tolerance.

Development of diversity array technology (DArT) markers should overcome the constraint associated with the development of high-density linkage maps. The discovery of single nucleotide polymorphism (SNP), which is relatively new in plant systems, has great potential for marker development. Rajesh and Muehlbauer (2008) estimated single nucleotide polymorphism (SNP) frequency at 1 in 94 bp in coding sequences and 1 in 74 bp in genomic regions in chickpea line FLIP 84-92C and wild relative *C. reticulatum* (PI 599072), two parental chickpea lines previously used to develop an interspecific linkage map.

A strategy for reverse genetics that is based on ethylmethyl sulfonate (EMS) mutagenesis was first described by McCallum et al. (2000) using the acronym targeted induced local lesions in genomes (TILLING). A specific advantage of EMS mutagenesis

is that the series of allelic mutations can serve as the basis of detailed structure-function studies. In addition, this has the potential to recover weak alleles with subtle changes in functionality of genes that would be lethal when more strongly affected. TILLING identifies individuals carrying point mutations in any gene of interest within a large population of EMS-mutagenized plants. In chickpea, M_2 seeds from approximately 9,000 individual M_1 plants of chickpea germplasm accession ICC12004 that had been treated with 0.2% EMS were obtained in the initial phases for development of a TILLING platform for chickpea. The estimated mutation frequency was determined through an analysis of 768 M_2 progenies using 20 targets comprising genomic DNA and cDNA sequences. There was a 100% success rate in primer design and screening of the mutants when using genomic sequence, and only a 7% success rate using cDNA sequence (Muehlbauer and Rajesh 2008).

4.7.1 Molecular Maps

Progress has been made in the development of genetic maps and the placement of genes for resistance to Ascochyta blight and Fusarium wilt as well as genes controlling agronomically important traits such as time to flowering, time to maturity, and podding habits. Two types of mapping populations have been used in chickpea to generate genetic linkage maps: the F_2 population and recombinant inbred lines (RILs) derived from interspecific as well as intraspecific crosses. RILs are preferred for genome mapping because of the distinct advantages it offers. The first integrated molecular map with 354 markers, including 118 STMSs, 96 DAFs, 70 AFLPs, 37 ISSRs, 17 RAPDs, eight isozymes, three cDNAs, and two sequence-characterized amplified regions (SCARs),which covered a distance of 2,077.9 cm was the result of an international collaborative effort (Winter et al. 2000).

Santra et al. (2000) used an RIL population from an interspecific cross of *C. areitinum* × *C. reticulatum* to generate a map of nine linkage groups with 116 markers (isozymes, RAPDs, and ISSRs) covering a map distance of 981.6 cm with an average distance of 8.4 cm between markers. In order to identify blight resistance in chickpea as well as genomic regions associated with blight resistance on intraspecific genetic linkage maps, F_7-derived RILs from the intraspecific cross of PI 359075 (blight susceptible) × FLIP84-92C(2) (blight resistant) were used by Cho et al. (2004). An intraspecific genetic linkage map comprising 53 STMS markers was constructed to identify genomic associations with blight resistance on the RIL population from a cross between PI359075(1) and FLIP84-92C(2). A major QTL for resistance to pathotype II of *A. rabiei* and two QTLs for resistance to pathotype I were identified.

Flandez-Galvez et al. (2003) established an intraspecific linkage map of chickpea genome using STMS markers on a F_2 population of chickpea cultivars with contrasting reaction to Ascochyta pathogen. Fifty-one out of 54 STMS markers (94.4%), three ISSR markers (100%), and 12 resistance gene analog (RGA) markers (57.1%) mapped on eight linkage groups. Chickpea-derived STMS markers were distributed throughout the genome, while RGA markers clustered with ISSR markers on the linkage groups LG 1, II, and III. Intraspecific linkage map spanned 534.5 cm, with an average interval of 8.1 cm between markers.

Madrid et al. (2008) identified a gene that controls resistance to chickpea rust in an RIL population derived from an interspecific cross between chickpea and *C. reticulatum*. A QTL for 31% of the phenotypic variance was located on the LG 7 of the chickpea genetic map. Two STMS markers were detected flanking the resistance gene.

A composite linkage map was constructed using RILs from a cross between *C. arietinum* and *C. reticulatum*. The mapping population segregated for resistance to Ascochyta blight, Fusarium wilt, and rust diseases. RGA markers have mapped loci that confer resistance to Ascochyta blight and Fusarium wilt. Association was detected between RGAs and genes that controlled resistance to Fusarium wilt caused by races 0 and 5 (Palomino et al. 2009).

The genetic map published by Taran et al. (2007) was generated from 135 primer pairs including 134 SSRs and was based on a population of 186 F_2 plants from an intraspecific cross of "desi" cultivar ICCV 96029 and "kabuli" cultivar CDC Frontier. Markers reported in this map were assigned to eight linkage groups with a combined linkage distance of 1,285 cm. The average linkage distance between markers in all linkage groups was 8.9 cm. Common markers in these maps with SSR primer pairs could lead to the development of a high-density genetic map of chickpea to

identify tightly linked flanking markers for genes of interest, which will ultimately be helpful in marker-assisted selection (MAS) and positional cloning of agronomically important genes.

4.8 Directions for Future Research

Taking examples from rice and wheat, it is clear that a particular wild *Cicer* species may not possess many of the desirable traits needed for chickpea improvement. One of the major advantages of utilizing wild relatives is the resultant reshuffling/recombination in the genome, leading to the appearance of novel characters not found in parental species. With a large collection of molecular markers available for chickpea, it may not be too difficult to select against unwanted characters from the wild species brought along because of linkage drag.

Several wild species are poorly represented in gene banks either because of developmental activities in the areas of their origin or because of the inaccessibility of the region due to geographical or political reasons. Collection of additional accessions of each annual and perennial wild species is needed to widen the genetic base in the *Cicer* gene pool. Specifically, major emphasis should be placed on collection of *C. reticulatum* in the center of origin of chickpea, most importantly in Turkey, where the species is found in abundance. Collection could be followed by transfer of the agronomically important genes such as Ascochyta blight, botrytis gray mold, *H. armigera* to the cultigen by classical breeding methods. It will be useful if focused attempts in wide crosses are initially de-linked from the active breeding programs (often termed as pre-breeding) to utilize the available variation in the wild species. This will remove the pressure of agronomic performance for the segregants from the wide crosses. Although variation in resistance to some of the stresses is available in chickpea, it is not up to the desired level, and the use of genes from the wild species will broaden their genetic base and likely improve stability of resistance. Increased emphasis also needs to be placed on the collection of *C. echinospermum* as some of the accessions have shown variation for economically important traits. It is important to evaluate the available wild gene pool for various biotic and abiotic constraints to tap the potential of the available germplasm.

Low biomass production is often cited as one reason for low seed yields of chickpea. Increasing biomass of cultivated chickpea varieties appears to be possible with the use of *C. reticulatum* and *C. echinospermum* as progenies of both species in crosses with *C. arietinum* have had an apparent increase in biomass and overall plant vigor. Perennial *Cicer* also has potential for higher biomass but currently is unavailable for breeding purposes due to cross-incompatibility with the cultigen.

Advanced molecular marker technology should be exploited to estimate genetic diversity and phylogenetic relationships among annual and perennial wild species. Molecular markers may also improve our genetic understanding of the traits, and genetic mapping and QTL analysis will provide useful information on the locations of important genes and markers that can be used for gene introgression. The desired genes can eventually be transferred from wild species to the cultigen using marker-assisted breeding, which will ultimately facilitate chickpea crop improvement.

The presence of biochemical compounds with antifungal activity toward botrytis gray mold and antifeedant activity toward *H. armigera* opens up avenues for molecular breeding for fungal and insect resistance in chickpea coupled with biochemical markers.

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