GENETIC RESOURCES, CHROMOSOME ENGINEERING, AND CROP IMPROVEMENT

Grain Legumes

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RAM J.SINGH AND PREM P.JAUHAR

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CHAPTER 7
Chickpea (*Cicer arietinum* L.)

F.Ahmad, P.M.Gaur, and J.S.Croser

7.1 INTRODUCTION

Chickpea (*Cicer arietinum* L.), commonly called gram, Bengal gram, or garbanzo bean, is the most important food grain legume of South Asia and the third most important in the world after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). Chickpea is a diploid with $2n=2x=16$ chromosomes and a genome size of approximately 750 Mbp (Arumuganathan and Earle, 1991). Chickpea is one of the first grain crops cultivated by man and has been uncovered in Middle Eastern archaeological sites dated to the eighth millennium BC (Zohary and Hopf, 2000). Two distinct market type classes, *desi* and *kabuli,* are recognized in chickpea (Pundir, Rao, and van der Maesen, 1985). The *desi* types that account for about 85% of chickpea area usually have small, angular-shaped, dark-colored seeds with a rough surface, pink flowers, anthocyanin pigmentation on the stems, and either semi-erect or semi-spreading growth habit. The *kabuli* type, which cover the remaining 15% area, usually have large "rams head"-shaped smooth surface seeds, lack of anthocyanin pigmentation, and semi-spreading growth habit.

It has become increasingly clear during the last few decades that meeting the food needs of the world's growing population depends, to a large extent, on the conservation and use of the world's remaining plant genetic resources. Conservation without use has little point and use will not come without evaluation. Genetic resources encompass all forms of the cultivated species, as well as their related wild species (Harlan, 1984). That is a general concept to which chickpea is no exception. In reviewing genetic resources and their multifaceted applications in chickpea genetic improvement, we have placed more emphasis on the wild genetic resources of the cultivated chickpea, while providing a brief overview of resources available in the cultivated species.

7.1.1 Global Chickpea Production and Distribution

Chickpea is grown in more than 40 countries, but the main growing region is South and Southeastern Asia (ca. 70%), where India (ca. 60%) and Pakistan (ca. 10 to 15%) are major contributors. West Asia accounts for approximately 16% of global chickpea area, with Iran, Turkey, and Syria being the largest producers in the region. Africa accounts for

5%, mostly from Ethiopia, Malawi, and Tanzania in Eastern Africa and Morocco in North Africa. In remaining parts of the world, about 3% area is contributed by North America (mainly Canada), 2% by Australia, and 1% by Europe (mainly Spain).

The global chickpea area has remained almost stagnant during the past 40 years; it was 11.8 m/ha during 1961–1965 and 11.0 m/ha during 1996–2000. Though there has not been much change in the global chickpea area, the production has increased from 7 m/t in 1961–1965 to 8.4 m/t during 1996–2000, due to enhancement in yield levels from 599 to 791 kg/ha^{-1} during this period. During 2000–2002, chickpea production was 7.5 m/t from an area of 9.7 m/ha (average of three years).

There has been more than a sevenfold increase in the global trade of chickpea during the past four decades. Trade increased from 0.13 m/t during 1961–1965 to 0.66 m/t during 1996–2000 and reached its highest level of 1 m/t during 2001. India has been the world's largest chickpea importing country during the past 15 years. It had a record import of 0.52 m/t during 2001. Pakistan has emerged as the second largest importer of chickpea during this period, its imports increasing from 5,000 t during 1981–1985 to 0.1 m/t during 2001. Other countries that have had sizable chickpea import during recent years are Spain, Saudi Arabia, Italy, Jordan, Tunisia, Lebanon, Turkey, Sri Lanka, and Colombia (Gowda and Gaur, 2004).

7.1.2 Importance of the Crop

Chickpea has one of the highest nutritional compositions of any dry edible grain legume and does not contain significant quantities of any specific major antinutritional factors. On an average, chickpea seed contains 23% of highly digestible protein, 64% total carbohydrates, 47% starch, 5% fat (primarily linoleic and oleic acids), 6% crude fiber, 6% soluble sugar, and 3% ash. The mineral component is high in phosphorus (343 mg/100 g), calcium (186 mg/100 g), magnesium (141 mg/100 g), iron (7 mg/100 g), and zinc (3 mg/100 g) (Williams and Singh, 1987). Used in a variety of ways, chickpea is not only good for human health but also for soil health. It meets 80% of its nitrogen (N) requirement from a symbiotic rhizobial interaction, which enables the crop to fix up to 140 kg N ha^{-1} from atmosphere (Saraf et al., 1998). It leaves substantial amount of residual nitrogen behind for subsequent crops and adds much needed organic matter to maintain and improve soil health, long-term fertility, and sustainability of the ecosystems. In recent years, chickpea has also gained popularity in broad-acre cropping systems in developed countries, particularly Australia and Canada (Siddique and Sykes, 1997). A dryland crop requiring minimal inputs, chickpea is a boon to the resource-poor marginal farmers in the tropics.

7.2 ORIGIN OF CROP AND ITS TAXONOMY

7.2.1 Origin of Chickpea

The available evidence suggests that chickpea originated in the fertile crescent region of southeastern Turkey and adjoining Syria (van der Maesen, 1987). The proposed wild progenitor and several other annual *Cicer* species occur there (Ladizinsky, 1975). Further evidence of its origin comes from seeds dated to about 5450 BC, unearthed from archaeological excavations at Hacilar near Burdur in Turkey (Helbaek, 1970).

It is believed that chickpea diverged from Turkey in two directions—into the western parts where it is grown in the spring and summer and into the eastern and southern parts where it is grown in the cool dry seasons. The majority of the wild *Cicer* species are found in the West Asia and North Africa region covering Turkey in the north to Ethiopia in the south, and Pakistan in the east to Morocco in the west. Botanically, the cultivated chickpea has been split into two groupings, *microsperma* and *macrosperma,* corresponding to seed size and in much the same way as it has been done for lentils (Cubero, 1987). From a practical point of view, chickpea is also classified into *kabuli* and *desi* types. The terms *desi* and *kabuli,* however, do not overlap with *microsperma* and *macrosperma*. The *kabuli* types are now grown predominantly in the countries of the Mediterranean region, West Asia, North Africa, Australia, and North America, while the *desi* types are grown mostly in South Asia, Iran, Ethiopia, Mexico, and Australia. Other than morphological differences, all forms of the cultivated species share the same genome.

The wild annual progenitor of chickpea has been repeatedly identified as the annual species *C. reticulatum* Lad. (Ladizinsky and Adler, 1976b; Ahmad, Gaur, and Slinkard, 1992; Iruela et al., 2002). It is believed that one of the crucial steps in the origin of cultivated chickpea was the change from the perennial to annual life cycle (Gupta and Bahl, 1983). The perennial species have not been extensively studied, however, the available evidence suggests *C. anatolicum* Alef. to be the probable perennial progenitor of *C. arietinum* (Gupta and Bahl, 1983; Ahmad, 1989; Tayyar and Waines, 1996). It appears that domestication and, thus, evolution of the cultivated species followed the usual process of artificial selection, which favored large palatable seeds, reduced pod dehiscence, nondormancy, synchronous ripening, earliness, and diversity of forms (van der Maesen, 1987).

7.2.2 Taxonomy of *Cicer*

The cultivated chickpea species has been taxonomically placed in the genus *Cicer,* which belongs to the family Fabaceae and its monogeneric tribe Cicereae Alef. (Kupicha, 1981). Presently, the genus *Cicer* consists of 43 species (Table 7.1), divided into 4 sections, *Monocicer, Chamaecicer, Polycicer,* and *Acanthocicer,* based on their morphological characteristics, life cycle, and geographical distribution (van der Maesen, 1987; http://singer.cgiar.org/Search/SINGER/search.htm). Eight

Table 7.1 List of All Known Species in the Genus *Cicer*

Annual Species

*C. arietinum** L.
*C. bijugum** K.H.Rech.
*C. chorassanicum** (Bge.) M.Pop.
*C. cuneatum** Hochst. ex Rich.
*C. echinospermum** P.H.Davis
*C. judaicum** Boiss.
*C. pinnatifidum** Jaub. & Sp.
*C. reticulatum** Ladiz.
*C. yamashitae** Kitamura

Perennial Species

C. acanthophyllum Boriss.
*C. anatolicum** Alef.
C. atlanticum Coss. ex Maire
C. balcaricum Galushko
C. baldshuanicum (M.Pop.) Lincz.
*C. canariense** Santos Guerra & Lewis
C. fedtschenkoi Lincz.
C. flexuosum Lipsky
*C. floribundum** Fenzl.
C. graecum Orph.
C. grande (M. Pop.) Korotk.
*C. heterophyllum** Contandr et al.
C. incanum Korotk.
*C. incisum** (Willd.) K.Maly
*C. isauricum** P.H.Davis
C. kermanense Bornm.
C. korshinskyi Lincz.
C. macracanthum M.Pop.
*C. microphyllum** Benth.
C. mogoltavicum (M. Pop.) Koroleva
*C. montbretii** Jaub. & Sp.
C. multijugum van der Maesen
C. nuristanicum Kitamura
C. oxyodon Boiss. & Hoh.
C. paucijugum (M. Pop.) Nevski
*C. pungens** Boiss.
C. rassuloviae Lincz.
C. rechingeri Podlech
*C. songaricum** Steph. ex. DC.
C. spiroceras Jaub. & Sp.
C. stapfianum K.H.Rech.
C. subaphyllum Boiss.
C. tragacanthoides Jaub. & Sp.

Unspecified

C. laetum Rassulova & Sharipova

*Species with confirmed somatic chromosome number of 2n=16
Source: The CGIAR System-Wide Information Network for Genetic Resources (SINGER; http://singer.cgiar.org/Search/SINGER/search.htm) and van der Maesen, 1987. Adapted from Croser et al. 2003a.

of these *Cicer* species, sharing the annual growth habit with chickpea, are of particular interest to breeders. Of the nine annual *Cicer* species, eight are classified within the *Monocicer* section, and one, *C. chorassanicum,* within the *Chamaecicer* section (Kazan and Muehlbauer, 1991; Muehlbauer, Kaiser, and Simon, 1994). Thirty-three of the remainder species are known to be perennial, while *C. laetum* Rass. & Sharip has an unspecified life cycle (van der Maesen, 1987).

7.3 GERMPLASM COLLECTION, MAINTENANCE, AND EVALUATION

Due to its importance as an affordable protein source in developing nations, it is crucial to obtain and maintain higher yields of the chickpea crop. Effective genetic means are, thus, needed to reach such key objectives. The effectiveness of selection in any crop depends upon the extent and nature of phenotypic and genotypic variability present in different traits of the population (Arora, 1991). Unfortunately, the chickpea crop is susceptible to a range of biotic and abiotic stresses, which can be devastating to crop yield. A low level of genetic variability within *C. arietinum* has hampered chickpea breeders in their efforts to develop widely adapted cultivars with resistance to biotic and abiotic stresses (Ladizinsky and Adler, 1975; Tuwafe et al., 1988; Kazan and Muehlbauer, 1991; Ahmad et al., 1992; Ahmad and Slinkard, 1992; Udupa et al., 1993; van Rheenen et al., 1993; Labdi et al.,1996; Tayyar and Waines, 1996; Simon and Muehlbauer, 1997; Siddique et al., 2000). Recently, this low level of genetic variability has been attributed to a series of genetic bottlenecks in the domestication of chickpea, including the restricted distribution of the wild progenitor, the founder effect associated with domestication, and the shift from winter to summer cropping (Abbo et al., 2003). To compound this problem, genetic erosion of *C. arietinum* intraspecific resources is occurring due to the loss of local ecotypes, through diseases, insects, and environmental stresses, as well as for economic and strategic reasons (Malhotra et al., 2000; Abbo et al., 2003). Breeders are therefore looking to the wild relatives of chickpea as an alternative genetic resource for crop improvement.

The world collection of annual wild *Cicer* species is very limited and currently consists of 593 entries held in nine genebanks around the world. These represent only 285 known separate accessions of the eight annual wild *Cicer* species (Berger et al., 2003). Of these, only 120 have been collected independently from wild populations, with the remainder being selections from the original material. There is a single original accession of *C. cuneatum,* 2 of *C. chorassanicum,* 13 of *C. echinospermum,* 18 of *C. reticulatum,* 21 of *C. bijugum,* 28 of *C. pinnatifidum,* three of *C. yamashitae,* and 34 of *C. judaicum*. Thus, only a small proportion of the habitat and genetic diversity that is potentially available in wild populations is present in *ex situ* collections. It is essential to widen genetic diversity of the world collection of annual wild *Cicer* species by conducting targeted missions, which address the multitude of gaps in the current collection (Berger et al., 2003).

The world germplasm collection of perennial *Cicer* species is even more limited. Compared to the annual species, screening has been difficult due to the inherent problems with growing these species so far from their adapted climatic conditions. Malhotra et al.

(2000) have suggested that they should be conserved *in situ* by preserving their original habitats. Recent progress in determining adequate growing conditions for some of the perennial species, and subsequent establishment of perennial *Cicer* nurseries in eastern Washington state should go some way to improving this situation in the future (Kaiser et al., 1997). Approximately 12 of the 34 perennial species have been maintained, with various degrees of success, at The Germplasm Resources Information Network-United States Department of Agriculture (Pullman, WA).

7.4 TRAITS OF ECONOMIC SIGNIFICANCE WITHIN THE WILD *CICER* GENE POOL

Comprehensive screening of wild *Cicer* collections at the International Centre for Agricultural Research in Dry Areas (ICARDA), Syria, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, and other national institutions have identified germplasm with resistance to various diseases and insects. Table 7.2 lists the traits of economic significance currently identified within the wild annual and perennial species of *Cicer*.

Wild *Cicer* species are the only source of resistance so far found for bruchid (*Callosobruchus chinesis* L.) and cyst nematode. They have a higher level of resistance than the cultivated species for fusarium wilt, leaf miner, phytophthora root rot, drought, ascochyta blight, pea streak carlavirus, and cold. Perhaps even more importantly, several accessions of the wild *Cicer* species are resistant to three or more stresses (Robertson et al., 1996). Particularly promising are the following accessions that are resistant to four or five different stresses, viz. *C. reticulatum* (ILWC 81, 112), *C. echinospermum* (ILWC 39, 181), *C. bijugum* (ILWC 32, 62, 73, 79), *C. judaicum* (ILWC 46), and *C. pinnatifidum* (ILWC 236). Such accessions are obvious targets for interspecific hybridization efforts. Overall, the species containing accessions with the highest levels of resistance to the most stresses in order of performance are: *C. bijugum, C. pinnatifidum,* and *C. judaicum* (Singh et al., 1994; Singh et al., 1998).

Accessions of the eight wild annual *Cicer* species have also been evaluated for various morphological traits at ICARDA and a catalogue was prepared (Robertson et al., 1995). Robertson et al. (1997) have reported useful variations for vegetative and reproductive characters in the annual wild species. Not surprisingly, *C. arietinum* showed greater intraspecific morphological variability compared with the wild species, particularly for characters like leaf area, growth habit, plant height, first pod height, pod dehiscence, and 100-seed weight. Among the wild species, *C. reticulatum* showed the largest overall morphological variability, followed by *C. pinnatifidum, C. echinosper-*

Table 7.2 Desirable Traits Identified in Wild Annual and Perennial *Cicer* Species

Trait	Species with Resistance/Tolerance	Reference(s)
Ascochyta blight	*C. anatolicum, C. bijugum, C. cuneatum, C. echinospermum, C. judaicum, C. montbretti, C. pinnatifidum,* C. reticulatum	Haware et al., 1992; Singh and Reddy, 1993; Stamigna et al., 1998; Singh et al., 1998; Collard et al., 2001; Collard et al., 2003a
Botrytis grey mould	*C. bijugum*	Haware et al., 1992
Bruchid	*C. bijugum, C. cuneatum, C. echinospermum,*	Singh et al., 1994; Singh et al., 1998
	C. judaicum, C. pinnatifidum, C. reticulatum	
Cold	*C. bijugum, C. echinospermum, C. judaicum, C. microphyllum, C. pinnatifidum, C. reticulatum*	van der Maesen and Pundir, 1984; Singh et al., 1990,1995; Singh et al., 1998; Toker, 2004
Cyst nematode	*C. bijugum, C. pinnatifidum, C. reticulatum*	Singh et al., 1989; Singh and Reddy, 1991; Singh et al., 1994, 1996; Singh et al., 1998; Di Vito et al., 1996
Crenate broomrape	*C. bijugum, C. canariensis, C. echinospermum, C. judaicum, C. macracanthum, C. multijugum, C. oxyodon, C. pinnatifidum, C. reticulatum, C. songaricum, C. yamashitae*	Rubiales et al., 2004
		C. bijugum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum C. bijugum, C. canariense, C. cuneatum,
Drought	*C. microphyllum*	Chandel, 1984 .... implied based on distribution
Fusarium wilt	*C. bijugum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum*	Nene and Haware, 1980; Haware et al., 1992; Singh et al., 1994; Kaiser et al., 1994; Infanito et al., 1996; Porta-Puglia and Infantino, 1997
Helocoverpa pod borer	*C. bijugum, C. canariense, C. cuneatum, C. echinospermum, C. judaicum, C. macracanthum, C. microphyllum, C.*	Kaur et al., 1999, Sharma et al., 2002, 2004

	pinnatifidum, C. reticulatum	
Leaf miner	*C. bijugum, C. chorassanicum, C. cuneatum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum*	Singh and Weigand, 1994; Singh et al., 1994, 1998
Pea streak carlavirus	*C. anatolicum, C. canariensis, C. microphyllum, C. oxyodon*	Kaiser et al., 1993
Phytophthora root rot	*C. echinospermum*	Singh et al., 1994; Knights et al., 2003
Trypsin inhibitor	*C. chorassanicum*	Ahmad and Kollipara, 2004

mum, and *C. bijugum*. Of interest was the variability for wide leaflets in *C. chorassanicum,* number of branches in *C. bijugum,* and *C. reticulatum* and early flowering in *C. judaicum*.

In terms of nutritional value, there is a wide variation in seed protein and amino acid content in the annual *Cicer* species (Singh and Pundir, 1991). Seed protein ranges from 168 g/kg in *C. cuneatum* to 268 g/kg in *C. pinnatifidum*. Despite this, prospects for upgrading the nutritional value of chickpea by introgression of genes for high protein content are low, as the variability in the wild accessions falls within the range for the cultivated chickpea (Ocampo et al., 1998). Further, chickpea seed extract is known to inhibit trypsin and chymotrypsin (Saini et al., 1992), however, preliminary data for trypsin protease inhibitor indicate that there is no such activity in *C. chorassanicum* (Ahmad and Kollipara, 2004).

7.5 SPECIES RELATIONSHIPS AND INTERSPECIFIC HYBRIDIZATION

7.5.1 Species Relationships within the Genus

Traditionally phenotypic traits (Nozzolillo, 1985; De Leonardis et al., 1996; Robertson et al., 1997; Hassan, 2000; Javedi and Yamaguchi, 2004a), hybridization success (Ladizinsky and Adler, 1976a, 1976b; Pundir and van der Maesen, 1983; Verma et al., 1990; Pundir et al., 1992; Sheila et al., 1992; Pundir and Mengesha, 1995; Badami et al., 1997; Singh et al., 1999a, 1999b; Stamigna et al., 2000), analysis of chromosome pairing in hybrids (Ladizinsky and Adler, 1976a, 1976b; Ahmad et al., 1987; Ahmad, 1988), and the study of chromosome structure (Ohri and Pal, 1991; Ocampo et al., 1992; Tayyar et al., 1994; Ahmad, 2000) have been widely used methods for analysis of genomic relationships and the construction of phylogenies among *Cicer* species. Over the past 15 years, electrophoretic data based on seed storage protein (Ladizinsky and Adler, 1975; Vairinhos and Murray, 1983; Ahmad and Slinkard, 1992) and isozymes (Kazan and

Muehlbauer, 1991; Ahmad et al., 1992; Labdi et al., 1996; Tayyar and Waines, 1996; Gargav and Gaur, 2001, Sudupak and Kence, 2004) have also been applied to systematic studies in *Cicer*. More recently, DNA-based techniques (Patil et al., 1995; Sharma et al., 1995; Ahmad, 1999; Choumane et al., 2000; Iruela et al., 2002; Sudupak et al., 2002; Rajesh et al., 2003; Javedi and Yamaguchi, 2004a, b; Nguyen et al., 2004; Sudupak, 2004; Sudupak et al., 2004) have provided many new approaches to compare aspects of genome relationships in ways not previously possible.

Among the annual *Cicer* species, there is a consensus that *C. reticulatum* and *C. echinospermum* are the wild species most closely related to the domesticated *C. arietinum. Cicer bijugum, C. pinnatifidum,* and *C. judaicum* show a closer relationship among themselves and appear to form a group that is next closest to the first group containing the cultivated species. The remainder of the annual species, viz. *C. chorassanicum, C. yamashitae,* and *C. cuneatum* share an even more distant relationship with the cultivated species. This relationship only generally holds true, as the five annual species that are not part of the group with the cultivated species appear to change positions for relatedness to each other and to *C. arietinum*. Perennial *Cicer* species have only recently been subjected to phylogenetic analysis (Tayyar and Waines, 1996; Gargav and Gaur, 2001; Iruela et al., 2002; Sudupak et al., 2002; Rajesh et al., 2003; Javedi and Yamaguchi, 2004a, b; Nguyen et al., 2004; Sudupak, 2004; Sudupak and Kence, 2004; Sudupak et al, 2004). Again, with some exceptions, the perennial species in general have shown a distant relationship and are far removed from the cultivated species. The relationship between the perennial species *C. anatolicum* and domesticated chickpea remains a contentious issue, with some studies placing this species as a close relative of the cultivated species, while others indicating it to be far removed (Kazan and Muehlbauer, 1991; Staginnus et al., 1999; Choumane et al., 2000; Rajesh et al., 2003; Nguyen et al., 2004; Sudupak, 2004; Sudupak and Kence, 2004).

7.5.2 Gene Pools and Interspecific Hybridization

Ladizinsky and Adler (1976a, 1976b) and van der Maesen (1987) adopted the classical definition of the primary, secondary, and tertiary gene pools as proposed by Harlan and de Wet (1971) for classification of the *Cicer* wild relatives. According to this definition, the primary gene pool includes all species that hybridize freely, show good chromosome pairing leading to gene exchange, and produce viable hybrids. The secondary gene pool includes species that can be used as germplasm resources; however, hybridization with the cultivated species is difficult because of genetic barriers or chromosome alterations, and some degree of sterility is associated with the first-generation hybrids. Members of the tertiary gene pool are difficult to utilize, and sterility is always associated with hybrids. Fertility can sometimes be restored, but usually the percentage of recovered viable zygotes is extremely small.

On the basis of Harlan and de Wet's (1971) definition, and in consideration of the results obtained from crossability, biochemical and molecular diversity, and karyotypic studies, a recently revised model of the wild annual *Cicer* gene pools has been proposed (Croser et al., 2003a). However, if one were to follow Harlan and de Wet's (1971) definition alone, the primary gene pool of *Cicer* would consist of *C. arietinum* and only one wild species, the wild annual progenitor *C. reticulatum*. Although the other closely

related species, *C. echinospermum,* crosses quite readily with the cultivated species, it shows varying levels of sterility in the F_1 and F_2 generations. The secondary gene pool, thus, consists of *C. echinospermum* only. *Cicer bijugum, C. pinnatifidum,* and *C. judaicum,* which have been reported to give hybrids when crossed with the cultivated species (Verma et al., 1990; Singh et al., 1994; Singh et al., 1999a, 1999b), have been placed in the secondary gene pool by Croser et al. (2003b). This, however, is an issue that is not resolved yet and calls for some discussion. Although successful crosses of *C. bijugum, C. pinnatifidum,* and *C. judaicum* with *C. arietinum* have been reported, the authors have as yet failed to produce (except the *C. arietinum*×*C. judaicum* hybrid) (Verma et al., 1995) conclusive proof of hybridity by any cytogenetical, biochemical, or DNA-based approach. Indeed, there is a growing skepticism in the scientific community about the authenticity of these apparent partially fertile hybrids and their resulting progenies (Pundir, personal communication, 1994; Cubero and Ocampo, personal communication, 2004; Muehlbauer, personal communication, 2004). Considering the ongoing scientific dilemma and the lack of success, despite repeated attempts by various researchers globally utilizing embryo and ovule culture procedures and spanning more than two decades, we conclude that partially fertile interspecific hybrids and their progenies as claimed by the above research groups (Verma et al., 1990; Singh et al, 1994; Singh et al., 1999a, 1999b) are highly questionable. We would, thus, propose that the above three species should be placed in the tertiary gene pool of chickpea, along with the remaining annual species *C. chorassanicum, C. yamashitae,* and *C. cuneatum*. The lack of availability of perennial *Cicer* species has greatly restricted the assessment of their crossability with the cultivated species. Given the current situation with perennial *Cicer* species (Croser et al., 2003a), and until proven otherwise, we feel these species should be appropriately placed in the tertiary gene pool along with the six other annual wild species.

It is obvious from crossability studies of the cultivated species with the wild *Cicer* species that technical difficulties in obtaining hybrids beyond those within the primary and secondary gene pools remain a major obstacle. Apart from a few rather surprising exceptions (Verma et al., 1990; Singh et al., 1994; Singh et al., 1999a, 1999b), postfertilization incompatibility barriers (Bassiri et al., 1987; Ahmad et al., 1988; Stamigna et al., 2000; Ahmad and Slinkard, 2004) have restricted successful hybridization (using conventional crossing techniques) exclusively to the species of the primary and secondary gene pools. Given the fact that the tertiary gene pool species remain crossincompatible with the cultivated species, bridging crosses deserve further attention in chickpea in light of the known close relationships of *C. arietinum* with the wild annual species *C. reticulatum* and *C. echinospermum*. Ahmad and Slinkard (2003) have recently shown that, provided reciprocal crosses are used, there are at least no prefertilization barrier(s) limiting the hybridization of *C. reticulatum* and *C. echinospermum* with the other annual wild *Cicer* species. If the above-mentioned three species can be crossed with any one of the wild annual or perennial species, then it would be possible to transfer traits of interest to *C. arietinum*.

Rescuing abortive interspecific embryos and their *in vitro* culture has been proposed as an effective strategy for overcoming species barriers (van Rheenen, 1991; Muehlbauer et al., 1994; Singh et al. 1994; Robertson et al., 1996; Singh and Ocampo, 1997). In *Cicer,* there has been limited success from attempts to develop tissue culture techniques

to enable hybridization between chickpea and the more distantly related annual wild species *C. cuneatum, C. pinnatifidum,* and *C. bijugum* (Singh and Singh, 1989; Swamy and Khanna, 1991; Verma et al., 1995; Badami et al., 1997; van Dorrestein et al., 1998; Mallikarjuna, 1999, 2001). To date, a technique that enables the reproducible rescue and growth of embryos before eight days after pollination is lacking in *Cicer*. Thus, there is a clear need to identify and fine-tune information regarding the physiological, nutritional, and hormonal requirements of developing chickpea embryos (Croser, 2002; Croser et al., 2003a). An international collaboration has been established between Canadian, Australian, and Indian researchers to develop techniques for efficient interspecific hybridization between the cultivated and wild *Cicer* species.

7.6 CYTOGENETICS AND LINKAGE MAPS OF CHICKPEA

7.6.1 Cytogenetics

Chickpea is a crop that is not amenable to cytogenetic studies. Hence, most studies are limited to chromosome counts and feulgen-stained studies of karyotypes because of the small size and sticky nature of chromosomes. No cytogenetic stocks, other than tetraploids, are available in chickpea (Bahl, 1987; Gupta and Sharma, 1991) and except for one (Vlacilova et al., 2002), linkage groups have not yet been associated with respective chromosomes. *Cicer arietinum* has been the subject of a considerable number of karyotypic studies, while the wild annual species have received less attention (Croser et al., 2003a).

The nine annual and nine of the remainder 34 species are confirmed diploids with $2n=2x=16$ chromosomes (Table 7.1) (Ladizinsky and Adler, 1976a, 1976b; Ahmad, 1989; Pundir et al., 1993; Ohri, 1999; Ahmad, 2000; Ahmad and Chen, 2000). The karyotypes of the primary gene pool species *C. arietinum, C. reticulatum, C. echinospermum,* and the tertiary gene pool species *C. songaricum* and *C. anatolicum* show a high degree of similarity to each other and form the first group, while the second group contains all of the remaining six annual species (Ahmad, 2000; Croser et al., 2003a). Karyotypic details of the other species in the genus are not known. The crossability of these two perennial species with *C. arietinum* remains largely unknown. Whether a karyotypic similarity-crossability equation holds true in the *Cicer* genus needs additional research, but broad karyotypic and crossability studies of the cultivated chickpea with perennial *Cicer* species for alien gene introgression appear warranted.

Our understanding of the cytogenetic map in chickpea and related *Cicer* species is very much lacking. Individual chromosomes of chickpea are reported to be identifiable by C-banding and fluorochrome staining (Galasso and Pignone, 1992; Tayyar et al., 1994; Galasso et al., 1996). The related annual wild species have also been subjected to a solitary C-banding analysis (Tayyar et al., 1994), and the general conclusion drawn is that the heterochromatic C-bands are located proximally around the centromere with only occasional bands in intercalary and distal positions. Pachytene chromosome analysis of the cultivated species (Ahmad and Hymowitz, 1993) has further corroborated such distribution of heterochromatin. While the authors (Tayyar et al., 1994) were able to

identify individual pairs of chromosomes within a species, its applicability in an interspecific hybrid situation remains to be explored.

Karyotype analyses have shown that only one chromosome pair is associated with the nucleolar organizing regions, and is thus considered satellited (Ocampo et al., 1992; Ahmad, 2000). The satellite is present on the longest first chromosome in *C. arietinum, C. reticulatum,* and *C. echinospermum,* and an extra satellite in the case of *C. reticulatum* may also be present on the second chromosome. Unlike the somatic karyotype, detailed pachytene chromosome analysis had shown the third chromosome to be clearly satellited in *C. arietinum* (Ahmad and Hymowitz, 1993). Such refined pachytene analysis has made individual chromosome identification more conclusive and adds a further dimension to chickpea cytology (Ahmad and Hymowitz, 1993).

Given the poor state of chickpea cytology, there has been commendable progress in molecular cytogenetics in the genus. Individual chickpea chromosomes have been successfully sorted by flow cytometry (Vlacilova et al., 2002) and utilized for mapping specific DNA sequences and genes to individual chromosomes. Thus, specific genes (coding for various rRNA loci), major random repetitive DNA sequences, STMS markers, microsatellites, En/Spm-like transposon sequences, simple sequence repeats, and *Arabidopsis-type* telomeric sequences have been successfully hybridized to and localized on the chickpea chromosomes by fluorescent *in situ* hybridization (FISH) (Abbo et al., 1994; Galasso et al., 1996; Gortner et al., 1998; Staginnus et al., 1999, 2001; Vlacilova et al., 2002; Valarik et al., 2004). Using polymerase chain reaction and FISH, Vlacilova et al. (2002) have successfully associated two STMS markers (belonging to linkage group 8 of Winter et al., 2000) to the shortest chromosome of the chickpea genome. More recently, FISH analysis on super-stretched flow-sorted chickpea chromosomes has revealed spatial resolution of neighboring loci that has not been obtained by any other method (Valarik et al., 2004). It is expected that further technical advances will lead to the development in genome mapping of chickpea and the association of all genetic linkage groups to specific well-defined chromosomes.

7.6.2 Genetic Linkage Mapping

Development of high-density integrated genetic linkage maps based on morphological, biochemical, and DNA markers is a prerequisite for use in marker-assisted selection, positional cloning, and mapping of quantitative trait loci of agronomically important traits in a crop species. Although an impressive amount of progress has been achieved in linkage mapping, chickpea researchers are still awaiting a sufficiently dense genetic linkage map. Intraspecies polymorphism in cultivated chickpea for commonly used molecular markers is extremely low, therefore, interspecific crosses (*C. arietinum*×*C. reticulatum, C. arietinum*×*C. echinospermum*) have been exploited to develop genetic linkage maps with a higher number of markers (Gaur and Slinkard, 1990a, 1990b; Simon and Muehlbaur, 1997; Winter et al., 2000; Tekeoglu et al., 2002; Pfaff and Kahl, 2003). A genetic map constructed from an interspecific cross may not represent the true recombination distance map order of the cultivated genome due to uneven recombination of homoeologous chromosomes and distorted genetic segregation ratios (Flandez-Galvez et al., 2003a). A genetic linkage map constructed from a cross within the cultivated gene pool (Cho et al., 2002; Flandez-Galvez et al., 2003a), especially in the framework of

targeting traits of breeding importance, would therefore be more desirable. The genetic linkage maps developed to date with morphological, biochemical, and DNA-based molecular markers are summarized in Table 7.3.

The beginnings of linkage map development in chickpea were based on morphological and isozyme loci. However, their small numbers and the fact that expression of these markers is often influenced by the environment, makes them unsuitable for routine use. Thus, these maps were sparse and represented less than 30 loci mapped in a very small portion (about 250 cM) of the chickpea genome (Gaur and Slinkard, 1990a, 1990b; Kazan et al., 1993). Later developments on genetic linkage maps in chickpea that started with the work of Simon and Muehlbauer (1997) relied heavily on DNA-based molecular markers. In the lack of more recently available molecular markers, Simon and Muehlbauer (1997) employed RFLP and RAPD markers that show limited polymorphism in the cultivated species (Udupa et al., 1993; Banerjee et al., 1999).

Two independent interspecific-derived populations have been extensively employed for genetic linkage map development in chickpea (*C. arietinum* ICC 4958×*C. reticulatum* PI 489777 at the University of Frankfurt, Germany, and *C. arietinum* FLIP 84–92C×*C. reticulatum* PI 599072 at Washington State University, Pullman, WA), with the one developed in Germany being denser than the other. The 982 cM distance linkage map developed by Santra et al. (2000), which consisted of 89 RAPD, 17 ISSR, 9 isozyme, and 1 morphological markers was further expanded (Rajesh et al., 2002b; Tekeoglu et al., 2002) by integrating sequence tagged microsatellite sites (STMS) and resistance gene analog (RGA) loci. It covers a distance of 1175 cM. The STMS markers have proved very useful in linkage mapping and formed the basis for the map initially developed by Winter et al. (1999) that spanned a distance of 613 cM and consisted of 120 STMS markers. This map was greatly extended by Winter et al. (2000) and more recently by Pfaff and Kahl (2003) with the addition of 47 defense response (DR) genes. It covers a distance of 2500 cM arranged in 12 linkage groups and represents the most extensive linkage map in chickpea (Table 7.3). Relatively smaller maps derived from intraspecific (within *C. arietinum*) crosses, are also being developed (Cho et al., 2002; Flandez-Galvez et al., 2003a).

Combining these maps into a consensus linkage map is in progress and is being facilitated by the International Chickpea Genomics Consortium (Rajesh et al., 2004; http://www.icgc.wsu.edu/). Although steady progress is being made in extending the genetic linkage map in chickpea, there has been little effort in relating these maps to the eight pairs of chromosomes in the cultivated chickpea. Using flow-sorted chromosomes and molecular cytogenetic analysis, a recent report (Vlacilova et al., 2002) has linked STMS markers GAA46 and TS45 (linkage group 8 of the linkage map developed by Winter et al., 2000) to chromosome number 8 of chickpea.

Table 7.3 Genetic Resources Utilized in the Evolution of Genetic Linkage Map Development in Chickpea*

Publication	Mapping Population	Remarks (map size, markers)
Gaur and Slinkard, 1990a,	F_2, intraspecific *(C. reticulatum)*	200 cM, 7 linkage groups
1990b	F_2, interspecific *(C. arietinum×C. reticulatum)* and F2, interspecific *(C. arietinum×C. echinospermum)*	3 morphological and 26 isozymes
Kazan et al., 1993	F_2, intraspecific *(C. arietinum)*	257 cM, 8 linkage groups
	F_2, interspecific *(C. arietinum×C. reticulatum)* and F2, interspecific *(C. arietinum×C. echinospermum)*	5 morphological and 23 isozymes
Simon and Muehlbauer, 1997	F_2, interspecific *(C. arietinum×C. reticulatum)* and F2, interspecific *(C. arietinum×C. echinospermum)*	550 cM, 10 linkage groups
		9 morphological, 27 isozyme, 10
		RFLP and 45 RAPD
Winter et al., 1999	RIL, interspecific *(C. arietinum×C. reticulatum)*	613 cM, 11 linkage groups 120 STMS
Winter et al., 2000	Same as Winter et al.,1999	2078 cM, 16 linkage groups 118 STMS, 96 DAF, 70 AFLP, 37 ISSR, 17 RAPD, 8 isozyme, 3 cDNA, 2 SCAR and 3 morphological
Santra et al., 2000	RIL, interspecific *(C. arietinum×C. reticulatum)*	982 cM, 9 linkage groups 89 RAPD, 17 ISSR, 9 isozyme, and 1 morphological
Hajj-Moussa et al., 2001	RIL, interspecific *(C. arietinum×C. reticulatum)*	23 linkage groups RAPD, ISSR, and morphological
Rajesh et al., 2002b	Same as Santra et al., 2000	Addition of RGA Potkin 1–2 171 to Linkage group 5 of Santra et al. (2000)
Tekeoglu et al., 2002	Same as Santra et al., 2000	Extended map of Santra et al. (2000) to 1,175 cM

		50 STMS and 1 RGA was integrated
Cho et al., 2002	RIL, intraspecific *(C. arietinum)*	297 cM, 14 linkage groups 68 STMS, 34 RAPD, 4 ISSR, and 5 morphological
Pfaff and Kahl, 2003	Same as Winter et al. 1999; 2000	Incorporated 47 DR gene specific markers to Winter et al. (2000)
		2500 cM, 12 linkage groups
Flandez-Galvez et al., 2003a	F_2, intraspecific *(C. arietinum)*	535 cM, 8 linkage groups 51 STMS, 3 ISSR, 12 RGA
Collard et al., 2003b	F_2, interspecific *(C. arietinum*×*C. echinospermum)*	570 cM, 8 linkage groups 14 STMS, 54 RAPD, 9 ISSR, 6 RGA

* RIL: Recombinant Inbred Line, RFLP: Restriction Fragment Length Polymorphism, RAPD: Random Amplified Polymorphic DNA, STMS: Sequence Tagged Microsatellite Site, DAF: DNA Amplification Fingerprint, AFLP: Amplified Fragment Length Polymorphism, SCAR: Sequence Characterized Amplified Region, ISSR: Inter Simple Sequence Repeat, RGA: Resistance Gene Analog, DR: Defense Response.

7.7 GERMPLASM ENHANCEMENT

7.7.1 International Efforts in Chickpea Research

Systematic international efforts on chickpea research started after the establishment of two future harvest Centers of Consultative Group on International Agricultural Research (CGIAR). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was established in 1972, with its headquarters in Patancheru, India, and the International Center for Agricultural Research in the Dry Areas (ICARDA) was established in 1977, with its headquarters in Aleppo, Syria. ICRISAT works on both *desi* and *kabuli* chickpeas, while ICARDA concentrates on *kabuli* chickpea. In collaboration with National Agricultural Research Systems (NARS), these centers have made extensive efforts on collection, characterization, and conservation of chickpea germplasm. The genebank of ICRISAT has more than 17,000 accessions of the cultivated species and 136 accessions of 18 wild species; similarly the genebank of ICARDA has more than 12,000 accessions of the cultivated species and 260 accessions of 8 wild species. These precious assets held, in trust for humanity, by these centers are provided freely to research and development specialists around the world. More than 116,000 seed samples of germplasm accessions from ICRISAT and more than 19,000 from ICARDA have been disseminated to requesting NARS. Given the large number of accessions available in the genetic resource collection, it becomes a daunting task to identify germplasm that could be used in a crop improvement program. A chickpea core collection, which is a chosen subset of large germplasm collection that generally contains about 10% of the total accessions and represents the genetic variability of the entire germplasm collection, as

recently developed by Upadhyaya and Ortiz (2001) and Upadhyaya et al. (2001), should greatly facilitate global chickpea breeding and genetic improvement.

Fortunately, in chickpea, 15 genebank accessions supplied by ICRISAT and 17 supplied by ICARDA have been directly released as varieties by national authorities in different countries. Additionally, ICRISAT and ICARDA have been helping the chickpea breeding programs of NARS around the world by making available segregating materials and advanced breeding lines. A total of 50 varieties released in 10 countries are from ICRISAT-supplied breeding materials. Similarly, 97 varieties in 25 countries are from ICARDA-supplied breeding material.

7.7.2 Conventional Breeding Efforts

7.7.2.1 Breeding Methods

The breeding methods used in chickpea are not different from other self-pollinated food legumes. In the early phase of chickpea breeding, most varieties were developed through selection from the landraces collected in the country or through germplasm introduction, evaluation, and selection. Later, the emphasis gradually shifted to hybridization for increasing genetic variation in the breeding materials. Most recent varieties have been developed through hybridization.

Single, three-way, double, or multiple crosses are used, depending on the number of traits to be combined and their spread in the parents. It is well established that selection for yield in early segregating generations is not effective in chickpea because of its indeterminate growth habit. Thus, many scientists prefer to select crosses rather than plants within crosses in early segregating generations (F_2 and F_3). The pedigree method, earlier used at many institutes, including ICRISAT, is not practiced widely in its original form, because it is cumbersome and only a limited number of crosses can be handled by this method. The bulk method, variously modified, is now the most common selection method used after hybridization in chickpea. Single-seed descent (SSD) method, a modification of bulk method, is widely used for development of recombinant inbred lines (RILs) for genome mapping studies. Backcross method is commonly used to incorporate one or few traits from a germplasm line, sometimes a wild species, to a well-adopted variety. A population improvement method that involves intercrossing of selected plants in F_2s or F_3s has been suggested for legumes for enhancing the chances of recombination in segregating generations (Muehlbauer et al., 1988). van Rheenen et al. (1991) proposed a method called polygon breeding, whereby segregating populations and selections are shared and exchanged between breeders. This method has been used by ICRISAT in collaboration with some state agricultural universities in India.

Attempts have been made to define chickpea ideotypes for different growing conditions. In the drought-prone environments, traits that help plants to escape or tolerate drought should be considered in the ideotype. Saxena and Johansen (1990) suggested that an ideotype for drought-stress environments should have early maturity, a deep root system, and a smaller leaf size. In the Mediterranean climate, the crop experiences a cool and wet winter followed by rapid warming in spring, leading to terminal drought. Thus, the ideotype for the Mediterranean environments should

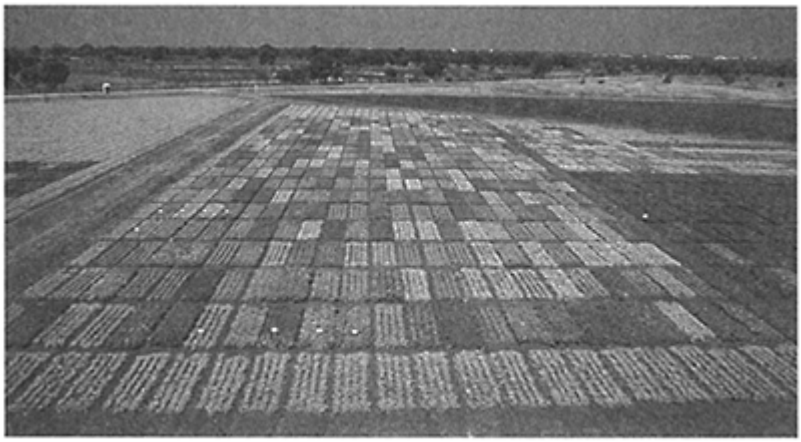

Figure 7.1 (See color insert following page 178) Breeding chickpea for early maturity at ICRISAT. Short-duration varieties have helped adaptation of chickpea in tropical environments.

include early flowering and tolerance to cold during flowering (Sedgley et al., 1990). It has also been suggested that a compact plant type with erect growth habit and short internodes could help resist excessive growth in high input conditions (Dahiya and Lather, 1990). A spontaneous mutant with short internodes and compact growth habit, E100YM, has been identified (Dahiya et al., 1984) and used in ideotype breeding. Promising progenies with compact growth habit have been obtained, which can be grown at high plant density (Lather, 2000).

7.7.2.2 Breeding for Tolerance to Abiotic Stresses and Widening of Adaptation

Drought is the most important constraint to yield in chickpea accounting for 40 to 50% yield reduction globally. Four approaches are being pursued: (1) high root mass, (2) smaller leaf area, (3) osmoticum adjustment, and (4) early-maturing short-duration varieties. Lines with a greater degree of drought tolerance have been developed by combining large root traits of ICC 4958 with fewer pinnules trait of ICC 5680 (Saxena, 2003). A recent screening of the mini-core collection has identified several other lines with large root traits (Krishnamurthy et al., 2003). Genotypic variation for osmotic adjustment has been observed in chickpea (Morgan et al., 1991; Leport et al., 1999; Abbo et al., 2002b). The heritability of osmotic adjustment has been found to be low (h^2 =0.20 to 0.33), indicating that gains from selection for increased osmotic adjustment are likely to be small (Abbo et al., 2002b). ICRISAT has placed a high emphasis on development of short-duration varieties (Figure 7.1), as these can escape terminal drought. The first

breakthrough was the development of an extra-short-duration *kabuli* variety, ICCV 2, from a multiple cross involving five parents (2 *kabuli* and 3 *desi*), which has helped the expansion of *kabuli* chickpea cultivation to tropical environments. Now ICRISAT has developed super-early chickpea lines (ICCV 96029, 96030) in *desi* chickpea, and these are being used extensively in crossing programs by NARS in India and Canada for development of early varieties.

Singh (1990) listed several advantages of winter chickpea in comparison to spring chickpea, including higher germination rate, less incidence of fusarium wilt, increased nitrogen fixation, better utilization of available moisture, mechanization of harvesting due to increased crop height, and increased protein and grain yield per hectare. The effects of low-temperature stress and its implications for chickpea improvement were reviewed recently by Croser et al. (2003b). Most chickpea cultivars are susceptible to chilling temperature at flowering (Croser et al., 2003b). The germplasm line ILC 8262, the mutant ILC 8617, and the breeding line FLIP87–82C were the best sources of cold tolerance in cultigen, with a consistent score of 3 (on a 1-to-9 scale) over years and locations (Singh et al., 1990, 1995). A number of varieties combining cold tolerance and resistance to ascochyta blight have been released in several countries from the breeding material supplied by ICARDA. A pollen selection method, as described by Clarke et al. (2004), has also been successfully used to transfer cold tolerance from ICCV 88516 (CTS 60543) to the popular varieties Amethyst and Tyson in Australia (Clarke and Siddique, 2004).

Legumes, in general, are sensitive to salinity, and within legumes, chickpea, faba bean, and field pea are more sensitive than other grain legumes. Saline soils are very common in West and Central Asia and Australia, where chickpea is widely grown. Soil salinity adversely affects germination, resulting in poor plant stand. Only salt tolerant cultivars can be grown successfully in soils having ECe higher than 4.0 dS/m. The levels of salinity tolerance identified in chickpea are low to moderate. Several tolerant sources have been identified in India and Pakistan (Singh and Singh, 1984; Dua and Sharma, 1995; Kathiria et al., 1997). The salt-tolerant lines CSG 88101and CSG 8927 identified by Dua and Sharma (1995) had lower Na^+ in root than the sensitive genotypes. A salinity tolerant *desi* chickpea variety Karnal Chana 1 (CSG 8963) has been released in India for salt-affected soils of northwestern parts.

7.7.2.3 Breeding for Resistance to Biotic Stresses

7.7.2.3.1 Diseases

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. and represented by several pathotypes, is a highly devastating foliar disease of chickpea in West and Central Asia, North Africa, North America, and Australia. The problem of fungal variability and the existence of races appear to be complex. A standard set of well-characterized genotypes, a common inoculation technique, and a well-defined disease-rating methodology should be used by workers who wish to determine the extent and distribution of variability in *A. rabiei* in different geographic regions (Haware, 1998). Considerable efforts have been made on identification of chickpea genetic resources resistant to AB and breeding for AB resistance. Multilocation evaluation of chickpea

germplasm indicated that *kabuli* germplasm generally shows higher resistance to AB than *desi* germplasm (Reddy et al., 1992; Haware et al., 1995). Extensive germplasm screening has identified many resistant *desi* and *kabuli* lines (Reddy and Singh, 1984; Singh et al., 1984b). Using the breeding material supplied by ICARDA and ICRISAT, more than 100 AB tolerant varieties have been released in 25 countries. The national breeding programs in the U.S., Canada, Australia, Pakistan, India, Europe, and Western Asia have also made good progress in development of AB-resistant varieties.

Fusarium wilt (FW) is the most important root disease of chickpea. It has been reported from almost all the chickpea-growing regions of the world. Seven races of FW have been reported worldwide (Phillips, 1988; Jimenez-Diazet, et al., 1989). Effective field, greenhouse, and laboratory procedures for resistance screening have been developed (Nene et al., 1981) (Figure 7.2), and good progress has been made in identifying sources of resistance (Haware et al., 1990; van Rheenen et al., 1992). A number of varieties with absolute resistance to FW are available in many countries. In most cases, the resistance to FW has been stable. Some of the varieties released in India about two decades ago, such as JG 315 and JG 74, still maintain a high level of resistance.

Botrytis gray mold (BGM), caused by the necrotrophic fungus *Botrytis cinerea* Pres., is an important foliar disease of chickpea in northern India, Nepal, Bangladesh, and Pakistan (Haware and McDonald, 1992) and has been reported from more than 15 countries (Nene et al., 1996), including Australia (Corbin, 1975) and North America (Kharbanda and Bernier, 1979). High levels of resistance have not been found in the cultivated species (Haware and Nene, 1982; Haware and McDonald, 1993). Some accessions with erect plant type, such as ICCL 87322 and ICCV 88510, were found to be less affected by the disease (Haware and McDonald, 1993).

Figure 7.2 A Fusarium wilt screening nursery at ICRISAT.

Three types of root rot diseases (dry root rot, collar rot, and phytophthora root rot) are known to affect chickpea. High soil moisture, the presence of undecomposed organic matter on the soil surface, low soil pH, and a high temperature favor collar rot. There has been limited research toward the identification of races of dry root rot, even though the existence of different races has been implicated (Than et al., 1991). Pundir et al. (1988) provided a list of 47 tolerant accessions available in the genebank of ICRISAT. A few germplasm lines and cultivars have been identified to have low to moderate levels of resistance. These include the cultivars SAKI 9516 (Dua et al., 2001), breeding lines RSG 130, 132, and 191 (Chitale et al., 1990), and germplasm accessions ICC 1696, ICC 4709, and ICC 14391 (Singh, personal communication, 2003). The breeding efforts have led to release of a number of moderately resistant varieties, such as ICCC 37, ICCV 10, JG 130, and WCG 1 (Dua et al., 2001). Phytophthora root rot, caused by *Phytophthora medicaginis* Hansen, is the major disease of chickpea in northern New South Wales and Queensland (Knights et al., 2003). Genotypic differences in resistance to Phytophthora root rot have been identified (Brinsmead et al., 1985; Dale and Irwin, 1991), and cultivars less susceptible to the disease, such as Jimbour, have been developed. The levels of resistance available in the cultivated species are low as compared with the wild species (Knights et al., 2003).

7.7.2.3.2 Root Nematodes and Insect Pests

The major nematodes known to affect chickpea are root knot nematodes (*Meloidogyne* spp.), cyst-forming nematodes (*Heterodera* spp.), and lesion nematodes (*Prathylenchus* spp.). None of the 8000 accessions of cultivated chickpea (7000 *kabuli* type and 1000 *desi* type) screened at ICARDA against cyst nematodes was found to have even a moderate level of resistance (Di Vito et al., 1988). However, good sources of resistance have been identified in the wild species (Robertson et al., 1995).

Pod borer (*Helicoverpa armigera* Hubner) is the most important insect pest of chickpea worldwide. Several techniques are available for screening of chickpea genotypes for resistance to pod borer (Sharma et al., 2003). Only low to moderate levels of resistance for pod borer have so far been identified in the cultivated and wild *Cicer* species (Lateef and Pimbert, 1990; Sharma et al., 2002). Unavailability of a high level of resistance to pod borer in the cultivated and cross-compatible wild species has been the major limitation in developing pod borer resistant varieties. Limited progress has been made in breeding for resistance to leaf miner (Malhotra et al., 1996; Robertson et al., 1995). Seed beetle or bruchid (*Callosobruchus* spp.) is the most important storage pest of chickpea. No source of resistance to bruchid has been identified in the cultivated species. Some of the accessions of wild species showed no damage by this insect (Robertson et al., 1996). It is yet to be established whether the resistance is due to seed morphology or chemical properties.

7.7.2.4 *Introgression of Economically Valuable Traits through Wide Crosses*

Interspecific hybridization has played an important role in genetic enhancement of many crop species by facilitating transfer of useful traits to the cultivated species from wild,

weedy forms. However, the progress in chickpea is still in its infancy (Croser et al., 2003a). Although the wild annual *Cicer* species have been extensively evaluated, their exploitation in chickpea breeding programs has not yet been widely realized. No perennial species has yet been successfully hybridized with the cultivated species.

The exploitation of the wild *Cicer* species in breeding programs has been limited to cyst nematode (*Heterodera ciceri* Vovlas, Greco & Di Vito) resistance (Di Vito et al., 1996; Singh et al., 1996; Erskine et al., 2001; Malhotra et al., 2002). Selections derived from the interspecific hybrid of *C. arietinum*×*C. echinospermum* and possessing resistance to root lesion nematode, enhanced phytophthora resistance, and moderate resistance to ascochyta blight are also being considered for release in Australia (Knights et al., 2002).

In addition to the genes for resistance and tolerance, heterosis for yield has been observed in populations from crosses between *C. arietinum, C. reticulatum,* and *C. echinospermum* (Jaiswal et al., 1987; Singh and Ocampo, 1993, 1997). While, most introgressions have involved the two crosscompatible species, *C. reticulatum* or *C. echinospermum,* Singh et al. (1994), and Verma et al. (1995) are the only ones reporting F_2 recombinants with a very large number of secondary branches, high pod number, and yield from a cross between *C. arietinum* and *C. judaicum,* or *C. pinnatifidum,* or *C. bijugum*. Some progress has also been made in producing early maturing segregants, following interspecific hybridization. Transgressive segregants for days to flowering have been isolated from the interspecific cross *C. arietinum*×*C reticulatum* (Singh et al., 1984a).

An interesting perspective has been brought forth by Abbo et al. (2002a), who recently presented evidence indicating that some wild *Cicer* relatives have a vernalization requirement. This may be useful in certain environments to avoid early flowering and consequent frost damage, etc.; however, it also can be an unfavorable trait in other environments. Introgression of genes from the wild species back into chickpea may result in reintroduction of these vernalization-sensitive alleles into the cultivated species and should be considered with caution in any hybridization program.

7.7.2.5 Mutation Breeding

Most studies on polymorphism of molecular markers in chickpea indicate presence of limited genetic variability in the cultivated species, and this has forced researchers to use interspecific crosses for genome mapping (Gaur and Slinkard, 1990a, 1990b; Kazan et al., 1993; Simon and Muehlbauer, 1997; Winter et al., 1999, 2000). Kenneth J.Frey (Iowa State University, Ames, IA) called chickpea "a recalcitrant crop species," as it has not been very amenable to genetic improvement in spite of extensive breeding efforts during the past three decades (van Rheenen et al., 1993). It was suggested that mutation breeding and interspecific hybridization should be used for increasing genetic variability and yield advancement of chickpea (van Rheenen et al., 1993).

A large variability is seen in chickpea germplasm for morphological traits, but it could be a reflection of the expression of a limited number of mutant genes, as a single mutant gene may cause marked changes in the appearance of the plant (Gaur and Gour, 2003). Though a variety of physical and chemical mutagens have been used, the most commonly used mutagen is γ-rays, with doses ranging from 10 to 40 kR. There are reports

suggesting that *kabuli* chickpeas are more sensitive to mutagens than the *desi* type (Khanna and Meharchandani, 1981; Kharkwal, 1998).

At least two institutes, the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan and the Indian Agriculture Research Institute (IARI), New Delhi, India, have had strong programs on mutation breeding for incorporating disease resistance for chickpea improvement. The first of the three *desi-type* Ascochyta blight-resistant varieties, CM 72 from the NIAB program, was released in 1983, which was then followed by CM 88 in 1994, and CM 98 in 1998 (Haq et al., 1999). A *kabuli* variety, CM 2000, from this program was released in 2000. In India, at least six chickpea varieties have been developed through mutation breeding. Of these, three were developed by IARI. The mutation breeding program of IARI has had a major focus on resistance to diseases. The varieties, Pusa 408 (Ajay) and Pusa 413 (Atul), were moderately resistant to AB, whereas Pusa 417 (Girnar) was resistant to wilt and moderately resistant to stunt and root rots (Kharkwal et al., 1988; Micke, 1988; Dua et al., 2001). Three other chickpea mutants, RS 11, RSG 2 (Kiran), and WCG 2 (Surya), were developed by agricultural universities (Micke, 1988; Dua et al., 2001).

Mutation breeding has also helped to improve the nutritional quality of chickpea. The Bangladesh Institute of Nuclear Agriculture, Mymensingh, obtained a high-yielding and high-protein mutant of chickpea cultivar Faridpur-1 through γ-rays induced mutation. The mutant had 20% higher yield and 20% higher protein than the parental cultivar Faridpur-7. The mutant was released as a cultivar by the name "Hyprosola" in 1981 by the National Seed Board of Bangladesh (Oram et al., 1987). These examples clearly indicate that mutation breeding has played important role in chickpea improvement. In addition to the release of 11 mutants as cultivars, several mutants have been used as parents in breeding programs.

7.7.2.6 Development of Hybrids Using Cytoplasmic Male Sterility (CMS) System

Male sterility has been reported in chickpea (Chaudhary et al., 1970; Sethi, 1979). Genetic studies indicated that the male sterility was under the control of a monogenic recessive gene (Chaudhary et al., 1970; Reddy and Reddy, 1997). It has also been possible to induce male sterility (up to 80% pollen sterility) through gametocide (Mathur and Lal, 1999).

Utilization of male sterility for hybrid seed production requires an efficient mechanism for pollen dispersal by the male parent and reception of pollen by the stigma of the male sterile line. This is the biggest hurdle in chickpea, where cross-pollination has been reported to be less than 1% (Tayyar et al., 1995) due to the cleistogamous nature of the flower. Thus, the availability of CMS and fertility restorer systems will not be enough in chickpea for production of hybrid seed. A significant change in floral morphology is needed in the A, B, and R lines. It basically requires open flowers in which stigma and anthers are not enclosed by the petals. A male sterile open flower mutant has been reported in chickpea (Pundir and Reddy, 1988). It may be used as female parent. A similar, but male fertile, mutant is needed for use as male parent. A fertile outwardly curved-wing mutant has been reported (Gaur and Gour, 2003), but the keel petal still encloses the anthers in this mutant.

The commercial cultivation of hybrid chickpea may still be far from reality, even if we have the appropriate CMS system and the required changes in floral morphology. It will be difficult to produce a large quantity of hybrid seed as each pod produces only one or two seeds, and seed-to-seed multiplication is notoriously low in chickpea and other cool season grain legumes, compared to cereals and canola. Most chickpea cultivation (95%) is in developing countries where the majority of farmers cannot afford expensive hybrid seed every year. As there is no immediate hope of utilization of a male sterility system for development of commercial hybrids in chickpea, apomixis can be explored as an alternative system for exploitation of heterosis in this important food legume. So far, however, there is no report detailing the occurrence of apomixis in chickpea.

7.7.3 Genetic Transformation

The advancement in recombinant DNA technology and availability of efficient transformation and regeneration systems in plants have made it possible to transfer genes from any organism to plants with optimized expression. Targeted transfer of genes from the wild *Cicer* species into the cultivated species would represent a very elegant application of transformation technology. The progress in molecular mapping and, to some extent, chickpea transformation, now considered a routine procedure in chickpea, has brought the application of this type of technology much closer to reality in chickpea (Fontana et al., 1993; Hamblin et al., 1998; Chakrabarty et al., 2000; Krishnamurthy et al., 2000; Sharma and Ortiz, 2000; Jaiwal et al., 2001; Senthil et al., 2004). The applicability of this technology will, however, depend on the identification of key genes, the number of genes conferring a particular character, and public acceptance of cultivars resulting from transformation technology. In chickpea, transgenic technology is being exploited primarily for insectpests and disease resistance, drought tolerance, and quality enhancement.

An efficient and reproducible tissue culture regeneration system is a prerequisite for development of transgenics. Many earlier studies on chickpea tissue culture encountered problems in rooting or establishment of plants in the soil. One option tried was to graft the *in vitro* germinated shoot on scions of pre-germinated seedling (Krishnamurthy et al., 2000), although such a tedious method would be inefficient for routine use. Novel rooting systems that give a rooting frequency of 90% or more have been reported. These involve either placing elongated shoots on a filter paper bridge immersed in liquid rooting medium (Jayanand et al., 2003) or placing nodal segments in an inverse polarity in a specially formulated tissue culture medium (Fratini and Ruiz, 2003).

Several studies have been conducted on transformation of chickpea during the past decade. Reports are now available on successful transformation of chickpea and regeneration of transformed plants. The most commonly used method has been the *Agrobacterium-mediated* transformation using embryo axes as explants (Fontana et al., 1993; Kar et al., 1996; Chakrabarty et al., 2000; Krishnamurthy et al., 2000; Chandra and Pental, 2003). However, bombardment with accelerated tungsten particles has also been successfully used (Hussain et al., 1997; Kar et al., 1997; Tewari-Singh et al., 2004). Except Kar et al. (1997), all these studies used only marker genes like *uidA* and *npt II* for transformation. Sommers et al. (2003) are of the opinion that, in addition to the limited availability of an efficient transformation protocol, a rapid and reliable selection strategy

has also impeded the uptake of this technology in chickpea improvement programs. An efficient and reliable nonantibiotic selection strategy using the phosphinothricin-acetyltransferase and aspartate kinase genes, has been recently developed for the production of transgenic chickpea (Tewari-Singh et al., 2004).

One of the areas, where traditional chickpea breeding methods have yet to make progress is the development of cultivars resistant to the devastating pest helicoverpa pod borer. It is simply because sources of a high level of resistance are not available in the cultivated or cross-compatible wild species. Thus, development of transgenic chickpea seems to be the only hope to host-plant resistance to this insect-pest. There is a report (Kar et al., 1997) on development of transgenic chickpea plants for *BtCry1A(C)* gene derived from the bacterium *Bacillus thuringiensis* (Bt). The transformation method used was particle bombardment of embryo axis (devoid of root and shoot meristems) from mature seeds. The transformation was confirmed through molecular analysis. Insect feeding assay indicated inhibition of development of feeding larvae. There is no further report available on field testing of these transgenics.

ICRISAT has produced transgenics for resistance to pod borer by using *BtCry1Ab* and *SbTi* (soybean trypsin inhibitor) genes. The molecular characterization and insect bioassays are currently ongoing (Sharma, personal communication, 2003). New constructs with combinations of different *Cry* genes are being developed in collaboration with CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) France, besides the artificial synthesis of some of these genes for optimal expressions in plants (Lavanya et al., 2003).

Research at the Scottish Crops Research Institute, U.K., has shown that immature raspberry fruit contains a polygalacturonase inhibitory protein (PGIP) effective against endopolygalactoseuronases produced constitutively by *Botrytis cinerea* (Johnston et al., 1993). Polygalactuseuronases are the key enzymes in the invasion of plant tissues by many facultative fungal pathogens. At ICRISAT, PGIP gene and other antifungal genes, such as chitinases and glucanases, are being introduced into chickpea for resistance to fungal diseases (Sharma and Ortiz, 2000). Efforts are also being made to identify and clone tissue-specific promoters for more controlled expression of these potential transgenes. A project funded under the Indo-Swiss Collaboration in Biotechnology (ISCB) is aimed at developing transgenic chickpea for tolerance to drought and low-temperature stresses by using genes with regulatory functions, such as drought responsive elements (DREs), and osmoregulation, such as *codA* and *P5CSF* (Sharma, personal communication, 2003). Research in progress in this area is expected to improve chickpea for the traits that are difficult to improve through traditional breeding methods. The applicability of this technology will, however, depend on the identification of key genes, the number of genes conferring a particular character, and public acceptance of cultivars resulting from transformation technology.

In addition, Sagare and Krishnamurthy (1991) have proposed fusion of protoplasts as an alternative means of enabling interspecific hybridization between widely related *Cicer* species. This technique is currently limited by the absence of protocols for the regeneration of whole plants from single cells or protoplasts in any of the *Cicer* species. Recent progress toward the development of a microspore culture system in *Cicer* (Croser et al., 1999; Lülsdorf et al., 2001; Croser, 2002; Croser and Lülsdorf, 2004) resulting in

embryogenesis from single-celled microspores in liquid culture may assist in the future development of this technique.

7.7.4 Molecular Marker-Assisted Selection

The underlying principle of molecular marker-assisted selection is that if a gene(s) is linked to an easily identifiable genetic marker, then it may be more efficient to select in a breeding program. This effectiveness, however, will depend on the strength of linkage of the marker to the locus controlling the character of interest. Such an approach can be the most effective means of enhancing the transfer of a desired gene(s) by a backcross program, reducing the linkage drag resulting from a wide cross, and for pyramiding genes and alleles of various combinations. The successful application of marker technologies, thus, requires (1) a dense saturated intraspecific map linking traits of agronomic importance to highly polymorphic, co-dominant markers in sufficiently close proximity to allow marker-assisted selection in offspring derived from intraspecific crosses, and (2) tailoring of markers and reactions in combination with high-thoroughput screening techniques to speed up and facilitate the application of marker technology.

The history of developing and identifying molecular markers for marker-assisted selection in chickpea, understandably reflects a mirror image of the increasing understanding of genetic linkage maps in the crop. The first of such markers was not identified until 1997 (Mayer et al., 1997), however, recent progress has resulted in many markers being identified (Table 7.4). Indeed, the developments in chickpea have lagged considerably behind those in cereals (Gupta et al., 1999). Other than the molecular markers identified for double podding trait (Cho et al., 2002; Rajesh et al., 2002a), leaf traits, and erect growth habit (Banerjee et al., 2001), only genes conferring resistance to fusarium wilt and ascochyta blight have been tagged. Most of the populations utilized in genetagging experiments have been derived from interspecific crosses with the wild annual species *C. reticulatum* and, to a limited extent, *C. echinospermum,* as well as some intraspecific crosses within the cultivated species. Among the various types of molecular markers that are available, the STMS markers are most amenable for multiplex PCR and electrophoresis, as shown for more than 600 STMS markers in soybean (Cregan et al., 1999; Narvel et al., 2000). In chickpea, the number of STMS markers is still too low for such applications. Marker development is in progress at the various research institutions working on chickpea, however, there is no application of a robust

Table 7.4 Potentially Useful Markers for Marker-Assisted Selection in Chickpea Improvement

Publication	Marker	Remarks
Mayer et al., 1997	RAPD marker UBC-170_{550}	7 cM from fusarium wilt resistance to race 1
Ratnaparkhe et al., 1998b	RAPD marker UBC-855_{500}	Linked in repulsion, 5.2 cM from fusarium wilt resistance to race 4
Ratnaparkhe, et al.,1998b	ISSR marker UBC-825_{1200}	5.0 cM from fusarium wilt resistance to race 4
Tullu et al., 1998	RAPD marker CS-27_{700}	9 cM from fusarium wilt resistance to race 1
Tullu et al., 1999	RAPD marker CS-27_{700}	Fusarium wilt resistance to race 4
Santra et al., 2000	2 RAPD marker	10.9 cM apart and flanking major QTL-1 for ascochyta blight resistance
	1 ISSR and 1 isozyme	5.9 cM apart and flanking major QTL-2 for ascochyta blight resistance
Banerjee et al., 2001	RAPD markers	Linked to QTL for leaf length, leaf width, and erect plant habit
Cho et al., 2002	PCR marker Tr44	7.8 cM from gene for double podding trait
	QTL marker Ta130s	Explaining 31% of the variation for seed number per plant
Rajesh et al., 2002a	STMS marker T-80	4.84 cM from gene for double podding trait
Benko-lseppon et al., 2003	SCAR marker R-2609–1	2.0 cM from fusarium wilt resistance to race 4
Collard et al., 2003b	QTL marker $CS44a_{1150}$	Significant QTL for seedling resistance to ascochyta blight
Flandez-Galvez et al., 2003b	6 QTL markers	For ascochyta blight resistance
Idnani and Gaur, 2003	Isozyme marker *Amy*	6.2 cM from leaf necrosis gene *(nec)*
Millan et al., 2003	19 RAPD, 1 ISSR markers	Distributed in an 18.8 cM region, and resistance to ascochyta blight,
	QTL marker	marker OPAC/1200 present most frequently
Rakshit et al., 2003	DAF marker OPS06–1	Significant QTL for ascochyta blight resistance

Rubio et al., 2003	RAPD marker $OPJ20_{600}$	6 cM from fusarium wilt resitance to race 0
Sharma et al., 2004	STMS markers TA96, TA27	0.6 cM from fusarium wilt resistance to race 3

molecular marker system for selection within a large breeding program. We anticipate that this technology is not far away, and it holds great potential for targeted introgression of genes from wild *Cicer* into chickpea.

7.8 SUMMARY

The rich diversity for various biotic and abiotic stresses present in the annual and perennial wild related species has to be exploited for genetic enhancement of chickpea. Wide hybridization is currently limited by reproductive barriers and simultaneous transfer of undesirable characters. The perennial species, in particular, are poorly evaluated and in many ways an unknown quantity. There is a clear need to develop methods for the maintenance and creation of *ex situ* collections of perennial species to enable their evaluation for important agronomic traits. An efficient embryo rescue protocol to enable crosses between chickpea and the more distantly related tertiary gene pool *Cicer* species will be crucial to the utilization of traits from these wild relatives. Recent advances in biotechnology techniques, including embryo rescue for interspecific hybrids, linkage mapping, marker-assisted breeding, and transformation, hold much promise for successful introgression of genes from wild annual species into cultivated chickpea in the future. The development and commercialization of genetically transformed chickpea, however, should be approached with caution, keeping in mind their possible rejection by consumers. There is little doubt that wild related *Cicer* species will play an important role in the future improvement of chickpea varieties. The task at hand is to identify the novel genes and find the best strategy to utilize them in a breeding program. The challenges facing the utilization of *Cicer* genetic resources are global and call for a wider collaborative research initiative for maximizing resource management and gains.

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