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Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species

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Abstract Random amplified polymorphic DNA markers were used to distinguish between nine different *Cicer* taxa representing the cultivated chickpea and eight other related annual wild species. Of the 75 random10-mer primers tested, only 8 amplified genomic DNA across all the species. A total of 115 reproducibly scorable RAPD markers were generated, all except 1 polymorphic, and these were utilized to deduce genetic relationships among the annual *Cicer* species. Four distinct clusters were observed and represented *C. arietinum*, *C. reticulatum* and *C. echinospermum* in first cluster followed by *C. chorassanicum* and *C. yamashitae* in the second cluster, while *C. pinnatifidum*, *C. judaicum* and *C. bijugum* formed the third cluster. *Cicer cuneatum* did not cluster with any of the species and was most distantly placed from the cultivated species. Except for the placement of *C. chorassanicum* and *C. yamashitae*, deduced species' relationships agreed with previous studies. In addition, species-diagnostic amplification products specific to all the nine species were identified. The results clearly demonstrate a methodology based on random-primed DNA amplification that can be used for studying *Cicer* phylogeny and chickpea improvement.

Key words *Cicer* · Species relationships · DNA fingerprinting · RAPD · Chickpea

Introduction

The genus *Cicer* L. belongs to the family Leguminosae, subfamily Papilionaceae, tribe Cicereae Alef and comprises 43 species: nine annuals, 33 perennials and one unspecified (van der Maesen 1987). The annual species consist of nine taxa: *C. arietinum* L., *C. bijugum* K.H. Rech., *C. chorassanicum* (Bge.) M. Pop., *C. cuneatum* Hoechst ex. Rich., *C. echinospermum* P.H. Davis, *C. judaicum* Boiss, *C. pinnatifidum* Jaub&Spp., *C. reticulatum* Ladiz. and *C. yamashitae* Kitamura. The cultivated species, *C. arietinum*, is grown from the Meditteranean region to Myammar, Australia, Ethiopia, Mexico, Chile, the cooler parts of the tropics and North America, while the annual *Cicer* species are distributed from Turkey to Central Asia and can be found in patches in Ethiopia, Sudan and Egypt (Robertson et al. 1997).

World chickpea germplasm lacks traits needed for effective improvement of the crop (Robertson et al. 1997). Furthermore, the genetic erosion of chickpea resources due to biotic and abiotic stresses as well as economic and strategic reasons is a persistent process. It has been recognized that interspecific hybridization will increase the variation and can be useful for plant breeding purposes in a "recalcitrant" crop like chickpea (Singh et al. 1994; van Rheenen et al. 1993). This is further exemplified by the utilization of two related wild species, *C. reticulatum* and *C. echinospermum*, in plant breeding programmes (Singh and Ocampo 1993, 1997; Singh et al. 1994).

Wild related species have been exploited for the transfer of genes for biotic and abiotic stress resistance to the cultivated species in several cultivated crops (Stalker 1980). However, wild species have not been exploited in food legumes, including chickpea. Annual *Cicer* species have been evaluated and useful germplasm identified for resistance to ascochyta blight, fusarium wilt, grey mold, cyst nematode, leaf minor, seed beetle and cold (Muehlbauer et al. 1994; Singh et al. 1998). Attempts to introgress useful genes from wild related species to the cultivated chickpea has been hampered by a limited understanding of species relationships (Tayyar and Waines 1996) as well as by strong post-fertilization crossability barriers (Ahmad et al. 1988). In the past, the question of species' relationships have been investigated by plant morphology (Robertson et al. 1997), karyotype analysis (Ocampo et al. 1992; Tayyar et al. 1994), crossability studies (Ladizinsky and Adler 1976; Ahmad et al. 1987; Singh and Ocampo 1993), seed storage protein fractionation (Ahmad and Slinkard 1992) and isozyme analysis (Kazan and Muehlbauer 1991; Ahmad et al. 1992; Tayyar and Waines 1996). All of these approaches suggest *C. reticulatum* to be the progenitor species of

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cultivated chickpea. Except for one study using restriction fragment length polymorphisms (RFLPs) (Patil et al. 1995), none of the various DNA-based diagnostic procedures have yet been utilized towards gaining an understanding species' relationships in the genus. The development and application of randomly amplified polymorphic DNA (RAPD) markers generated by the polymerase chain reaction (PCR) using arbitrary primers has resulted in alternative molecular markers for the detection of nuclear DNA polymorphisms. The technical simplicity of the RAPD technique has facilitated its use in the analysis of phylogenetic relationships, cultivar identification, genetic diversity, parentage determination and marker-assisted selection in several plant genera (Kawchuk et al. 1994; Abo-elwafa et al. 1995; Sharma et al. 1995a; Friesen et al. 1997; Wolff and MorganRichards 1998).

The objectives of the study presented here were (1) to assess the feasibility of the PCR-based DNA amplification in all the 9 annual *Cicer* species, (2) to detect interspecific genetic variation at the DNA level for understanding species relationships in the genus and (3) to compare phylogenetic relationships deduced by RAPD analysis with those inferred by other approaches.

Materials and methods

Plant material

Single accessions of each of the nine annual *Cicer* species, which included the cultivated species and eight wild related species, were utilized in this study (Table 1). Plants were grown in the field and DNA was isolated separately for five plants of each accession. Bulked DNA was used for RAPD analysis for the purpose of establishing species' relationships, and individual plant DNA was used for determining inter-plant genetic variation.

DNA isolation

DNA was isolated from young healthy leaves while plants were still in their vegetative stage by a modified method of Rogers and Bendich (1988). Leaf material was ground to a fine powder in liquid nitrogen, transferred to Eppendorf tubes and an equal volume of hot (65°C) 2 × CTAB was added to it. The mixture was incubated at 65° for 10 min, then mixed with an equal volume of chloroform/isoamyl alcohol (24 : 1) and centrifuged at 13,000 *g* for 5 min. The aqueous phase was transferred to another tube and 1/10 volume of 10 × CTAB was added, mixed and treated with an equal volume of chloroform/isoamyl alcohol. After 5 min of centrifugation, the supernatant was treated three times with chloroform/isoamyl alcohol. An equal volume of CTAB precipitation buffer was added to the aqueous phase to precipitate the DNA. The DNA pellet was rehydrated in high-salt TE buffer, and the DNA reprecipitated with 2 volumes of chilled ethanol. Finally, the DNA pellet was air-dried, rehydrated in 0.1 × TE buffer and treated with RNAse. DNA concentration was measured both by running it on agarose gels as well as by quantification with a UV spectrophotometer.

DNA amplification

Seventy-five 10-mer oligonucleotides (UBC #1-1 through UBC #1-75), obtained from Dr. John Carlson (University of British Columbia, B.C., Canada), were tested as single primers for the amplification of RAPD sequences. PCR was carried out in 25-µl reaction volumes containing 10 m*M* TRIS-HCl (pH 9.0), 50 m*M* KCl, 0.1% Triton X-100, 1.5 m*M* $MgCl_2$, 0.1 m*M* of each dATP, dCTP, dGTP, and dTTP, 2 m*M* primer, 0.5 units of *Taq* DNA polymerase (Promega) and 25 ng template DNA. The reaction mixture was overlaid with a drop of liquid wax. Amplifications were carried out in a thermo-cycler (Thermolyne) programmed for 40 cycles with an initial strand separation at 94°C, 1 min a 37°C and 2 min at 72°C. After 40 cycles, there was a final extension step of 5 min at 72°C.

Amplification products were electrophoresed in 1.8% agarose gels and detected by staining with ethidium bromide. Standard molecular-weight markers were also used in each electrophoretic run. UV *trans*-illuminated gels were photographed on polaroid film.

Data collection and analysis

The amplified bands were scored as 1 (present) or 0 (absent). Faintly stained bands that were not clearly resolved were not considered in the data collection. A data matrix of 1's and 0's was prepared from the 115 bands scored. The characters were treated as binary characters, and a pairwise genetic distance matrix was prepared based on the commonality of the amplified fragments. The similarity indices were calculated according to Gelfand by utilizing a computer programme developed by Angus et al. (1988). Gelfand's similarity indices for all pairwise combinations were calculated as:

$$S = 1 - \frac{1}{k}\sum_{t=1}^{k} |X_t - Y_t|.$$

Table 1 Accession, source and origin/collection site of the annual *Cicer* species used for RAPD analysis

Species	Accession	Source[a]	Origin/collection site
C. arietinum	PI 203142	USDA	Jordan
C. reticulatum	ICCW 9	ICRISAT	Mardin Province, Turkey (12 km to Savur, in/near Pinardere)
C. echinospermum	PI 599041	USDA	Diyarbakir, Turkey (20 km east of Karacadag)
C. bijugum	ICCW 7	ICRISAT	Savur, Turkey (14 km to Egypt)
C. judaicum	ICCW 36	ICRISAT	Gully, Israel (10 km east of Zinchon Yaakov)
C. pinnatifidum	ICCW 37	ICRISAT	Izmir, Turkey
C. chorassanicum	ILWC 23	ICARDA	Bamiyan, Afghanistan (45 km to Bamiyan, east of Bandi-Amir)
C. yamashitae	ILWC 3	ICARDA	Kabul, Afghanistan
C. cuneatum	ICCW 47	ICRISAT	Ethiopia

[a] USDA, United States Department of Agriculture, Plant Introduction Station, USA; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India; ICARDA, International Centre for Agricultural Research in Dry Areas, Aleppo, Syria

Table 2 DNA sequence of random decamer oligonucleotide primers used for annual *Cicer* species DNA amplification, their %G+C content and approximate size range of amplified product

Primer number	Primer DNA sequence	Percentage G+C content	Approximate range of fragment size (kb)
UBC #1-4	5′ CCTGGGTTCC 3′	70	0.4–1.2
UBC #1-19	5′ GCCCGGTTTA 3′	60	0.3–1.7
UBC #1-23	5′ CCCGCCTTCC 3′	80	0.4–2.2
UBC #1-28	5′ CCGGCCTTAA 3′	60	0.4–0.7
UBC #1-29	5′ CCGGCCTTAC 3′	70	0.5–1.8
UBC #1-30	5′ CCGGCCTTAG 3′	70	0.4–1.7
UBC #1-34	5′ CCGGCCCCAA 3′	80	0.3–1.5
UBC #1-70	5′ GGGCACGCGA 3′	80	0.3–2.1

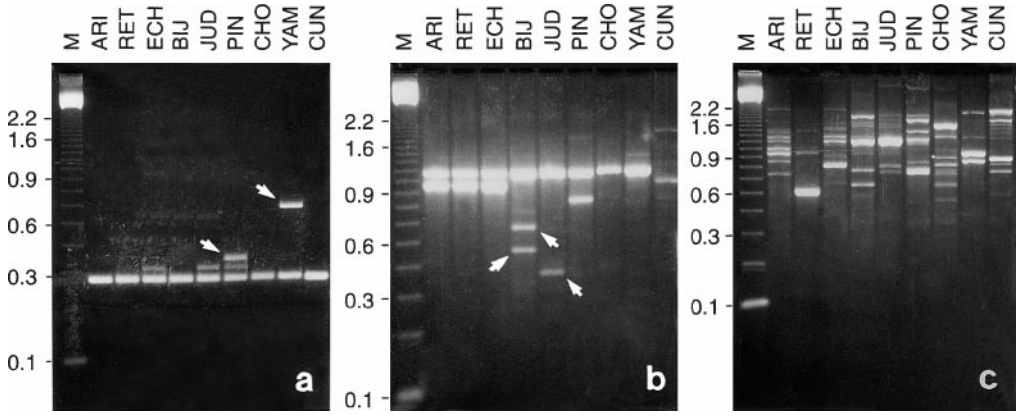

Fig. 1a–c Genomic DNA amplification pattern in nine annual *Cicer* species with 10-mer random primer UBC #1-28 **a**, UBC #1-4 **b** and UBC #1-23 **c**. Representative species-specific amplifications are indicated by *arrows*. *M* Molecular-weight marker, *ARI C. arietinum*, *RET C. reticulatum*, *ECH C. echinospermum*, *BIJ C. bijugum*, *JUD C. judaicum*, *PIN C. pinnatifidum*, *CHO C. chorassanicum*, *YAM C. yamashitae*, *CUN C. cuneatum*

where, X_t and Y_t represent the frequencies (0 or 1) of the '*t*'th band in species *X* and *Y*, respectively, and '*k*' is the total number of amplified bands observed.

The genetic relatedness among species was accomplished by clustering genetic distance data with Unweighted Pair Group Mean Average (UPGMA, Sneath and Sokal 1973) and represented in the form of a dendrogram.

Results

After testing several concentrations of $MgCl_2$ (0.5, 1.0, 1.5, 2.0 and 2.5 m*M*), template DNA (12.5, 25, 50, 75 and 100 ng per 25-µl reaction) and *Taq* DNA polymerase (0.2, 0.5, 0.8, and 1 U per 25-µl reaction), I was able to determine the optimum reaction mixture. In the present experiment for *Cicer* genomic DNA amplification, 1.5 m*M* $MgCl_2$, 25 ng template DNA and 0.5 U *Taq* polymerase per 25-µl reaction was found to give the most consistent and reproducible amplification results and, therefore, these parameters were kept constant for the entire experimental analysis.

A total of 75 decamer random primers (UBC #1-1 through UBC #1-75) were tested in each of the nine annual *Cicer* species accessions for examining RAPD pattern. Of these, only 8 primers amplified across all the species. The sequence of these successful primers, their %G+C content and the approximate amplified fragment size are presented in Table 2. In all, 115 reproducible fragments were amplified, although different primers amplified varying numbers of fragments (Fig. 1a–c, Table 2). Primer UBC #1-28 amplified the least number of bands (4), while UBC #1-19 amplified as many as 21 fragments. All, except 1 amplified band (UBC #1-28-300) of an approximate size of 0.3 kb, were polymorphic in the nine species studied. Thus, there was a high degree of genetic polymorphism present among the annual *Cicer* species. Varying numbers of bands were amplified in different species. The maximum number of bands (29) were observed in *C. reticulatum* and *C. echinospermum*, while *C.* chorassanicum showed the least number of amplified bands (18). No RAPD polymorphism was observed between different plants of a species' accession.

Pairwise genetic distances within *Cicer* species, as revealed by RAPD analysis, are shown in Table 3. It ranged from as low as 0.113, or 13 character differences, between *C. arietinum* and *C. reticulatum* to as high as 0.435, or 50 character differences between *C. cuneatum* and *C. echinospermum* (Table 3). Genetic distances between *C. arietinum* and other related species (without considering inter-relatedness among wild species) was the lowest for *C. reticulatum*, followed by *C. echinospermum*, and the largest distance was shown by *C. cuneatum*, *C. pinnatifidum* and *C. bijugum*.

Cluster analysis of the genetic distance values was performed to generate a dendrogram showing overall genetic relatedness between *Cicer* species (Fig. 2). Four distinct clusters could be identified. The first cluster

Table 3 Pairwise genetic distances between various *Cicer* species based on RAPD analysis

Species[a]	ARI	RET	ECH	BIJ	JUD	PIN	CHO	YAM	CUN
ARI	0.000								
RET	0.113								
ECH	0.287	0.313							
BIJ	0.339	0.400	0.400						
JUD	0.296	0.339	0.357	0.287					
PIN	0.339	0.365	0.365	0.348	0.304				
CHO	0.261	0.322	0.322	0.287	0.278	0.322			
YAM	0.278	0.357	0.287	0.322	0.330	0.357	0.261		
CUN	0.339	0.400	0.435	0.348	0.374	0.330	0.287	0.339	0.000

[a] ARI, *C. arietinum*; RET, *C. reticulatum*; ECH, *C. echinospermum*; BIJ, *C. bijugum*; JUD, *C. judaicum*; PIN, *C. pinnatifidum*; CHO, *C. chorassanicum*; YAM, *C. yamashitae*; CUN, *C. cuneatum*

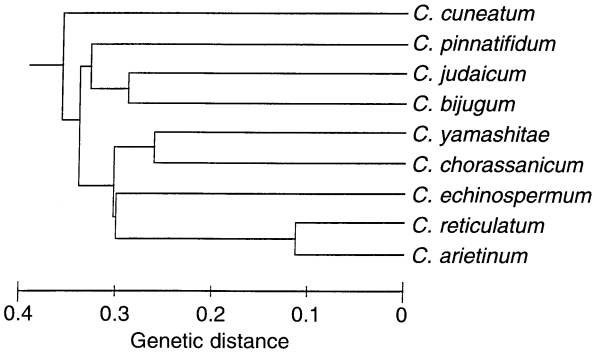

Fig. 2 Dendrogram of genetic relationships between nine annual *Cicer* species. *Scale* indicates the genetic distance derived from RAPD markers data

comprised *C. arietinum*, *C. reticulatum* and *C. echinospermum*, whereas *C. yamashitae* and *C. chorassanicum* formed the next higher genetic distance cluster. The third cluster was formed by *C. pinnatifidum*, *C. judaicum* and *C. bijugum*, while *C. cuneatum* did not cluster with any species and remained separate by itself (Fig. 2). Species placed within a cluster were more closely related to each other than to species present in different clusters. Thus, *C. arietinum*, *C. reticulatum* and *C. echinospermum* were genetically closer to each other than to other species, as revealed by RAPD markers at the DNA level.

Certain amplified bands were found to be specific to a given species, i.e. they were present in only one species but absent from the remaining species. These bands could be used for species identification purposes (Fig. 1a,b). There was only one species-specific diagnostic amplified band present in *C. arietinum* and *C. reticulatum*, while as many as 10 bands characterized *C. bijugum*. *Cicer judaicum* and *C. yamashitae* had 8 characteristic bands, while *C. cuneatum*, *C. echinospermum*, *C. pinnatifidum* and *C. chorassanicum* could be identified by 9, 7, 5 and 4 bands, respectively.

Discussion

RAPD markers represent an efficient and inexpensive way to generate molecular data and, thus, have been used successfully in various taxonomic and phylogenetic studies (Abo-elwafa et al. 1995; Sharma et al. 1995a; Friesen et al. 1997; Wolff and Morgan-Richards 1998). One of the objectives of this work was to assess the suitability and reliability of RAPD analysis for inferring phylogenetic relationships among *Cicer* species. Two criteria were considered to be relevant: first, the suitability of the technique to detect a high level of polymorphism that was reproducible and, second, the reliability of this approach to reconstruct phylogenies. Both of these were successfully met in the present study. The rationale for using RAPD analysis in the present study is the underlying assumption that genetic divergence between two DNA sequences is inversely correlated with the proportion of shared DNA restriction fragments. It was assumed that the same relationship exists for amplified DNA fragments while realizing, however, that restriction fragments are codominant in nature, whereas amplified fragments are primarily dominant. In the present study, the estimate of genetic distance measures and the clustering obtained by UPGMA have depicted the extent of genetic relatedness among annual *Cicer* species and their relationships.

A total of 115 RAPD markers were scored from only 8 primers that were used to amplify the annual *Cicer* species' genomic DNA. This data set provided a broader survey of the genome than was previously available in any single study (up to 30 isozyme loci, Kazan and Muehlbauer 1991) and, therefore, offers improved resolution in classifying annual *Cicer* species. Large genetic distances were observed between genetically different species, suggesting that it was clearly possible to detect relatively more polymorphism by random amplification than was detected using isozymes and a limited number of RFLP probes. The possibility that different-sized DNA fragments

contained homologous DNA sequences or that similar-sized products were non-homologous could not be ruled out based on the present study. In addition, no attempt was made to confirm that the amplified fragments were exclusively generated from nuclear DNA. Measures of similarity based on single-linkage cluster analysis were used to examine data.

The 75 primers used initially in this study differed greatly in their efficiency for revealing polymorphism. Many of them failed to amplify DNA. These primers may have special requirements for amplification in terms of PCR reagents or temperature profile. Since all of the reaction parameters were identical for all primers, differences in the clarity of the banding patterns are likely due to the specific requirements of a primer. Weeden et al. (1992) also reported variation in the efficiency of primer amplification. Fritsch et al. (1993) demonstrated the importance of the G+C content of primers on the PCR yield of detectable on amplified products. However, no correlation between G+C content and the clarity of the banding pattern was noted in the present study. In wheat, Devos and Gale (1992) found that the optimal concentration of DNA template differed with the primer used. They also speculated that the efficiency of a primer's amplification varied as a result of the absence of suitable priming sites in the genomic DNA. Consequently, PCR parameters should ideally be adjusted for each primer to optimize the amplification. The extra work, however, would considerably limit the RAPD technique by increasing the time required for generating markers. In this study, results were highly reproducible if the reaction were performed under the same amplification conditions (reagents and thermocycler parameters).

The presence of unique composite RAPD markers among the various *Cicer* species indicate the utility of the approach for fingerprinting purposes. RAPD fingerprinting has a number of potential applications and includes the determination of cultivar purity, efficient use and management of a genetic resource collection, particularly the identification of mis-labelled accessions, and the establishment of property rights (plant variety protection and patenting). This study represents only the first step in using DNA-based markers as a tool to implement studies of molecular systematics in this large genus of globally important food legumes. The inclusion of additional accessions and perennial species and the use of an increased number of primers may provide a greater resolution of the affinities among these taxa.

Previous reports on phylogenetic relationships among the annual *Cicer* species have indicated that *C. chorassanicum* and *C. yamashitae* are farther removed from *C. arietinum* as compared to *C. judaicum*, *C. pinnatifidum* and *C. bijugum* (Kazan and Muehlbauer 1991; Ahmad and Slinkard 1992: Ahmad et al. 1992; Tayyar and Waines 1996). However, the results obtained in present study, dealing with phylogenetic differences directly at the DNA level, disagree with those from previous studies and place *C. yamashitae* and *C. chorassanicum* in the cluster adjacent to the cluster containing *C. arietinum*. Such a relationship has not been shown before and could very well be true as it is based on a rather large number of molecular markers that directly reflect genetic differences at the DNA level. The implications of this new information is that perhaps it might be relatively less experimental to utilize these two species for cultivated chickpea improvement. Interestingly, *C. arietinum* has not yet been successfully crossed with either *C. chorassanicum* or *C. yamashitae*. On the other hand, hybrids of cultivated chickpea with *C. pinnatifidum*, *C. judaicum*, *C. bijugum* and *C. cuneatum* have been reported either by using in vitro immature seed culture (Singh and Singh 1989; Badami et al. 1997) or, surprisingly enough, without using any in vitro culture methods (Verma et al. 1990; Singh et al. 1994). However, the ability to interbreed may not be treated as the sole factor determining the degree of relatedness between species. Closely related species commonly lose the ability to interbreed and become genetically isolated due to chromosomal structural mutations (Tayyar and Waines 1996). In this respect, the position of *C. cuneatum* is very interesting. In both the present study and previous studies this species has been placed quite distant from all other species. It has a very distinct RAPD amplification pattern (present study), isozyme profile (Kazan and Muehlbauer 1991; Ahmad et al. 1992; Tayyar and Waines 1996), seed protein profile (Ahmad and Slinkard 1992) and peculiar morphological features (Robertson et al. 1997). It is the only species that has a climbing growth habit, leaves that end in branched tendril, elliptical to obtuse pods and round seeds that lack the characteristic beak (Robertson et al. 1997). In spite of these differences, an uncharacterized hybrid between this species and the cultivated chickpea has been reported (Singh and Singh 1989).

Studies using data from crossability, karyotype analysis, isozyme polymorphism and seed protein have indicated close genetic relationships between *C. arietinum*, *C. reticulatum* and *C. echinospermum* (Ladizinsky and Adler 1976; Ahmad and Slinkard 1992; Ocampo et al. 1992; Tayyar and Waines 1996). Such a relationship is further proven by the present study at the DNA level. In addition, the known overlapping of the geographical distribution of the two wild species, *C. reticulatum* and *C. echinospermum*, in south-east Turkey with that of the origin and domestication of the cultivated chickpea also supports their close relationship (van der Maesen 1987). Of these two wild related species, *C. reticulatum* was found to be closer to the cultivated species than *C. echinospermum* (Ladizinsky and Adler 1976; Ahmad et al. 1992; Patil et al. 1995). Although both species show a close morphological resemblance to the cultivated species, hybrids with *C. reticulatum* show normal chromosome pairing and

complete fertility (Ladizinsky and Adler 1976; Singh and Ocampo 1993), whereas those with *C. echinospermum* show a reciprocal translocation difference and partial to complete sterility (Ladizinsky and Adler 1976). Clearly, in being the closest relative to the cultivated species, *C. reticulatum* is the prime candidate for being the progenitor species.

The implication of *C. reticulatum* as the progenitor species, however, should be considered only at the secondary level, since it is believed that the transition from a perennial state to the annual state represents the primary level of steps in the evolution of chickpea (Ahmad et al. 1992). The question as to which of the perennial species is involved at the primary level remains largely unknown. Of the perennial species only two, *C. anatolicum* and *C. soongaricum*, have been studied for isozyme polymorphism in comparison with the annual species (Kazan and Muehlbauer 1991; Tayyar and Waines 1996). While Kazan and Muehlbauer (1991) reported a close relationship between *C. anatolicum*, *C. reticulatum*, *C. echinospermum* and the cultivated species, Tayyar and Waines (1996) contradicted such a close relationship between *C. anatolicum* and the other three annual species. Additionally, Tayyar and Waines (1996) also found a distant relationship between *C. soongaricum* and the three annual species. The discrepancy in the status of *C. anatolicum* between the two studies is rather surprising. However, as indicated by Tayyar and Waines (1996), it could have resulted from the small size of the sample surveyed in both studies, in addition to variation in the source of seeds, in the enzyme systems studied and in the procedure of sample analysis.

It appears that in the genus *Cicer* karyotypic similarities do reflect on genetic similarities and crossability (Ahmad 1989; Ocampo et al. 1992). Thus, the close relationship of cultivated chickpea, *C. reticulatum* and *C. echinospermum* is also supported by their karyotypic similarities. Among the perennial species, only *C. anatolicum* has been subjected to karyotypic analysis (Ahmad 1989) and the karyotypic similarity observed between this species and the above-mentioned three species has been corraborated by the genetic similarity of isozyme loci as reported by Kazan and Muehlbauer (1991). There is clearly a need to study the remainder of the perennial *Cicer* species before any conclusion can be drawn about the species involved at the primary level in the origin of cultivated chickpea.

An important goal in chickpea research remains the construction of detailed genetic linkage maps. However, in order to detect significant polymorphism, previous researchers using isozymes, RFLPs, isozymes and morphological markers have focussed on interspecific crosses of cultivated chickpea with the wild species *C. echinospermum* and *C. reticulatum* (Gaur and Slinkard 1991; Kazan et al. 1993; Simon and Muehlbauer 1997). In these cases of interspecific-derived mapping populations, the segregation of markers sometimes deviated significantly from expected ratios (Gaur and Slinkard 1991; Kazan; et al. 1993). Chickpea has been characterized as a species with poor genetic variability (Ahmad et al. 1992; van Rheenen 1992; Simon and Muehlbauer 1997), but it has recently been shown that ample genetic variability exists for short sequence tandem repeats (Weising et al. 1992; Sharma et al. 1995b). Thus, it is expected that the utilization of a large number of accessions and the increased resolution associated with the larger number of potential arbitrary primers available to the RAPD approach may provide sufficient markers to construct genetic linkage maps between carefully chosen cultivated chickpea accessions. Skewed segregation could thus be avoided and linkage data of more immediate relevance to chickpea breeding be provided.

References

Abo-elwafa A, Murai K, Shimada T (1995) Intra- and Inter-specific variations in *Lens* revealed by RAPD markers. Theor Appl Genet 90:335–340

Ahmad F (1989) The chromosomal architecture of *Cicer anatolicum* Alef., a wild perennial relative of chickpea. Cytologia 54:753–757

Ahmad F, Slinkard AE (1992) Genetic relationships in the genus *Cicer* L. as revealed by polyacrylamide gel electrophoresis of seed storage proteins. Theor Appl Genet 84:688–692

Ahmad F, Slinkard AE, Scoles GJ (1987) The cytogenetic relationship between *Cicer judaicum* Boiss. and *Cicer chorassanicum* (Bge.) M. Pop. Genome 29:883–886

Ahmad F, Slinkard AE, Scoles GJ (1988) Investigations into barrier(s) to interspecific hybridization between *Cicer arietinum* L. and eight other annual *Cicer* species. Plant Breed 100:14–18

Ahmad F, Gaur PM, Slinkard AE (1992) Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L. Theor Appl Genet 83:620–627

Angus RA, Hardwick SW, Cline GB (1988) A computer program that calculates various similarity coefficients between electropherograms. J Hered 79:74–75

Badami PS, Mallikarjuma N, Moss JP (1997) Interspecific hybridization between *Cicer arietinum* and *Cicer pinnatifidum*. Plant Breed 116:393–395

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:194–200

Friesen N, Fritsch R, Bachmann K (1997) Hybrid origin of some ornamentals of *Allium* subgenus Melanocrommyum verified with GISH and RAPD. Theor Appl Genet 95:1229–1238

Fritsch P, Hanson MA, Spore CD, Pack PE, Riseberg LH (1993) Constancy of RAPD primer amplification strength among distantly related taxon of flowering plants. Plant Mol Biol Rep 11:10–20

Gaur PM, Slinkard AE (1991) An isozyme gene map of chickpea. Int Chickpea Newsl 24:25–28

Kawchuk LM, Lynch DR, Hachey J, Bains PS, Kulcsar F (1994) Identification of a codominant amplified polymorphic DNA marker linked to the verticillium wilt resistance gene in tomato. Theor Appl Genet 89:661–664

Kazan K, Muehlbauer FJ (1991) Allozyme variation and phylogeny in annual species of *Cicer* (Leguminosae). Plant Syst Evol 175:11–21

Kazan K, Muehlbauer FJ, Weeden NW, Ladizinsky G (1993) Inheritance and linkage relationships of morphological and isozyme loci in chickpea (*Cicer arietinum* L.). Theor Appl Genet 86:417–426

Ladizinsky G, Adler A (1976) Genetic relationships among the annual species of *Cicer* L. Theor Appl Genet 48:197–203

Muehlbauer FJ, Kaiser WJ, Simon CJ (1994) Potential for wild species in cool season food legume breeding. Euphytica 73 : 109–114

Ocampo B, Venora G, Errico A, Singh KB, Saccardo F (1992) Karyotype analysis in the genus *Cicer*. J Genet Breed 46 : 229–240

Patil PB, Vrinten PL, Scoles GJ, Slinkard AE (1995) Variation in the ribosomal RNA units of the genera *Lens* and *Cicer*. Euphytica 83 : 33–42

Robertson LD, Ocampo B, Singh KB (1997) Morphological variation in wild annual *Cicer* species in comparison to the cultigen. Euphytica 95 : 309–319

Rogers SO, Bendich AJ (1988) Extraction of DNA from plant tissues. In: Glevin SB, Schilperoort RA (eds) Plant molecular biology manual. Kluwer Academic Publ, Dordrecht, pp 1–10

Sharma SK, Dawson IK, Waugh R (1995a) Relationships among cultivated and wild lentils revealed by RAPD analysis. Theor Appl Genet 91 : 647–654

Sharma PC, Winter P, Bunger T, Huttel B, Weigand F, Weising K, Kahl G (1995b) Abundance and polymorphism of di-, tri- and tetra-nucleotide tandem repeats in chickpea (*Cicer arietinum* L.). Theor Appl Genet 90 : 90–96

Simon CJ, Muehlbauer FJ (1997) Construction of a chickpea linkage map and its comparison with maps of pea and lentil. J Hered 88 : 115–119

Singh KB, Ocampo B (1993) Interspecific hybridization in annual *Cicer* species. J Genet Breed 47 : 199–204

Singh KB, Ocampo B (1997) Exploitation of wild *Cicer* species for yield improvement in chickpea. Theor Appl Genet 95 : 418–423

Singh KB, Malhotra RS, Halila MH, Knights EJ, Verma MM (1994) Current status and future strategy in breeding chickpea for resistance to biotic and abiotic stresses. Euphytica 73 : 137–149

Singh KB, Ocampo B, Robertson LD (1998) Diversity for abiotic and biotic stress resistance in the wild annual *Cicer* species. Genet Res Crop Evol 45 : 9–17

Singh RP, Singh BD (1989) Recovery of rare interspecific hybrids of gram *Cicer arietinum* × *C. cuneatum* L. through tissue culture. Curr Sci 58 : 874–876

Sneath PHA, Sokal RR (1973) Numerical taxonomy. The principles and practice of numerical classification. W.H. Freeman, San Francisco

Stalker HT (1980) Utilization of wild species for crop improvement. Adv Agron 33 : 111–147

Tayyar RI, Waines JG (1996) Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation. Theor Appl Genet 92 : 245–254

Tayyar RI, Lukaszewski AJ, Waines JG (1994) Chromosome banding patterns in the annual species of *Cicer*. Genome 37 : 656–663

van der Maesen LJG (1987) Origin, history and taxonomy of chickpea. In: Saxena MC, Singh KB (eds) The chickpea. CAB Int Publ, UK, pp 11–34

van Rheenen HA (1992) Biotechnology and chickpea breeding. Int Chickpea Newsl 26 : 14–17

van Rheenen HA, Pundir RPS, Miranda JH (1993) How to accelerate the genetic improvement of a recalcitrant crop species such as chickpea. Curr Sci 65 : 414–417

Verma MM, Sandhu JS, Rrar HS, Brar JS (1990) Crossability studies in different species of *Cicer* (L.). Crop Improv 17 : 179–181

Weeden NF, Timmerman GM, Hemmat M, Kneen BE, Lodhi MA (1992) Inheritance and reliability of RAPD markers. In: Application of RAPD technology to plant breeding. Crop Science Society of America, Madison, Wis., pp 12–17

Weising K, Kaemmer D, Weigand F, Epplen JT, Kahl G (1992) Oligonucleotide fingerprinting reveals various probe-dependent levels of informativeness in chickpea (*Cicer arietinum*). Genome 35 : 436–442

Wolff K, MorganRichards M (1998) PCR markers distinguish *Plantago* major subspecies. Theor Appl Genet 96 : 282–286