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Genetic relationships among perennial and annual *Cicer* species growing in Turkey assessed by AFLP fingerprinting

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Abstract AFLP markers were used to assess genetic relationships among *Cicer* species with distribution in Turkey. Genetic distances were computed among 47 *Cicer* accessions representing four perennial and six annual species including chickpea, using 306 positions on AFLP gels. AFLP-based grouping of species revealed two clusters, one of which includes three perennial species, *Cicer montbretii*, *Cicer isauricum* and *Cicer anatolicum*, while the other cluster consists of two subclusters, one including one perennial, *Cicer incisum*, along with three annuals from the second crossability group (*Cicer pinnatifidum*, *Cicer judaicum* and *Cicer bijugum*) and the other one comprising three annuals from the first crossability group (*Cicer echinospermum*, *Cicer reticulatum* and *Cicer arietinum*). Consistent with previous relationship studies in the same accession set using allozyme and RAPD markers, in AFLP-based relationships, *C. incisum* was the closest perennial to nearly all annuals, and *C. reticulatum* was the closest wild species to *C. arietinum*. Cluster analysis revealed the grouping of all accessions into their distinct species-clusters except for *C. reticulatum* accessions, ILWC247, ILWC242 and TR54961; the former was found to be closer to the *C. arietinum* accessions while the latter two clustered with the *C. echinospermum* group. Small genetic distance values were detected among *C. reticulatum* accessions (0.282) and between *C. reticulatum* and *C. arietinum* (0.301) indicating a close genetic similarity between these two species. Overall, the AFLP-based genetic relationships among accessions and species were congruous with our previous study of genetic relationships using allozymes. The computed level of AFLP variation and its distribution into within and between *Cicer* species paralleled the previous report based on RAPD analyses. AFLP analysis also confirmed the presence of the closest wild relatives and previous projections of the origin of chickpea in southern Turkey. Results presented in this report indicate that AFLP analysis is an efficient and reliable marker technology in determination of genetic variation and relationships in the genus *Cicer*. Obviously, the use of AFLP fingerprinting in constructing a detailed genetic map of chickpea and cloning, and characterizing economically important traits would be promising as well.

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Introduction

The genus *Cicer* (Fabaceae), containing cultivated chickpea (*Cicer arietinum* L.) along with 42 wild species, is represented with 11 wild species in Turkey (Davis 1973; Van der Maesen 1987). As consistent with the distribution of the closest wild relatives, southeastern Turkey has been recognized as the primary center of origin for chickpea (Ladizinsky and Adler 1976; Van der Maesen 1987; Singh 1997). Thus, the chickpea germplasm diversity and genetic relationships in the genus *Cicer* in Turkey warrants investigation.

Description of relationships and genetic diversity in the genepools of crop species is a pre-requisite for crop improvement studies. Molecular markers have been the major tools in addressing these issues of plant genetics (Karp et al. 1997; Westman and Kresovich 1997; Kumar 1999). The last decade witnessed the development of a number of new PCR-based DNA fingerprinting techniques, which appear to be more efficient and reliable. AFLP fingerprinting (Zabeau and Vos 1993) is one of which has found wide-spread application in numerous studies ranging from genetic mapping to diversity surveys in plants and other organisms (Hill et al. 1996; Lu et al.

1996; Sharma et al. 1996; Heun et al. 1997; Hongtrakul et al. 1997; Eujayl et al. 1998; Pejic et al. 1998; Mueller and Wolfenbarger 1999; Masiga et al. 2000). AFLP fingerprinting combines both robustness and reproducibility of classical restriction fragment-length polymorphism (RFLP, Botstein et al. 1980)-based fingerprinting with no prior sequence requirement and the abundance of PCR-based fingerprinting with the arbitrary primers such as random amplified polymorphic DNA (RAPD) (Williams 1990), DNA amplification fingerprinting (DAF) (Caetano-Anollés et al. 1991) and arbitrarily primed-PCR (AP-PCR) (Welsh and McClelland 1991). AFLP fingerprinting is considerably informative allowing the survey of variation in more than 50 co-amplified restriction fragments in each AFLP reaction. The complexity observed in banding patterns appears to be related to both genomic complexity of the organism under study, and combination, composition and the number of selective nucleotides employed at the 3′ end of the primers.

Several groups have studied the genetic diversity and relatedness among annual *Cicer* species by means of hybridization (Ladizinsky and Adler 1976), and the electrophoresis of the seed storage proteins and isozymes (Kazan and Muelbauer 1991; Ahmad and Slinkard 1992; Ahmad et. al. 1992; Labdi et al. 1996; Tayyar and Waines 1996). Recently, our analysis of allozyme variation at 12 loci in a germplasm collection, representing six annual and four perennial *Cicer* species, also confirmed the groupings given in the cited studies for the six annual species, and revealed the relationships among four perennials with these annual species. In addition, this study suggested that *Cicer incisum* is the closest perennial to six annual species (Sudupak and Kence 2002). In a number of studies, DNA-based approaches were utilized in *Cicer* and showed a considerable amount of microsatellite variation present in chickpea and its two close relatives, while the levels of RAPD and RFLP variation generally paralleled the allozyme variation (Weising et al. 1992; Sharma et al. 1995; Simon and Muehlbauer 1997; Udupa et al. 1999). Recently, RAPD markers were employed to determine phylogenetic relationships in the genus *Cicer* (Ahmad 1999; Iruela et al. 2002; Sudupak et al. 2002). Sudupak et al. (2002) have studied the genetic relationships with RAPD markers using nearly the same set of *Cicer* accessions. Groupings in that study indicated that *Cicer echinospermum*, *Cicer reticulatum* and *C. arietinum* are closely related species forming a distinct group in the dendrogram, while four perennial (*Cicer montbretii*, *Cicer isauricum*, *Cicer anatolicum* and *Cicer incisum*) and three annual species (*Cicer judaicum*, *Cicer bijugum*, and *Cicer pinnatifidum*) clustered together and were differentiated from this group. Consistent with the allozyme-based study, relationships inferred from RAPD analysis also suggested that *C. incisum* is the closest species to annuals, and *C. reticulatum* was the closest wild species to chickpea. The goal of the present study was to use AFLP markers to assess the intra and interspecific genetic diversity and relationships among accessions of four perennial and six annual species found in Turkey.

Materials and methods

Plant material

The plant material used in this study was essentially the same accession set used in RAPD analysis for our previous work (Sudupak et al. 2002). Only four accessions (TR54963, ILWC242, Desi and İzmir 92) were added to that set. *C. reticulatum* accession TR54963 was collected from Siirt province by the Aegean Agricultural Research Institute, İzmir, Turkey (AARI), while the locality of ILWC242 was from Adıyaman province and provided by the International Center for Agricultural Research in Dry Areas (ICARDA), Aleppo, Syria. The chickpea in the collection was represented by six Turkish chickpea varieties, five of which were Kabuli type (Eser 87, Aydın 92, Diyar 95, İzmir 92 and Canitez 89) and one Desi type provided by the Anatolian Agricultural Research Institute, Eskişehir, Turkey. The list and other details regarding accessions are not given here.

A single genotype from each of 47 accessions representing ten *Cicer* species; four perennials (*C. montbretii*, *C. isauricum*, *C. anatolicum*, and *C. incisum*) and six annuals (*C. judaicum*, *C. bijugum*, *C. pinnatifidum* and *C. echinospermum*, *C. reticulatum*, and *C. arietinum*) was subjected to AFLP analysis. DNA extraction from *Cicer* genotypes were carried out according to the CTAB DNA extraction procedure using the youngest leaves of the seedlings, as described in Sudupak et al. (2002). DNA concentration in samples was determined by running on a 1% agarose gel with 1 µg of *Hin*dIII-digested λ DNA.

AFLP procedure

AFLP protocol was performed as described in Vos et al. (1995) using both an AFLP analysis system I kit from Invitrogen (GIBCO BRL, Cat nos. 10544-013, 10483-014) and commercially synthesized AFLP adapter and primer sets (Qbiogene, Evry, France). The amount of genomic DNA used for template preparation was approximately 250 ng. Each DNA sample was digested with 2.5 units of both *Eco*RI and *Mse*I, and adapters were ligated to the resulting restriction-fragment ends. Preamplification reactions were carried out in a Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany) using 50 ng of each *Eco*RI and *Mse*I primers with a single selective nucleotide at their 3′ end (E-A and M-C) in a 50-µl reaction cocktail containing 10 mM of Tris-HCI, pH 8.8, 1.5 mM of $MgCI_2$, 50 mM of KCI 200 µM of each dNTPs, 1 U of*Taq* DNA polymerase (MBI, Fermentas) and 5 µl of ten-times-diluted ligation mixture as a template. The time and temperature profile of the cycles were 30 s at 94°C, 60 s at 56°C, 60 s at 72°C for 20 cycles. Following preamplification, the reaction mixtures were diluted 50 times with TE buffer and stored at −20°C. Selective amplification reactions were carried out using 5 ng of *Eco*RI and 30 ng of *Mse*I primers having three selective nucleotides at their 3′ end in a 20-µl reaction mixture, containing the same components and amounts as in the preamplification step except for 0.5 U of *Taq* DNA polymerase (MBI, Fermentas) and 5 µl of the diluted preamplification product as a template. PCR in selective amplification was performed for one cycle 30 s at 94°C, 30 s at 65°C, and 60 s 72°C followed by 12 cycles of touch-down phase in that the annealing temperature was lowered 0.7°C in each cycle. This was followed by a further 24 cycles with a time/temperature profile of 30 s at 94°C, 30 s at 56°C, and 60 s at 72°C. Following PCR, products mixed with the loading dye (a 2:1 ratio containing 98% formamide, 10 mM EDTA, 0.025% bromophenol blue and 0.025% xylene cyanol), denatured for 3 min at 90°C, and 4 µl was loaded onto pre-heated 4.5% denaturing polyacrylamide gels containing 7 M urea. Samples were electrophoresed in 1×TBE (89 mM Tris borate and 1 mM EDTA, pH 8.2) for 2 h at 50 W until xylene cyanol

has migrated for two-thirds of the gel. AFLP fingerprints were visualized by silver staining as described in Bassam et al. (1991) using the Promega silver-staining kit. Dried gels were pictured using a digital gel documentation system (EDAS290LE, Eastman Kodak Company, NY), and images were processed in an IBM-PC.

A total of 80 combinations were screened on a sample of accessions containing one from each species, and three of the informative combinations were used in the measurement of AFLP variation and the determination genetic relationships.

Data analysis

Screening 47 *Cicer* accessions with three primer combinations yielded 306 gel positions (loci). Each locus was treated as a separate character, and the amplification products were scored as either 1 (present) or 0 (absent) and compiled into a binary data matrix of 47×306. Computations of several population-genetic parameters from the data matrix were carried out using the POPGENE program (Ver. 1.32, Yeh et al. 1997). Computations of Nei and Li's (1979) coefficients and cluster analysis were performed using the MVSP-pc (Ver. 3.1 h, 2000; Kovach Computing Services, Wales, UK). Before the cluster analysis, coefficients in the similarity matrix were converted into genetic distances (GD) using the formula;

$$GD_{ab} = -\ln(S_{ab}),$$

where S_{ab} is Nei and Li's (1979) genetic similarity between accessions a and b defined as $S_{ab}=2N_{ab}/N_a+N_b$, where N_{ab} is the number of common bands present in accessions a and b, and N_a and N_b are the sum of the scored bands in accessions a and b, respectively. The distance matrix was then used for cluster analysis of accessions using the unweighted pair-group method using the arithmetic average (UPGMA) procedure. Nei and Li's (1979) genetic distances among species were computed by averaging pairwise-distance coefficients among the respective accessions for each species, and the same procedure as in accessions was utilized to construct a dendrogram for species.

Results

Screening 47 *Cicer* genotypes with three primer combinations revealed PCR products at 306 gel positions detected with silver staining (Table 1). In the data

Table 1 Primer combinations and the number of gel positions scored

Primer pairs	Number of gel positions scored
E-ACT/M-CAC	111
E-ACC/M-CTA	114
E-ACG/M-CCA	81
Total	306

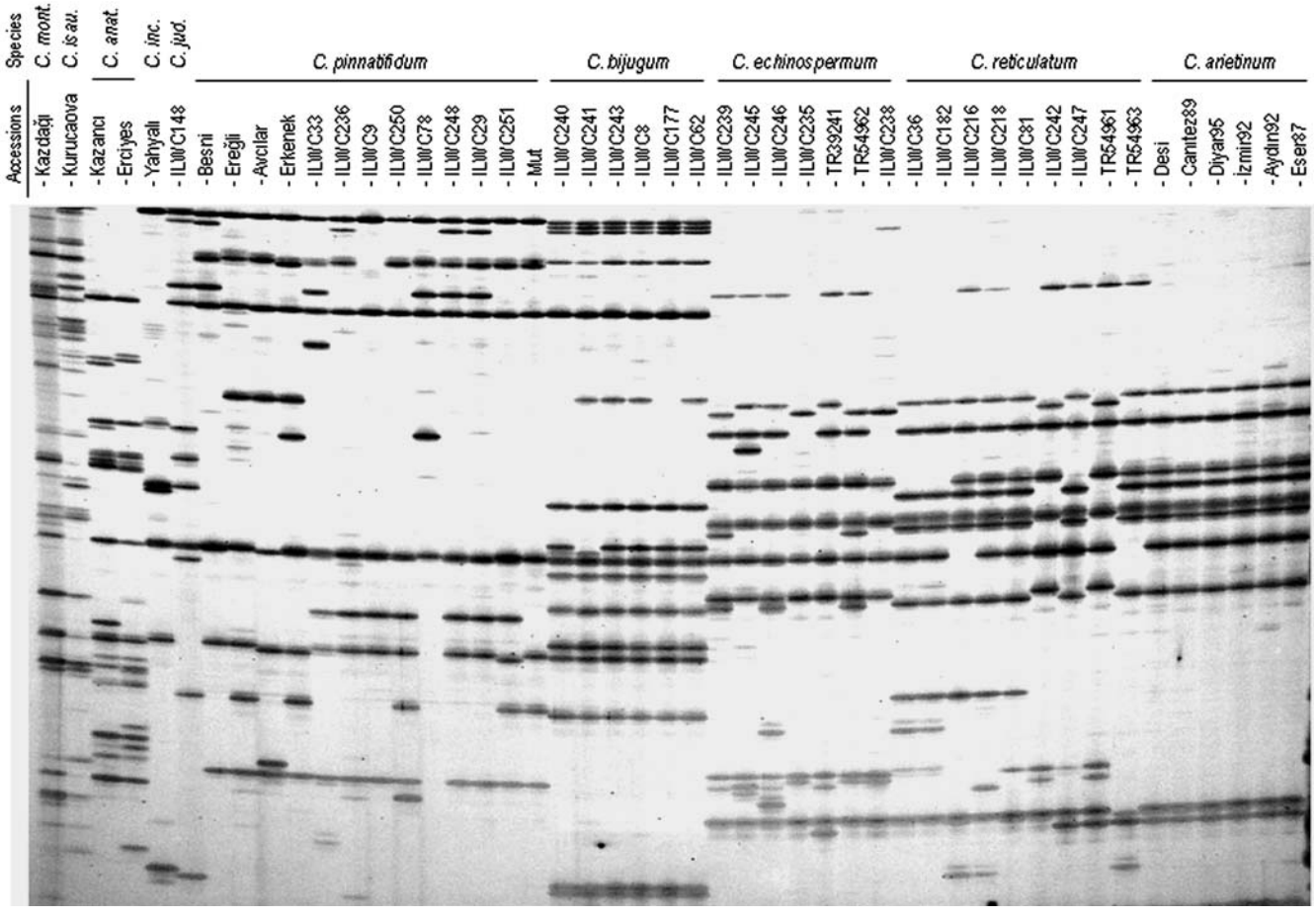

Fig. 1 AFLP amplicon profiles detected in *Cicer* accessions using the primer combination, E-ACT/M-CAC. Only the middle portion is given in the figure; upper and lower 10 cm of the gel are not shown

Table 2 AFLP-based genetic variability computed for six *Cicer* species

Species	Number of accessions	Combinations	Number of polymorphic bands	Percent of polymorphic loci	Mean gene diversity[a]
C. anatolicum	2	E-ACT/M-CAC	7	6.31	0.032Â±0.120[b]
		E-ACC/M-CTA	8	6.96	0.035Â±0.130
		E-ACG/M-CCA	0	0.0	0.000Â±0.000
		For 306 AFLPs	15	4.9	0.025Â±0.108
					G_{ST}=0.86
C. pinnatifidum	13	E-ACT/M-CAC	22	19.82	0.0584Â±0.130
		E-ACC/M-CTA	37	32.17	0.1062Â±0.170
		E-ACG/M-CCA	21	26.25	0.0680Â±0.130
		For 306 AFLPs	80	26.14	0.0789Â±0.149
					G_{ST}=0.56
C. bijugum	6	E-ACT/M-CAC	5	4.50	0.0185Â±0.087
		E-ACC/M-CTA	8	6.96	0.0290Â±0.109
		E-ACG/M-CCA	2	2.50	0.0090Â±0.058
		For 306 AFLPs	15	4.90	0.0200Â±0.089
					G_{ST}=0.88
C. echinospermum	7	E-ACT/M-CAC	14	12.61	0.0449Â±0.125
		E-ACC/M-CTA	13	11.30	0.0390Â±0.115
		E-ACG/M-CCA	9	11.25	0.0378Â±0.110
		For 306 AFLPs	36	11.76	0.0408Â±0.117
					G_{ST}=0.77
C. reticulatum	9	E-ACT/M-CAC	22	19.82	0.0770Â±0.159
		E-ACC/M-CTA	22	19.13	0.0588Â±0.129
		E-ACG/M-CCA	19	23.75	0.0747Â±0.147
		For 306 AFLPs	63	20.59	0.0696Â±0.145
					G_{ST}=0.62
C. arietinum	6	E-ACT/M-CAC	2	1.80	0.0065Â±0.049
		E-ACC/M-CTA	2	1.74	0.0048Â±0.037
		E-ACG/M-CCA	3	3.75	0.0125Â±0.065
		For 306 AFLPs	7	2.29	0.0074Â±0.050
					G_{ST}=0.96
Total	43		231	75.49	0.1812Â±0.175
For 47 accessions			305	99.67	0.1934Â±1597

[a] Nei's (1973) mean gene diversity
[b] Standard deviation

collection, each gel position was treated as a bi-allelic locus, and the products having the same size were considered to be homologous (same allele). Comparisons of the prescreening amplification patterns with screening gels revealed extremely high reproducible amplicon profiles with considerably high multiplex ratios. The number of amplified loci among primer combinations varied from 81 to 114 (Table 1) with the approximate fragment sizes ranging from 50 to 600 bp including adapter sequences. Polymorphism among species was common (99.7%) and only one locus was monomorphic across all accessions. A number of fragments were found to be species specific, and the majority of the primer combinations revealed banding patterns with which *Cicer* species could be differentiated. An example of AFLP amplicon profiles in *Cicer* accessions obtained using the primer combination, E-ACT/M-CAC is given in Fig. 1.

Genetic variability in terms of the percent of polymorphic loci, and the mean gene diversity for each primer combination and for 306 AFLP markers in the species, which were represented with more than one accession in the analysis, is given Table 2. Among the species, *C. pinnatifidum* was the most polymorphic one followed by *C. reticulatum*. *C. arietinum* had the least genetic variation. Although there were some exceptions, AFLP variation computed for each primer combination, overall, revealed similar levels of variation in each species (Table 2). In general, while relatively low AFLP variation was detected within species, it was considerably high among species as indicated by the G_{ST} values (Table 2).

Based on the computed pairwise genetic distance coefficients, a dendrogram for accessions was constructed (Fig. 2). In general the dendrogram had two main clusters, one containing accessions representing the three perennial species (*C. montbretii*, *C. isauricum* and *C. anatolicum*) and the other one comprising accessions belonging to the six annual species and the perennial species *C. incisum* accession (Fig. 2). Within this cluster, accessions belonging to three annual species *C. pinnatifidum*, *C. judaicum*, *C. bijugum* and *C. incisum* formed a group, while three closely related species *C. echinospermum*, *C. reticulatum* and *C. arietinum* grouped together. Overall, accessions grouped into their distinct species clusters in the dendrogram, except for three *C. reticulatum* accessions, ILWC242, TR54961 and ILWC247. Accessions ILWC242 and TR54961 had band profiles similar to *C.*

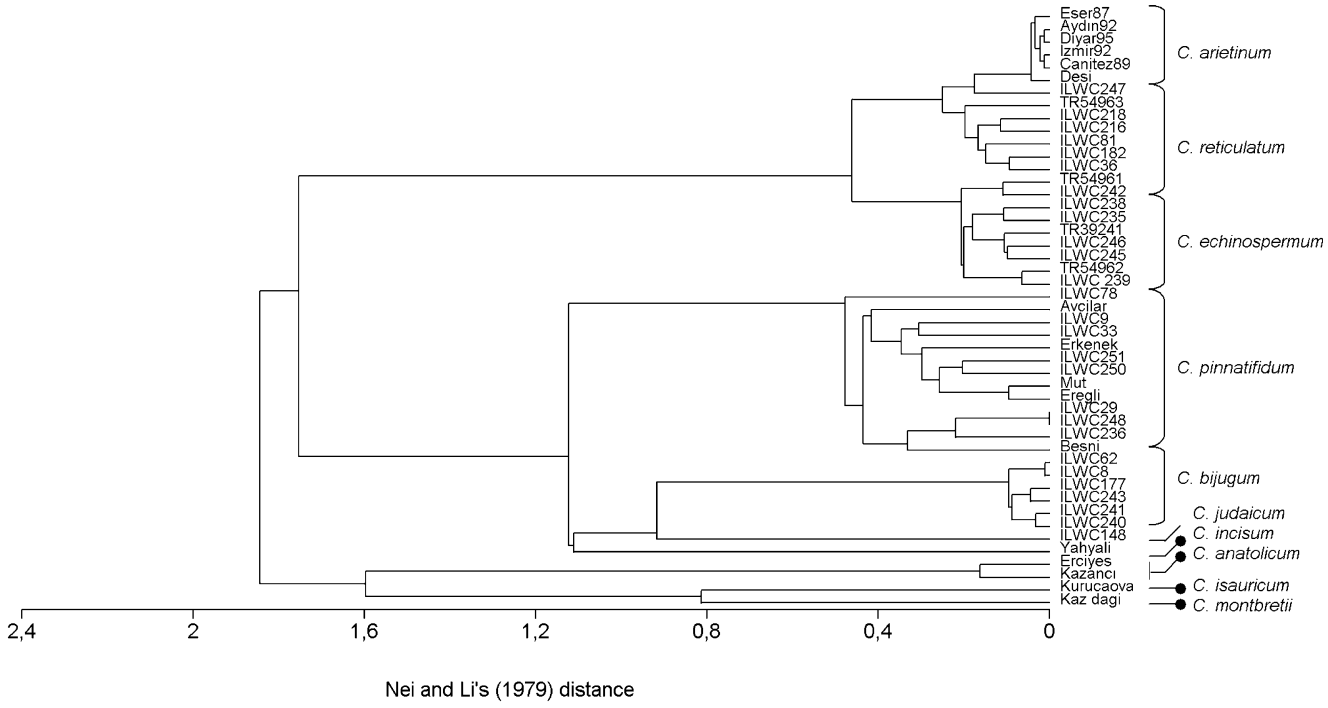

Fig. 2 AFLP-based UPGMA dendrogram of *Cicer* accessions

echinospermum accessions (Fig. 1) and thereby clustered together with the accessions of this species while the accession ILWC247 grouped with the *C. arietinum* group. When *C. arietinum* accessions were compared, one accession named as Desi (Desi type) had relatively small genetic distance values to both *C. echinospermum* and *C. reticulatum* accessions, and clustered in the outer layer of the *C. arietinum* group (Fig. 2, see Table 4).

AFLP-based mean genetic distances among species are given in Table 3. Among the perennials, *C. incisum* had the lowest genetic distance values near to all annuals, and closely clustered with the species in the second crossability group of Ladizinsky and Adler (1976). Among wild species, *C. reticulatum* was the closest species to cultivated chickpea (0.301). This distance was comparable to the mean distance within *C. reticulatum* (0.282). In addition, when compared with other species, pairwise genetic distances detected among the first crossability group species were considerably lower (Table 3). Regarding the distance coefficients within species, *C. pinnatifidum* had the highest value (0.387), which was followed by *C. reticulatum* (0.282), and *C. arietinum* had the lowest distance within species (0.030).

Discussion

In the present study, AFLP markers were successfully used to survey genetic variation and relationships among six annual and four perennial species with the distribution in Turkey. Overall, the dendrogram developed from the AFLP fingerprints is congruous, with our previous analysis of relationships in the same *Cicer* collection using allozymes in terms of species relationships (Sudupak and Kence 2002) and partially with the RAPD-based dendrogram (Sudupak et al. 2002), and in agreement with the relationships reported for the six annual species (Ladizinsky and Adler 1976; Kazan and Muelbauer 1991; Ahmad et al. 1992; Ahmad and Slinkard 1992; Labdi et al. 1996; Tayyar and Waines 1996; Ahmad 1999; Iruela et al. 2002). The topology of the dendrogram obtained in AFLP analysis varies significantly with some primer combinations; and in our analysis, some primer combinations confirmed the RAPD-based grouping of the accessions while others corroborated the allozyme-based grouping. Among these three primer combinations, when only the primer combination E-ACT/M-CAC (Fig. 1; two other combinations were not included in the analysis) is considered in the analysis, the dendrogram closely resembled the dendrogram obtained in the RAPD analysis given in Sudupak et al. (2002).

AFLP-based grouping observed among three species *C. echinospermum*, *C. reticulatum* and *C. arietinum* was fully consistent with the groupings obtained using RAPD markers (Ahmad 1999; Iruela et al. 2002; Sudupak et al. 2002) while *C. reticulatum* was found to be genetically closer to *C. echinospermum* than *C. arietinum* in seed protein and allozyme-based studies (Kazan and Muehlbauer 1991; Ahmad and Slinkard 1992; Labdi et al. 1996; Tayyar and Waines 1996; Sudupak and Kence 2002), except for one study of Ahmad et al. (1992). Likewise, relationships between this group and other annuals and perennials differed in different studies. Although in nearly all allozyme-based studies, including ours, species in the

Table 3 Nei and Li's (1979) genetic distance estimates among *Cicer* species computed from AFLP data. The values given in parenthesis are ranges

Item	*C. mont.*	*C. isau.*	*C. anat.*	*C. inc.*	*C. jud.*	*C. pin.*	*C. bij.*	*C. ech.*	*C. ret.*	*C. ari.*
C. montbretii	0 (0.00–0.00)									
C. isauricum	0.812 (0.81–0.81)	0 (0.00–0.00)								
C. anatolicum	1.27 (1.23–1.32)	1.924 (1.85–1.99)	0.161[a] (0.16–0.16)							
C. incisum	1.505 (1.50–1.50)	1.743 (1.74–1.74)	2.399 (2.27–2.53)	0 (0.00–0.00)						
C. judaicum	1.234 (1.23–1.23)	1.802 (1.80–1.80)	1.767 (1.75–1.78)	1.252 (1.25–1.25)	0 (0.00–0.00)					
C. pinnatifidum	1.380 (1.23–1.53)	1.848 (1.58–2.1)	1.732 (1.41–2.09)	1.121 (0.984–1.32)	1.083 (1.01–1.25)	0.387 (0.0–0.65)				
C. bijugum	1.525 (1.51–1.59)	2.101 (2.1–2.11)	2.012 (1.85–2.11)	1.085 (1.05–1.11)	0.916 (0.87–0.95)	1.127 (0.91–1.39)	0.079 (0.0–0.11)			
C. echinospermum	1.599 (1.52–1.66)	2.208 (2.1–2.41)	1.979 (1.80–2.11)	2.180 (1.95–2.27)	1.461 (1.27–1.69)	1.796 (1.54–2.19)	1.595 (1.41–1.69)	0.169 (0.063–0.25)		
C. reticulatum	1.44 (1.36–1.58)	2.058 (1.85–2.15)	1.994 (1.73–2.28)	1.922 (1.68–2.27)	1.341 (1.26–1.43)	1.749 (1.47–2.22)	1.567 (1.40–1.68)	0.377 (0.13–0.52)	0.282 (0.09–0.49)	
C. arietinum	1.547 (1.50–1.58)	2.187 (2.09–2.24)	2.105 (1.92–2.27)	1.910 (1.88–1.92)	1.681 (1.61–1.73)	1.844 (1.55–2.22)	1.946 (1.78–2.09)	0.491 (0.38–0.63)	0.301 (0.13–0.58)	0.030 (0.01–0.06)

[a] Mean genetic distance within species

first crossability group were found to be closer to the species in the second crossability group. In a recent study, Ahmad (1999) showed that species in this group are closer to the annuals containing *Cicer yamashita* and *Cicer chorassanicum.* Although these species were not included in Iruela et al. (2002) and Sudupak et al. (2002), using RAPD markers, they obtained similar groupings detected in allozyme-based studies. Similar conflicting reports of relationships are also present between annuals and perennials; while Kazan and Muehlbauer (1991) reported a close association between species in the first crossability group and *C. anatolicum*, a number of other reports including our allozyme, RAPD and AFLP analyses, did not reveal a similar association and grouped *C. anatolicum* with other perennials (Tayyar and Waines 1996; Ireula et al. 2002; Sudupak and Kence 2002; Sudupak et al. 2002). On the other hand, our allozyme analysis suggested that *C. incisum* is the closest perennial species to the annuals considered in this study, and this similarity was confirmed in RAPD analysis, and AFLP analysis narrowed this similarity to the species of the second crossability group (Sudupak and Kence 2002; Sudupak et al. 2002). Regarding the grouping of *C. judaicum*, contrary to our RAPD-based grouping, AFLP analysis indicated a close similarity between *C. bijugum* and *C. judaicum* consistent with the RAPD-based results of Ahmad (1999) and Ireula et al. (2002), while nearly all of the protein-based studies revealed that it is the closest species to *C. pinnatifidum*.

Genetic diversity detected by AFLP markers within and between *Cicer* species paralleled the RAPD and allozyme values reported for selfing the species (Table 2). In general, there is a correlation between the reproductive system and the distribution of genetic variation with low levels of intra- and higher levels of inter-species variation characterizing the selfing species (Nybom and Bartish 2002). Our AFLP-based estimates of variation and its distribution within and among species are in agreement with this aspect. Even though there were some exceptions, the amount of variation detected by each primer combination was generally similar, and the combined estimates were closer to the RAPD variation values reported in Sudupak et al. (2002).

Grouping of *C. reticulatum* accessions indicated that accessions ILWC242 and TR54961 are closer to *C. echinospermum* than *C. reticulatum*, which suggests that some accessions may be intermediate between these two species (Fig. 2; Table 4). In our RAPD analysis, only TR54961 was studied, however, consistent with the observation here; Iruela et al. (2002) have reported a RAPD-based similarity between ILWC242 and *C. echinospermum* accessions, and explained the basis of this similarity as intercrossing possibly occurring within natural populations of *C. reticulatum* and *C. echinospermum*. Accession TR54961 was included in both allozyme and RAPD analysis of relationships, and allozyme-based similarity of this accession to *C. echinospermum* was not detected. Although it was not very evident in RAPD-based groupings of *C. reticulatum* accessions, this acces-

Table 4 AFLP-based Nei and Li's (1979) mean genetic distance estimates between two *Cicer* species and *C. reticulatum* accessions having small genetic distances to either *C. echinospermum* or *C. arietinum*

Accessions	Species	TR54961	*C. echinospermum*	*C. reticulatum*	*C. arietinum*	
					Desi type	Kabuli type
ILWC242	*C. ret.*	0.108	0.196	0.390	0.470	0.498
TR54961	*C. ret.*	0.000	0.216	0.402	0.504	0.534
ILWC247	*C. ret.*	0.396	0.375	0.276	0.134	0.182
ILWC36[a]	*C. ret.*	0.457	0.445	0.241	0.316	0.299
ILWC81[a]	*C. ret.*	0.425	0.397	0.231	0.171	0.165
ILWC239[a]	*C. ech.*	0.168	0.168	0.385	0.529	0.560
Desi type	*C. ari.*	0.504	0.468	0.294	0.000	0.042
Kabuli type	*C. ari.*	0.534	0.496	0.302	0.042	0.000

[a] Randomly chosen accessions included for comparison purposes

sion had band profiles similar to *C. echinospermum* accessions (Sudupak et al. 2002). Similarly, accession ILWC247 is clustered with *C. arietinum* accessions. Comparatively, accession ILWC247 was also closer to *C. echinospermum* suggesting that in the evolution of *C. arietinum*, *C. echinospermum* might have played a role as speculated by Tayyar and Waines (1996) because of the small genetic distances observed between these two species. Equally, being the most polymorphic species, *C. reticulatum* or a common ancestor could be the progenitor species giving rise to this group.

AFLP fingerprints detected with a majority of primer combinations in the first crossability group (*C. echinospermum*, *C. reticulatum* and *C. arietinum*) indicated a close genetic similarity and a dramatic differentiation of this group from other species (Fig. 1, Table 3). Similar striking pattern shifts of these three species were also observed in our analysis of RAPD variation (Sudupak et al. 2002). Thus, as discussed in the previous paragraph, species in this group presumably have a common origin and a different genetic background when compared to other *Cicer* species studied here. The observed small distance values within this group support this interpretation and suggest that they have diverged recently. AFLP-based species relationships in this group coincide with previous hybridization (Ladizinsky and Adler 1976), protein-based (Ahmad et. al. 1992) and DNA-based studies (Ahmad 1999; Iruela et al. 2002; Sudupak et al. 2002), and evolution of chickpea from these species in southeastern Turkey. In addition, AFLP-based grouping of the chickpea accessions suggests that Kabuli- and Desi-forms can be differentiated, and AFLP markers could be used to fingerprint chickpea varieties. Furthermore, observed relationships between two chickpea types also support the hypothesis that the Kabuli type may have originated from the Desi type.

AFLP markers have been proven useful for assessing genetic differences among individuals, populations and species, with its capacity to simultaneously screen many loci distributed randomly in the genome (Mueller and Wolfenbarger 1999). Chickpea is a crop characterized with limited genetic variability, and in constructing linkage maps; either inter-species crosses or inter-species derived recombinant inbred lines were utilized even in mapping SSR loci (Kazan et al. 1993; Simon and Muelbauer 1997; Winter et al. 1999). One of the major limitations in dissecting and characterizing crop genomes such as chickpea is the lack of a suitable marker system that can be easily and reproducibly assayed, and a virtually unlimited number of markers covering the entire genome can be generated. AFLP technology has this capacity and overcomes the majority drawbacks associated with other marker systems. Thus, it can be used to evaluate chickpea germplasm collections in combination with suitable screening procedures to identify and characterize economically important traits in both chickpea and its close relatives, and the transfer of these traits into elite chickpea varieties can be accelerated with the marker-assisted selection techniques.

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