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ORIGINAL ARTICLE

Comparison of genetic variation and differentiation among annual *Cicer* species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers

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Abstract Three molecular markers, including start codon targeted (SCoT) polymorphism, directed amplification of minisatellite-region DNA polymerase chain reaction (DAMD-PCR), and inter simple sequence repeat (ISSR) markers, were compared in terms of their informativeness and efficiency for analysis of genetic relationships among 38 accessions of eight annual *Cicer* species. The results were as follows: (1) the highest level of detected polymorphism was observed for all three marker types; (2) the rate of diversity for the three marker techniques was approximately equal, and the correlation coefficients of similarity were statistically significant for all three marker systems; (3) the three molecular markers showed relatively similar phylogenetic grouping for examined species. Diversity analysis showed that *Cicer reticulatum* is the closest wild species to the cultivated chickpea, and this finding supports the hypothesis that *C. reticulatum* is the most probable progenitor of the cultivated species. *C. bijugum*, *C. judaicum*, and *C. pinnatifidum* were clustered together, and in other clusters *C. yamashitae* and *C. cuneatum* were grouped close together. To our knowledge, this is the first detailed comparison of performance among two targeted DNA region molecular markers (SCoT and DAMD-PCR) and the ISSR technique on a set of samples of *Cicer*. The results provide guidance for future efficient use of these molecular methods in genetic analysis of *Cicer*.

Keywords *Cicer* · Genetic diversity · SCoT · ISSR · DAMD-PCR

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Introduction

The phylogeny of the genus *Cicer* has attracted considerable attention because it harbors the third most important grain legume crop worldwide—the chickpea. Chickpea (*C. arietinum* L.) is the only cultivated species belonging to the *Cicer* genus, which is classified in Fabaceae, tribe Cicereae Alef (van der Maesen 1987). The genus *Cicer* contains 42 wild species including nine annuals and 33 perennials with chromosome number $2n = 2x = 16$ in almost all the species (Labdi et al. 1996). Chickpea is an important self-pollinated grain legume crop, grown mainly in West Asia, North Africa, and the Indian Subcontinent, where it is a basic component of the human diet. Vavilov (1926) was the first to identify areas with similar physiographic characteristics with maximum variability for the major cultivated species. He recognized the Near Eastern, Central Asian, Indian, and Mediterranean regions as the probable centers of origin for chickpea. *C. arietinum* is an economically important crop in Iran, India, the Middle East, North Africa, and Ethiopia, and is the third most important pulse crop in the world next to *Phaseolus vulgaris* and *Pisum sativum*. However, the average annual yield worldwide (0.78 ton/ha) is considered to be somewhat lower than its potential yield (Singh et al. 1994; Sudupak et al. 2002). Therefore, many chickpea breeding programs are focused on improving the genetic potential

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both to increase yield and to provide protection against abiotic and biotic stresses. It has been recognized that interspecific hybridization will increase the variation and can be useful for plant breeding purposes in a recalcitrant crop such as 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 programs (Singh and Ocampo 1993, 1997; Singh et al. 1994). Wild relatives of chickpea were reported to bear traits conferring resistance to a number of biotic and abiotic stresses and have potential to improve yield (Singh et al. 1994; Muehlbauer et al. 1994; Singh and Ocampo 1997; Akem et al. 2000). Investigation of genetic variation and relationships among accessions collected from diverse geographic regions where the closest wild relatives exist and were presumed to be first domesticated is critical for genetic improvement of the chickpea. Most of the interspecific relationship studies in *Cicer* have been carried out using plant morphology (Robertson et al. 1997), karyotype (Ocampo et al. 1992; Tayyar et al. 1994), crossability data (Ladizinsky and Adler 1976; Pundir and Vander Maesen 1983; Ahmad et al. 1987), restriction fragment length polymorphism (RFLP) (Patil et al. 1995), seed storage protein analyses (Vairinhos and Murray 1983; Ahmad and Slinkard 1992), allozyme markers (Kazan and Muehlbauer 1991), amplified fragment length polymorphism (AFLP) markers (Nguyen et al. 2004), inter simple sequence repeat (ISSR) markers (Sudupak 2004), and more recently random amplified polymorphic DNA (RAPD) markers (Talebi et al. 2009). In addition, allelic variation at a microsatellite locus [(TAA)n] (Udupa et al. 1999) and also in *Ty1-copia*-like retrotransposon sequence (Sant et al. 2000) have been utilized to study diversity in *Cicer*. In recent years, many new alternative and promising marker techniques have emerged. These techniques include start codon targeted (SCoT) polymorphism (Collard and Mackill 2009) and directed amplification of minisatellite-region DNA polymerase chain reaction (DAMD-PCR) to direct the amplification of tandemly repeated region of a genome (Karaca et al. 2002; Kang et al. 2002). However, to the best of our knowledge, there has been no application of SCoT and DAMD-PCR in *Cicer*. Coupled with the rapid growth of genomics research, there has been a trend away from random DNA markers towards gene-targeted markers (Andersen and Lubberstedt 2003; Gupta and Rustgi 2004). Genome sequence data offers enormous potential for the development of new markers in diverse plant species (Collard and Mackill 2009). The present study was carried out to test the utility of the SCoT and DAMD-PCR markers in comparison with ISSR markers to determine intra- and interspecies genetic diversity and relationships among accessions representing eight annual *Cicer* species.

Materials and methods

Plant materials

Thirty-eight accessions, representing eight annual *Cicer* species, originating from the main *Cicer* centers of the world diversity, were obtained from the Australian Temperate Field Crops Collection (ATFCC) at the Victorian Institute for Dryland Agriculture (VIDA), Horsham, Australia and International Center for Agricultural Research in the Dry Areas (ICARDA), Alepo, Syria (Table 1).

Genomic DNA extraction

DNA was extracted from 2 g of young leaves collected from 10-day-old seedling plants of each accession using the cetyltrimethylammonium bromide (CTAB) method according to Lassner et al. (1989) with the modification described by Torres et al. (1993). The DNA final concentration was determined by agarose-gel electrophoresis using a known concentration of uncut λ DNA as standard.

ISSR and DAMD-PCR

For ISSR analysis, a set of 15 primers representing di-, tri-, tetra-, and pentamer repeats (UBC set #9) was procured from the Biotechnology Laboratory, University of British Columbia, Canada. Following the optimization of polymerase chain reaction (PCR) conditions and prescreening of the first 15 primers on a sample set that included each species, 10 primers providing clear and informative amplicon profiles across species were selected to survey ISSR variation in the accessions listed in Table 2. PCR reactions were composed as described previously by Rajesh et al. (2002). DAMD-PCR markers [originally derived from the repeat elements of weed rice (Kang et al. 2002)] were used in this study. Of 10 primers screened, 6 with GC content of 50–60 % were selected to generate the DNA fingerprint profiles of all the accessions at 48 °C annealing temperature. PCR reactions were performed in a volume of 20 µl in Eppendorf thermocyclers. PCR products were separated on 1.5 % agarose gels, stained with ethidium bromide, and scored for presence or absence of bands.

SCoT PCR

Start codon targeted primers were designed from consensus sequences derived from the studies by Collard and Mackill (2009). For primer design, the ATG codon (+1, +2, and +3), G at position +4, and A, C, and C at positions +7, +8, and +9, respectively, were fixed (Table 2). All primers were 18-mer and ranged in GC content between 50 and 72 % (Table 2). Primers were checked for dimers and

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

Number	Species	Accession	Source	Origin
1	*C. bijigum*	TN04522	ATFCC[a]	Turkey
2		TN04683	ATFCC	Turkey
3	*C. cuneatum*	TN04478	ATFCC	Ethiopia
4		TN04629	ATFCC	Ethiopia
5		TN04631	ATFCC	Ethiopia
6		TN4676	ATFCC	Ethiopia
7	*C. echinospermum*	TN04674	ATFCC	Turkey
8		TN04678	ATFCC	Turkey
9		TN04681	ATFCC	Turkey
10		TN04686	ATFCC	Turkey
11		TN04687	ATFCC	Turkey
12		ILWC235	ICARDA[b]	Turkey
13		ILWC181	ICARDA	Turkey
14		PI599069	ATFCC	Turkey
15	*C. judaicum*	TNO4498	ATFCC	Syria
16		TN04507	ATFCC	Syria
17		TN04546	ATFCC	Ethiopia
18		TN04551	ATFCC	Palestine
19		TN04556	ATFCC	Palestine
20		TN04607	ATFCC	Syria
21		TN04611	ATFCC	Syria
22		PI510662	ATFCC	Jordan
23		ILWC20	ICARDA	Syria
24		PI458559	ATFCC	Syria
25	*C. reticulatum*	PI599092	ATFCC	Turkey
26		ILWC242	ICARDA	Turkey
27	*C. yamashitae*	ILWC3	ICARDA	Afghanistan
28		ILWC215	ICARDA	Afghanistan
29		ILWC55	ICARDA	Afghanistan
30	*C. pinnatifidum*	ILWC263	ICARDA	Syria
31		ILWC236	ICARDA	Syria
32		ILWC225	ICARDA	Turkey
33	*C. arietinum*	ICCV3309	ICARDA	Syria
34		FLIP91-96C	ICARDA	Syria
35		ILC588	ICARDA	Syria
36		Arman	IRAN	Iran
37		Hashem	IRAN	Iran
38		Pirooz	IRAN	Iran

[a] Australian Temperate Field Crops Collection

[b] International Center for Agricultural Research in the Dry Areas

hairpin loops. Using the FAST PCR program (Kalendar 2007). PCR was optimized for 10 primers as described previously by Collard and Mackill (2009). PCR reactions were performed in volume of 20 µl in Eppendorf thermocyclers. PCR products were separated on 1.2 % agarose gels, stained with ethidium bromide, and scored for presence or absence of bands.

Data analysis

DNA bands obtained with all the markers were scored visually for presence (1) or absence (0) of bands for all 38 accessions. DARwin version 5.0 was used for calculating pairwise genetic distances and for constructing the dissimilarity matrix (Perrier et al. 2003). The dissimilarity

Table 2 Primers used in ISSR, URP, and SCoT analyses

Marker	Sequence (5′–3′)	GC (%)	Annealing temperature (°C)
ISSR			
UBC807	AGAGAGAGAGAGAGAGT	47	48
UBC828	TGTGTGTGTGTGTGTGA	47	50
UBC878	GGATGGATGGATGGAT	50	48
UBC899	CATGGTGTTGGTCATTGTTCCA	45	52
UBC822	TCTCTCTCTCTCTCTCA	47	48
UBC874	CCCTCCCTCCCTCCCT	75	52
UBC816	CACACACACACACACAT	47	48
DAMD-PCR			
URP1F	ATCCAAGGTCCGAGACAACC	55	48
URP2F	GTGTGCGATCAGTTGCTGGG	60	48
URP6R	GGCAAGCTGGTGGGAGGTAC	65	48
URP4R	AGGACTCGATAACAGGCTCC	55	48
URP9F	ATGTGTGCGATCAGTTGCTG	50	48
URP13R	TACATCGCAAGTGACACAGG	50	48
URP17R	AATGTGGGCAAGCTGGTGGT	55	48
SCoT			
SCOT22	AACCATGGCTACCACCAC	55	50
SCOT13	ACGACATGGCGACCATCG	61	50
SCOT28	CCATGGCTACCACCGCCA	66	50
SCOT35	CATGGCTACCACCGGCCC	72	50
SCOT20	ACCATGGCTACCACCGCG	66	50
SCOT36	GCAACAATGGCTACCACC	55	50
SCOT11	AAGCAATGGCTACCACCA	50	50
SCOT2	CAACAATGGCTACCACCC	55	50
SCOT1	CAACAATGGCTACCACCA	50	50

matrix thus obtained was subjected to cluster analysis using the unweighted neighbor-joining analyses (UNJ) (Gascuel 1997), followed by bootstrap analysis with 1,000 permutations to obtain a dendrogram for all 38 accessions (Perrier et al. 2003). Mantel statistic was used to compare the similarity matrices as well as the dendrograms produced by the ISSR, URP, and SCoT techniques. All these procedures were performed by appropriate routines in NTSYSpc version 2.0 (Rohlf 1997). Polymorphic information content (PIC) values were calculated for each ISSR primers according to the formula: $\text{PIC} = 1 - \sum (P_{ij})^2$, where P_{ij} is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers (Botstein et al. 1980). Partitioning of variation within and among the groups by each marker system was achieved by analysis of molecular variance (AMOVA) using Arlequin version 2000 software as described by Excoffier et al. (1992). The Mantel test of significance (Mantel 1967) was also used to compare each pair of similarity matrices produced.

Results

DNA fingerprint database has been reported using the three different PCR-based molecular marker (ISSR, DAMD-PCR, and SCoT) systems for 38 *Cicer* accessions belonged to eight annual species. Our results indicated that primers which were obtained from the different regions of genomic DNA successfully amplified accession template DNAs (Fig. 1). All three molecular markers used in this study were able to distinguish and identify each of 38 accessions to referred species. Salient features of the fingerprint database obtained using the different markers are given below.

ISSR analysis

A total of 81 bands were detected using seven ISSR markers, of which 78 were polymorphic (Table 3). ISSR DNA bands varied between 15 (UBC828) and 6 (UBC874), with an average of 11.5 ± 0.9 per primer. Percent polymorphism ranged from 85.7 % to as high as 100 % with average polymorphism of 96.75 % across all accessions. ISSR markers showed relatively high level of polymorphism in the examined germplasm; the PIC value for ISSRs was calculated in the range of 0.29–0.49 with an average of 0.4 across the genotypes assayed (Table 3). A significant correlation ($r = 0.938$; $P < 0.01$) was observed between the total number of bands and the number of polymorphic bands. As seen in Table 3, dinucleotides $(AG)_8$, $(TG)_8$, $(TC)_8$, and $(CA)_8$ with single-nucleotide anchor T/A at the 3′ end were found to be the most polymorphic compared with all others.

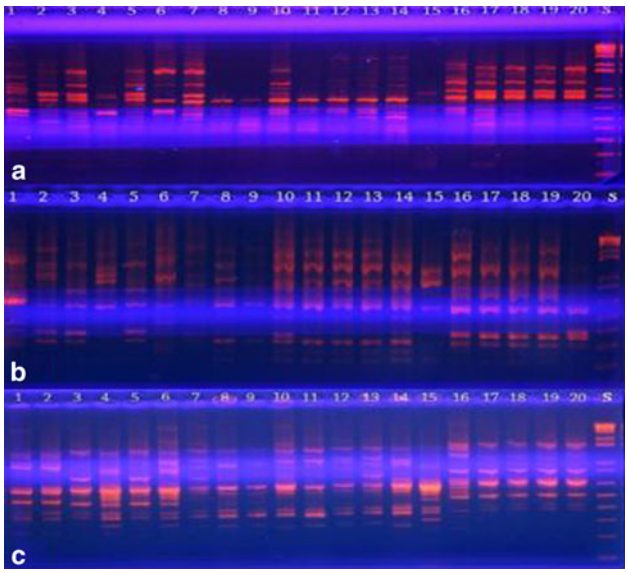

Fig. 1 Amplification profile obtained with URP13R (*a*), SCoT28 (*b*), and UBC807 (*c*) primers detected in *Cicer* accessions

Table 3 Polymorphism and PIC values in annual *Cicer* species as revealed by ISSR, URP, and SCoT markers

Primer	No. of amplified bands	No. of polymorphic bands	Polymorphism (%)	PIC value
ISSR				
UBC807	14	12	85.7	0.48
UBC828	15	15	100	0.43
UBC878	10	10	100	0.33
UBC899	14	14	100	0.38
UBC822	12	11	91.6	0.49
UBC874	6	6	100	0.41
UBC816	10	10	100	0.29
DAMD-PCR				
URP1F	13	13	100	0.31
URP2F	15	15	100	0.23
URP6R	14	14	100	0.43
URP4R	12	12	100	0.41
URP9F	15	15	100	0.29
URP13R	11	11	100	0.51
URP17R	12	12	100	0.60
SCoT				
SCOT22	13	13	100	0.61
SCOT13	17	17	100	0.43
SCOT28	15	14	93.3	0.57
SCOT35	15	15	100	0.48
SCOT20	11	11	100	0.42
SCOT36	8	8	100	0.62
SCOT11	11	11	100	0.36
SCOT2	15	13	86.6	0.78
SCOT1	7	7	100	0.39

DAMD-PCR analysis

A PCR-based approach involving the DAMD with seven minisatellite core sequences as primers was used to analyze diversity in 38 annual *Cicer* accessions. Analysis indicated that the PCR profile and the optimized chemical concentrations resulted in reproducible and reliable DNA amplification (Fig. 1). In the accessions of *Cicer* species the number of amplified DAMD-PCR products varied from 11 to 15 fragments depending on the primers used. A total of 92 bands were scored, all of which were polymorphic (Table 3). PIC values ranged from 0.23 to 0.60, with an average value of 0.39 per locus. Based on the independent replications of DAMD-PCR, we observed that reproducible DNA markers were amplified and also noted that all DAMD-PCR primers used in this study produced RAPD-like results, but the numbers of bands were sharp and clear. The relatively high PCR stringencies in DAMD-PCR application effectively limited the PCR artifacts which commonly occur in RAPDs (Karaca et al. 2002; Ince et al. 2009).

SCoT analysis

A total of 112 bands were detected among 38 accessions belonging to eight annual *Cicer* species using nine SCoT markers, of which 109 were polymorphic (Table 3). The number of bands ranged from 17 (SCoT13) to 7 (SCoT1) with an average of 12.4 per primer. The overall size of amplified products ranged from 220 to 2,250 bp. Percent polymorphism ranged from 86.6 % to as high as 100 % with average polymorphism of 97 % across all accessions. PIC values ranged from 0.39 to 0.78, with an average value of 51.7 per primer. Evaluation using a representative set of *Cicer* species has indicated that SCoT primers generate a DNA fingerprint similar to those generated by RAPD markers, but the bands were sharp, clear, and 100 % polymorphic (Fig. 1).

Correlation between the similarity values measured using the three marker systems

The values of the Mantel test showed a positive correlation between the three marker types. The correlation coefficient (r) was 0.629 between ISSR and DAMD-PCR, 0.650 between ISSR and SCoT (significant, $P > 0.01$), and 0.50 (significant, $P > 0.05$) between DAMD-PCR and SCoT (Table 4). The AMOVA showed apparent differences in partitioning of variation within and between species accomplished by the three different marker types (Table 5). All three marker types showed greater variance between species than within species. SCoT and ISSR markers showed relatively greater variance between the species, while DAMD-PCR markers showed greater variance among species compared with other marker types (Table 5).

Interrelationships among species

All three marker types showed high similarity in dendrogram topologies. The dendrogram constructed by DARwin

Table 4 Mantel test correlation coefficients among similarity matrices obtained using ISSR, URP, and SCoT markers

	ISSR	DAMD-PCR	SCoT
ISSR	1		
DAMD-PCR	0.629**	1	
SCoT	0.65**	0.503*	1

* and ** indicates significant at 5 and 1 % probability level, respectively

Table 5 Partitioning of variance within and between different *Cicer* species from the analysis of molecular variance for data derived from ISSR, DAMD-PCR, and SCoT markers

Percent variance	ISSR	DAMD-PCR	SCoT
Among species	17.21	23.2	21.83
Between species	88.21	68.33	89.92

(version 5.0) software using an unweighted neighbor-joining method. Cluster analysis using ISSR data grouped genotypes into five distinct clusters (Fig. 2). Group I included *C. arietinum*, *C. reticulatum*, and *C. yamashitae*, group II included *C. echinospermum*, and group III included *C. pinnatifidum*. *C. bijugum* and *C. cuneatum* were grouped in cluster IV. Group V had *C. judacium* and *C. cuneatum*. Generally, all accessions were positioned into species clusters. The dendrogram constructed by SCoT markers demonstrated that four main groupings existed in these accessions (Fig. 3). Group I [the primary and secondary crossability groups documented by Ladizinsky and Adler (1976)] clustered *C. arietinum* and *C. reticulatum* close together, and *C. yamashitae* and *C. pinnatifidum* also grouped in this cluster. Group II and group III included accessions belonging to *C. echinospermum* and *C. judcium*, respectively. In the fourth cluster *C. bijugum* and *C. cuneatum* were grouped close together. In cluster analysis based on DAMD-PCR markers, all accessions that belonged to *C. arietinum* and *C. echinospermum* grouped in cluster I, with *C. judacium*, *C. bijugum*, and *C. cuneatum* in the second cluster (Fig. 4). Also *C. yamashitae* and *C. pinnatifidum* were grouped in one cluster very close together (Fig. 4).

Discussion

For genetic analysis, it is of utmost importance to know which type of markers and how many of them truly represent variation in the entire genome and should be used to derive reliable estimates of diversity. There have been a number of efforts to transfer agro-economically important genes from wild species into cultivated species through conventional breeding practices; For example, interspecific hybridizations were performed to introgress

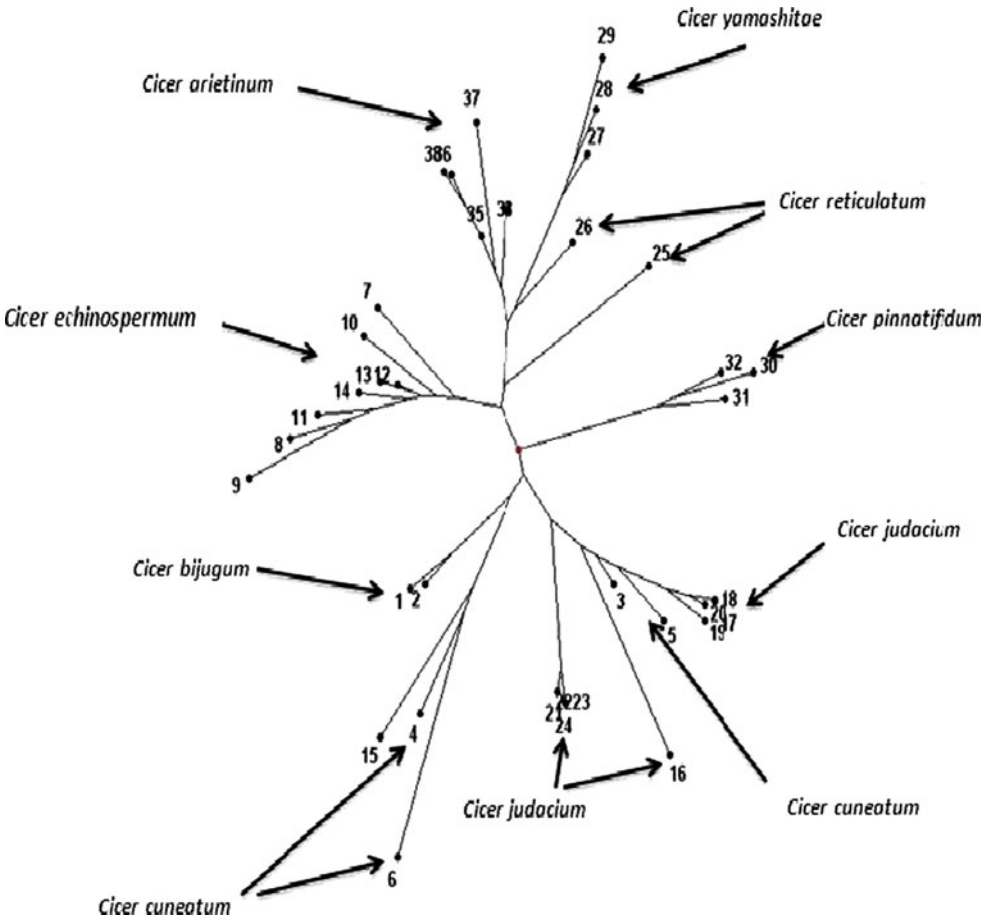

Fig. 2 Dendrogram of the annual *Cicer* species based on the similarity matrix developed using ISSR markers

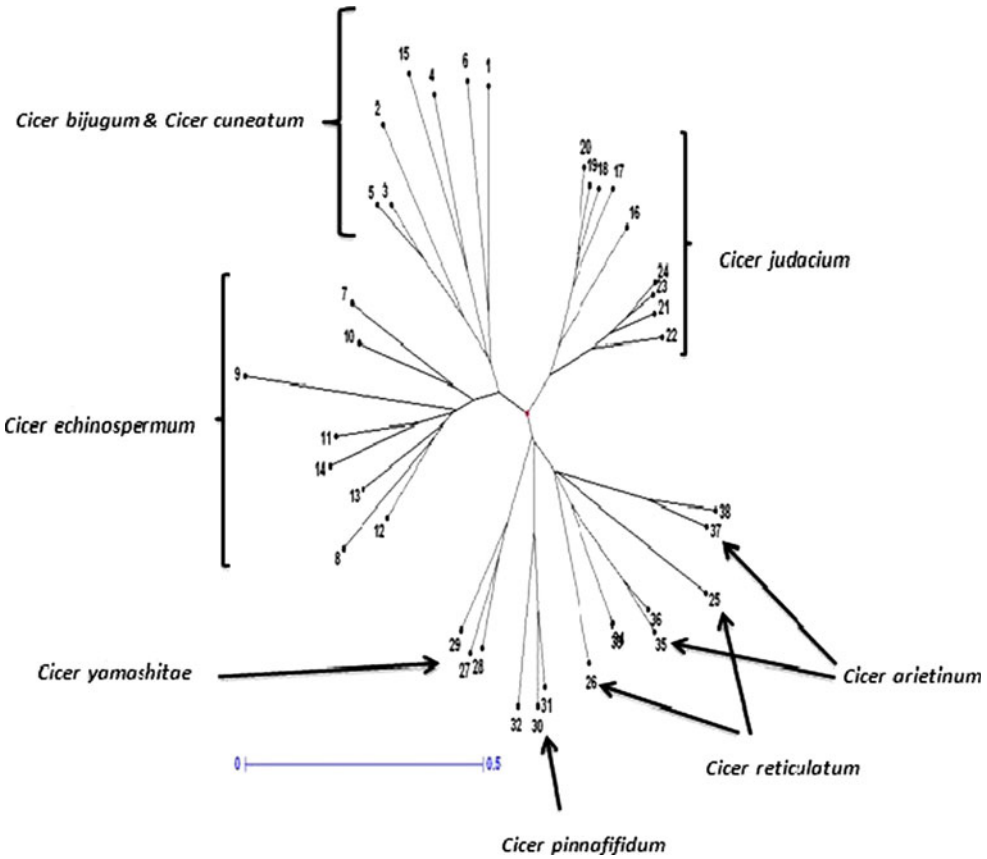

Fig. 3 Dendrogram of the annual *Cicer* species based on the similarity matrix developed using SCoT markers

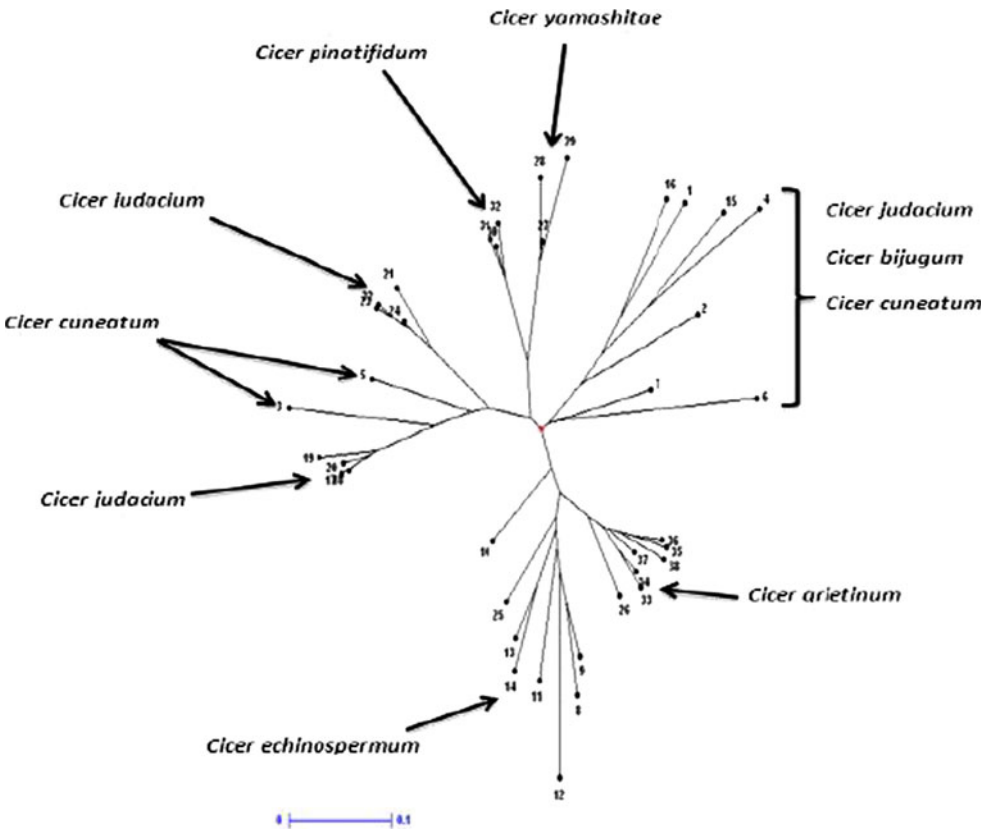

Fig. 4 Dendrogram of the annual *Cicer* species based on the similarity matrix developed using DAMD-PCR markers

perennial germplasm into cultivated alfalfa (McCoy and Echt 1993) and a nematode resistance gene in peanut (Garcia et al. 1996). However, similar attempts were not successful to develop hybrids between *C. arietinum* and any wild species of *Cicer* except *C. reticulatum* and *C. echinospermum*. A few efforts using plant tissue culture techniques such as embryo rescue to develop hybrids between *C. arietinum* and *C. pinnatifidum* were reported (Anonymous 1995). High hybrid vigor was obtained in F_1 and F_2 generations, when *C. arietinum* was crossed with *C. reticulatum* and *C. echinospermum* (Singh and Ocampo 1997), confirming the potential of increasing the chickpea yield using interspecific hybridization. However, knowledge of genetic relationships among various wild species is necessary for successful and efficient exploitation of genetic diversity present in the wild species, and such information is very poorly available in the genus *Cicer*, especially using molecular markers. In this study, we compared the marker datasets produced using three different marker systems, namely ISSR, DAMD-PCR, and SCoT, to define genetic relationships within a set of 38 genotypes representing eight annual *Cicer* species, and to determine whether these marker systems can be effectively used and/or complement identification of *Cicer* species. This technique was more informative than previously used biochemical and molecular methods to study variation and genetic relationships in *Cicer* such as isozymes, storage proteins (Ahmad et al. 1992; Labdi et al. 1996), and RAPD markers (Sudupak et al. 2002; Talebi et al. 2009). All three molecular markers detected abundant polymorphisms at both inter- and intraspecies level, implying that all of them could be applied to germplasm identification and genetic diversity assessment in the genus *Cicer*. Results of the three types of markers in this study corroborated the first and second crossability groups of Ladizinsky and Adler (1976), and grouping in the first crossability group revealed that *C. reticulatum* is the closest wild species to *C. arietinum* while *C. echinospermum* is relatively distant from both, consistent with allozyme- (Ahmad et al. 1992), RAPD- (Talebi et al. 2009; Sudupak et al. 2002; Iruela et al. 2002), and AFLP-based groupings (Nguyen et al. 2004). Groupings of the accessions of the second crossability group species indicated that *C. pinnatifidum* is closer to *C. judaicum*, and *C. bijugum* was the next closer species to this group, consistent with results obtained with different markers (Sudupak et al. 2002; Rajesh et al. 2002; Nguyen et al. 2004). It is interesting to note that all three (ISSR, DAMD-PCR, and SCoT) datasets showed high levels of correlation (Table 4). This is not surprising as these markers are known to target different genomic fractions involving repeat and/or unique sequences, which may have been differentially evolved or preserved in due course of natural or human selection. The levels of genetic variation detected by these three types of markers within and between *Cicer* species were relatively higher than the amount computed from RAPD and AFLP markers (Sudupak et al. 2002; Nguyen et al. 2004). This can be attributed to the hypervariable nature of these markers, which are expected to reveal higher levels of variation. Consistent with our previous analyses of RAPDs, *C. pinnatifidum* was the most polymorphic species followed by *C. reticulatum*, while *C. arietinum* had the least amount of RAPD variation. Results obtained from these three marker systems also confirm that chickpea has a narrow genetic base. Previous studies have already shown that studies of DNA polymorphisms across multiple regions of the genome enable discrimination between closely related cultivars or accessions (Saini et al. 2004; Aruna et al. 2008). Although a larger number of species and accessions are needed to identify species-specific DAMD-PCR and SCoT, and genetic relationships, our studies indicated that accessions represented their corresponding species in *Cicer*. Our results demonstrate that high genetic diversity exists between the investigated accessions belonging to eight annual species. As the examined species are diploid and have relatively similar pedigree, this range of diversity is interesting. Estimates of genetic diversity were nearly the same using SCoT, ISSR, and DAMD-PCR markers. Although the level of diversity for the three marker techniques was approximately equal, we anticipate that the source of detected diversity is different, as each technique targets different regions of the genome. For precise phylogeny and evolutionary analysis of *Cicer*, studies with more perennial and annual species and with a large number of accessions of each species are required. However, the scarcity of seeds of *Cicer* species and their poor growing ability at various locations are the major limitations to carrying out this exercise. In conclusion, in this study we provide additional data for identification of accessions and species in *Cicer* in addition to previous analyses of morphological characteristics and RAPD, AFLP, and isozyme studies. We have demonstrated that DAMD-PCR and SCoT markers can be used as reliable techniques for detecting levels of DNA polymorphism and genetic relationship in *Cicer*.

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