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Genetic relationships among annual wild *Cicer* species using RAPD analysis*

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Chickpea (*Cicer arietinum* L.) is a very important cool season legume crop, grown in at least 44 countries of the world. It is a major source of protein for the vegetarian masses. However, its average annual yield world-wide is lower than its potential yield. Biotic and abiotic stresses are the limiting factors to achieve the higher yield. Therefore, inter-specific and intra-specific hybridization is employed to obtain high-yielding, biotic and abiotic stress-tolerant segregants because genetic diversity is limited within the cultivated germplasm. On the other hand, accessions of wild *Cicer* species are known to possess genes for resistance to many biotic and abiotic stresses as proposed by Muehlbauer *et al.* (1994). Prior to performing inter-specific crosses, it is necessary to know the exact pattern of genetic variation within and between the cultivated and wild *Cicer* accessions. Use of DNA markers is becoming increasingly popular to examine genetic diversity among various accessions. In the present study, random amplified polymorphic DNA (RAPD) primers were used to assess genetic diversity among cultivated and wild accessions of 6 *Cicer* species. The genetic relationship determined by RAPD analysis would facilitate identification of diverse parents for inter-specific and intra-specific crosses to achieve the desired objectives

The plant material consisted of 13 wild accessions, procured from International Center for Agricultural Research in the Dry Areas (ICARDA, Syria), and 4 released cultivars of cultivated species, which together represented 6 *Cicer* species (Table 1). The material was grown at the experimental field of the University, Ludhiana during winter (*rabi*) 2006.

Genomic DNA was extracted from immature leaf twigs of 4–6 week old plants using CTAB method of Taylor *et al.* (1995). Thirty RAPD (10-mer) primers (obtained from Operon Technologies, USA) were used to detect polymorphism between *Cicer* accessions. Each PCR reaction mixture contained 2 µl of template DNA (40 ng), 2 µl of 0.25 mM dNTPs (Biogene, USA), 2 µl of 10 × Taq buffer (Biogene), 0.4 µl of 50 mM $MgCl_2$ (Invitrogen), 2 µl of primer, 0.2 µl of Dr. Taq DNA polymerase (Biogene) and 11.4 µl of double distilled autoclaved water to make total volume to 20 µl. PCR was run following a modification of the protocol of Croft *et al.* (1999). For this, an initial denaturation at 94°C for 3 min., followed by 40 cycles of 94°C for 30 s, 37°C for 30 s and 72°C for 1 min: the final extension at 72°C was held for 5 min. Each PCR product was separated on 1.5% agarose gel (stained with ethidium bromide) in 0.5 × concentration TBE electrophoresis buffer and visualized under UV light. The resulting band patterns were visually scored as either present (1) or absent (0) for each accession and each primer combination. The binary matrix was used to construct a dendrogram based on the unweighed pair-group method with arithmetic averages (UPGMA), which depicted genetic relationships between *Cicer* accessions.

A total of 30 primers were assessed, of which 6 (20%) detected polymorphism across 17 accessions (Table 2). Same set of RAPD primers was used in *Cicer* earlier by Collard *et al*. (2003) and genetically diverse parents were determined. In total, 73 reproducible and clearly scorable bands were produced from 6 primers. All were polymorphic. The most informative primer was OPBB 05, which produced as many as 17 polymorphic bands accounting for 23% of the total polymorphic bands examined in the study.

Genetic variation was measured within each of the 6 *Cicer* species assessed (Table 1). The most genetically diverse species was *C. judaicum*, for which highest number of polymorphic loci (36) were detected among 6 accessions. This may be due to comparatively more number of *C. judaicum* accessions included. The lowest genetic variation

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Table 1 Number and names of accessions assessed within each *Cicer* species

Species	Number of accessions	Accessions	Number of polymorphic loci
Cicer arietinum (cultivated)	4	'GPF 2', 'PBG 5', 'PDG 4', 'L 550'	28
C. reticulatum	1	'ILWC 129'	None
C. echinospermum	2	'ILWC 35', 'ILWC 179'	15
C. pinnatifidum	2	'ILWC 9', 'ACC 188'	13
C. judaicum	6	'ILWC 95', 'ACC 182', 'ACC 185', '185W', 'ACC 185A', 'ACC 185B'	36
C. yamashitae	2	'ILWC 3', 'ICC 17157'	6

was detected within *C. yamashitae* between 2 accessions, with only 6 polymorphic loci detected. For the cultivated species *C. arietinum*, 28 polymorphic loci were detected among a group of 4 accessions. Thus, the ability to detect genetic variation among species at the molecular level was directly related to the number of polymorphisms detected and their reproducibility. The amount of genetic variation detected within *C. arietinum* was less than that detected within *C. judaicum* and *C. echinospermum*. Shan *et al*. (2005) using AFLP markers and Rao *et al*. (2008) using ISSR markers also revealed that *C. arietinum* has narrowest genetic variation in comparison to its wild relatives. Therefore, the wild species *C. judaicum* is a novel source of genetic variation, which may be introduced into cultivars to broaden their genetic base through inter-specific hybridization. The genetic variation within wild species *C. echinospermum* and *C. pinnatifidum* was less than that of the cultivated species, which might be due to relatively lesser number of wild species' accessions evaluated (2 in each case).

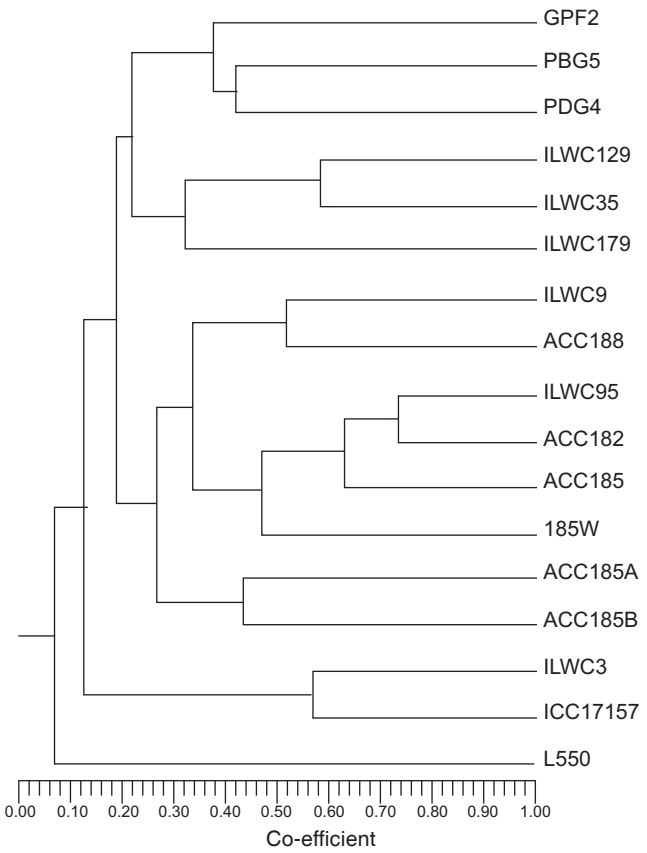

Fig 1 Dendrogram of 17 *Cicer* accessions consutructed on the basis of UPGMA clustering. The scale indicates genetic similarity between accessiens

The dendrogram constructed by the UPGMA method demonstrated that 3 main groups existed in the collection (Fig 1). Group I included *C. arietinum*, *C. reticulatum* and *C. echinospermum.* Within this group, as compared to *C. echinospermum* accessions 'ILWC 35' and 'ILWC 179', *C. reticulatum* accession 'ILWC 129' was nearer to *C. arietinum* cultivars 'PDG 4', 'PBG 5' and 'GPF 2' indicating that *C. reticulatum* is the progenitor of the cultivated species. This is in conformity with the earlier reports (Sudupak *et al*. 2002, Iruela *et al*. 2002, Rajesh *et al*. 2002). Again, in this group, one of the two *C. echinospermum* accessions 'ILWC 35' shared more genetic similarity (58%) with *C. reticulatum* than with the second *C. echinospermum* accession 'ILWC 179' (32.5%). Group II contained *C. pinnatifidum* and *C. judaicum*. In a study (Nguyen *et al*. 2004), it has been reported that *C. pinnatifidum*, *C. judaicum* and *C. bijugum* formed one group. Group III included *C. yamashitae* accessions.

Generally, all accessions were positioned into either one or the other group with one exception: all accessions of *C. arietinum* clustered in the vicinity of *C. reticulatum* accession 'ILWC 129' except 'L 550', the only *kabuli* accession in a group of *desi* accessions, which was placed at the opposite

Table 2 RAPD primers used for fingerprinting *Cicer* species and their sequences

Primer	Sequence (5'-3')	Number of polymorphic bands
OP B17	AGGGAACGAG	8
OP F05	CCGAATTCCC	13
OP BA03	GTGCGAGAAC	10
OP BA07	GGGTCGCATC	13
OP BA20	GAGCGCTACC	12
OP BB05	GGGCCGAACA	17
Total		73

end. It might be due to the fact that the *kabuli* type chickpea has originated from the *desi* type through natural mutation.

Based on Jaccard similarity co-efficient, all the 6 *Cicer* species (except *C. arietinum* accession 'L 550') were at the most 7% genetically similar (Fig 1). First of all, group III came into existence at 13% similarity, in which *C. yamashitae* accessions 'ILWC 3' and 'ICC 17157' were 57% genetically alike. Groups I and II came into picture at 19% similarity. At 22% similarity, group I got differentiated into 2 sub-groups. Sub-group 1 comprised *C. arietinum* accessions 'GPF 2', 'PBG 5' and 'PDG 4'. Second subgroup comprised *C. reticulatum* ('ILWC 129') and *C. echinospermum* accessions 'ILWC 35' and 'ILWC 179'. Genetic similarity within *C. arietinum* accessions ranged from 38–41.5%. *C. echinospermum* accessions were 32.5% similar.

At 27% similarity, group II further divided into 2 subgroups. Sub-group 1 included *C. pinnatifidum* accessions 'ILWC 9' and 'ACC 188', and 4 *C. judaicum* accessions 'ILWC 95', 'ACC 182', 'ACC 185' and '185W'. Sub-group 2 included the remaining two *C. judaicum* accessions 'ACC 185A' and 'ACC 185B'. The latter were 43% genetically alike. Further, sub-group 1 got differentiated into 2 sections at 33.5% genetic similarity. One section comprised *C. pinnatifidum* accessions, which shared 52% similarity. Second section consisted of *C. judaicum* accessions, where genetic similarity ranged between 47 and 73%.

The most suitable *C. arietinum* parents for intra-specific crossing were 'GPF 2' and 'PDG 4'. Parents suitable for inter-specific hybridization with *C. arietinum* for the generation of inter-specific mapping population were 'ILWC 35' (*C. echinospermum*), 'ILWC 9' (*C. pinnatifidum*), 'ILWC 95' (*C. judaicum*) and 'ILWC 3' (*C. yamashitae*), which were most closely related accessions to the cultivated species. However, before germplasm from wild species can be utilized in chickpea breeding, the barriers preventing inter-specific hybridization need to be overcome.

SUMMARY

Genetic diversity analysis was carried out using random amplified polymorphic DNA (RAPD) primers among 4 cultivated and 13 wild accessions, representing 6 *Cicer* species. Thirty primers were assessed, out of which 6 (20%) were polymorphic across above accessions. The genetic variation within a species was highest in *C. judaicum* and lowest in *C. yamashitae*. Based on UPGMA clustering, 3 species groups were identified. Group I included *C. arietinum*, *C. reticulatum* and *C. echinospermum.* In this group, as compared to *C. echinospermum* accessions 'ILWC 35' and 'ILWC 179', *C. reticulatum* accession 'ILWC129' was closer to *C. arietinum* cultivars 'PDG 4', 'PBG 5' and 'GPF 2', indicating that *C. reticulatum* is the progenitor of the cultivated species. Group II contained *C. pinnatifidum* and *C. judaicum,* while group III included *C. yamashitae* accessions. The most suitable *C. arietinum* parents for intra-specific crossing were 'GPF 2' and 'PDG 4'. Parents suitable for inter-specific hybridization with *C. arietinum* were 'ILWC 35' (*C. echinospermum*), 'ILWC 9' (*C. pinnatifidum*), 'ILWC 95' (*C. judaicum*) and 'ILWC 3' (*C. yamashitae*), which were most closely related accessions to the cultivated species.

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