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# RELATIONSHIP AMONG ANNUAL *CICER* SPECIES USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

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## ABSTRACT

**The present investigation further demonstrates the potential of RAPD finger printing in detecting polymorphism among cultivars and wild accessions of chickpea. The random amplified polymorphic DNA (RAPD) technique was applied for species and strain identification. Large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. Though the reproducibility of RAPD profiling in the polymorphy is still debated, yet the technique has become invaluable due to its being inexpensive and faster for developing large number of DNA markers without involving sophisticated instrumentation. DNA was isolated from young healthy leaves of total twenty four cultivars and wild varieties of *Cicer* using the CTAB extraction method, with minor modification, followed by the PCR procedure. Detection of genetic relationships between 19 *Cicer arietnum* cultivars and their five wild accessions of *Cicer reticulatum* were investigated by using RAPD markers. On an average, six bands per primer were observed in RAPD analysis. The wild accessions shared 77.8% polymorphic bands with other *Cicer arietnum* cultivars. On analysis 51.7% and 50.5% polymorphic bands were observed among wild and other cultivars respectively. The results indicate that the RAPD technique is useful for distinguishing polymorphisms in these species and can therefore provide useful information for the management of genetic resources. The genetic data produced through RAPD can also be used to correlate the relationships based on pedigree data and morphological traits. This is to minimize the individual inaccuracies in chickpea. The results here show that RAPD markers can be employed for confirming the relationships among various taxonomic units in the genus *Cicer*.**

**KEYWORDS**: Chickpea, *Cicer arietinum*, DNA, RAPD

Grain legumes rank second only to cereals in importance as staple protein food. The genus *Cicer* L. belongs to the family leguminosae, subfamily Papilionaceae. Chickpea (*Cicer arietinum* L.) is a self pollinated diploid (2n=2x=16) annual grain legume and is traditionally grown in many parts of the world. Analysis of genetic polymorphism in cultivars of chickpea based on morphological traits, seed protein profile and isozyme studies have shown few polymorphic loci. A low level of unstable polymorphic loci in this crop has limited the linkage analysis of the various genes and thus assignment of important traits in linkage map. Few wild annual and perennial species of *Cicer* have been examined using isozymes pattern. These have shown many polymorphic isozyme loci useful for studying the genetic relationships amongst these (Tayyar and Waines, 1996). Interspecific hybridizations have also been performed between the cultivated accessions and various wild *Cicer* species which includes *Cicer reticulatum*; presumably being progenitor of the present day chickpea . This is done with a new to introduce various disease resistance genes into chickpea cultivars.

The development of molecular marker technique like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD) and Microsatellites has helped in the identification of useful polymorphic loci in various crop species (Williams et al., 1990; Winter and Kahl, 1995). RFLP analysis in chickpea accessions has shown a very low level of polymorphism and therefore, a narrow genetic base in an important grain legume (Udupa et al., 1993; Van Rheenen 1992; Banerjee et al., 1999). However, oligonulotide finger printing with short microsatellite motifs has revealed considerable variation in chickpea at the interspecific level ( Sharma et al., 1995).

Studies have shown the role of random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) in the detection of DNA polymorphism (Williams et al., 1990.). Variations among individuals for RAPD profile

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mostly arise from base-pair substitution or insertions/deletions that modifies and eliminates the primer site to a distance that will not permit amplification. RAPD markers have been demonstrated to be useful for the studies of taxonomic identities, systematic relationship, population genetic structure, species hybridization, and parentage identification (Ahmad, 1999). Though the use of RAPD markers for molecular typing can be affected, careful optimization of the RAPD protocol has been shown to increase the reproducibility of the RAPD data .The present study has therefore, been performed with an objective to survey the usefulness of RAPD markers for varietals identification in chickpea and also to determine genetic similarities between chickpea breeding lines and the cultivars.

## MATERIALS AND METHODS

### Plant Material

Sample of dry mature seeds of the Twenty four annual *Cicer* species which included 19 cultivated species and five wild related species were utilized in this study. The seed materials were generously provided by ICRISAT. Plants were grown in the field and DNA was isolated from each of these. Bulked DNA was used for RAPD analysis for the purpose of establishing species relationship. Individual plant DNA was used for determining inter-plant genetic variation.

### DNA Isolation

DNA was isolated from young healthy leaves of Twenty four above mentioned cultivars using the CTAB extraction method of Doyle and Doyle (1987), with minor modification. Leaf material was ground to powder in liquid nitrogen and was then transferred to eppendorf tubes. 100mg leaf tissue was ground in 1ml CTAB extraction buffer (100mM Tris pH 8.0; 1.4M NaCl; 20mM EDTA pH 8.0; 0.2% (v/v) β-mercaptoethanol; 2 %(v/v) CTAB) and heated at 60°C for 30 min. DNA was extracted with one volume of a chloroform: isoamyl alcohol mix (24:1), and then centrifuged at 12000 rpm for 15 min at 4°C. Supernatant was taken and further mixed with one volume of chloroform: isoamyl. This was again centrifuged at 12000 rpm for 15 min at 4°C and then precipitated with isopropanol to 40% v/v final concentration. The DNA pellet was washed with 5mM ammonium acetate and 70% ethanol, dried and dissolved in 100µl of TE buffer (19mM Tris-HCl pH 8.0; 1mM EDTA pH 8.0).

### DNA Electrophoresis and Quantification

The electrophoresis of the soluble DNA was performed on 1% agarose gel in TAE buffer (Tris acetateEDTA) and stained with ethidium bromide (0.5µg $ml^{-1}$). Operations were carried out at 100 V for 45 min. Visualization was done under a UV transilluminator. The quantification has been done by comparisons to lambda DNA mass marker (MBI, Fermentas, Richlands B. C., Qld). The quality was evaluated by measuring absorbance ratio of 260 and 280 nm.

### RAPD Assay

Sixty RAPD primers (OPERON Tech.Inc. Almeda, USA) dissolved in sterile TE buffer at a concentration of 15µg $ml^{-1}$ were used for RAPD analysis. The PCR procedure of Williams et al., (1990) was followed with modification as follows: PCR was carried out in 25 µl reaction volumes containing 10mM Tris-HCl pH 9.0; 50mM KCl; 0.1% TRITON X-100; 1.5mM MgCl; 0.1mM dNTP; 2mM primer; 0.5 unit of Taq DNA polymerase (promega) and 25 ng template DNA. Amplification were carried out in a thermo-cycler programmed for 35 cycles with an initial melting at 94°C for 4 min, followed by denaturation at 94°C for 1 min. The annealing was performed at 37°C for 1 min, which was then followed by polymerization at 72°C for 2 min. Final extension step was at 72°C for 7 min.

Amplification products were then subjected to electrophoresis on 1.8% agrose gel run in 1X TAE buffer and detected by staining with ethidium bromide. Standard molecular weights were used as markers. Each electerophoretic run was visualized under a trans-illuminator and also photographed on Gel doc.

## RESULTS AND DISCUSSION

Agronomic, morphological, biochemical (e.g. storage proteins, isozymes), and molecular characteristics are either direct or indirect representations of genetic variability at the DNA level. These are therefore, expected to provide an inter/intra specific information about genetic relationships. The assessment of genetic diversity is

important not only for crop improvement but also for efficient management and conservation of germplasm resources. 20 reproducible RAPD markers were used to ascertain the genetic polymorphism and inter genetic relationship amongst the 19 cultivars of *C. arietinum* and five different accessions of its wild relative *Cicer reticulatum*.

RAPD analysis revealed considerable polymorphism among chickpea cultivars. Sixty random with twenty each of OP- (AS), OP-(I) and OP-(F) primers were taken and of these only 29 primers were found to be polymorphic. 6 bands on an average per primer of the 29 polymorphic primers therefore, numbering 176 bands were observed showing around 74.4% polymorphy of the total 236 bands. Fig.1 shows the RAPD banding pattern of chickpea cultivars and their wild accession along with the markers. The RAPD data as collected from the banding patterns showed 49.4% and 50.5% of common and polymorphic bands respectively amongst the chickpea cultivars. Similarly wild accessions showed 48.3% and 51.7% of common and polymorphic bands respectively. Maximal bands, numbering 11 were shown by the primer 3

**Table 1: List of *Cicer* accessions used in RAPD analysis**

| *S.No* | *Cicer* species | Accession | Seed type | Source |
|---|---|---|---|---|
| 1 | *C. arietinum* | ICC1400 | desi | ICRISAT |
| 2 | *C. arietinum* | ICC1468 | desi | ICRISAT |
| 3 | *C. arietinum* | ICC1532 | desi | ICRISAT |
| 4 | *C. arietinum* | ICC3996 | desi | ICRISAT |
| 5 | *C. arietinum* | ICC4475 | desi | ICRISAT |
| 6 | *C. arietinum* | ICC5124 | kabuli | ICRISAT |
| 7 | *C. arietinum* | ICC12952 | desi | ICRISAT |
| 8 | *C. arietinum* | ICC76 | desi | ICRISAT |
| 9 | *C. arietinum* | ICC12004 | kabuli | ICRISAT |
| 10 | *C. arietinum* | ICC6328 | kabuli | ICRISAT |
| 11 | *C. arietinum* | ICC13077 | kabuli | ICRISAT |
| 12 | *C. arietinum* | ICC15518 | kabuli | ICRISAT |
| 13 | *C. arietinum* | ICC6263 | desi | ICRISAT |
| 14 | *C. arietinum* | ICC 11944 | desi | ICRISAT |
| 15 | *C. arietinum* | ICC1180 | kabuli | ICRISAT |
| 16 | *C. arietinum* | ICC 6537 | desi | ICRISAT |
| 17 | *C. arietinum* | ICC 7554 | kabuli | ICRISAT |
| 18 | *C. arietinum* | ICC 6306 | desi | ICRISAT |
| 19 | *C. arietinum* | ICC637 | desi | ICRISAT |
| 20 | *C. reticulatum* | ICC17164 | kabuli | ICRISAT |
| 21 | *C. reticulatum* | ICC17163 | kabuli | ICRISAT |
| 22 | *C. reticulatum* | ICC17123 | kabuli | ICRISAT |
| 23 | *C. reticulatum* | ICC17121 | kabuli | ICRISAT |
| 24 | *C. reticulatum* | ICC17141 | kabuli | ICRISAT |

of OP-F (OPF-3), whereas minimum of 2 bands were shown by primer 12 of the same series (OPF-12).

Using the NTSYS-PC (version 2.02e) software on UPGMA cluster analysis to generate a RAPD dendogram presented two major clusters amongst the wild and cultivars of chickpea. The similarity coefficient varied from 0.84 to 0.96 and 0.71 to 0.80 in the cultivars and the wild accessions respectively.

Despite being self-pollinated, the cultivars seem to present an excessive genetic variation amongst themselves. The reason for the same could be explained on the basis of heterozygocity at certain marker loci in specific accessions. Earlier also RAPD markers were successfully used to identify genetic variation in *Cicer* (Iruela et al., 2002). In the present analysis of cultivars, the variations identified were greater as compared to analysis on the basis of isozymes or on the basis of seed storage proteins reported earlier on annual *Cicer* species ( Labdi et al., 1996; Tayyer and Wainess, 1996). In any case, the existence of large genetic variation amongst chickpea cultivars and its wild accession can therefore, be exploited constructively for gene tagging and mapping. It can further be employed to introduce disease and insect resistance into cultivated genotypes.

The present investigation further demonstrates the potential of RAPD finger printing in detecting polymorphism among chickpea and their wild accessions. *C. reticulatum* cultivars had the narrowest genetic variation while its wild *C. reticulatum* accessions had much higher genetic variation. This could be used in strategies for the chickpea improvement. However, the genetic diversity was unbalanced and varies considerably in amongst the species of the world collections consisting of the wild annual *Cicer* germplasm. The number of accessions and genetic diversity in the wild annual *Cicer* germplasm overall is very limited compared to other major cereal collections, viz wheat, barley and rice (Virk et al., 1995; Abbo et al., 2003). The markers generated by RAPD assays can also provide practical information for the proper management of genetic resources in the chickpea by selecting good parental genome for breeding programs; this can be further improved substantially by incorporating pedigree data and morphological traits so as to minimize the individual inaccuracies of the cultivars.

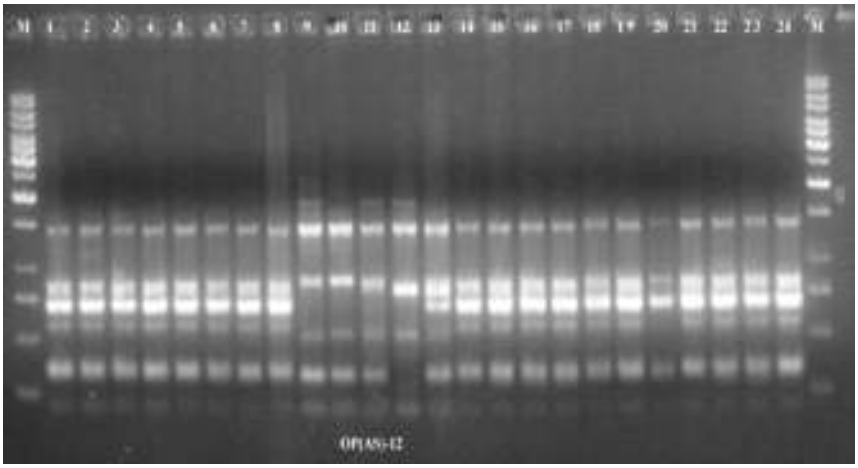

**Fig. 1: Agarose gel showing the amplified product using OP (AS) 12 primer. M; I kb ladder**

Lane 18;, ICC1400, ICC1468, ICC1532, ICC3996, ICC4475, ICC5124, ICC12952, ICC76
Lane 913; ICC 17121, ICC 17123, ICC 177141, ICC 17164 and ICC17163,
Lane 1424; ICC-12004, ICC-6328, ICC13077, ICC15518, ICC6263, ICC 11944, ICC1180, ICC 6537, ICC 7554, ICC 6306, ICC637

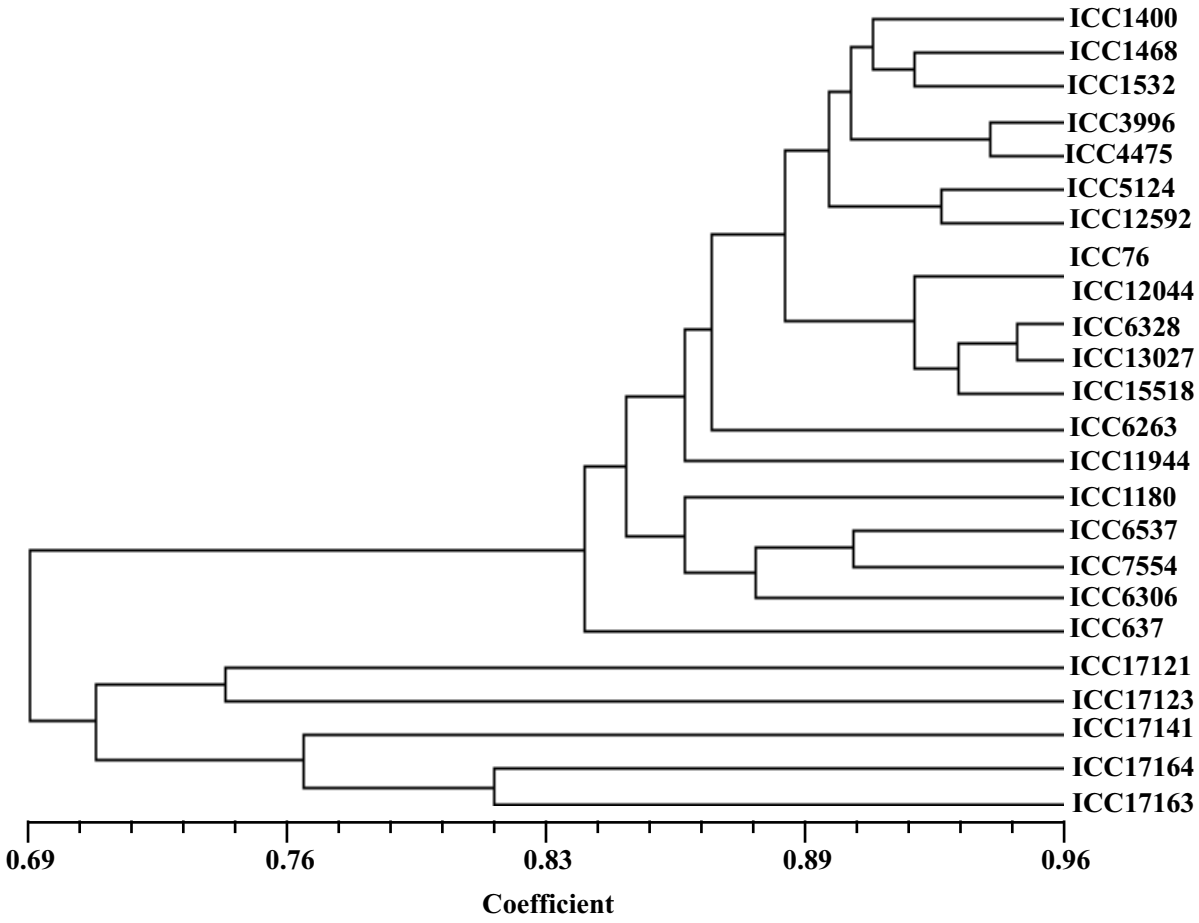


**Fig.2: Dendogram showing the relationship coefficient amongst the 24 accession of chickpea by RAPD analysis**

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**REFRENCES**

Ahmad F.,1999. Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among the annual *Cicer* species. Theor Appl Genet., **98**: 657-663.

Banerjee H., Pai R.A. and Sharma R.P.,1999. Restriction fragments length polymorphism and random amplified polymorphic DNA analysis of Chick pea accession. Biol. Plant, **42**: 197-208.

Doyle J.J. and Doyle J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue.Phytochem Bull., **19**:11-15.

Iruela M., Rubio J., Cubero J.I., Gil J. and Milan T. ,2002. Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers.Theor Appl Genet., **104**:643-651.

Labdi M., Robertson L.D., Singh K.B. and Charrier A., 1996. Genetic diversity and phylogentic relationships among the annual Cicer species as revealed by isozyme polymorphism. Euphytica, **88**:181-188

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

Tayyar R.I. and Waines J.G. ,1996. Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation. Theor Appl Genet., **92**: 245-254.

Udupa S.M., Sharma A., Sharma A.P. and Pai R.A. ,1993. Narrow genetic variability in *Cicer arietinum* L. as revealed by RFLP analysis. J Plant Biochem Biotechnol., **2**:83-86.

Van Rheenen H.A. ,1992. Biotechnology and chickpea breeding. Int Chickpea Newsl, **26**: 14-17.

Virk P.S., Newbury H.J., Jacckson M.T. and Ford-Lioyd B.V. ,1995. The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. Theor Appl Genet., **90**:1049-1055.

Willams J.G.K., Kubelik A.R., Livak K.J., Rafalski J.A. and Tingey S.V. ,1990. DNA polymorphism amplified by arbitraray primers are useful as genetic markers. NuceicAcids Res .,**18**:6531-6535.

Winter P. and Kahl G. ,1995. Molecular marker technology for plant improvement. World J Microb Biotechnol., **11**: 438-448.