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GENETIC DIVERSITY IN FIVE *CICER* GENOTYPES USING OPM-05 PRIMER

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ABSTRACT

Five genotypes of Cicer (Dhawal, Ujjwal, Shubhra, DCP-92-3 and IPC-04-20) were analyzed to estimate genetic diversity by using OPM-05 primer. A total of 21 bands were detected, all of them were polymorphic. Similarity index calculated was found highest between Ujjwal and IPC-04-20 (37.5%) and lowest between Shubhra and IPC-04-20 (22.22%). Dice's similarity coefficient ranged from 0.00000 to 0.25000. A dendrogram constructed based on UPGMA revealed two clusters. Cluster 1 consists of three accessions-S1, S3 and S5 while as cluster 2 consists of two accessions- S2 and S4. It is evident from the dendrogram that accession DCP-92-3 and IPC-04-20 are genetically dissimilar; hence it is recommended that these should be used for future breeding programs to create higher amount of genetic variability in chickpea.

Keywords: Genetic diversity, *Cicer*, RAPD, UPGMA dendrogram

INTRODUCTION

The genus *Cicer* includes 33 perennials, eight annuals and one unspecified wild species as well as the cultivated chickpea (Van der Maesen 1987). Chickpea is the second most important cool season pulse crop in the world and is grown in atleast 33 countries including central and west Asia, South Europe, Ethiopia, North Africa, North and South America and Australia (Ladizinsky and Adler 1976; Singh and Ocampo 1997).

Chickpea is the most important pulse crop in India. It is commonly grown in Uttar Pradesh, Panjab, Maharashtra, Rajasthan, Bihar and Madhya Pradesh. These together accounts for more than 90% of the total area under it. There are two broad groups of gram-brown and white. The brown or desi type is most widely grown. The white or kabuli is characterized by larger seeds.

In India, gram is sown as Rabi crop at the end of the rainy season. Sowing takes place from September to November, and harvesting from February to April.

India is the largest producer of chickpea, accounting for 66% of the world production (FAO 2004). The average annual yield world wide (0.78 ton/ hectare) is considered to be somewhat lower than its potential yield (Singh *et al.*, 1994; Sudupak *et al.*, 2002).

Assessment of the extent of genetic variability within chickpea is fundamental for chickpea breeding and the conservation of genetic resources and is particularly useful as a general guide in the choice of parents for breeding hybrids. Criteria for the estimation of genetic diversity can be different which include morphological traits (Upadhaya *et al.*, 2007) or molecular markers (Sharma *et al.*, 1995). Molecular markers have proved to be useful tools in the characterization and evaluation of genetic diversity within and between species and populations. A no. of DNA based markers is now available for the effective quantification of

genetic variation in plant species. Restriction fragment length polymorphism (RFPL) and Amplified fragment length polymorphism (AFLP) have been applied successfully and have provided considerable genetic information in a no. of plant species (Vos *et al.*, 1995, Xu *et al.*, .2000). These techniques are slow and expensive and are not amenable for assessment of genetic variation in large scale population genetic studies. More recently PCR based RAPD and simple sequence repeat (SSR) markers requiring small amounts of DNA have also been developed (Williams *et al.*, 1990). SSR markers have been proved to be polymorphic but require nucleotide information for primer design (Sun *et al.*, 1998). RAPD methodology overcomes this limitation; considerable polymorphic markers can be obtained with relative ease from minute amounts of genomic DNA without prior knowledge of sequence information

RAPD is a PCR based technique first developed by Williams *et al.*, (1990) widely used in molecular biology. Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus don't require prior knowledge of a DNA sequence. Low expense, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment has made the RAPD technique valuable although reproducibility of the RAPD profile is still the centre of debate (Bardakcl F 2001).

MATERIALS AND METHODS

The germplasm were obtained from Indian Institute of Pulse Research, Kanpur (U.P.) India. During the present study, five accessions of *Cicer* were used to estimate genetic diversity by using RAPD analysis. The details of five accessions are given in table 1.

Table1: Five experimental accessions of *Cicer*

S. No.	Accession name	Sample name	Source	Seed size
1	Dhawal	S1	IIPR, Kanpur	Extra Bold
2	Ujjwal	S2	Same	Bold
3	Shubhra	S3	Same	Bold
4	DCP-92-3	S4	Same	Small
5	IPC-04-20	S5	Same	Small

RAPD analysis

For RAPD, 1g seed of each accession was used to isolate the genomic DNA

Genomic DNA isolation

The problem of DNA extraction is still an important issue in the field of plant molecular biology. Various plants contain high levels of polysaccharides and many types of secondary metabolites affecting DNA purification (Zidani *et al.*, 2005). CTAB procedure based on the protocol of Doyle and Doyle (1990) is the method of choice for obtaining good quality DNA from many plant species and also from fungi (Weising *et al.*, 1991). CTAB is a cationic detergent which solubilizes membranes and forms a complex with DNA. One gram seed of each accession was ground to a fine powder in liquid nitrogen then transferred to PCR tubes and an equal volume of hot (65^0C) 2X CTAB was added to it. The mixture was incubated at 65^0C for 10 minutes and mixed it with an equal volume of chloroform/isoamyl alcohol (24: 1) and centrifuged at 13000 g for 5 minutes. The aqueous phase was then transferred to another tube and 1/10 volume of 10 X CTAB was added to it, mixed and treated with an equal volume of chloroform/isoamyl alcohol. An equal volume of CTAB precipitation buffer was added to the aqueous phase to precipitate the DNA. The DNA pallet was rehydrated in high salt TE buffer and the DNA reprecipitated with 2 volumes of chilled

ethanol. Finally the DNA pallet was air dried rehydrated in 0.1 X TE buffer and treated with RNase.

Selection of the primer

The decamer primer OPM-05 (5'GGGAACGTGT 3') used for RAPD analysis in chickpea was selected on the basis of prior study. It showed high polymorphism in the earlier studies also as revealed by Talebi et el 2009 and other workers.

Polymerase Chain reaction

PCR stand for polymerase chain reaction is a technique used to selectively amplify in-vitro a specific segment of the total genomic DNA a billion fold. The most essential requirement of PCR is the availability of a pair of short oligonucleotide called primers has sequences complementary to either end of the target DNA segment to be synthesized in large amounts (Ahmad *et al.,* 2010). Amplification are carried out in a themocycler for about 35-40 cycles with an initial strand separation at 94^0C, 1 minute at 37^0C and 2 min. at 72^0C.

Amplification products were electrophoresed in 2% agarose gels and detected by staining with ethiduim bromide. After electrophoresis, the RAPD patterns were visualized with UV transilluminator. RAPD markers were scored as DNA fragments present or absent. The bands scored were used for the construction of dendrogram by using UPGMA (Unweighted Pair Group Mean and Arithmetic Average) method to know the phylogenetic relationships among the five accessions of *Cicer*.

RESULTS AND DISCUSSION

The RAPD analysis of five Cicer genotypes was carried out to investigate genetic diversity using OPM-05 primer.RAPD markers represent an efficient and inexpensive way to generate molecular data, thus have been used successfully in various taxonomic and phylogenetic studies (Aboelwafa et al., 1995; Sharma et al., 1995; Friesen et al., 1997., Wolff and Morgan-Richards 1998). Only clearly scorable bands were included in the analysis. Minor bands which could not be scored reliably were not included in the analysis. In total 21 visible bands were scored in all accessions corresponding to an average of 4.2 bands per accessions. All bands were polymorphic (Table 2, Figure 1). Similar results were observed by Ahmad F (1999) in chickpea by using 75 random decamer primers. Several other workers also observed high polymorphism by using different random primers in different species. Sudupak et al., (2002) used seven decamer primers in chickpea and observed 96.82% polymorphism. Sharma et al., (2000) also recorded 100% polymorphism in *Podophylum haxandrum* by using seven decamer primers and SDS-PAGE. Jaya prakash et al., (2006) recorded 97.4% polymorphism in sorghum genotypes by using 64 RAPD primers. Aboelwafa et al., (1995) used 40 primers and recorded 90% polymorphism in Lens.Verma et al., (2009) used 20 random primers and observed 96.8% polymorphism in *Trichodesma indicum*. Iqbal et al., (2010) worked on *Jatropa curcas* L. and recorded 93.90% polymorphism by using 50 random primers. Chickpea genotype Dhawal produced the maximum number of bands (9), while Ujjwal gave the minimum number (4) of bands (Table 2, Figure 1).

Table 2: Binary scoring of bands in five accessions of *Cicer* using RAPD

Band No.	Rf value	Base pairs	S1	S2	S3	S4	S5
1	0.21	1185	0	0	1	0	0
2	0.26	1110	1	0	0	0	0
3	0.37	945	0	0	0	1	0
4	0.41	885	1	0	1	0	0
5	0.42	870	0	0	0	0	1
6	0.43	855	0	1	0	0	0
7	0.47	795	1	0	1	0	1
8	0.50	750	0	0	0	1	0
9	0.53	705	1	0	0	0	0
10	0.55	675	0	1	0	0	0
11	0.58	630	0	0	1	0	0
12	0.60	600	1	1	0	0	0
13	0.63	555	1	0	1	0	0
14	0.65	525	0	0	0	1	0
15	0.70	450	0	0	1	1	1
16	0.73	405	1	0	0	0	0
17	0.81	285	0	0	0	0	1
18	0.83	255	1	1	0	1	0
19	0.90	150	0	0	0	0	1
20	0.96	60	0	0	0	0	1
21	0.98	30	1	0	0	0	0

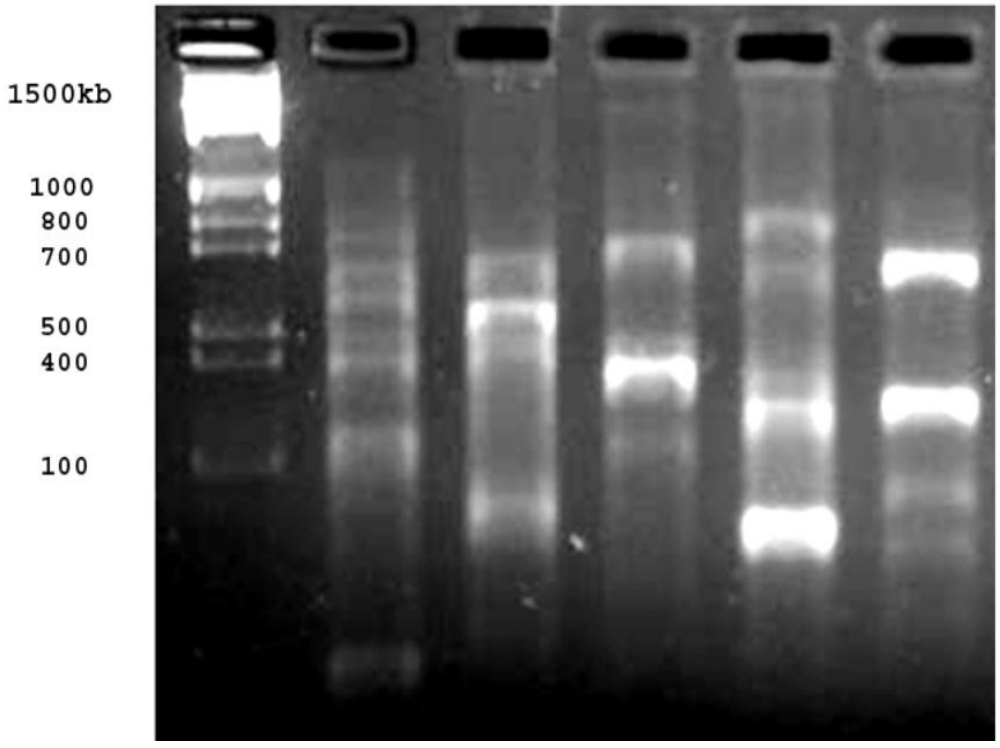

Figure 1: RAPD profile of five *Cicer* accessions

Dice's similarity coefficient

	S1	S2	S3	S4	S5
S1					
S2	0.18182				
S3	0.25000	0.00000			
S4	0.07692	0.12500	0.10000		
S5	0.07143	0.00000	0.20000	0.10000	

The dendrogram obtained from the UPGMA method is shown in Figure 2. Cluster analysis divided the five accessions into two clusters. Cluster I comprising three accessions (Dhawal, Shubhra and IPC-04-20) while as the cluster II consists of two accessions (Ujjwal and DCP-92-3). It is evident from the dendrogram that Dhawal and Shubhra and Ujjwal and DCP-92-3 are very close to each other while as IPC-04-20 and DCP-92-3 are genetically dissimilar. Closely related species commonly lose the ability to interbreed and become genetically isolated due to chromosomal structural mutations (Tayyar and Wainess 1996). IPC-04-20 and DCP-92-3 are genetically distant, hence it is recommended that these two accessions should be used in future breeding programs to create higher amount of genetic variability in chickpea.

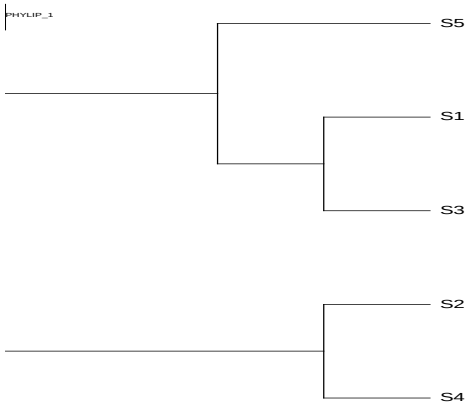

Figure 2: UPGMA dendrogram depicting genetic relationships among *Cicer* accessions based on RAPD.

CONCLUSION

RAPD analysis is efficient in revealing usable level of DNA polymorphism among cultivars of chickpea. By this study, we can show how much diversity and similarity level is present in five cultivars of chickpea. We can also be able to identify genetic makeup of the variety to protect the breeder's right. We can estimate the genetic distance among the cultivars of chickpea and calculate the similarity coefficient among the cultivars. It is suggested on the basis of RAPD analysis that chickpea cultivars DCP-92-3 and IPC-04-20 will produce higher hybrid vigour when they are utilized in breeding programs because they are genetically distinct from other cultivars of chickpea and have minimum genetic similarity.

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