

Original Research Article

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Characterization of Chickpea (*Cicer arietinum* L.) Genotypes through RAPD Analysis

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ABSTRACT

Keywords

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Chickpea (*Cicer arietinum* L., $2n=16$) is a legume of family *Fabaceae*, subfamily *Faboideae*. Chickpea is the second largest grown food legume of the world (Gaur *et al.*, 2008). Genetic divergence among 28 genotypes of *Cicer arietinum* L. was assessed through RAPD molecular markers. RAPD analysis showed 93.36 per cent of polymorphism. A total of 159 amplified bands were obtained that ranged between 150 bp to 3200 bp, using 22 RAPD primers only 15 showed amplification. The 153 out of 159 bands were polymorphic. The average PIC (polymorphic information content) of RAPD was found 0.259. Jaccard's similarity coefficient for RAPD lay between 0.31 and 0.79 (31% to 79%). Dendrogram based on genetic distance calculated by UPGMA method segregated the 28 chickpea genotypes into three RAPD major clusters, respectively. Two and three dimension principal component analysis (PCA) showed similar clustering of 28 genotypes as evident from cluster tree analysis with dice similarity coefficients ranged from 0.58 to 0.85 (58% to 85%) for RAPD analysis. Based upon RAPD analysis of 28 genotypes with 15 primers genotype PBC-509, PBC-511, Pratap channa-1, Dohad yellow and Pratap Rajchana were identified as genetically diverse. Therefore, the results of the present study can prove helpful for implementation of chickpea improvement programmes.

Introduction

Chickpea (*Cicer arietinum* L., $2n=16$) is a legume of family *Fabaceae*, subfamily *Faboideae*. Chickpea is the second largest grown food legume of the world (Gaur *et al.*, 2008). Chickpea cultivation in India covers an area of about 81.17 lakh hectares with a production of 59.01 lakh tonnes grains (FAOSTAT data, 2016). To develop high yielding varieties, assessment of variability is

a first step in any breeding programme. Substantial diversity exists among cultivated chickpea genotypes for various morphological, physiological and agronomic traits (Stalker, 1992). Greater the diversity in germplasm, better are the chances of improvement, provided the heritability is high and genetic advance is more.

Many DNA based molecular marker techniques are present for characterization of

genetic diversity in plant species (Weising *et al.*, 2005; Nybom *et al.*, 2014). Among the available markers, randomly amplified polymorphic DNA (RAPD) has been most widely used for diversity studies, DNA fingerprinting, map construction and linkage analysis.

The enormous attraction of RAPDs is due to the non-requirement for DNA probes, or for any sequence information for the design of specific primers. The procedure involves no blotting or hybridizing steps (Williams *et al.*, 1990).

The power of RAPD is that it is a fast technique, easy to perform and comparatively cheap. It is immediately applicable to the analysis of most organisms because universal sets of primers are used without any need for prior sequence information (Hallden *et al.*, 1996).

This marker system was used in many different applications involving the detection of DNA sequence polymorphisms, mapping in different types of populations (Carlson *et al.*, 1991; Reiter *et al.*, 1992), isolation of markers linked to various traits or specific targeted intervals (Giovannoni *et al.*, 1991; Michelmore *et al.*, 1991) and applications such as variety identification and analysis of parentage (Tinker *et al.*, 1993; Mailer *et al.*, 1994). In self pollinating crop species RAPD marker analysis is very useful technique with a relatively low level of intraspecific polymorphism, such as chickpea.

Considering the importance of chickpea in the State's economy in terms of area stability, production, productivity and its large consumption, the present study was proposed using 28 diverse elite genotypes to analyze genetic diversity critically at molecular level with by using RAPD analysis.

Materials and Methods

Plant material

In the present investigation, leaf samples of 21 days old plants from 28 genotypes of chickpea (*Cicer arietinum* L.) were procured from field of Agricultural Research Station (ARS), MPUAT, Borwat Farm, Dahod Road, Banswara (Raj.) – 327001.

RAPD analysis

The genomic DNA was extracted from 21 days old leaves of 28 genotypes of chickpea by CTAB extraction method of Doyle and Doyle (1990), with slight modifications. A set of 22 random decamer primers (Bangalore Genei, India) with more than 60% GC content were selected out of which 15 primers were selected for data analysis. The best amplified products were obtained using 20 µl of reaction mixture which specifically contained 50 ng template DNA, 0.5µM primer, 200µM of dNTPs mix, 2.5 mM MgCl₂, 1X Taq Polymerase buffer, 1 U Taq Polymerase. PCR amplification was performed to fulfill 35 cycles after an initial denaturation at 94 °C for 5 min. RAPD patterns were analysed by scoring presence (1) or absence (0) of bands for estimation of similarity among all tested samples. The matrix of similarity (Jaccard) and similarity of coefficients were calculated and the dendrogram obtained by clustering according to the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) using NTSYS-pc software. Principal component analysis along with 2D and 3D plots were constructed using eigen values and vectors.

Results and Discussion

An attempt has been made to estimate molecular diversity of chickpea genotypes, which would help in framing an effective

breeding programme. Isolated and purified DNA was subjected to PCR based molecular marker (RAPD) for assessment of genetic diversity in the genotypes of *C. arietinum*. DNA fingerprinting has become an essential tool for studying evolution and identification of crop germplasm, since this technological intervention is more precise, economical and least affected by environmental factors.

Out of 22 primers only 15 showed amplification. A total of 159 amplified bands were obtained out of which 153 were polymorphic, thus showing 93.36% polymorphism (Table 1 and Fig. 1).

The DNA amplicon size and polymorphism generated among the twenty eight genotypes

of *Cicer arietinum* using RAPD primers are presented in Table 1. The total number of amplified bands varied between 5 (primer OPP-05) and 16 (primer OPF-19) with an average of 10.6 bands per primer.

The polymorphism amongst 28 genotypes of *Cicer arietinum* was 93.36% and the overall size of PCR amplified products ranged between 150 bp to 3200 bp. The per cent polymorphism ranged from as low as 66.67 (OPP-01) to as high as 100 (OPA-01, OPA-02, OPA-04, OPD-05, OPD-12, OPF-13, OPF-17, OPF-19, OPP-02, OPP-07, OPP-10). The average PIC was 0.2591 ranging from 0.1508 to 0.408. The lowest and the highest PIC values were recorded for primers OPA-03 and OPF-17, respectively.

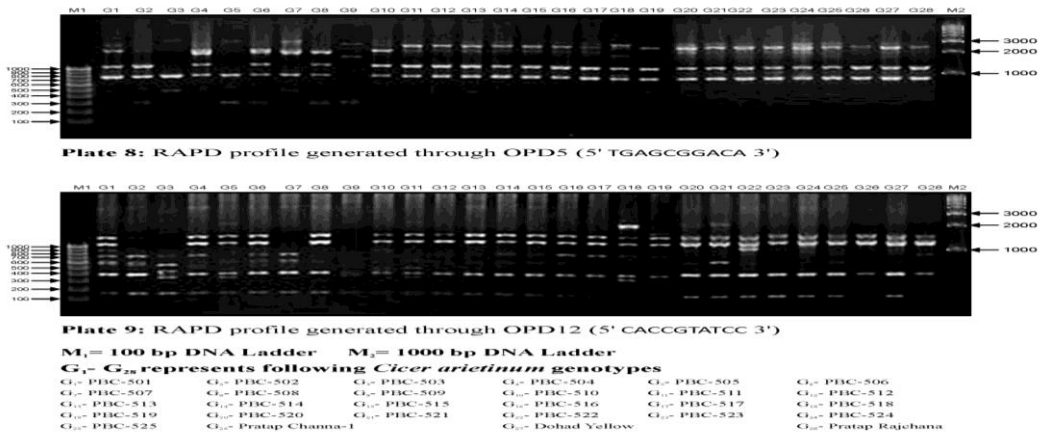
Table.1 DNA amplification pattern and polymorphism generated in *Cicer arietinum* using 15 RAPD primers

S. No.	Primer code	Molecular weight range (bp)	Total number of scorable bands (a)	Total number of polymorphic band (b)	Polymorphism (%) b/a X 100	PIC value
1.	OPA-01	400-1800	7	7	100	0.3008
2.	OPA-02	325-1600	15	15	100	0.1761
3.	OPA-03	150-1500	9	8	72.00	0.1508
4.	OPA-04	250-2000	13	13	100	0.2774
5.	OPD-05	350-2600	8	8	100	0.3037
6.	OPD-12	150-1800	12	12	100	0.2831
7.	OPF-13	175-2100	13	13	100	0.2772
8.	OPF-17	150-1800	9	9	100	0.408
9.	OPF-19	200-3100	16	16	100	0.3642
10.	OPP-1	450-1500	6	4	66.67	0.1518
11.	OPP-2	300-2500	9	9	100	0.2288
12.	OPP-3	200-2300	11	9	81.81	0.2467
13.	OPP-05	500-1800	5	4	80.00	0.1664
14.	OPP-07	175-2700	13	13	100	0.3063
15.	OPP-10	450-3200	13	13	100	0.2448
	Total		159	153	93.36	0.2591

Table.2 Molecular weight range and unique alleles obtained using RAPD primers

S. No.	Primer code	Total number of unique bands	Genotype	Size of bands (bp)
1.	OPA-01	2	PBC-525	1800
2.	OPA-02	4	PBC-502	1400
			PBC-508	1250
			PBC-511	600
			PBC-504	450
3.	OPP-02	2	PBC-518	1900, 2500
Total		08		

Fig.1 RAPD profiles generated by primer OPD-05 and OPD-12. The numbers (1-28) corresponding to genotypes at the top of each lane



Lane M1 (100bp) & M2 (1000bp) are the Molecular Weight Markers

Fig.2 Dendrogram generated for *Cicer arietinum* genotypes for RAPD using UPGMA cluster analysis based on Jaccard similarity coefficient

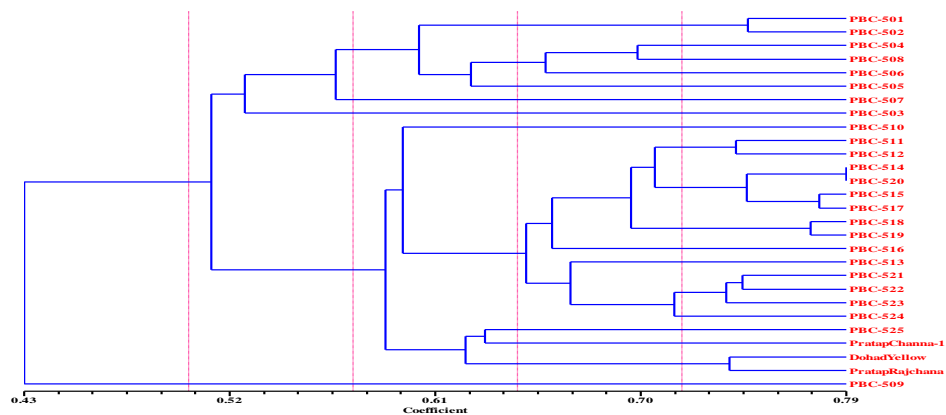
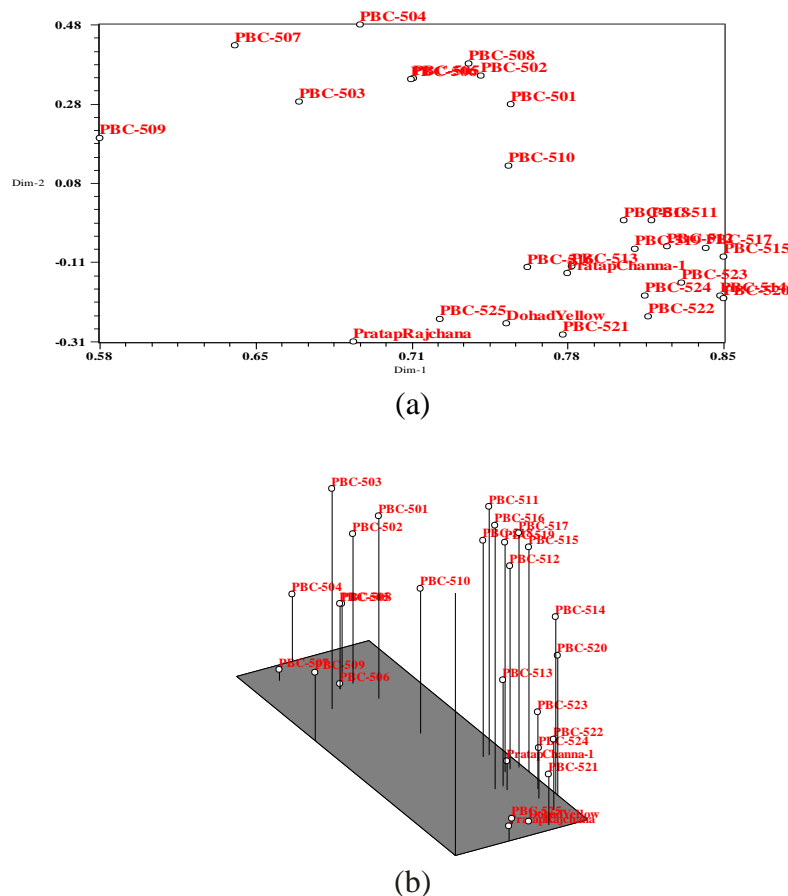


Fig.3(a,b) A two dimensional and three dimensional plot of principal component analysis based on RAPD primer in chickpea genotypes



A total of 8 unique bands (band which is present only in one genotype but absent in rest of the genotypes) were detected in six genotypes. The size of these unique bands ranged from 450-2500 bp (Table 2). Reddy *et al.*, (2008) narrated that cultivars with sharp DNA profiles are probable to contain the greatest number of novel alleles. Therefore, these genotypes are likely to uncover the largest number of unique and potential agronomically useful alleles. The value of similarity coefficient ranged from 0.31 to 0.79, *i.e.* 31-79% on the basis of RAPD similarity data matrix. Maximum similarity value of 0.79 was observed between genotypes PBC-514 and PBC-520. The RAPD cluster tree analysis of 28 *C. arietinum*

genotypes showed that they could be divided into 3 major clusters at a similarity coefficient of 0.51 (Fig. 2). Two and three dimension PCA based on RAPD data (Fig. 3) showed similar clustering of 28 genotypes, as evident from the cluster tree analysis. Dice similarity coefficients for 28 genotypes ranged from 0.58 to 0.85. Most of the genotypes tended to cluster mainly into 5 clusters.

In conclusion the RAPD patterns obtained from our study can serve as a vital input to the conventional method of varietal identification, future germplasm management, and marker assisted selection to improve the efficiency of new cultivar development in future breeding programs that relies solely on morphological

characters. Interestingly collections originating from various parts of the country did not form well defined distinct groups and were interspersed with each other, indicating no association between RAPD pattern and the geographic origin of accession.

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