

Original Research Article

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## Molecular Characterization of Heat Tolerance Chickpea (*Cicer arietinum* L.) Genotypes using Random Amplified Polymorphic DNA (RAPD) Markers

Devendra Vasht<sup>1\*</sup>, S.K. Dwivedi<sup>1</sup>, Yogendra Singh<sup>2</sup>, R.K. Samaiya<sup>1</sup>,  
A. Babbar<sup>2</sup>, S.K. Pooniya<sup>3</sup> and Vinod Kumar<sup>2</sup>

<sup>1</sup>Department of Plant Physiology, <sup>2</sup>Department of Plant Breeding & Genetics, <sup>3</sup>Department of Biotechnology, Jawaharlal Nehru KrishiVishwaVidyalaya, Jabalpur (MP), India

\*Corresponding author

### ABSTRACT

Chickpea (*Cicer arietinum* L.) is most important pulse crop of India in term of area and production and is extensively grown from human consumption mainly in South Asia and Middle East. Chickpea (*Cicer arietinum* L.) is a cool season legume crop and is grown in several countries worldwide as a food source. Molecular markers and variation in genomic DNA sequences have played a major role in varietal characterization and improvement of many crop species and have contributed to our abilities to assess biodiversity, reconstruct accurate phylogenetic relationships and transferring of particular traits. The polymorphism % ranged from 33.33 % (OPX-2) to 100% (OPB-6, OPX-4, OPB-12, OPA-17, OPA-11). The dendrogram was generated and 20 genotypes were aligned in 3 groups i.e. A has 10 genotypes, B has only one genotype and C has 9 genotypes. For molecular characterization of 20 genotypes, 10 RAPD primers showing polymorphism were used.

#### Keywords

DNA Isolation,  
RAPD primers,  
Polymorphism

#### Article Info

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### Introduction

Chickpea (*Cicer arietinum* L.) commonly known as 'Bengal gram or garbanzo' is one of the world's most important grain legume, the seed is a major source of plant-based dietary protein, carbohydrates and minerals especially for the human. Chickpea is a dry and cool season crop largely grown rained on residual soil moisture after the rainy season. The center of origin of chickpea is South East Turkey and Syria. Chickpea is the third most

important food legume crop India is the largest producer contributing to 65% of world's chickpea production. Chickpea is an essential crop for arid and semi-arid regions and is mostly cultivated in dry and rainfed areas (Varshney *et al.*, 2009). According to the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) chickpea seeds contain on average- 23% protein, 64% total carbohydrates (47% starch, 6% soluble sugar), 5% fat, 6% crude fiber and 3% as The chickpea genotypes can be evaluated for heat

tolerance in the semi-arid tropics environment by delaying the sowing time as the reproductive growth in late sown crops often coincides with high atmospheric temperature. Molecular markers and variation in genomic DNA sequences have played a major role in varietal characterization and improvement of many crop species and have contributed to our abilities to assess biodiversity, reconstruct accurate phylogenetic relationships and transferring of particular traits. Molecular genetic markers have brought phenomenal changes in the area of plant biotechnology by their ability to produce unique DNA profiles in various crops. The invention of the polymerase chain reaction (PCR) to amplify short segments of DNA gave rise to second generation of faster and less expensive PCR-based markers. RAPD-PCR technique employed for testing of genetic diversity and it's rapidly being used in research field crops improvement.

The technique is useful in examining genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of crop specie (Koutu *et al.*, 2017, 2019). The aim of present study was molecular characterization in chickpea genotype employing RAPD markers.

## **Materials and Methods**

### **Plant material**

A total of 20 genotypes (Table 1) were collected from Research Farm Adhartal, Department of Plant Breeding and Genetics, JNKVV, Jabalpur (M.P.). Leaf samples were obtained from plants (Table 1) for genomic DNA isolation.

### **Genomic DNA isolation**

DNA was isolated from young leaves of chickpea using CTAB Protocol (Saghai-Maroo *et al.*, 1984) with some modifications.

Chemical used for the DNA isolation 100mM Tris-HCl (pH 8.0), 20mM EDTA (pH 8.0), 0.5M NaCl, 24:1 Chloroformisoamyl alcohol (IAA), 3M sodium acetate (pH4.8), Isopropanol (-20°C), 70% ethanol, 5M NaCl. DNA quality was tested by (0.8%) agarose gel electrophoresis and visualized under UV light.

### **PCR Amplification and data analysis**

The PCR amplification of DNA components and their concentration used in the RAPD analysis. PCR amplification reactions volume of 20µl 2µl of PCR buffer 1X, 2.4µl of MgCl<sub>2</sub> 2.5mM, 0.2µl of Tag Polymerase (5Unit/µl) 0.5µl of dNTPs10mM, 2µl of Primer 10pM, 2µl of genomic DNA 50ng and nuclease free water was used to make up the total volume 20µl. PCR cycling parameters for RAPD-PCR an initial denaturation step at 94°C for 4 min followed by 45 cycles at 94°C for 45 second, 38°C for 1 min annealing and 72°C for 2 min elongation. In the final cycle, the elongation step at 72°C was extended by 5 min. PCR product RAPD primers were scored on the agarose gel as presence (1) or absence (0) of bands of molecular weight size and dendrogram generated by DARwin software (2006).

## **Results and Discussion**

RAPD analysis revealed DNA polymorphism among the 20 chickpea genotype (Table 1) were used for molecular characterization with each primer yielded distinct and easily detectable alleles of variable intensity of 10 RAPD markers (Table 2). The average number of bands per primer was 4.6 and maximum number of 9 alleles was produced by OPX-03 and OPD-11. While minimum numbers of alleles were produced by OPX-02, OPX-04 and OPA-04.H. The polymorphism % ranged from 33.33 % (OPX-2) to 100% (OPB-6, OPX-4, OPB-12, OPA-17, and OPA-11) (Fig. 1). The genetic similarity matrix was further analyzed using UPGMA clustering

algorithm by software programme DARwin 5.0.128 (Figure 2). The radial clustering clearly delineated the genotypes into three major clusters with sub groups. A having 10 genotypes (JG12XJG16-1, JG12XICC07110, BDNGXNARSINGPUR BOLD, JAKI9218XJG14, PA003 (303), JG2017-49, JG 36, JG74XJG11551, JG 24, JG12XJG16-3), B have only one genotype (JG 14 X JG 226) and C having 9 genotypes (JG 12, JG74XJG315-2, JG16XICC9029, ICCV15118, ICC96029XJG11551, JG 14, JG74XJG14, JG12X14, JG11XJG14).

Genetic fingerprinting in chickpea, for a long time was hampered by low variability in the chickpea genome. To have more compared data-sets by using combination of more competent markers has become a priority. More so, being a self-pollinated winter crop, available germplasm of cultivated chickpea shows a low genetic variation profile. The present study determines DNA polymorphism in chickpea accessions as revealed by RAPD markers. Overall the study ascertained that RAPD marker provide powerful tools in revealing genetic diversity in chickpea.

**Table.1** List of genotypes

S. No.	Genotypes
1	JG74XJG11551
2	JG12XJG16-3
3	ICCV15118
4	ICC96029XJG11551
5	JG14XJG226
6	PA003(303)
7	JG74XJG14
8	JG11XJG14
9	BDNGXNARSINGPUR BOLD
10	JG16XICC9029
11	JAKI9218XJG14
12	1JG12XICC07110
13	JG12XJG16-1
14	JG74XJG315-2
15	JG12X14
16	JG2017-49
17	JG24
18	JG12
19	JG14
20	JG36

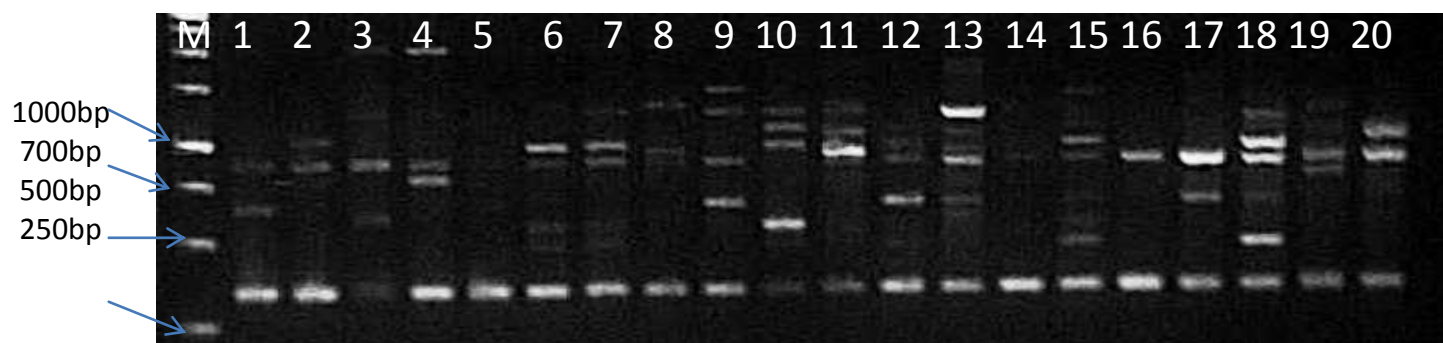
**Table.2** Number of alleles obtaining using RAPD markers

S.No.	Name of Primer	No. of Monomorphic band	No. of Polymorphic band	% of Polymorphism
1	OPX-2	2	1	33.33%
2	OPX-3	2	7	77.77%
3	OPB-6	0	4	100%
4	OPX-4	0	2	100%
5	OPD-11	1	8	88.88%
6	OPB-12	0	4	100%
7	OPD-7	2	2	00.00%
8	OPA-4	1	2	66.66%
9	OPA-17	0	3	100%
10	OPA-11	0	5	100%

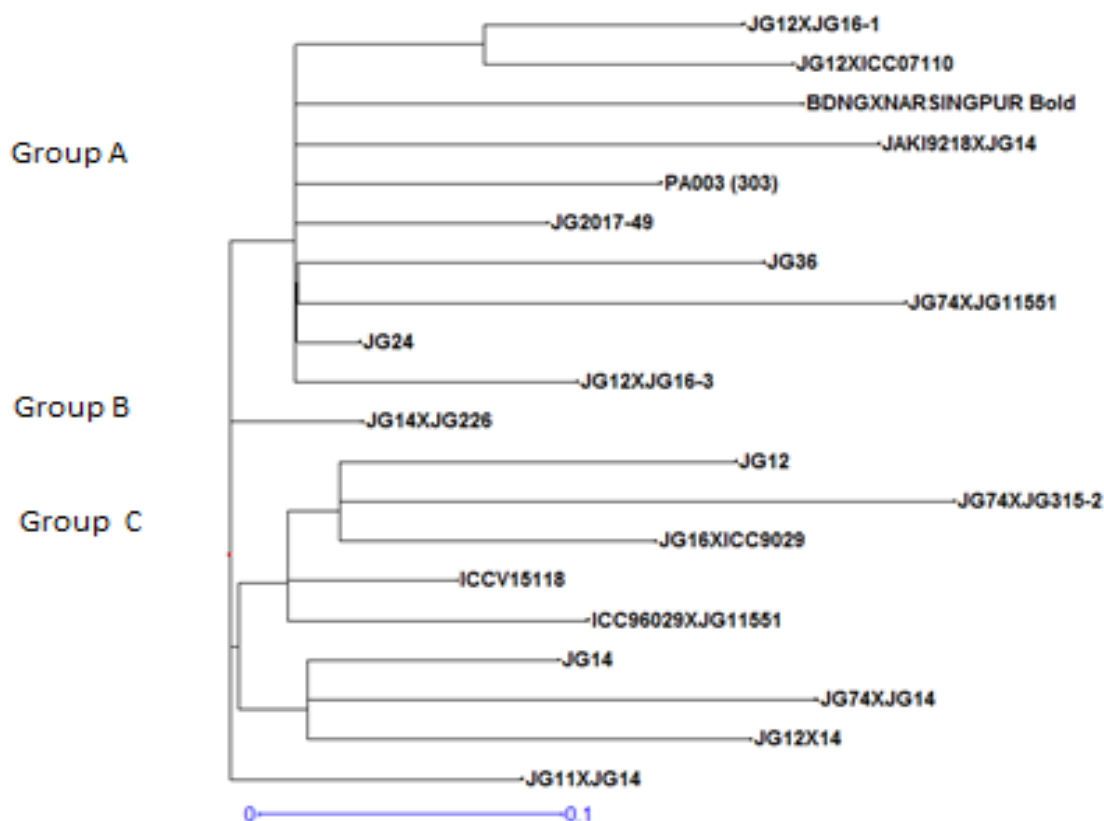
**Table.3** RAPD primers used in this study

S. No.	Primers	Sequences (5'-3')	Temperature (oC)
1	OPX-2	TTCCGCCACC	36
2	OPX-3	TGGCGCAGTG	36
3	OPB-6	TGCTCTGCCC	36
4	OPX-4	CCGCTACCGA	36
5	OPD-11	AGCGCCATTG	36
6	OPB-12	CCTTGACGCA	36
7	OPD-7	AGCGCCATTG	36
8	OPA-4	AATCGGGCTG	36
9	OPA-17	GACCGCTTGT	36
10	OPA-11	CAATCGCCGT	36

**Fig.1** RAPD PCR amplifications of 20 genotypes using OPX-3 marker



**Fig.2** Dendrogram of chickpea genotypes based on RAPD marker



The RAPD markers detect variation at DNA level in chickpea accession. Such genetic variation obtained from RAPD markers may be useful in discriminating chickpea accessions which needs to be complemented with the morphological traits data. Further, the genetic variation that exists between these chickpea accessions can efficiently be used as directives in planning future chickpea breeding programmes. Such genetic diversity data can provide practical information for selection of parental material and thus may assist in forecasting chickpea breeding strategies.

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