

International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

Visit: www.ijirset.com

Vol. 7, Issue 8, August 2018

Genetic Diversity Analysis of Chickpea (*Cicer arietinum* L.) by using RAPD Marker

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ABSTRACT: The present investigation was undertaken to analyze the genetic diversity in 6 chickpea varieties viz, Vijay, Vihar, Kripa, Vishal, Digvijay and Virat using RAPD analysis. Amplification of genomic DNA of 6 chickpea varieties by RAPD analysis, four primers generated 16 amplicons, of which 15 were polymorphic with an average of 3.75 amplicons per primer. A dendrogram was constructed by using the UPGMA that was based on similarity coefficients. The Jaccard's similarity coefficient ranged from 0.39 to 0.67 among the 6 chickpea genotypes. Maximum similarity coefficient 0.67 was observed between Vijay and Vishal. Six chickpea varieties were clustered in two distinct groups that is cluster A and cluster B. Cluster A consists of 5 varieties viz Vijay, Vishal, Digvijay, Vihar and Kripa and cluster B consist of only single varieties. The results from this study may be useful to maximize the selection of diverse parent cultivars and to broaden the germplasm base in the future for chickpea breeding programs.

KEYWORDS: RAPD, Chickpea varieties, Genetic diversity, Polymorphism, Cluster analysis, UPGMA

I. INTRODUCTION

Pulse occupies unique position in Indian Agriculture because of their characteristics of maintaining and restoring soil fertility beside high nutritive value. Pulse restores soil fertility through biological nitrogen fixation with the help of symbiotic bacteria *Rhizobium* in roots. Among the pulse, chickpea is important Rabi crop of India

Like other pulse crop, chickpea has multiple functions in tradition farming system in many developing countries. It is an important human food and animal feed and it also helps in the management of soil fertility.

Chickpea (*Cicer arietinum* L.) is traditionally grown in many parts of the world since ancient time both in Asia and Europe. It is known to have originated in western Asia. It belongs to genus *cicer* and tribe *Cicereae* and sub family *popilionaceae* of family *leguminaceae*. It is relatively small genus with 39 known species distributed mainly in central and western Asia of which two are found to be cultivated in India. They are *Cicer arietinum* ($2n=16$) cultivated in western temperate and Alpine region of Himalaya.

Chickpea seed contain about 23 to 24 percent protein and 2 to 5 percent oil and is consumed in variety of ways. Chickpea is of two types, Desi and Kabuli. The desi type are generally small seeded with colored seed coat and angular seed shape, while kabuli are generally large seeded with dirty white or creamy seed color and ram head shape, the two type are botanically similar but there are strong consumer preference for one or the other. In general kabuli type chickpea have higher type protein contain than desi type but they are low yielder. The main constituent of protein is globulin. It is mostly used in the form of 'Dal'. About 75 per cent of the total production is consumed as dal in India. The whole grain is also eaten raw or boiled and roasted. Green grains also eaten raw as snacks. Floor and meal used frequently mixed with wheat flour to produce unleavened bread or used in preparation of various confectionary products.

In India chickpea was grown on 8.75 million ha area with total production of 8.25 million tons with an average productivity of 983 kg hectare. In case of Maharashtra chickpea was grown on 13.95 lakh ha area with total

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production of 13.01 lakh tones with an average productivity of 9333 kg/ha. Chickpea occupies 41 per cent of total area under pulse and producing about 55 per cent of total pulse production in India.

Through India is largest producer of this crop, it's is productivity is low as compared to that in countries like Italy, Turkey, Iran etc. The important genetic factor like photo and thermo sensitivity, low harvest index, flower and pod drop, pour stability of present cultivars, susceptibility to disease and pest and management factor like predominantly cultivated on receding soil moisture and marginal land inadequate plant production, low use of organic and inorganic fertilizers and inadequate availability of quality seeds, limits the productivity of chickpea. Among the factors listed above susceptibility to major biotic strict factors namely fusarium wilt, pod borer and abiotic stress namely drought, heat, salt and cold.

Genetically diverse parent having better combining ability are more likely to give raise to transgressed segregantes. Germplasm is the reservoir for diversity in yield and yield components. Hence knowledge of genetic diversity and relatedness in the germplasm is prerequisite for crop improvement programme. Both morphological traits and isoenzyme marker (Kazan and Maebinauer, 1991) have been used for this purpose.

Molecular marker has proved to be valuable tool in the characterization and evaluation of genetic diversity within and between species and populations. A number of DNA based markers are now available for the effective quantification of genetic variation in plant species. Molecular marker has proved to be valuable tool in the characterization and evaluation of genetic diversity within and between species and populations. Chickpea breeding aims at developing high yielding cultivars with tolerance to abiotic stresses such as drought, cold and salt. Molecular markers are being widely used in various areas of plant breeding as an important tool for evaluating genetic diversity and determining cultivars identity (molecular fingerprinting). Establishment of a molecular marker and phenotypic assessment database of crop germplasm has helped breeders to trace down the origins and degrees of relatedness of many land races and cultivars. Considering the potential of molecular markers, the crop breeders extended their hands to use these to supplement other tools currently exploited in their crop breeding activities.

Molecular markers are biochemical constituents (e.g. secondary metabolites in plants) and macromolecules, viz., proteins and deoxyribonucleic acids (DNA) that play a very important role in taxonomy, physiology, embryology, plant breeding, ecology, genetic engineering etc. Analysis of secondary metabolites is restricted to those plants that produce a suitable range of metabolites which can be easily analyzed and which can be distinguished between varieties. These metabolites which are being used as markers should be ideally neutral to environmental changes. Thus among all molecular markers, DNA markers are more suitable and ubiquitous to most of the living organism.

PCR-based RAPD markers requiring small amounts of DNA have also been developed (Williams *et al.* 1990). 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 technique is highly polymorphic and useful in studies on chickpea for genetic diversity.

Although DNA and RNA finger printing could be accomplished by several methods, RAPD analysis is appealing because it does not require genomic sequence or radio labeling and can distinguish variability not only in organisms but even between different strains or isolates of the same organism. RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD polymorphism is detected by using oligonucleotide, usually 10 bases long of random sequence as primer in PCR reaction. In a strain that has in its genomic DNA sequence complementary to primer oligonucleotide, PCR product will be detected in gel. While those strains that do not have the complementary sequence, no product will be detected. Some of these bands are present in some and absent in other strums. These bands constitute RAPD loci. Thus RAPD generated by specific oligonucleotides are used in PCR reaction.

The importance of research work is tool for evaluating genetic diversity and determining cultivars identity (molecular fingerprinting), plant breeder can succeed in their breeding program by relying on the diversity or variation in plant population, it also important to study phylogenetic relationship among the species and subspecies.

The objective of present study was: to study genetic diversity of chickpea genotypes using RAPD marker.

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II. IMPORTANCE OF RESEARCH WORK

- It is important tool for evaluating genetic diversity and determining cultivars identity (molecular fingerprinting).
- Plant breeder can succeed in their breeding program by relying on the diversity or variation in plant population.
- It also important to study phylogenetic relationship among the species and subspecies.

Development of cultivars with multiple resistances to biotic and abiotic stresses is an important breeding objective for chickpea. The analysis of genetic diversity and relatedness between individuals within a species or between different species of populations are phylogenetic studies which were initially conducted using qualitative and quantitative traits, which are mostly morphological. These analyses are mostly based on quantitative traits that are highly influenced by environmental effects and require tedious statistical procedures.

III. MATERIAL AND METHOD

Plant Materials:

The plant material used for these study included seeds of 6 different varieties of chickpea collected from All India Co-ordinated Pulses Improvement Project, MPKV, Rahuri, having different agronomic characters and phenotypic variation. The list is given bellow

Table.1 List of chickpea varieties and their characteristics

Sr. No	Varities	Year of Realease	State	Ave.Yield (Q/ha.)	Days to maturity	Remark
1	Vijay	1994	MP,MH	19-21	105-110	Resistant to wilt, Tolerant to terminal stress.
2	Vihar	2002	Orisa, AP,TN	16-18	90-100	Resistant to wilt, seed bold.
3	Kripa	2009	MH, MP	20	107-108	Resistant to wilt, seed bold.
4	Vishal	1996	MH, MP	20	110-115	Resistant to wilt, tolerant to pod borer, early maturity.
5	Digvijay	2006	MH	19	105-110	Resistant to fusarium wilt.
6	Virat	2002	MH	20	108-118	Resistant to wilt.

DNA Isolation:

The plant genomic DNA was extracted by following the modified CTAB method described by Saghai-Marroof *et al.* (1984).of all six genotypes.

RAPD analysis

Out of ten randomly selected primers screened four primers produced reproducible and scorable bands with high degree of polymorphism (Fig.1). Sixteen amplicons were obtained with the four primers. Fifteen were found to be polymorphic and the level of polymorphism was 93% % (Table 3).

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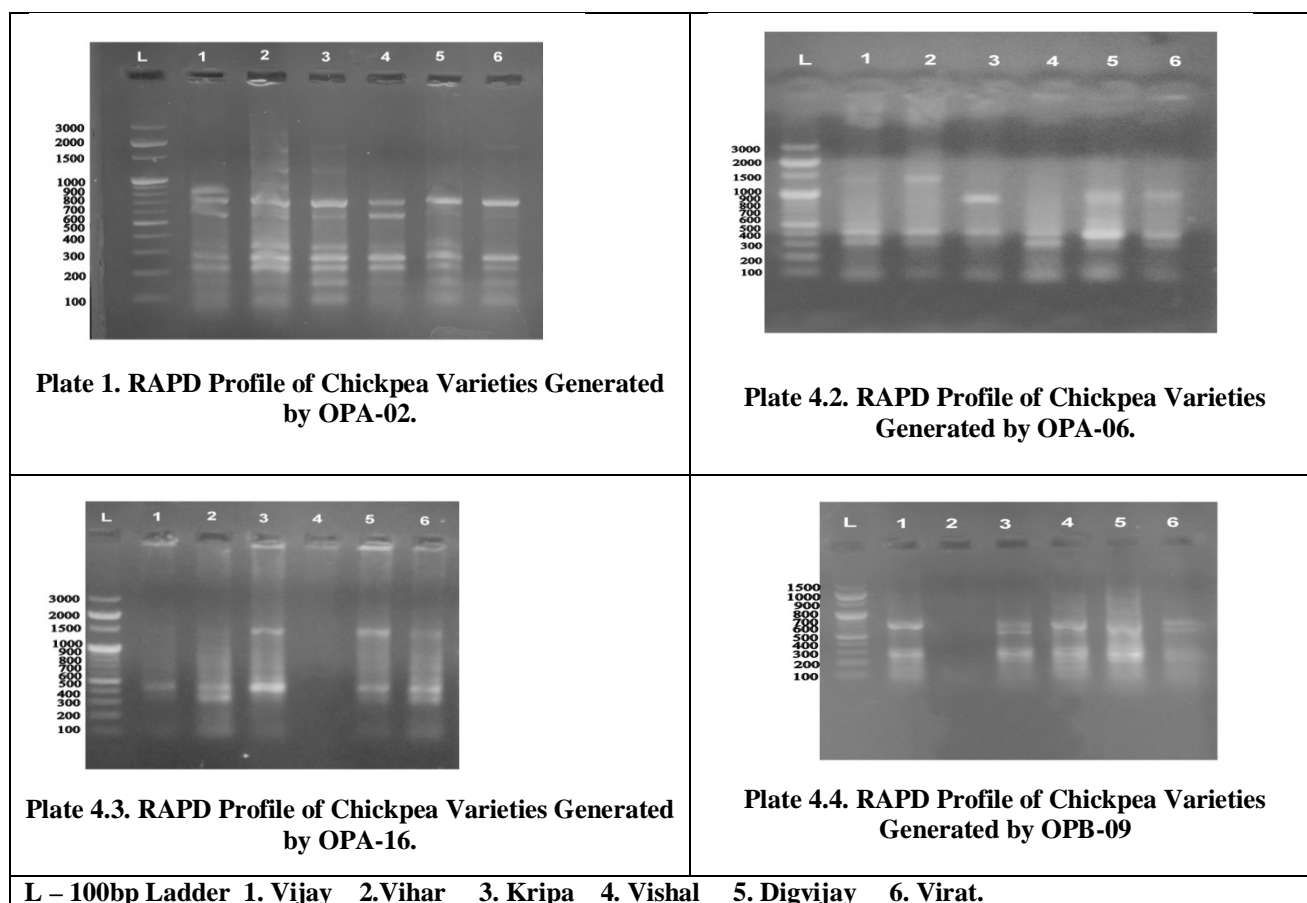
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Table.2 List of primers along with their sequences used for RAPD analysis.

Sr. No.	Primer	Primer sequences	Result
1	OPA-02	5' TGCCGAGCTG 3'	Obtained
2	OPA-06	5' GGTCCTAAC 3'	Obtained
3	OPA-16	5' AGCCAGCGAA 3'	Obtained
4	OPB-09	5' GTGGTCCGCA 3'	Obtained
5	OPF-13	5' GGCTGCAGAA 3'	Not Obtained
6	OPB-01	5' GTTTCGCTCC 3'	Not Obtained
7	OPB-18	5' CCACAGCAGT 3'	Not Obtained
8	OPB-17	5' AGGGACGAG 3'	Not Obtained
9	OPA-04	5' ATCGGGCTG 3'	Not Obtained
10	OPW-05	5' CGCGGATAAG 3'	Not Obtained

IV. EXPERIMENTAL RESULTS

Fig.1 Banding pattern of RAPD



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Table.3 List of RAPD primers and polymorphic amplicons generated.

Sr.No.	Primer code	Total no of amplicons	Total no of polymorphic amplicons	Percent polymorphism
1	OPA-02	5	4	80%
2	OPA-06	4	4	100%
3	OPA-16	3	3	100%
4	OPB-09	4	4	100%
TOTAL		16	15	93.75%

Average number of amplicons per primer = 4

Average number of polymorphic amplicons per primer = 3.75

Total percent polymorphism by RAPD marker = 93.75%

Four RAPD primers were screened which successfully discriminated the *Cicer* species. The screened primers were able to generate 16 amplicons with an average 4 amplicons per primer (Table no.3). Out of total amplicons, 15 amplicons were found polymorphic. They showed per cent polymorphism and the average number of polymorphic bands per primer was 93.75% (Table 3). Different primers produced a different level of polymorphism among the different varieties

Table. 4 Jaccard's Binary similarity matrix for RAPD analysis.

	Vijay	Vihar	Kripa	Vishal	Digvijay	Virat
Vijay	1.00					
Vihar	0.56	1.00				
Kripa	0.39	0.60	1.00			
Vishal	0.67	0.50	0.53	1.0		
Digvijay	0.52	0.56	0.48	0.57	1.00	
Virat	0.44	0.44	0.41	0.39	0.41	1.00

In present study, the similarity coefficient value ranged from 0.39 to 0.67 across six varieties indicating high degree of genetic variation. This ultimately means high range of genetic diversity among the varieties studied. The highest genetic similarity to an extent of 0.67 was recorded between Vijay and Vishal varieties followed by 0.57 similarity between Digvijay and Vishal varieties. Least genetic similarity 0.39 was observed in between Kripa and Vijay.

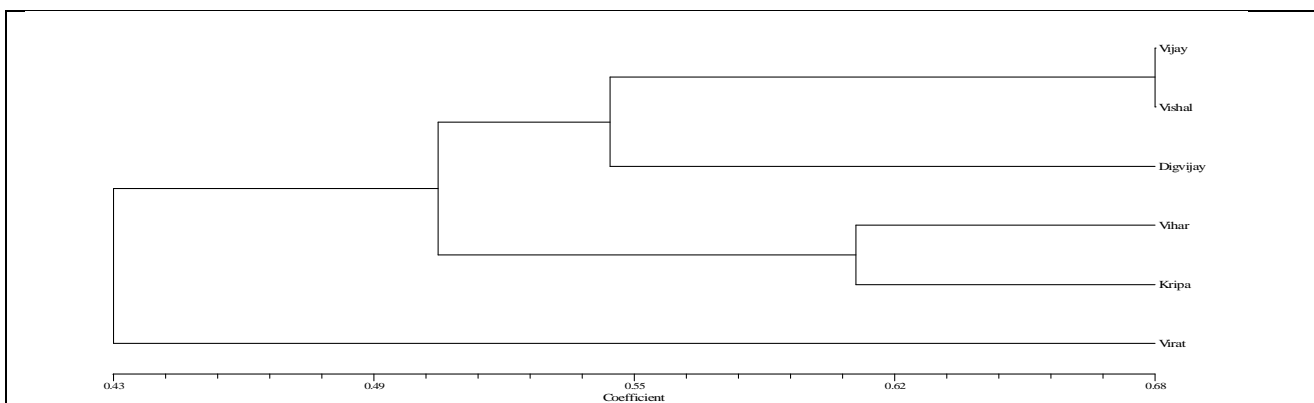
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Fig.5 The UPGMA dendrogram of six genotypes of chickpea based on Jaccard's similarity coefficient RAPD



The dendrogram RAPD analysis shows that six chickpea varieties can be grouped into two major clusters viz. A and B. Cluster A divided into two sub clusters namely cluster A1 and Cluster A2. Sub cluster A1 shows three varieties, Vijay, Vishal, and Digvijay. Out of these, Vijay and Vishal shows 0.67 similarity coefficient, whereas Digvijay shows 0.53 similarity coefficient. Sub cluster A2 consist of cultivar Vihar and Kripa having 0.60 similarity coefficient.

The cluster B distinct from cluster A containing single cultivar Virat 0.43 similarity coefficient.

V. DISCUSSION

Jamina Hamdard. (2013) studied objective of this study to estimate the genetic diversity and to evaluate the between eight chickpea varieties that is the valuable source of breeding. Chickpea seed is a good source carbohydrates and proteins, constituting 80% of the total dry seed weight. The variable response of chickpea varieties was characterized by using the 15 RAPD primers. Minimum number of 28 RAPD products were recorded as unique or species specific and resulted in 98.1% of polymorphism.

In the present study, genetic diversity among *Cicer species* was analyses by four RAPD primers among six varieties. The similarity coefficient among all varieties ranged from 0.39 to 0.67. In present study polymorphic RAPD could clear the distinguish varieties. Dendrograms were generated based on data recorded by the one marker systems. The result of cluster analysis indicated the separation of different varieties within same species. Vijay and Kripa showed least similarity of 0.39% in RAPD marker analysis.

VI.CONCLUSION

The varieties with distinct DNA profiles provided useful information for selection of parents to develop new chickpea hybrids. Show how much diversity and similarity level is present in six varieties of chickpea. Able to identify genetic makeup of the variety to protect the breeder's right. Estimate the genetic distance among the varieties of chickpea and calculate the similarity coefficient among chickpea varieties.

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