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Determination of Genetic Relationship among Wilt Resistant and Susceptible Varieties of Chickpea by RAPD Markers

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A B S T R A C T

The molecular evaluation of twelve chickpea (*Cicer arietinum* L.) varieties (screened for their resistance to fusarium wilt) at the Department of Plant Pathology and Agriculture Microbiology in 2014-15 was conducted to assess the genetic diversity and relationship of chickpea genotypes using RAPD. Twenty five primers of RAPD were used of which 15 primers gave amplification products. A total 349 amplicons were obtained of which 331 amplicons were polymorphic with 93.64 % level of polymorphism was observed. Cluster analysis by RAPD and ISSR markers revealed clear distinct diversity between genotypes. The similarity coefficient ranged from 0.64 to 0.92 showed high genetic variability. Digvijay revealed the highest dissimilarity comparing with the other varieties. JG 62 and Vikas showed more similarity than others varieties. The results showed that RAPD analysis for diversity can provide practical information for the management of genetic resources in chickpea breeding program.

Introduction

Chickpea (*Cicer arietinum* L.), as the second most important cool season food legume in the world after dry beans and peas (FAO, 2006), is a diploid, with 2n = 2x = 16 (Aru Muganathan *et al.*, 1991) and has a genome size of approximately 931 Mbp. Moreover, chickpea pod covers and seed coats can also be used as fodder. In grain legumes, proteins are an important seed component and are responsible for their relevant nutritional a socioeconomic importance. The chickpea seed is a good source of carbohydrates and proteins, which together constitute 80% of the total dry seed weight (Talebi *et al.*, 2008). Two main types of chickpea cultivars are grown globally kabuli and desi, representing two diverse gene pools. The knowledge of genetic diversity is a useful tool in gene bank management and breeding experiments like tagging of germplasm, identification and/or elimination of duplicates in the gene stock and establishment of core collections Genetic diversity among the parents is a rerequisite to improve the chances of selecting better segregates for various characters (Dwevedi *et al.*, 2009). Differences between genotypes with regard to susceptible and resistant reaction to the wilt disease and molecular

characteristics are either indirect or direct representations of differences at the DNA level and are therefore expected to provide 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. For this purpose 6 wilt resistant and 6 wilt susceptible varieties of chickpea were analyzed by using random amplified polymorphic DNA (RAPD) markers. Polymerase chain reaction (PCR) method, using RAPD primers, has been widely utilized in the last 20 years. DNA markers have proved valuable in crop breeding, especially in studies on genetic diversity and gene mapping.

The RAPD technique, based on the PCR, is one of the most commonly used molecular markers. RAPD markers are amplification products of anonymous DNA sequence using single, short and arbitrary oligonucleotide primer; thus, they do not require prior knowledge of 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 (Bardakci, 2001). RAPD identification techniques can be used at any stage of plant development and they are not affected by environment factors (Lisek *et al.,* 2006). The reproducibility of the RAPD techniques can be influenced by variable factor, such as concentration of MgCl2, DNA template; DNA polymerase (Iqbal *et al.,* 2002); number of primer; primer sequence; number of PCR cycles (Nkongolo *et al.,* 2002) and annealing temperature (Schiliro *et al.,* 2001).

The aim of this study is to evaluate the genetic diversity of wilt resistant and susceptible chickpea varieties by RAPD markers.

Materials and Methods

Plant material

Six wilt resistant varieties of chickpea *viz.* WR 315, Digvijay, Vishal, Virat, Vihar, Vijay and Six wilt susceptible varieties of chickpea *viz* Vikas, Vishwas, Phule G-12, ILC 'O', L550, JG 62 were used in this study (Table 2).

All varieties were obtained from All India Pulses Improvement Project, MPKV, Rahuri. Healthy seeds with identical dimensions were selected by visual observation.

Genomic DNA extraction and purification

Seeds were planted in a pot for three weeks at the Dept. of Plant Pathology and Agriculture Microbiology. Watering was done once a day and, after three weeks, healthy leaves were harvested. Total DNA was extracted from three weeks young chickpea leaves following the CTAB procedure (Cingilli *et al.*, 2005).

RAPD analysis

Twenty five primers were used in this study, only fiften primers gave the products (Table 1).

The reaction mixture (25 µl) contained 10× assay buffer, 2.5 mM MgCl2, 400 µM dNTP's (Fermantas), 5 pmoles of primer, 100 ng template DNA and 1 U of Taq DNA Polymerase (Fermantas). Amplification was carried out in a thermo-cycler (Master cycler) for 40 cycles, each consisting of a denaturation step at 94 °C for 1 min, annealing at 32, 34 and 36 °C for 50 second and an extension step at 72 °C for 2 min. An initial denaturation step at 94 °C for 5 min, and a final synthesis step of 6 min at 72 °C were also included. Amplification products were separated on 1.5% agarose gel in 1X TAE (Tris base, acetic acid and EDTA) buffer

Data analysis

Following Lynch and Milligan (Lynch *et al.,* 1994) assumptions, each amplified product was treated as an independent locus and assigned numbers in order of decreasing molecular weight. DNA fragment profiles representing a consensus of two independent replicates were scored in a binary fission with '0' indicating the absence and '1' indicating presence of band. Using the binary data, a similarity matrix was constructed using the Jaccard coefficient (Jaccard, 1908), which was further subjected to clustering analysis and a dendrogram was generated. A cophenetic matrix was constructed using the matrix that was used to generate the clusters. A correlation between the cophenetic matrix and the similarity matrix was determined by using SPSS version 18 (Masumbuko *et al.*, 2003).

Results and Discussion

The Twelve Varieties of chickpea *viz.* WR 315, Digvijay, Vishal, Virat, Vihar, Vijay, Vikas, Vishwas, Phule G-12, ILC 'O', L550 and JG 62 were screened in wilt sick plot for confirmation of resistant and susceptible for wilt. The results are shown in table 2.

RAPD analysis revealed a good polymorphism among chickpea varieties. (Figure 2) Twenty five random primers of RAPD were used in this study. From RAPD data 6.36 % of common bands and 93.64 (Table 3) of polymorphic bands were observed among chickpea varieties.

The primer UBC 701 gave rise to maximum bands (43) and UBC 709 showed the least number of bands (7) (Figure 2). Cluster analysis was carried out depending on the results of RAPD analysis using the SPSS analysis to find the diversity among the given varieties of chickpea as shown in the dendrogram (Figure 1). At Jaccard dissimilarity of distance Phule G 12 and L 550 showed more similarity than others varieties. These two varieties are susceptible to wilt. The resistant genotypes Vishal, Virat and WR 315 are grouped into one cluster while Vijay in another cluster. Digvijay showed more dissimilarity distance with the rest of the varieties.

Vijay and JG 62 showed more similarity and groped into one cluster though they have different in reaction to fusarium wilt. The similarity matrix varied from 0.64 to 0.92 in chickpea varieties. The highest value of similarity matrix was registered by phule G 12 and L 550 while the lowest value of similarity matrix was recorded by WR 315 and Digvijay (Table 4).

In this investigation, RAPD markers showed a high level of polymorphism and a high number of clearly amplified bands. The data reported in this study is in agreement with that obtained by other researchers. Extensive DNA polymorphism has been reported using RAPD markers in several other crops (Iruela *et al.*, 2002; Hou *et al.*, 2005).

The RAPD based dendrogram of chickpea genotypes displayed the genetic relationships between these accessions, which accorded with previous studies on chickpea (Ahmad *et al.*, 1992; Tayyar *et al.*, 1996 and Iruela *et al.*, 2002). Although the Cicer species are predominantly selfpollinating, more variation was observed among them.

The reason for this genetic variation could be that the specific accessions were heterozygous at some marker loci. Similar observations were reported in pea, lentil (Simon *et al.*, 1997), and chickpea (Moussa *et al.*, 1996; Sant *et al.*, 1999). Iruela *et al.*, 2002) showed that RAPD markers successfully identified genetic variation in Cicer.

Table.1 Sequences and Annealing temperature (ºC) of random primers used for RAPD Analysis

Sr.No	Primer Screened	Sequence	Annealing temp.
1	UBC701	CCC ACA ACC C	**34^0 C**
2	UBC702	GGG AGA AGG G	**34^0 C**
3	UBC703	CCA ACC ACC C	**34^0 C**
4	UBC706	GGT GGT TGG G	**34^0 C**
5	UBC709.	CCT CCT CCC T	**34^0 C**
6	UBC711	CCC TCT CCC T	**34^0 C**
7	UBC717	CCCACACCCA	**34 ^{0} C**
8	UBC729	CCC AAC CCA C	**34^0 C**
9	UBC751	CCC ACC ACA C	**34^0 C**
10	UBC763	CAC ACC ACC C	**34^0 C**
11	UBC764	CTC TCC TCC C	**34^0 C**
12	UBC771	CCCTCCTCCC	**36^0 C**
13	UBC778	CCA CAC CAC A	**32^0 C**
14	UBC783	GGT GGG TTG T	**32^0 C**
15	UBC790	GGA AGT CGC C	**34^0 C**
16	UBC 1-4	CCTGGGTTCC	**34^0 C**
17	UBC 1-19	GCCCGGTTTA	**32^0 C**
18	UBC 1-23	CCCGCCTTCC	**36^0 C**
19	UBC 1-28	CCGGCCTTAA	**32^0 C**
20	UBC 1-29	CCGGCCTTAC	**34^0 C**
21	UBC 1-30	CCGGCCTTAG	**34^0 C**
22	UBC 1-34	CCGGCCCCAA	**36^0 C**
23	UBC 1-70	GGGCACGCGA	**36^0 C**
24	OPA 05	AGGGGTCTTG	**32^0 C**
25	**OPA 07**	**GAAACGGGTG**	**32^0 C**

Table.2 Confirmation of resistance against wilt in chickpea genotypes under wilt sick soil

Sr.No.	Name of genotypes	Wilting %	Reaction
1	WR 315	0	Immune
2	Digvijay	2.71	Resistant
3	Vishal	3.84	Resistant
4	Virat	5.28	Resistant
5	Vihar	8.72	Resistant
6	Vijay	6.18	Resistant
7	Vikas	30	Susceptible
8	Vishwas	42.6	Highly Susceptible
9	Phule G-12	54.22	Highly Susceptible
10	ILC ‘O’	81.75	Highly Susceptible
11	L550	83.30	Highly Susceptible
12	JG 62	100	Highly susceptible

Table.3 Percent polymorphism observed in RAPD Primers

Sr. No.	Primers	Total No. of Bands	Polymorphic Bands	Monomorphic Bands	Percent Polymorphism
1	OPA 05	28	26	2	92.75
2	UBC 1-4	42	42	0	100
3	UBC 1-19	39	39	0	100
4	UBC 1-29	24	22	2	91.66
5	UBC 1-70	19	18	1	94.73
6	UBC 702	16	16	0	100
7	UBC 1-28	24	23	1	95.83
8	UBC 1-23	13	10	3	76.92
9	UBC 1-30	14	11	3	78.57
10	UBC 1-34	27	26	1	96.30
11	UBC 701	43	41	2	95.35
12	UBC 703	15	14	1	93.33
13	UBC 706	16	15	1	93.75
14	UBC 778	22	21	1	95.45
15	UBC 709	7	7	0	100
		349	**331**	**18**	**93.64% (Avg.)**

Table.4 Jaccard similarity coefficient showing the relationship among chickpea varieties based on RAPD data

	WR 315	Digvijay	Vishal	Virat	Vihar	Vijay	JG-62	Vikas	Vishwas	PhuleG12	ILC'O'	L550
WR 315	1											
Digvijay	0.64	1										
Vishal	0.807	0.76	1									
Virat	0.821	0.74	0.892	1								
Vihar	0.793	0.714	0.758	0.806	1							
Vijay	0.779	0.701	0.813	0.825	0.861	1						
JG-62	0.806	0.8	0.774	0.818	0.882	0.898	1					
Vikas	0.779	0.842	0.779	0.793	0.8	0.878	0.898	1				
Vishwas	0.784	0.816	0.862	0.836	0.771	0.793	0.819	0.793	1			
PhuleG12	0.880	0.72	0.88	0.905	0.8	0.862	0.833	0.827	0.84	1		
ILC'O'	0.777	0.692	0.814	0.827	0.8	0.819	0.843	0.754	0.754	0.807	1	
L550	0.827	0.714	0.896	0.903	0.843	0.892	0.882	0.83	0.877	0.928	0.866	1

Fig.1 Dendrogram of chickpea varieties showing the genetic similarity based on RAPD data by using cluster analysis

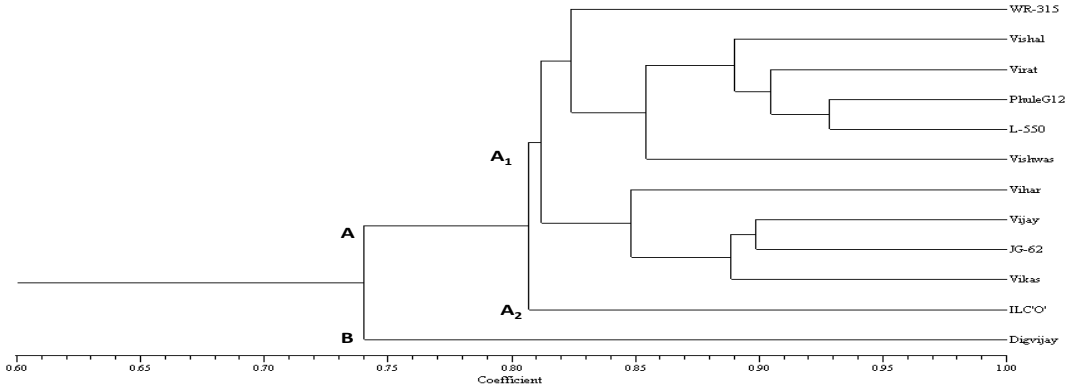
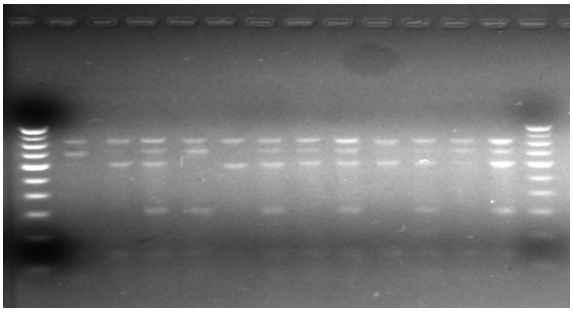
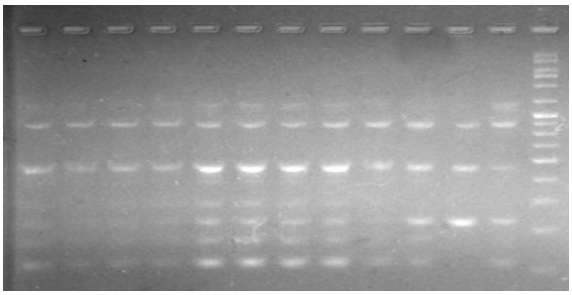


Fig.2

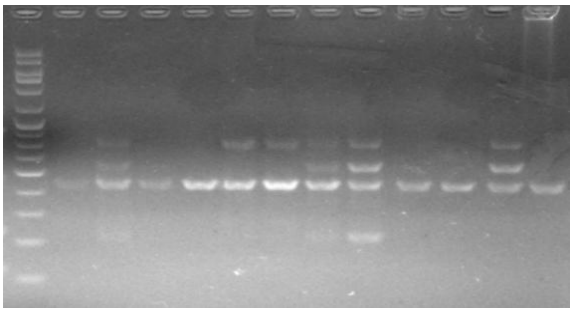


Primer OPA 05

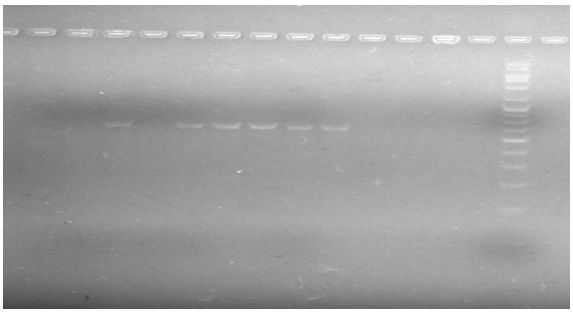
Figure 2 - Agarose gel (1.5%) showing the amplified product using RAPD primers, OPA 05 , Primer UBC 701, Primer UBC 709 and Primer UBC 703



Primer UBC 701



Primer UBC 703



Primer UBC 709

This observation was consistent with the study of Simon and Muehlbauer (1997), who detected variation within single *C. reticulatum* accession (PI 489777), used to generate an interspecific mapping population. Our results are in accordance with Iruela *et al.,* (2002). Iruela reported the genetic diversity among *C. arietinum* varieties using RAPD. Shan *et al.,* (2005) showed that a natural hybrid could be useful for bridging crosses to introduce genes to chickpea from incompatible species given that *C.reticulatum* was the wild progenitor of chickpea.

Further, large amount of genetic variation which exists between chickpea genotypes can be used efficiently for gene tagging and genome mapping of crosses to introgression the favorable traits such as high yield potential, disease resistance into the cultivated genotypes. Thus, RAPD markers were good indicators of morphological divergence.

In conclusion, the present investigation demonstrates the potential of RAPD fingerprinting in detecting polymorphism among chickpea varieties. Varieties Digvijay showed the highest dissimilarity comparing to others varieties. Genetic information obtained from RAPD markers can be used in discriminating chickpea varieties and can complement the genetic information generated from the morphological traits. Further, the genetic variation which exists between chickpea varieties can be used efficiently in plant breeding.

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