Electronic Journal of Plant Breeding, 1(4): 466-473 (July 2010)

Research Article

Genetic Diversity Analysis among Greengram genotypes using RAPD Markers

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Abstract :
Green gram is also one of the important pulse crops. Conventional breeding methods are very much difficult to utilize in the development of new genotypes. Hence incorporation of the molecular approaches along with the conventional techniques is most powerful method. Evaluation of the available wild accessions are more useful for selecting desirable gene sources. Genetic diversity analysis place an important role in this purpose. For this molecular analysis of selected 18 accessions in greengram (representing all nine clusters) was carried out through RAPD markers. Out of ten primers used nine were polymorphic in which the primer OPS-11 exhibited 100 per cent polymorphism. The value of similarity indices 0.72 to 0.91 indicates high genetic similarity among the selected accessions at molecular level.

Key words:
Greengram, RAPD,diversity analysis,polymorphism

Introduction

One of the basic activities in plant breeding is the search for sources of valuable genes (alleles) encoding resistance to various environmental and biotic stresses. These sources are usually lines, strains, cultivars or even wild relatives, which are difficult to include into the breeding process due to their possession of many unfavourable genes that decrease yield capacity. As greengram is concerned there is lack of variation existing in the accessions. The efficiency of direct selection based on the phenotype evaluation may be very low, if: 1) the trait under consideration is highly modified by external, environmental conditions (low heritability); 2) the trait under consideration is dominant or governed by more than one single gene; or 3) the method for trait evaluation is expensive, laborious and inconvenient for large scale selection. In any of these situations, an option to consider is the development of a molecular marker-based method of genotype identification, which would enable the performance of indirect, marker assisted selection (MAS). The aim of this paper is to know the variability among the18 accessions at the molecular level.

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Materials and methods

The Genomic DNA was extracted as per procedure outlined by Gawel and Jarret, (1991). RAPD analysis for all the 18 selected accessions was performed as per the procedures suggested

by Lakhanpaul *et al.*(2000). The binary matrix based on markers scores was subjected to cluster analysis. Hierarchical Cluster Analysis and Principal Component Analysis was adopted by Bhist et al.,(1998) for assessment of diversity among selected accession.

Results

Out of 15 primers surveyed, ten were selected for the analysis and the other five primers either gave sub optimal or monomorphic amplification products. The selected 18 accession from the core were subjected to PCR amplification using these ten primers. A total of 92 amplification products were scored in the 18 accessions with the ten primers which exhibited over all 42.39 percent polymorphism (Table 1). The average number of amplification products found was 92 with a maximum of 15 with the primer OPS-17 and minimum of 5 with the OPS-11. Out of ten primers only three primers exhibited more than 80 per cent polymorphism and as many as five showed less than 50 per cent polymorphism. The primer OPS-11 was found to be polymorphic not produced any amplification product.

The binary data scored from the RAPD profiles were subjected to UPGMA cluster analysis to establish the relationship among the 18 accessions selected from the core. The Jaccard's distance co efficient computed are presented in (Table 2). The value of Jaccard.s distance varied from 0.722 (AC 244) to 0.911 (AC 348).The dendrogram resulting from the UPGMA cluster analysis is depicted as fig 1. Altogether two distinct clusters were formed. Seven accessions namely PLS 267/2, AC 348, PLS 316, LM 294, LM 197, PLS (S) and PARJULA were grouped in first cluster while the cluster II comprised of 11 accessions namely LM 420 (B), AVT/RMI-6, AVT/RMI/6/1, 118891, CO5, LM196, AC 244, MDU 3484, LM 489, MDU 3405 and AC198. The results of principal component analysis were comparable to the cluster analysis. The grouping observed in the cluster analysis can be clearly distinguished in the three dimensional plot of PCA. A total of 16 amplification products were required to explain 99.22 per cent of total variation. This was indicative of the presence of low genetic diversity among the selected accessions (Table 3). The first three PCA explain 39.38 per cent of the total variation.

Discussion

Different methodological approaches such as morphological, isozyme, protein and DNA markers have employed in the course of years to evaluate the genetic diversity in crop plants (Panella and Gepts, 1992, Fotso *et al.,* 1994, Ehlers and Hall, 1996, Fatokun *et al.,* 1997, Mignouna *et al.,* 1998). Among them, the DNA based marker approach has been found to be superior, because of its capability to reveal more polymorphism (Mignouna *et al.,* 1998). Among the DNA markers, RAPD marker is highly cost effective (Zhang *et al.,* 1996) and it does not need prior knowledge of genomic nucleotide sequence to study variations (Williams *et al.,* 1990). Due to these relative merits, RAPD markers are being mostly employed in diversity analysis.

Considering the importance of genetic diversity, in the present study, genetic diversity among 18 selected genotypes from the core collection, were analyzed using RAPD markers. Out of 10 primers studied, OPS 11 exhibited 100 per cent polymorphism. Primers generated polymorphic fragments among the greengram genotypes, indicating the usefulness of RAPD analysis to disclose DNA polymorphism. The overall similarity between the genotypes varied from 0.72 to 0.91 showing the presence of high genetic similarity and narrow genetic base among the genotypes studied. There is a chance of high diversity when we use more number of primers. Investigation by Lakhanpaul *et al.* (2000) among 32 accessions of mung bean and Doldi *et al.* (1997) in 18 accessions of soybean demonstrated high level of genetic similarity among the accessions and they attributed this to the self pollinating characters of these species. The formation of only two clusters, through hierarchical cluster analysis of the RAPD data also confirmed that the presence of low diversity at molecular level among the selected accessions.

Conclusions

The formation of only two clusters, through hierarchical cluster analysis of the RAPD data also confirmed that the presence of low diversity at molecular level among the selected accessions. To obtain expected high diversity at molecular level in this study when we use more numbers of markers.

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Table 1 .Polymorphic RAPD profiles for primers across 18 diversified greengram core collection accessions

Sl.No	**Primer**	**Primer sequence (5^1-3^1)**	**Total no .of amplication products**	**No.of polymorphic products**	**Percentage of polymorphism**
1	OPS-11	AGTCGGGTGG	5	5	100.00
2	OPS-13	GTCGTTCCTG	9	5	55.55
3	OPS-16	AGGGGGTTCC	7	6	85.71
4	OPS-20	TCTGGACGGA	8	7	87.50
5	OPS-15	CAGTTCACGG	9	3	33.33
6	OPS-17	TGGGGACCAC	15	3	20.00
7	OPT-16	GGATGCCACT	9	1	11.11
8	OPT-19	GTCCGTATGG	10	6	60.00
9	OPT-15	GGATGCCACT	10	3	30.00
10	OPT-17	CCAACGTCGT	10	0	0.00
Total	10	10	92	33	42.39

Table 2. Jaccard.s similarity matrix based on RAPD markers among 18 diversified greengram accessionsfrom core collection

Name of the accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
PLS 267/2	1.00																	
LM 294	0.83	1.00																
PLS 316	0.80	0.75	1.00															
AVT/RMI-6	0.87	0.82	0.80	1.00														
PLS (S)	0.81	0.83	0.78	0.82	1.00													
AC 348	0.91	0.84	0.81	0.84	0.80	1.00												
LM 197	0.79	0.87	0.82	0.87	0.85	0.81	1.00											
118891	0.76	0.77	0.77	0.81	0.77	0.79	0.78	1.00										
AC 244	0.82	0.75	0.79	0.86	0.72	0.81	0.78	0.88	1.00									
LM 189	0.75	0.77	0.76	0.75	0.77	0.74	0.77	0.85	0.83	1.00								
AC 198	0.80	0.77	0.79	0.82	0.78	0.79	0.80	0.86	0.86	0.83	1.00							
CO5 (B)	0.80	0.81	0.79	0.81	0.77	0.81	0.78	0.90	0.83	0.81	0.83	1.00						
LM 196	0.83	0.78	0.80	0.80	0.77	0.82	0.77	0.84	0.85	0.80	0.83	0.87	1.00					
LM 420 (b)	0.79	0.78	0.78	0.87	0.81	0.77	0.81	0.84	0.84	0.77	0.84	0.89	0.83	1.00				
MDU 3405	0.77	0.77	0.81	0.79	0.79	0.76	0.82	0.85	0.81	0.83	0.83	0.81	0.80	0.84	1.00			
MDU 3484	0.83	0.78	0.82	0.84	0.78	0.82	0.81	0.82	0.89	0.84	0.82	0.84	0.83	0.85	0.84	1.00		
AVT/RMI/6 -1	0.84	0.77	0.77	0.85	0.79	0.78	0.75	0.85	0.85	0.74	0.81	0.85	0.84	0.86	0.81	0.82	1.00	
PARJULA	0.75	0.83	0.78	0.79	0.81	0.72	0.84	0.81	0.77	0.78	0.81	0.83	0.80	0.82	0.83	0.77	0.81	1.00

Table 3. Extraction of principal components for RAPD analysis

Sl. No.	Eigen value	Variance (%)	Cumulative variance (%)
1	6.06	15.54	15.54
2	5.27	13.53	29.07
3	4.02	10.31	39.38
4	3.25	8.34	47.72
5	3.10	7.95	55.68
6	2.96	7.61	63.29
7	2.22	5.70	68.99
8	2.14	5.49	74.49
9	1.97	5.06	79.55
10	1.83	4.70	84.26
11	1.60	4.12	88.38
12	1.17	3.01	91.39
13	0.99	2.54	93.94
14	0.79	2.05	95.99
15	0.71	1.84	97.83
16	0.54	1.39	99.22

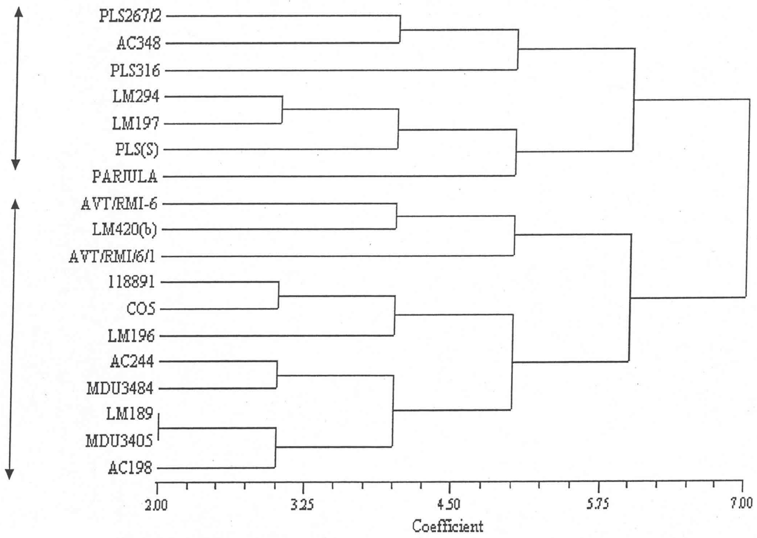
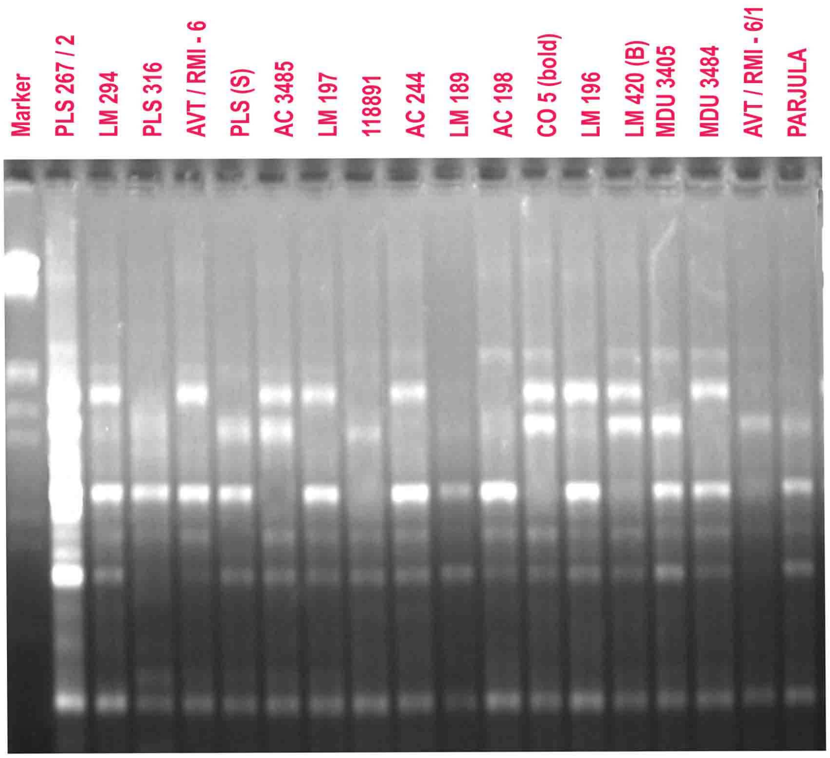
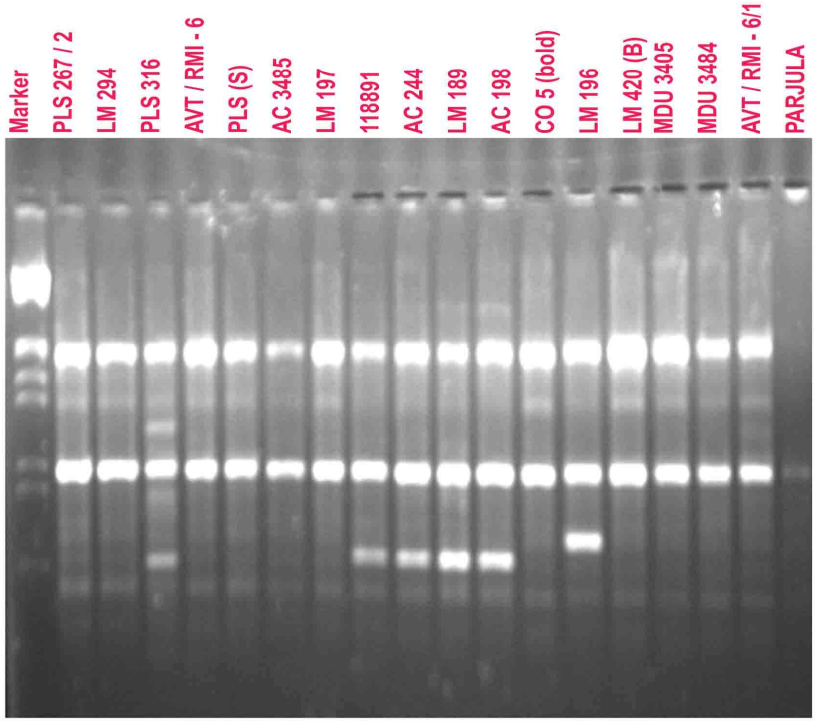


Fig 1 Phenetic tree depicting the clustering pattern of 18 diversified

core greengram accessions for RAPD analysis



B. RAPD profile generated by primer OPS - 16



C. RAPD profile generated by primer OPS - 20

Plate 33. RAPD profiles for 18 diverse germplasm greengram core collection