International Journal of Advanced Trends in Engineering and Technology (IJATET)
Impact Factor: 5.965, ISSN (Online): 2456 - 4664
(www.dvpublication.com) Volume 8, Issue 1, 2023

GENETIC DIVERSITY ANALYSIS AMONG GREEN GRAM GENOTYPES USING RAPD MARKERS

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Cite This Article: Pandiyan Muthaiyan, "Genetic Diversity Analysis Among Green Gram Genotypes Using RAPD Markers", International Journal of Advanced Trends in Engineering and Technology, Volume 8, Issue 1, Page Number 88-91, 2023.

Abstract:

Molecular analysis of selected 18 accessions (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

Introduction:

Greengram *Vigna radiata (L.) wilczek*, utilizing the variability available in the secondary and tertiary gene pools. There is no much variability in phenotypes in the primary gene pool. It is essential to know the variability in molecular level between the genotypes to develop hybrids for improvement in yield and yield components. It is essential to confirm the genotypic variation among greengam through RAPD analysis

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 (1998) for assessment of diversity among selected accession.

Results:

The pods and seeds of eighteen core collection accessions were depicted in the Plate 2. 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 most polymorphic while OPT-17 was least polymorphic primer showed none of the amplification product was polymorphic.

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 6. 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

S.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 accessions from core collection

Name of the Accessions	1	2	3	4	5	6	7	8	9
PLS 267/2	1.000								
LM 294	0.827	1.000							
PLS 316	0.800	0.774	1.000						
AVT/RMI-6	0.847	0.821	0.795	1.000					
PLS (S)	0.807	0.825	0.776	0.824	1.000				
AC 348	0.911	0.838	0.810	0.835	0.795	1.000			
LM 197	0.788	0.873	0.821	0.869	0.852	0.819	1.000		
118891	0.761	0.776	0.773	0.818	0.779	0.791	0.782	1.000	
AC 244	0.826	0.759	0.795	0.862	0.722	0.814	0.784	0.882	1.000
LM 189	0.756	0.771	0.767	0.753	0.774	0.744	0.776	0.855	0.835
AC 198	0.805	0.779	0.795	0.820	0.782	0.793	0.805	0.860	0.862
CO5 (B)	0.802	0.819	0.793	0.818	0.779	0.812	0.782	0.904	0.839
LM 196	0.835	0.788	0.805	0.809	0.770	0.824	0.773	0.849	0.851
LM 420 (b)	0.791	0.786	0.782	0.871	0.810	0.779	0.812	0.847	0.849
MDU 3405	0.779	0.774	0.812	0.795	0.798	0.767	0.821	0.857	0.816
MDU 3484	0.833	0.786	0.824	0.849	0.788	0.821	0.812	0.826	0.893
AVT/RMI/6 -1	0.843	0.774	0.770	0.859	0.798	0.788	0.759	0.857	0.859
PARJULA	0.756	0.838	0.788	0.793	0.817	0.724	0.841	0.812	0.773

Cond.,

Name of the Accessions	10	11	12	13	14	15	16	17	18
PLS 267/2									
LM 294									
PLS 316									
AVT/RMI-6									
PLS (S)									
AC 348									
LM 197									
118891									
AC 244									
LM 189	1.000								
AC 198	0.835	1.000							
CO5 (B)	0.812	0.839	1.000						
LM 196	0.802	0.830	0.871	1.000					
LM 420 (b)	0.779	0.849	0.892	0.837	1.000				
MDU 3405	0.831	0.837	0.814	0.805	0.845	1.000			
MDU 3484	0.843	0.828	0.847	0.837	0.857	0.845	1.000		
AVT/RMI/6 -1	0.747	0.816	0.857	0.847	0.867	0.812	0.824	1.000	
PARJULA	0.786	0.814	0.833	0.802	0.821	0.831	0.779	0.810	1.000

Table 3: Extraction of principal components for RAPD analysis

S.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