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GENETIC DIVERSITY ANALYSIS AMONG MUNGBEAN GENOTYPES BASED ON RAPD MARKERS

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ABSTRACT
The present study was done to investigate the genetic diversity among mungbean germplasm for identification of diversified parents to develop high yielding potential hybrids. Random amplified polymorphic DNA (RAPD) analysis of 40 markers was performed for twelve mungbean genotypes to study the DNA polymorphism. Amplification of genomic DNA of popular twelve genotypes with these RAPD primers yielded 58 RAPD fragments of which 37 were polymorphic, with an average of five polymorphic fragments per primer. Number of amplified fragments with random primers ranged from 10 (OPD 1) to 2 (OPD 2 &OPD 7). Percentage of polymorphism ranged from 28% (OPC 9) to 100% (OPB 1 & OPB 3) with an average of 63%. The Jaccard's similarity values ranged from 0.385 to 0.821 and genotypes subjected to UPGMA were grouped into three clusters. Our study has established that the RAPD markers are useful for studying genetic diversity among mungbean genotypes and thereby helps in selecting diverse parents for utilization in hybridization for the recovery of superior hybrids.

INTRODUCTION

Legumes, also referred to pulses, are the major source of proteins in Asia and constitute an important supplement to the predominantly cereal based diets. The major legumes in Asia are chick pea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* L.) and Mung bean (*Vigna radiata* L Wilczek). Mungbean (2n = 22) belonging to the papilinoid sub family is an important food legume that has inexpensive and low flatulence protein which makes it indespensible in Indian vegetarian diet (Roopa Lavanya *et al.,* 2008.). It is cultivated in tropical, sub tropical and temperate zones of Asia. Being a short duration crop (60-75 days), mungbean has an advantage to fit into several cropping systems besides as a sole crop. Owing to its importance, in daily life there is a need to breed and disseminate new varieties with high yield for different niches for which, an insight into the genetic base of mungbean varieties would provide valuable guidance to the breeders in planning future crossing programmme that helps in identification of transgressive seggregants.

Diversity analysis based on morphological studies (Gokulakrishnan *et al.,* 2012 and Abbas *et al.,* 2010), though made a great impact in crop improvement usually varies with the environment and evaluation of these traits requires growing plants to full maturity prior to identification. DNA based molecular markers are highly useful in this context since they are available in abundance and clearly allow the comparison of genetic material at juvenile phase avoiding any environmental influence on gene expression. Information regarding genetic diversity at molecular level could also be used to identify and develop genetically unique genotypes that compliments existing cultivars. Such molecular diversity analysis was found to be efficient in identifying superior diverse parents that can be employed in the hybridization programme. (Gokulakrishnan *et al.*, 2012 and Karuppanapandian *et al.*, 2006). Among several molecular markers possible, Random Amplified Polymorphic DNA (RAPD) profiles offer a rapid and reliable identification and characterization of genotypes (Williams *et al.,* 1990). The RAPD markers have become the markers of choice due to its speed, technical simplicity and higher frequency of polymorphism and they have been shown to be useful in assessing genetic variability in many crop species (Roopa Lavanya *et al.,* 2008). Realizing the importance and need for a comprehensive understanding of genetic diversity at molecular level in mungbean, the objective of the present study was to investigate the genetic diversity among the twelve mungbean genotypes using RAPD markers.

MATERIALS AND METHODS

Plant material and genome extraction

Seed material of twelve mungbean genotypes (AKM 9904, EC 396117, IPM-02-19, KM-8-657, LGG 450, MGG 347, MGG 350, MH 565, ML 145, PUSA 9531, VG 6097A and WGG 2) were collected and grown under laboratory condition at department of Genetics and Plant Breeding, S.V. Agricultural college, Tirupati. Total genomic DNA was isolated from two weeks old leaf tissue following the CTAB method described by Murray and Thompson (1980).

RAPD analysis

RAPD analysis was performed using 40 primers. Amplification reactions contained 2.5 μl of 10X Taq buffer, 1.0 μl of 10 mM dNTPs, 2.0 μl of 25 mM $MgCl_2$, 2.0 μl of Primer (20 picomoles/

μl), 0.3 μl of Taq polymerase enzyme (conc.1.5 U/μl) (New England Bio labs), 1.2 μl of Di Methyl Sulphoxide (DMSO), 3.0 μl of template DNA (15ng / μl) in a 25μl reaction mixture. Amplification reaction included an initial denaturation at 94°C for 2 min, and then amplification reactions were cycled 40 times at 94°C for 1 min, 36°C for 1min, 72°C for 2 min. A final amplification was allowed for 7 min at 72°C. Amplified products were resolved on 1 per cent agarose gel. The gel was stained with Ethidium bromide (4 μl/100ml of gel) and visualized by illumination under UV light in gel documentation system (Flour Chem™ Alpha innotech, USA). The size of the amplification products was determined by comparison with 1kb ladder.The details of RAPD primers used and their sequences are given in Table 1.

The resolved PCR amplified RAPD bands with 40 different primers were scored manually for the presence (1) or absence (0) of band for each primer in the binary data sheet. The data were used to generate Jaccard's similarity co-efficients (Jaccard, 1908). Unweighted Pair Group Method with Arthematic averages (UPGMA) was used for clustering the genotyped using the package, Package for Social Sciences (SPSS).

RESULTS AND DISCUSSION

Different methodological approaches such as morphological, Isoenzyme, protein and DNA markers have employed in the course of years to evaluate the genetic diversity in crop plants (Panella and Gepts, 1992 and Fosto *et al.,* 1998). Among them DNA based marker approach has been found to be superior, because of its capability to reveal more polymorphism (Mignouna *et al.,* 1998). In the current study the characterization of twelve mungbean genotypes was carried using 40 RAPD primers. Such RAPD primers based molecular characterization of redgram genotypes was also conducted by Parmar and Kathiria (2016). Out of forty, twelve primers gave scorable, clear and consistent amplification profiles with all the twelve mungbean genotypes.

A total of 58 amplicons were generated of which 37 are polymorphic, thus exhibiting a total of 63% polymorphism. Primers vary in their polymorphism content with the polymorphism varied between 28% (OPC 9) to 100% (OPB 1 and OPB 3). Earlier different levels of polymorphic bands generated with RAPD markers in mungbean were reported by Karuppanapandian *et al*. (2006) 80% and Sonia *et al*. (2012) 73%. Gupta *et al*. (2013) also reported similar type of higher polymorphism using SSR markers in black gram. Therefore, these findings clearly demonstrate the reliability of RAPD markers in revealing the genetic polymorphism among mungbean genotypes.

In the present investigation the number of bands generated by each primer varied from 2-10 corresponding to an average of 5 bands. The highest number of RAPD fragments were generated by the primer OPD 1 (10 fragments) followed by OPC 9 & OPD 8 (7 fragments) (Fig 1). Jaccard's genetic similarity values ranged from 0.375 to 0.821 (Table 2), indicating the presence of wide range of genetic diversity at molecular level among the twelve genotypes. Prakash G. Patil *et al*. (2015) also reported such wide range of diversity based on Jaccard similarity coefficient (0.16 to 0.96) in red gram using SSAP markers. The lowest similarity value (0.375) was present between genotypes, PUSA VISHAL and IPM-02-03 (0.385), while the highest similarity coefficient was observed between WGG 37 and LGG 528 (0.821) indicating that these genotypes showed maximum degree of similarity in their genetic makeup. Such similar results based on RAPD markers had been previously reported (Sai Rekha *et al*., 2015).

Through UPGMA (Unweighed Pair Group Method on Arithmetic average) dendrogram (Fig. 2), generated using Jaccards similarity coefficient values, cluster I and II had five genotypes each and cluster III had two genotypes. WGG 37 and LGG 528 of cluster I were closely related to each other indicating the presence of low genetic variation between them. Similarly, VG 7098A and PM 110, EC 396117 and ML 267 and IPM-02-03 and MGG 295 of cluster III were also found to be closely related.

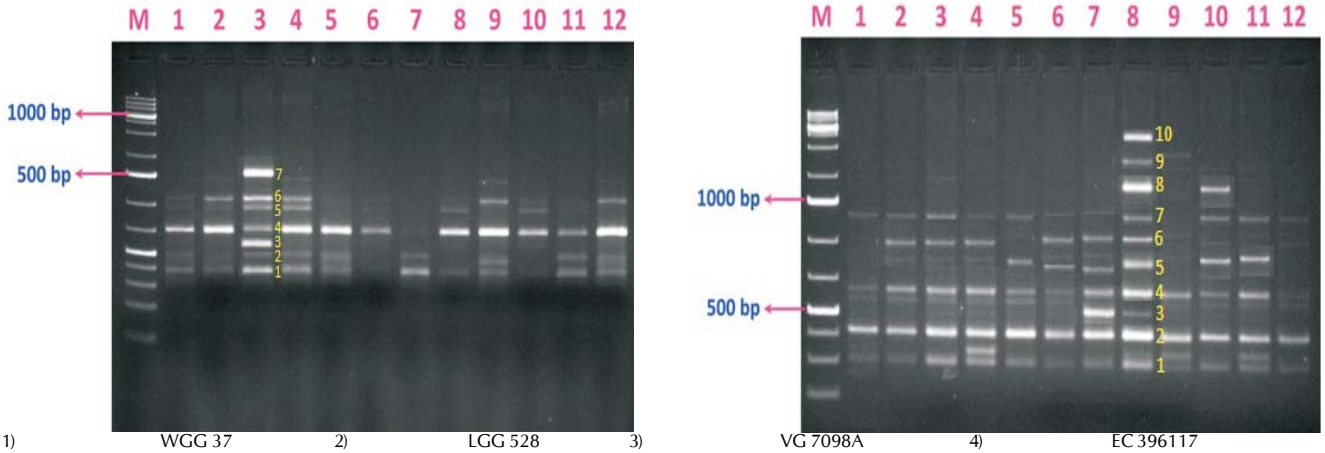
In conclusion, considering the diversity analysis, crossing between PUSA VISHAL (cluster II) X IPM-02-03(cluster III) and EC 396117 (cluster II) and IPM-02-03(cluster III) could be suggested for initiating breeding programme to develop high

Table 1: List of primers and their sequence

S. No.	Primer	Sequence (5 to3)
1	OPA-01	CAGGCCCTTC
2	OPA-02	TGCCGAGCTG
3	OPA-03	AGTCAGCCAC
4	OPA-0 4	AATCGGGCTG
5	OPA-05	AGGGGTCTTG
6	OPA -06	GGTCCCTGAC
7	OPA -07	GAAACGGGTG
8	OPA-08	GTGACGTAGG
9	OPA-09	GGGTAAGCCC
10	OPA-10	GTGATCGCAG
11	OPA- 11	CAATCGCCGT
12	OPA-12	TCGGCGATAG
13	OPA -14	TCTGTGCTGG
14	OPA-20	GTTGCGATCC
15	OPB-01	CTTTCGCTCC
16	OPB-02	TGATCCCTGG
17	OPB-03	CATCCCCCTG
18	OPB-06	TGCTCTGCCC
19	OPB-10	CTGCTGGGAC
20	OPB -12	CCTTGACGCA
21	OPB-13	TTCCCCCGCT
22	OPB-17	AGGGAACGAG
23	OPC-02	GTGAGGCGTC
24	OPC-04	CCGCATCTAC
25	OPC-05	GATGACCGCC
26	OPC-06	GAACGGACTC
27	OPC-08	TGGACCGGTG
28	OPC-09	CTCACCGTCC
29	OPC -10	TGTCTGGGTG
30	OPC-15	GACGGATCAG
31	OPC-16	CACACTCCAG
32	OPC-17	TTCCCCCCAG
33	OPC-20	ACTTCGCCAC
34	OPD-01	ACCGCGAAGG
35	OPD-02	GGACCCAACC
36	OPD-03	GTCGCCGTCA
37	OPD-05	TGAGCGGACA
38	OPD-07	TTGGCACGCG
39	OPD-08	GTGTGCCCCA
40	OPD-11	AGCGCCATTG

Table 2: Average similarity coefficients of 12 mungbean genotypes

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	
1.	WGG- 37	1.000											
2.	LGG- 528	0.821	1.000										
3.	VG 7098A	0.533	0.532	1.000									
4.	EC 396117	0.468	0.500	0.636	1.000								
5.	LGG – 460	0.576	0.618	0.553	0.622	1.000							
6.	TM-96-2	0.714	0.594	0.533	0.533	0.625	1.000						
7.	RMG- 492	0.677	0.719	0.587	0.553	0.647	0.677	1.000					
8.	PUSA VISHAL	0.537	0.571	0.615	0.680	0.523	0.615	0.595	1.000				
9.	IPM-02-03	0.594	0.543	0.500	0.385	0.486	0.417	0.447	0.375	1.000			
10.	ML- 267	0.523	0.458	0.692	0.692	0.511	0.489	0.511	0.608	0.489	1.000		
11.	PM- 110	0.649	0.641	0.708	0.673	0.667	0.605	0.667	0.652	0.488	0.538	1.000	
12.	MGG- 295	0.576	0.667	0.587	0.404	0.600	0.486	0.647	0.489	0.667	0.511	0.512	1.000



1) WGG 37 2) LGG 528 3) VG 7098A 4) EC 396117
5) LGG 460 6) TM96 7) RMG 492 8) PUSA VISHAL
9) PM-02-03 10) ML267 11) PM 110 12) MGG 295

Figure 1: RAPD gel profile of mungbean genotypes by using OPC 9 and OPD 1 primer respectively LaneM:1Kb ladder

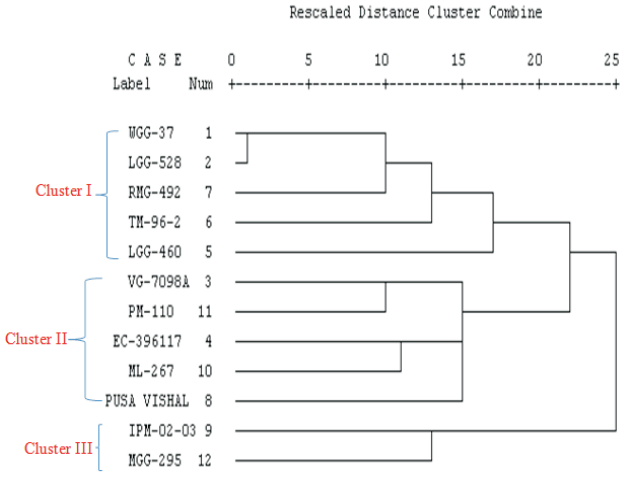


Figure 2: Dendrogram showing various genetic relationships among 12 mungbean genotypes

yielding mungbean genotypes, since these genotypes not only showed high molecular diversity but also high mean yield (data not shown). Therefore the diversity observed in this study may be exploited for further yield improvement by conducting planned hybridization

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