RESEARCH ARTICLE

RAPD ANALYSIS IN MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK]

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Received-16.03.2017, Revised-06.04.2017

Abstract: Molecular characterization is helpful in understanding the phylogenetic relationship among various germplasm to reveal the genetic diversity within a given taxonomic group. Evaluation of genetic diversity would promote the efficient use of genetic variations (Paterson *et al.,* 1991), effective conservation and purity of the genotype to be determined as well as utilization of germplasm in crop improvement .RAPD marker analysis was performed to detect relatedness and diversity among eight parental genotypes. Twenty five RAPD primers having 60% or more GC content were used for the present investigation. Out of 25 primers only 17 were amplified and produce total 391 amplified fragments (amplicon) ranged between 100 bp to 2500 bp. Out of 104 scorable bands, 91 were polymorphic that showed 88 per cent polymorphism. The average number of bands per primer was found to be 6.12 and average numbers of polymorphic bands per primer were 5.35. OPP-10 proved to be best primer in our investigation with total 52 fragments and eight highest scorable bands as well as 100 per cent polymorphism.

Keywords : Mungbean, RAPD Markers, Yield, Yield components

INTRODUCTION

Pulses constitute an important ingredient of the vegetarian diet in the Indian sub-continent and play a significant role in Indian farming because of their value in providing quality food to teeming million and restoring soil fertility through biological nitrogen fixation. India is the largest producer and consumer of pulses in the world accounting 33 per cent of the area and 25 per cent of the global out-put. Green gram [*Vigna radiata* (L.) Wilczek) is the most important legume (Pulse) crop in India after chickpea and pigeonpea. It contributes to about 14% of total pulses cultivation area and 7% of total pulses production in India. Among pulses, green gram has important place as it contains more digestible proteins. It contains 25.0 per cent proteins with all essential amino acids, which is almost three times more than that of cereals (Saini *et al.,* 2010). Besides being a rich source of protein, green gram enriches soil fertility through atmospheric nitrogen fixation with the help of rhizobium bacteria in nodules and humus thus, plays a crucial role in furthering sustainable agriculture. For any successful breeding programme to improve grain yield and component characters, it is essential to know precisely the genetic architecture of these characters under prevailing conditions.

Morphological and biochemical markers used for discriminating cultivars / varieties or parental material are not adequate as they are subject to environmental influences, whereas the molecular markers especially DNA based, have proven better. The latter may or may not correlate with phenotypic expression of a genomic trait. Varietal profiling methods that directly utilize DNA have been found to potentially address all the limitations associated with morphological and biochemical data. They offer numerous advantages over conventional, phenotype-based characters as they are stable and detectable in all situations regardless of growth, differentiation, development or defense status of the cell. Additionally, they are not confounded by environmental, pleotropic and epistatic effects. The DNA markers become the marker of choice for the study of crop genetic diversity, especially those based on DNA sequence variations which are increasingly being utilized in crops for construction of genetic maps and marker-assisted selection studies. Among the DNA markers, development of RAPD-PCR based DNA finger printing has proved to be versatile (Gherardi *et al*., 1998). RAPD markers have been used for the identification and assessing the genetic diversity among parental material of several crops like green gram (Saini *et al.,* 2010),. Moreover, RAPD-derived genetic information helps to compare each germplasm and to choose competent parents for hybridization. Among the various molecular markers, PCR based RAPD markers have become popular since their application does not need any prior information about the target sequences on the genome and is simple and fast. Application of molecular markers to plant breeding has established the need for information on variation in DNA sequence even in those crops in which little classical genetic and cytogenetic information is available.

MATERIAL AND METHOD

Eight diverse and well adapted genotypes of green gram were selected as parents for crossing programme, namely IPM-99-125, BM-4, ML-131, IPM 02-03, PDM-139, RMG-1035, RMG-344 and RMG-1045. Cross success percentage was very less

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in open field at normal environmental condition; therefore crosses were attempted at green house during *spring*, 2013-14 in diallel fashion (excluding reciprocals) to obtain 28 F_1 crosses. The experimental material for the present investigation comprised of 36 entries including 8 parents and their 28 F_1 crosses. These parents and F_1 were grown in randomized block design with three replications during *kharif*, 2014 at RCA college farm, MPUAT, UDAIPUR All recommended cultural practices and plant protection measures were adopted to raise a good crop.

Table 1. Experimental material and their pedigree

Parent	Pedigree	Source
IPM 99-125	PM 3 x APM 36	IIPR, Kanpur
BM 4	MUTANT of T44	ARS, Badnapur
ML 131	ML 1 x ML 23	ARS, Durgapura
IPM 02-03	IPM 99-125 x Pusa bold 2	IIPR, Kanpur
PDM 139	ML 20/19 x ML 5	IIPR, Kanpur
RMG 1035	RMG 492 x ML 818	ARS, Durgapura
RMG 344	MOONG SEL.1 x J 45	ARS, Durgapura
RMG-1045	RMG-62 x KM 2170	ARS, Durgapura

Molecular marker analysis was done exclusively for the parental material only to see the diversity present among the parental material. DNA extracted from different green gram cultivars were compared using RAPD methodology. DNA was extracted from young leaves (3–4 weeks old) using CTAB method and was amplified by using decamer random oligonucleotide primer in a DNA thermo cycler (Biometra). The amplified samples were separated on agarose gel electrophoresis (1.2%). The bands were scored for their presence or absence. The leaves were harvested after 21 days and DNA was isolated with the help of Doyle and Doyle, 1987 protocol.

The DNA was diluted to final concentration of 10.0 ng/µl in $T_{10}E_1$ buffer (10 mM Tris HCL, 1 mM EDTA, pH 8.0). A set of 30 decanucleotide RAPD primers were used for PCR amplification. PCR amplification conditions such as concentration of template DNA, primers, concentration of $MgCl_2$, *Taq* DNA polymerase and annealing temperature were optimized for RAPD primers. Reproducible and clear banding patterns were obtained in a reaction mixture of 20 µl containing 50 ng of template DNA, 2 µl of 10 X *Taq* DNA polymerase buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.30 µM of primer and 1 U of *Taq* DNA polymerase, at an annealing temperature of 37°C (RAPD) for PCR amplification. Similar findings reported by Khamassi *et al.* (2011).

Table 2. PCR reaction mixture content

Components	Final concentration	Single tube/20 (µl)
DNA template	50ng	2.00 µl
Master Mixture		
(i) dNTP MIX	200µM	1.6 µl
(ii) Taq polymerase	1 U	0.33µl
(iii) Reaction buffer (10x)	1X	2.00 µl
(iv) Primer	0.5 µM	1.00µl
(vi) dd H_2O		12.07µl

Agarose gel electrophoresis and Data analysis: Submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.5 per cent agarose gel. The gel was prepared in 1X TAE buffer containing [(0.50 µg/ml) of ethidium bromide] the samples and loading dye were mixed in 1:1 ratio and loaded with micropipette. Electrophoresis was carried out at 100 volt for 3 hrs. Photographs from ethidium bromide containing gel were used to score the data manually and independently for RAPD analysis. Presence of amplified product were scored as 1 and its absence as 0 for all genotypes and primer combinations. These data matrices were then entered into NTSYS-PC (Numerical Taxonomy and

Multivariate Analysis System Programme) developed by Rohlf (1993).

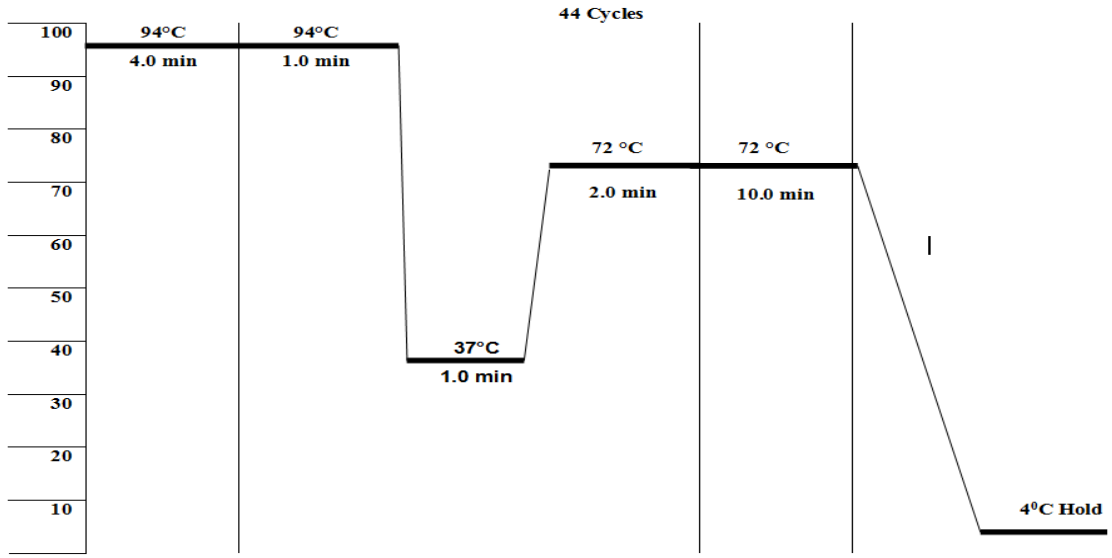

Figure 1. Protocol used for RAPD primers for PCR amplification

RESULT AND DISCUSSION

The amount of DNA isolated from various genotypes of *V. radiata* L. ranged from 757 to 1518 ng/µl (Table 4.23). The genotype IPM 02-03 yielded the highest amount of DNA (1518 ng/µl). Whereas the lowest amount of DNA (757 ng/µl) was obtained from genotype RMG-344. The ratio of absorbance (A260/A280) ranged from 1.70 to 1.89 revealing that the DNA obtained was free from contaminants like polysaccharides, protein and RNA. The quality of DNA as also checked by gel electrophoresis revealed a single discrete band in all genotypes showing that genomic DNA was intact and had high molecular weight, free from any mechanical or enzymatic degradation, free from RNA contamination and was of high quality.

Table 3. Quality and quantity of total genomic DNA of *V. radiata* L. isolated and purified by CTAB method

Genotypes	Parents' Name	Concentration (ng/ µl)	Ratio 260/280
P1	IPM 99-125	1420	1.81
P2	BM-4	968	1.77
P3	ML-131	1250	1.79
P4	IPM 02-03	1518	1.89
P5	PDM-139	1251	1.8
P6	RMG-1035	1012	1.81
P7	RMG-344	757	1.74
P8	RMG-1045	998	1.82

Twenty-five RAPD primers having 60% or more GC content were used for the present investigation. Out of 25 primers only 17 were amplified. A total of 104 amplified bands were obtained of which 91 were polymorphic and 13 monomorphic that showed 88 % polymorphism. The total number of amplified bands varied between 5 and 8. The average number of bands per primer was found to be 6.12 and average numbers of polymorphic bands per primer were 5.35. The polymorphism amongst all genotypes of *V. radiata* L. was 88% and the overall size of PCR amplified products ranged between 100 bp to 2500 bp. The per cent polymorphism ranged from as low as 60 % (OPA-15 and OPB-06) to as high as 100 % (OPA-09, OPA-10, OPA-08, OPB-03, OPB-07, OPE-03, and OPA-16). Similar result has been found by Datta *et al.* (2012), Undal *et al.* (2011) and Saini *et al*. (2010) etc.

Table 4. Details of the RAPD primers used for amplification of genomic DNA of green gram Genetic relationship and Cluster Tree Analysis

Total number of primers	25
Number of primers which showed amplification	17
Number of primer which showed polymorphism	17
Total number of monomorphic bands	13
Total number of polymorphic bands	91
Total number of bands	104
Total number of amplicon produced	391

The data obtained by using RAPD and ISSR primers were further used to construct similarity matrix of eight *V. radiata* L. genotypes using 'Simqual' sub-programme of software NTSYS-pc. Dendrograms were constructed using similarity matrix values as determined from RAPD data for *V. radiata* L. genotypes using unweighted pair group method with arithmetic average (UPGMA) sub-programme of NTSYS-pc software.

Similarity Matrix

Based on RAPD similarity matrix data, the value of similarity coefficient ranged from 0.34 to 0.57 . The average similarity across the eight parents was found out to be 0.46 showing that genotype were moderately diverse from each other. Maximum similarity value of 0.57 was observed between genotypes PDM-139 and RMG-1035; PDM-139 and RMG-1045 followed by PDM-139 x RMG-344 and RMG-1035 x RMG-344 with a similarity coefficient value of 0.54. Likewise, minimum similarity value of 0.34 was observed between genotypes IPM 99-125 and RMG-1045. The findings confirm with that of Saini *et al.* (2010) and Lavanya *et al*. (2008).

RAPD Marker Based Cluster Tree Analysis

The Jaccard'ssimilarity coefficient based on UPGMA displayed in the range of 0.34 to 0.57. The RAPD cluster tree analysis of eight *V. radiata* L. genotypes showed that they could be divided into 2 major clusters viz., cluster I and cluster II at a similarity coefficient of 0.40 . The dendrogram clearly indicated that cluster I included two genotypes IPM 99-125 and IPM 02-03 at 0.46 similarity coefficient. The cluster II was larger than cluster I, included six genotypes, viz. BM-4, ML-131, PDM-139, RMG-1035, RMG-1045 and RMG-344. Cluster II was divided into two sub-clusters, cluster II-A has only one genotype BM-4, where as cluster II-B has two sub-clusters, II B1 included genotype ML-131 and second sub cluster II B2 included four genotypes PDM-139, RMG-1035, RMG-1045 and RMG-344. II B2' has two sub-clusters, sub-cluster II B2'a included PDM-139 and RMG-1035 with 0.57 similarity coefficient, Looking to the morphological similarity both are having hard seed with approx. similar maturity time and yield potential and RMG-1045 included sub-cluster II $B2^1$ b. While, the genotype RMG-344 was grouped single in separate cluster (II B2"). Saini *et al.* (2010) and Datta *et al.* (2012) reported almost similar results.

Table 5. Jaccard'se Similarity Coefficient based on RAPD profiling

	IPM 99-125	**BM-4**	**ML-131**	**IPM 02-03**	**PDM-139**	**RMG-1035**	**RMG-344**	**RMG-1045**
IPM 99-125	1.00							
BM-4	0.40	1.00						
ML-131	0.46	0.41	1.00					
IPM 02-03	0.46	0.40	0.43	1.00				
PDM-139	0.40	0.51	0.51	0.40	1.00			
RMG-1035	0.38	0.49	0.51	0.41	0.57	1.00		
RMG-344	0.38	0.44	0.50	0.42	0.54	0.54	1.00	
RMG-1045	0.34	0.42	0.37	0.38	0.57	0.53	0.47	1.00

From the above discussion, it may be concluded that RAPD analysis revealed substantial polymorphism in parental mungbean genotypes. The technique may be used to obtain reasonably precise information on genetic relationship among mungbean genotypes. Such information may be useful for selecting diverse parents for hybridization purpose and also monitoring the genetic diversity periodically in the breeders working collection of mungbean. To achieve breakthrough in the yield and quality characters of *V. radiata* L., genetic divergence analysis should be attempted so that the highly diverse genotypes could be selected for molecular breeding programmes

Table 6. Polymorphism information of RAPD primers analyzed

Sl No	Primer	Total No of bands (a)	Total no. of polymorphic bands (b)	Polymorphism % (b/a X 100)	Range of band size
1	OPA-02	7	6	86	200-1000
2	OPA-05	6	5	83	300-2000
3	OPA-07	7	6	86	300-1000
4	OPA-08	7	7	100	400-2000
5	OPF-19	6	4	67	200-1500
6	OPP-03	5	4	80	300-1500
7	OPB-06	5	3	60	100-900
8	OPA-10	6	6	100	200-1000
9	OPP-10	8	8	100	200-1500
10	OPA-11	6	5	83	400-1500
11	OPA-14	NA	NA	NA	-
12	OPA-15	5	3	60	400-1000
13	OPC-01	NA	NA	NA	-
14	OPB-03	6	6	100	100-1500
15	OPA-09	7	7	100	200-2500
16	OPB-07	6	6	100	300-1000
17	OPC-05	NA	NA	NA	-
18	OPE-03	5	5	100	400-1500
19	OPA-16	6	6	100	400-2000
20	OPC-06	NA	NA	NA	-
21	OPB-02	6	4	67	400-2000
22	OPB-04	NA	NA	NA	-
23	OPB-05	NA	NA	NA	-
24	OPB-08	NA	NA	NA	-
25	OPB-10	NA	NA	NA	-
Total		**104**	**91**	**88**	-
Average		**6.12**	**5.35**		

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