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Molecular Diversity Analysis of Soybean Genotypes Using Molecular Markers

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A B S T R A C T

Soybean is legume crop having high protein (40%) and oil (20%) content. It has highest share in production among all oilseeds of the world. In present study 10 soybean genotypes were characterized using 14 RAPD primers. Total 103 amplicons were obtained out of which 75 were polymorphic and 28 were monomorphic. The percent polymorphism obtained was 72.81%. Highest average similarity coefficient was exhibited by the genotype MAUS-32 (0.675) and lowest similarity coefficient exhibited by MAUS-2 (0.584). The dendrogram of these genotypes grouped into two main clusters. These two clusters again divided into 6 sub-clusters having distinct morphological and physiological characteristics.

Introduction

Soybean (*Glycine Max* L. Merril) is annual legume crop originated in China. It ranks first among all oilseeds crops of world having 50 percent share in total oilseed production (Anonymous, 2009). In India large number of soybean varieties has been released for cultivation. To test genetic resources for their productivity, quality parameters and stress tolerance, field trials are usually time consuming therefore, molecular markers are used to assess diversity in the gene pool to identify genes of interest and to develop a set of markers for screening progenies (Karp *et al.*, 1997). Many types of molecular markers viz. RAPD, AFLP, RFLP, ISSR, SSRs are becoming increasingly important for cultivar identification and diversity analysis. In soybeans dense genetic maps were developed using RFLP and AFLP markers (Keim *et al.*, 1997). Also PCR based intraspecific RAPD map is built, however number of polymorphism was relatively small due to narrow genetic base of cultivated soybean (Abdelnoor *et al.*, 1995). There are several reports of using molecular markers for evaluation of genetic diversity, out of which RAPD markers have been shown to be a simple and effective means to evaluate variability; because they are technically simple, non radioactive, inexpensive and require small amount of DNA. In the view of above content, the present study has been carried out to analyse genetic diversity of selected accessions from Soybean Research Station, M.K.V., Parbhani (Maharashtra).

Materials and Methods

Ten Soybean genotypes exhibiting different phenotypic characters were used for this study (Anonymous, 2006) (Table 1). Seeds were germinated on germination papers kept at

37°C. After 7 days 1.2 to 2 g shoot portion of seedlings was used for DNA extraction. Genomic DNA was extracted by using modified CTAB method of DNA isolation (Bhat *et al.*, 1999; Saghai *et al.*, 1984). The extraction buffer consisted of EDTA (20 mM), Tris-HCL having pH 8.0 (100 mM), NaCl (1.4M), CTAB (2%), β-mercaptoethanol (0.2%). Leaf tissues were grounded to fine powder in liquid nitrogen (-196°C) with mortar and pestle. To the powdered tissue, 2 ml of extraction buffer was added and mixed well by gentle inversion and incubated at 65°C for 30 min in a water bath. The mixture was then subjected to centrifugation at 10,000 rpm at 4°C temperature for 10 min. The supernatant was taken and then mixed with equal volume of freshly prepared Chloroform: Isoamyl alcohol and recentrifuged at 10,000 rpm for 10 min. at 4°C temperature then collected the supernatants into a fresh tube. To the collected supernatants, 0.7 volume of chilled isopropanol was added mixed well and DNA was allowed to precipitate at 20°C for overnight. The DNA was pelleted by centrifugation at 10,000 rpm for 10 min at room temperature. Collected pellet was washed with 70 percent alcohol and dissolved in optimum quantity of TE buffer. DNase free RNase-A was added at a final concentration of 20 µg/ml and incubated at 37°C for 1 hour in hot water bath. To the incubated sample equal volume of phenol: chloroform (1:1) mixture was added and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was collected in fresh tube. To the aqueous phase 0.1 volume of sodium acetate and 2 volumes of ice cold absolute ethanol was added, mixed well and kept it at -20°C for 1 hour. The DNA was then repelleted by centrifugation at 10,000 rpm for 10 min at room temperature. The collected pellet was rewashed with 70 per cent alcohol air dried and dissolved in optimum quantity of TE buffer and stored at 20°C.

Fifty random primers from OPA, OPG, OPM, OPF, OPG, OPR, OPJ, OPX, OPH series (Bio Serve Biotechnologies, India Pvt. Ltd. Hyderabad) were screened out of them fourteen primers (OPA-6, OPA-10, OPA-13, OPX-11, OPX-14, OPM-05, OPM-20, OPG-09, OPG-12, OPF-10, OPF-12, OPR-04, OPH-20, OPJ-01) were selected for RAPD analysis. PCR was carried out in 25 µl reaction volume containing 10X PCR buffer (with KCL) 2.5 µl, dNTPs (10 mM) 0.5 µl, $MgCl_2$ (25mM) 1.5µl, primer (20 pm/µl) 1.5 µl, Taq DNA polymerase (1.5U/µl) 0.3 µl, template DNA (25 ng) 1.0 µl and sterile double distilled water 17.7 µl. Amplification was programmed for 35 cycles with initial denaturation at 94°C for 4 min., followed by cycling conditions of denaturation at 94°C for 1 min, annealing at 35°C at 1min. and extension at 72 °C for 2 min. After 35 cycles, there was a final extension step of 10 min at 72 °C. The amplicons were analyzed on 1.5% agarose gels at 100V for 4 hours and detected by staining with ethidium bromide. UV trans-illuminated gels were photographed with gel documentation system (Alphaimager TM 2200).

The amplified products were scored for presence (1), absence (0), missing and doubtful case was scored as 9. Band size was determined by comparison with 1 kb DNA ladder (MBT, Fermentas, U. K.) as standard.

The data was used for similarity based analysis using programme NTSYS-PC (Version 2.02) developed by (Rolf *et al.*, 1993) Jaccard's similarity coefficients (F') was calculated using the programme SIMQUAL. Similarity coefficients were used to construct UPGMA (unweighted pair group method with average) to generate dendrogram. The polymorphic percentage of the obtained bands were calculated by using following formula

Polymorphic % = (no. of polymorphic bands /Total bands) X 100

Results and Discussion

Total 103 amplicons were generated with an average of 7.4 amplicons per primer, out of them 28 were monomorphic and 75 polymorphic. The result showed average 72.81% polymorphism, highest i. e. 100% polymorphism was recorded in primer OPM-20, OPH-20 and OPX-11. Average monomorphic band were 2 (Table 2) while, average polymorphic bands were 5.3 (Fig. 2).

Genetic relationship was determined on the basis of jaccard's similarity coefficient values, these values ranged from 0 to 1 (Table 3 and 4). Average genetic similarity coefficient was 0.634. Cluster analysis revealed by dendrogram (fig. 1) shown these accessions into two super clusters 'A' and 'B' at 59 per cent similarity. Super cluster 'A' accommodates 6 genotypes having early maturity character. Super cluster 'B' contains 4 genotypes which are high yielding and having pest resistance. Super clusters 'A' divided into 6 sub clusters in which sub cluster-I contains only one variety MAUS-1 having early maturity as distinct character and which is also suitable for intercropping. Sub cluster-II contains two varieties MAUS-32 and MAUS-61-2 having early maturity and Non – shattering characters common, these two varieties having 76% similarity. Sub cluster-III contains two varieties MAUS-47 and MAUS-61 having about 81% similarity, these genotypes are having round seeds and early maturity. Sub cluster-IV contains only one genotype MAUS-2 which is having white colour flowers and high oil content and unique marker associated with this trait is OPA-13. Sub cluster-V contains two genotypes MAUS-71 and MAUS-81with about 72% similarities, these varieties are having good germination and high yielding character. Sub cluster-VI contains two varieties MAUS-158 and MAUS-162 with about 77% similarity are high yielding and having pest resistance.

Table.1 List of Soybean genotypes with characters

Sr. No.	Variety	Days to 50% flowering	Days to maturity	Flower colour	Characters
1	MAUS-1	40	95	Purple	Early, suitable for intercropping
2	MAUS-2	39	90	white	Early, high oil content
3	MAUS-32	40	90	purple	Early, Non- shattering,
4	MAUS-47	38	85	purple	Very early, round seeds, good germination
5	MAUS-61	40	95	purple	Early, round seeds, good germination
6	MAUS-61-2	39	95	Purple	Early, round seeds,Non-shattering
7	MAUS-71	38	100	Purple	Good germination, High yielding
8	MAUS-81	40	105	Purple	High yielding, good germination
9	MAUS-158	40	100	Purple	pest resistance
10	MAUS-162	42	100	Purple	pest resistance

Table.2 RAPD amplicons/bands produced by soybean genotypes

Sr. No.	Primer	Total no. of amplicons	No. of monomorphic amplicons	No. of polymorphic amplicons	Percent polymorphism
1	OPA-06	10	2	8	80
2	OPA-10	6	3	3	50
3	OPX-14	8	4	4	50
4	OPR-04	5	2	3	60
5	OPA-13	7	2	5	71.42
6	OPG-12	9	1	8	88.88
7	OPM-05	7	2	5	71.42
8	OPM-20	9	0	9	100
9	OPH-20	3	0	3	100
10	OPF-10	9	3	6	66.66
11	OPX-11	10	0	10	100
12	OPG-09	9	5	4	44.44
13	OPF-12	6	3	3	50
14	OPJ-01	5	1	4	80
Total		**103**	**28(Avg. 27.18 %)**	**75(Avg. 72.81 %)**	**Avg. 72.34 %**

Table.3 Average similarity Index of 10 soybean genotypes

Sr. No	Variety	Average similarity value	Sr. No	Variety	Average similarity value
1	MAUS-1	0.640	6	MAUS-61-2	0.654
2	MAUS-2	0.584	7	MAUS-71	0.603
3	MAUS-32	0.675	8	MAUS-81	0.640
4	MAUS-47	0.673	9	MAUS-158	0.614
5	MAUS-61	0.66	10	MAUS-162	0.603
				Average	**0.634**

Table 4 . Similarity matrix based on jaccard's similarity coefficient values obtained from RAPD analysis of 10 soybean genotypes

	MAUS-1	MAUS-2	MAUS-32	MAUS-47	MAUS-61	MAUS-61-2	MAUS-71	MAUS-81	MAUS-158	MAUS-162
MAUS-1	1									
MAUS-2	0.659	1								
MAUS-32	0.684	0.666	1							
MAUS-47	0.73	0.582	0.738	1						
MAUS-61	0.662	0.623	0.771	0.802	1					
MAUS-61-2	0.692	0.6	0.758	0.788	0.717	1				
MAUS-71	0.563	0.522	0.604	0.576	0.597	0.559	1			
MAUS-81	0.645	0.541	0.635	0.625	0.612	0.625	0.712	1		
MAUS-158	0.581	0.526	0.604	0.593	0.58	0.545	0.697	0.725	1	
MAUS-162	0.545	0.537	0.617	0.623	0.576	0.606	0.6	0.648	0.767	1

Figure.1 Dendrogram Generated by UPGMA analysis based on RAPD data showing relationship among soybean genotypes

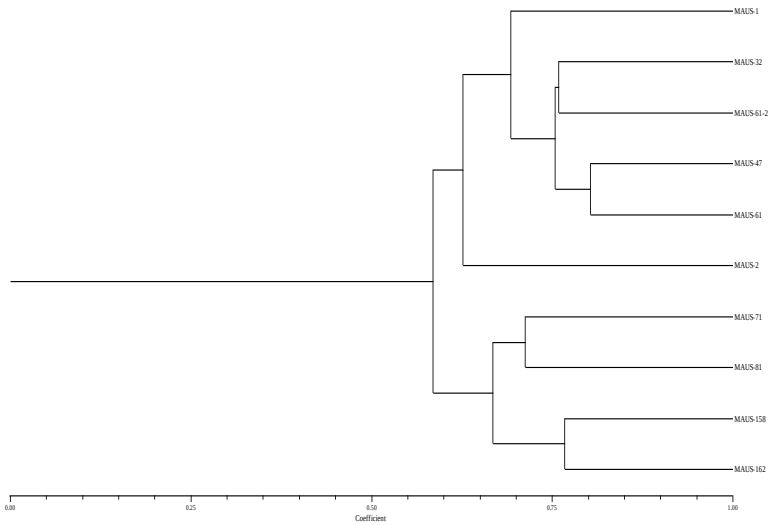

Fig 15.Dendrogram generated by UPGMA analysis based on RAPD data showing relationship among 10 soybean genotypes.

Figure.2 Representative results obtained with 14 RAPD primers

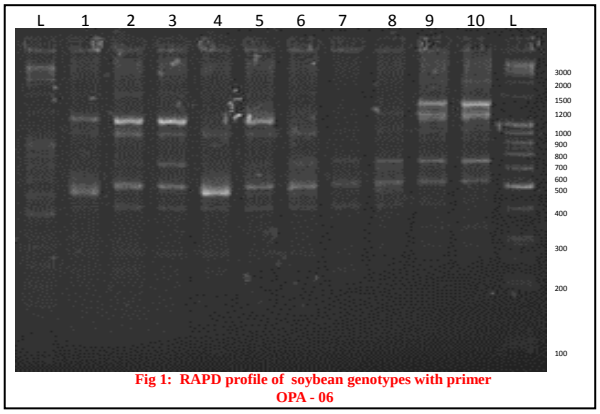

Fig 1: RAPD profile of soybean genotypes with primer OPA - 06

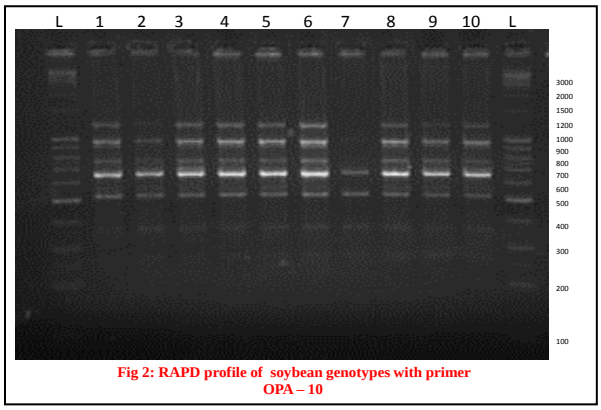

Fig 2: RAPD profile of soybean genotypes with primer OPA – 10

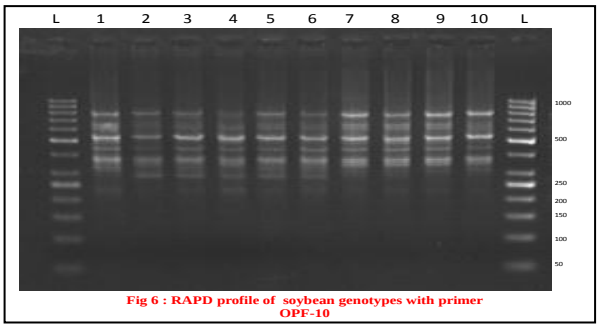

Fig 6 : RAPD profile of soybean genotypes with primer OPF-10

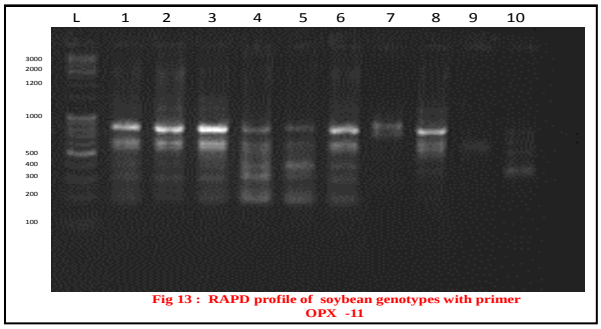

Fig 13 : RAPD profile of soybean genotypes with primer OPX -11

Sr. No	Variety	Sr. No	Variety
L	1 kb DNA Ladder	Lane 6	MAUS-61-2
Lane 1	MAUS-1	Lane 7	MAUS-71
Lane 2	MAUS-2	Lane 8	MAUS-81
Lane 3	MAUS-32	Lane 9	MAUS-158
Lane 4	MAUS-47	Lane 10	MAUS-162
Lane 5	MAUS-61		

Although there was low variation shown by previous studies; present study shows high variation in comparison, because polymorphism ratio is mainly affected by sequences of primers, types and number of lines being evaluated (Keim *et al.*, 1997). Thus, clusters analysis can help to confirm characters like maturity duration, flower colour, seed shape, oil content, pest and disease resistant and non-shattering habit as distinct characters. It clearly indicates that geographical origin and phenotypic characters play important role in cluster formation and genetic relationships (Zenglu *et al.*, 2001). Our results supported previous studied results wherein a very high level of genetic variability had been reported. These results showed 67.6 % genetic diversity in soybean (*Glycine max* L.) and wild soybean (*Glycine soja*) indicating inter varietal relationship of soybean have a narrow genetic base and between varieties are more closely related, while wild soybean is quite distantly related (Lee *et al.*, 1998).

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