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Randomly Amplified Polymorphic DNA (RAPD) Molecular Markers as a Valuable Tool to Confirm the Genetic Diversity in Soybean Germplasms (*Glycine max*)

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ABSTRACT

Background: This study was performed to understand the genetic base of soybean which mainly focused to assess the diversity among sixteen soybean germplasms namely (AMS 100-39, BAUS 102, DS 3108, DSB 34, MACS 1493, NRC 128, NRC 130, NRC 131, NRC 132, NRC 136, NRC 137, NRC SL 1, PS 1613, RSC 11-03, RSC 11-07 and SKF SP-11) with two cultivated checks (JS-335 and Bragg).

Methods: The genomic isolation was carried out using CTAB buffer and the diversity was estimated with the help of nine RAPD markers using percent polymorphism, PIC content, Jaccard's similarity index and represented in dendogram.

Result: The banding patterns were obtained with all the primer showed a total of 59 bands, out of the which, 17 bands were monomorphic, while other are polymorphic and the amplification ranged from 100 bp to 960 bp. The range of similarity coefficients varies from 0.87 to 0.36. Out of total amplification, products were scored, averages of 70.3% were polymorphic among all genotypes. The cluster clearly divided the whole germplasms into two four groups showing the clear-cut diversity profile of all germplasms. As a result, this study is very beneficial for understanding the diversity of different soybean germplasms and the application of this technique for the development of highly profitable crops.

Key words: *Glycine max*, Jaccard's similarity matrix, Polymorphism, RAPD markers, Similarity coefficients, Soybean germplasms.

INTRODUCTION

The wild ancestor of the cultivated soybean, *Glycine soja*, gave rise to *Glycine max* (Singh *et al.,* 2017). *Glycine max*, a member of the Leguminosae family, is the most important bean in the world economically. It provides 25% of the world's edible oil, which is used by millions of people, as well as ingredients for hundreds of chemical products and about two-thirds of the protein concentrate used to feed livestock (Agarwal *et al*., 2013). In India, soybeans were grown on an area of roughly 119.9 million ha and 118.9 million metric tons were produced (SOPA, 2022). The majority of abiotic and biotic variables have an impact on soybean productivity. Rainfall had an impact on soybean output due to abiotic causes, whilst insect pests attacked the crop during its growth due to biotic factors. This can be because no cultivars resistant to these biotic effects have been developed. Insect host selection has a great impact on agriculture and plants do have some specific genes to counteract the insect activity. Plant breeders have crossed insect resistance genes from wild relatives or land races of crops with agricultural varieties to develop new lines that are less attractive for oviposition and feeding by insect pests**.** The extent of genetic diversity among plants can be accessed through protein and genetic markers (Sharma *et al.,* 2018; Weiguo *et al.,* 2007). Among a variety of tools which have been used for genetic modification the RAPD (Random Amplified Polymorphic DNA) primer is one of the most vital parameters to identify the change in genetic constitutions of plants (Wahyudi *et al*., 2020). Pamidimarri *et al.* (2009) also reported that these polymorphic primers not only used for identification at molecular level but also specifically identified between two individuals of same species with different properties. In polymerase chain reaction (PCR), RAPD is primarily based on a variation of primer annealing sites and can be visualized through agarose gel. RAPD markers used to evaluate genetic diversity among various host plants with polymorphism studies among population which is determined through PIC value (Polymorphic Information Content) while the explanation of the relationship among

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germplasm is determined using cluster analysis (Thompson *et al.* 1998). Narvel *et al.* (2001) observed that RAPD primers are very specific and could distinguish the population into different clusters according to the similarity coefficient. Hence the study can provide a great characterization of genetic diversity which will help in the soybean crop improvement program.

In this study, sixteen soybean germplasms with two susceptible checks (JS-335 and Bragg) were subjected for the screening of molecular variation through nine RAPD molecular markers to evaluate the genetic relationships among these eighteen soybean testing plants. These germplasms show the morphological difference at their best level and considered as the best source of resistance against *Spodoptera litura* (Mathpal *et al.,* 2022). The finding of this study was expected to be useful in supporting the soybean breeding researches in India.

MATERIALS AND METHODS

In present study soybean germplasms from different sources were subjected to diversity analysis through RAPD markers. For this, sixteen germplasms (with two susceptible checks) were analyzed with the help of Randomly Amplified Polymorphic DNA (RAPD) markers. Procedures of this experiment were performed in the Cytogenetic laboratory at the Department of Plant Breeding and Genetics, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

Plant materials

Seeds of sixteen soybean germplasm namely (AMS 100-39, BAUS 102, DS 3108, DSB 34, MACS 1493, NRC 128, NRC 130, NRC 131, NRC 132, NRC 136, NRC 137, NRC SL 1, PS 1613, RSC 11-03, RSC 11-07 and SKF SP-11) with the check varieties (JS-335 and Bragg) were collected from the department of Genetics and Plant Breeding, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

Genomic DNA extraction and RAPD analysis

The DNA extraction from newly trifoliate leaf of each soybean germplasm was performed by CTAB method with slight modification (Cubero *et al.,* 1999). The fresh leaves were first ground on 2 ml pre-warmed (60°C) CTAB buffer (100 mM Tris HCl + 20 mM EDTA + 1.4M NaCl + 2% CTAB + 2% SDS + 2% β-mercaptoethanol) with the help of mortar-pestle. After centrifugation addition of P:C:I (25:24:1) was done in the supernatant then again centrifuged at 10,000 rpm for 15 min. The supernatant was then taken to new centrifugal tube and after adding chilled isopropanol, it was kept at -20°C. After 24 hrs a white DNA pellet was observed at the bottom of eppendorf tube when centrifuged for 18 min at 10,000 rpm which then was washed with 100% ethanol and then dried for 1-2 hrs. After complete drying 50 ml of TE buffer [1M Tris-HCl buffer (pH 8.0) + 0.5 M EDTA] was added then after quantification with the help of spectrophotometer and then stored it at -20°C for further use.

For variability analysis, nine Randomly Amplified Polymorphic DNA (RAPD) markers were obtained from Chromous Biotech, Bangalore. The primers namely OPC-14 to OPD-14 was obtained from (Wahyudi *et al.,* 2020) while GM-9 to GM-3 (Nautiyal, 2016) (Table 1). The reaction was then performed by taking 25 µl reaction mixture consisted of DNA, PCR mastermix (Hi-Media) and diluted RAPD primers. PCR amplifications were performed in a profile using Eppendorf Master Thermal Cycler programmed for an initial denaturation of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 35°C for 30 sec and primer extension at 72°C for 2 min, with a final extension (renaturation) at 72°C for 10 min.

Visualization of bands using gel electrophoresis

The amplified products of genomic DNA of various soybean germplasms with the help of RAPD markers were evaluated through 2.5% agarose gel stained with ethydium bromide in 1× TBE (Tris Base + Boric acid + 0.5 M EDTA). The PCR-RAPD amplified products were loaded in the wells with the help of pipette and then separated under 70 volts for 3 hrs then the bands were visualized under UV-doc (Fig 1).

Statistical analysis

The amplified DNA fragments bands were scored on the basis of presence (1) and absence (0), generating a matrix for nine RAPD markers used for all soybean germplasms. The percent polymorphism was calculated through the number of polymorphic bands to the total number of bands multiplied by hundred. Polymorphism information content (PIC) value was also calculated as:

$$PIC = \frac{\Sigma(1 - P^2i)}{n}$$

Where:

n = Number of band positions analyzed in the set of accessions.

Pi = Frequency of i^{th} pattern (Mokate *et al.,* 2017).

The score marker data of different germplasms and susceptible checks were estimated through Jaccard's similarity matrix (Jaccard, 1908) to compute the similarities between the germplasms. The matrix then subjected to the CLUSTER analysis on the basis of unweighted pair group arithmetic mean analysis (UPGMA) using NTSYS-PC 2.0 package (Rohalf, 1998) to generate a dendogram using average linkage procedure.

RESULTS AND DISCUSSION

Optimization of RAPD reaction and polymorphism

Nine RAPD primers were used to characterize the genetic diversity present among the soybean germplasm. The banding patterns were obtained with all the primer showed

a total of 59 bands (Table 2). The primers assorted greatly in their ability to resolve variability among the germplasms. Individually the range of the bands generated varied from 4 to 8 bands with an average of 6.5 bands per primer. Out of the 59 bands 17 bands were monomorphic *i.e.* it was present in all the isolates. The amplification ranged from 100 bp to 960 bp. The percentage of polymorphism was calculated by multiplying the total number of scored bands

Table 1: List of RAPD primers used for the varietal characterization and their sequences.

Primer name	Sequences	GC content	Tm (°C)
OPC-14	TGCGTGCTTG	60%	31
OPC-15	GACGGATCAG	70%	33
OPC-18	TGAGTGGGTG	60%	33
OPC-19	GTTGCCAGCC	70%	36
OPC-20	ACTTCGCCAC	60%	39
OPD-14	CTTCCCCAAG	60%	35
GM-9	ACAACGCGAG	60%	39
GM-11	AAAGTGCGGG	60%	35
GM-3	GGGGTGACGA	70%	36

Table 2: Analysis results of polymorphism and affectivity of RAPD primers.

Primer	Amplified product range (bp)	nB	nMB	nPB	PB%	PIC
OPC-14	100-800	7	2	5	71.43	0.19
OPC-15	280-620	4	2	2	50.00	0.16
OPC-18	100-650	4	1	3	75.00	0.26
OPC-19	150-700	6	2	4	66.67	0.18
OPC-20	150-850	8	3	5	62.50	0.39
OPD-14	100-900	8	3	5	62.50	0.49
GM-9	100-960	7	2	5	71.43	0.44
GM-11	180-800	7	1	6	85.71	0.49
GM-3	150-880	8	1	7	87.50	0.50
Average		6.56	1.89	4.11	70.30	0.34

nB= Total number of bands, nMB= Number of monomorphic band, nPB= Number of polymorphic bands, PB%= Percentage of polymorphic bands, PIC= Polymorphic information content.

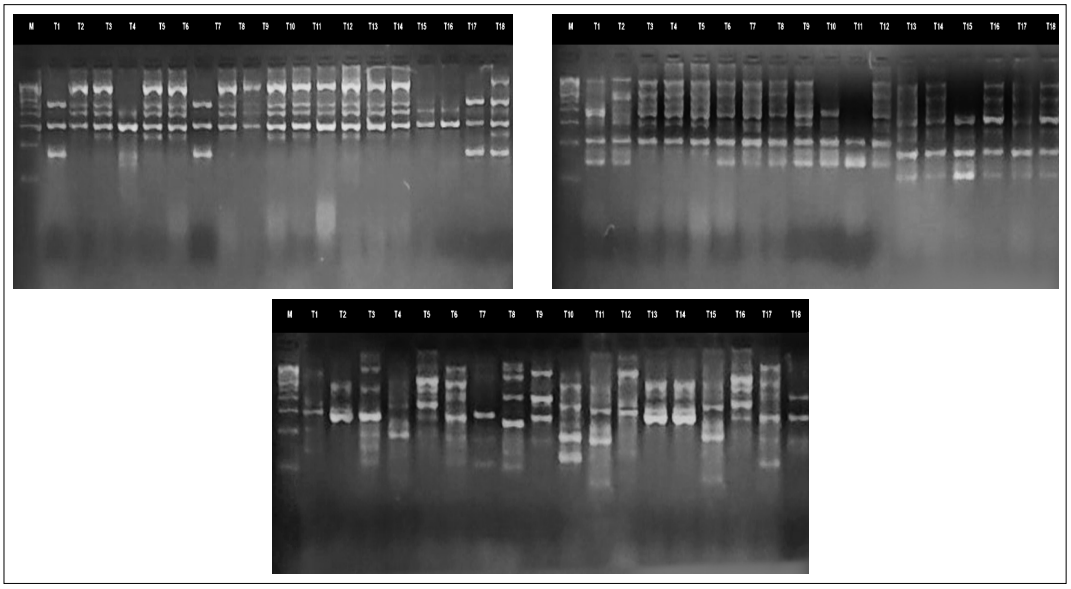

Fig 1: RAPD profile with different markers observed in UV gel doc.

by the number of polymorphic bands shown by each primer which was ranged between 50.00 (OPC-15) to 87.5 (GM-3). Polymorphism information content values provides the information of effectiveness of each primer and the maximum number of PIC for RAPD marker is 0.5 because of two alleles per locus are assumed in RAPD analysis. In this study the PIC value ranged from 0.16-0.50. The highest PIC value is depicted was 0.50 by GM-3, while the lowest by OPC-15 (0.16) total with an average of 0.34. Similarly, Thompson *et al.* (1998) used these markers to assess the diversity among soybean germplasms using SMC similarity matrix and cluster analysis. They found out that these results help in the exploration of soybean breeding to increase yield. This method to detect variability has been widely used by various researchers like Sharma *et al.* (2018) used RAPD marker to access the diversity among soybean varieties using cluster analysis. Similarly, Wahyudi *et al.* (2020) studied the diversity in a mutagenized soybean variety by calculating the percent polymorphism and by using Jaccard's similarity matrix and cluster analysis. Casas *et al.* (1999) was also found out that the RAPD results appear to play a vital role in the differentiation among different genotypes.

Evaluation of similarity matrix

The pair-wise Jaccard coefficients for the genetic similarities among the sixteen germplasms are presented in Table 3. The values of the coefficients are estimated on the basis of nine primers to ascertain the degree of genetic relationship among all. The range of similarity coefficients varies from 0.87 to 0.36. The highest similarity matrix was observed between germplasms namely DS 3108 and NRC 131 (0.91) followed by NRC 132 and NRC 136 (0.87) then, NRC 128 and NRC 130; NRC 130 and NRC 136; NRC 136 and NRC 137; NRC SL-1 and SKF-SP-11 (0.83), then by NRC 128 and NRC 132 (0.82) showing that all these germplasms have very similar genetic constituent with each other while lowest similarity was observed between germplasms DS 3108 and RSC 11-03 (0.36) followed by NRC 131 and RSC 11-03 (0.39) then NRC SL-1 and RSC 11-03 (0.41), JS-335 and RSC 11-03 (0.42) and RSC 11-07 and RSC 11-03 (0.44) showing farther relations of these germplasms with other germplasms indicating the difference amongst the soybean germplasms. Mokate *et al.* (2017) conducted an experiment to find out the comparison of divergence assessment through RAPD and ISSR molecular marker among 24 soybean genotypes. Through RAPD primer analysis they found out that the similarity matrix among soybean genotypes ranges from 0.41 and 1.00. The highest divergence was observed in only two genotypes KDS 753 and DS 228. The study reveals that among ISSR and RAPD, RAPD markers shows the target regions efficiently and target for a specific trait. Sharma *et al.* (2018) estimated the genetic diversity among eight soybean varieties through fourteen RAPD primers. In single RAPD marker they found highest level of polymorphism (80%) with most of the primers with Jaccard's similarity coefficient values ranges from 0.44 to 0.76. Dendogram based on cluster analysis showed relationship among soybean varieties shows clearly two groups in which group-I was further divided into two groups.

Cluster analysis showing similarity among soybean germplasms

The binary data matrix based on the PCR amplification results was then subjected to clustering and distance method

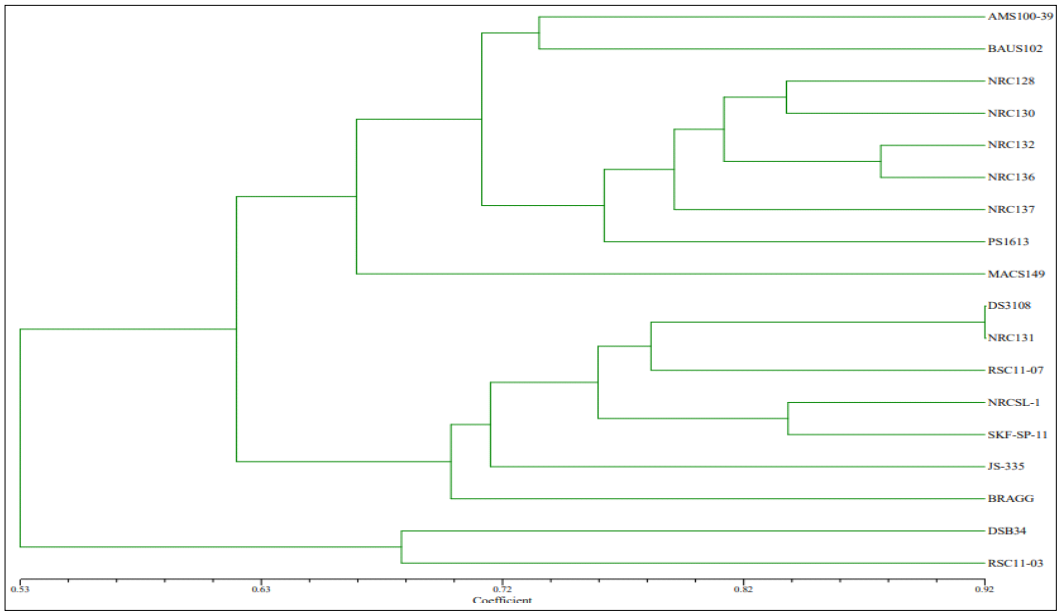

Fig 2: Dendogram of soybean germplasms constructed by RAPD primers using UPGMA analysis on similarity matrix data.

Table 3: Jaccard's coefficient similarity matrix among soybean germplasms based on RAPD-primer.

Soybean germplasm	AMS 100-39	BAUS 102	DS 3108	DSB 34	MACS 1493	NRC 128	NRC 130	NRC 131	NRC 132	NRC 136	NRC 137	NRC SL-1	PS 1613	RSC 11-03	RSC 11-07	SKF-SP-11	JS-335	BRAGG
AMS 100-39	1.00																	
BAUS 102	0.74	1.00																
DS 3108	0.60	0.47	1.00															
DSB 34	0.62	0.60	0.51	1.00														
MACS 1493	0.71	0.65	0.50	0.65	1.00													
NRC 128	0.74	0.72	0.65	0.60	0.65	1.00												
NRC 130	0.73	0.67	0.65	0.63	0.65	0.83	1.00											
NRC 131	0.65	0.52	0.91	0.52	0.54	0.67	0.66	1.00										
NRC 132	0.68	0.69	0.60	0.62	0.67	0.82	0.77	0.61	1.00									
NRC 136	0.74	0.68	0.65	0.60	0.65	0.80	0.83	0.67	0.87	1.00								
NRC 137	0.72	0.74	0.60	0.53	0.61	0.75	0.81	0.62	0.76	0.83	1.00							
NRC SL-1	0.67	0.65	0.75	0.47	0.53	0.69	0.68	0.76	0.63	0.69	0.72	1.00						
PS 1613	0.70	0.72	0.58	0.64	0.69	0.77	0.76	0.63	0.79	0.77	0.71	0.65	1.00					
RSC 11-03	0.52	0.64	0.36	0.68	0.58	0.54	0.51	0.39	0.55	0.47	0.53	0.41	0.60	1.00				
RSC 11-07	0.56	0.51	0.75	0.47	0.56	0.65	0.65	0.81	0.60	0.65	0.64	0.70	0.61	0.44	1.00			
SKF-SP-11	0.59	0.56	0.74	0.46	0.52	0.69	0.64	0.81	0.66	0.69	0.71	0.83	0.69	0.46	0.78	1.00		
JS-335	0.57	0.52	0.72	0.48	0.57	0.63	0.59	0.70	0.57	0.63	0.58	0.72	0.59	0.42	0.72	0.71	1.00	
BRAGG	0.55	0.53	0.65	0.53	0.61	0.60	0.63	0.70	0.58	0.64	0.63	0.65	0.60	0.46	0.77	0.72	0.70	1.00

using an unweighted pair group method with arithmetic mean (UPGMA) algorithm. A dendogram was constructed for all germplasms using data pooled from all the primers. The result showed distinction among all the germplasms (Fig 2) by dividing the germplasms into two major groups. The first major group consist sixteen germplasms and the second major group consisted two germplasms namely DSB 34 and RSC 11-03. Group one is further divided into two subgroups in which first subgroup consist nine germplasms namely AMS 100-39, BAUS 102, NRC 128, NRC 130, NRC 132, NRC 136, NRC 137, PS 1613 and MACS 1493 while the second subgroup consist DS 3108, NRC 131, RSC 11-07, NRC SL-1, SKF-SP-1, JS-335 and Bragg. Lakhanpaul *et al.* (2000) also analyzed the *Vigna radiata* cultivars through RAPD markers and found out that a total 267 amplification products were formed at an average of 12.71 per primer with an overall polymorphism of 64%, while Jaccard similarity coefficient values show range from 0.65 to 0.92. The cluster analysis resulted in three clusters revealing greater homology between cultivars released from the same source indicating that RAPD primers are the best source for cultivar improvement program. Macial *et al*. (2001) evaluated the variability among the *Phaseolus vulgaris* cultivars and a landrace of soybean with the help of RAPD markers. They found out a great variability with an average of 20.3 bands per primers with an average of 88.8% polymorphism among *Phaseolus* genotypes. The result of this analysis revealed that the diversity of the cultivars was certainly determined through cluster differentiation.

CONCLUSION

In order to keep cultivation of crop at economically viable level and realize full potential of crop's productivity regular varietals improvements are prerequisite. Molecular analysis revealed that all the RAPD markers amplified with a highest of 87% polymorphic band (GM-3) and a lowest of 50% (OPC-15). The primer combinations divide the clusters into two groups, first group consisted sixteen germplasm within two sub groups while second group consist two germplasms with a maximum similarity coefficient value of 0.91 in between DS 3108 and NRC 131. The analysis depicted that soybean germplasms used in the present study were moderately diverse. The study can be concluded by saying that the material though comes from diverse sources but had narrow genetic base. However, the germplasms may be used further to create new diversities and utilized in soybean crop improvement program.

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Conflict of Interest statement

All the authors hereby declare that there is no conflict of interest.

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