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Molecular characterization of soybean (*Glycine max* L.) genotypes by using RAPD marker

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Abstract
Soybean (*Glycine max*) is the primary source of protein, classified as an oilseed crop. RAPD molecular markers have been shown to be a simple and effective means to evaluate variability in crop. Genetic similarity estimate based on RAPD banding pattern used for cluster analysis to present genetic relationship in the form of dendrogram. Which gives polymorphism with highest percent. According to this data it can be concluded that highest similarity coefficient recorded in between AVT-23-96 and AVT-23-89 i.e. 0.96 whereas AVT-23-89 and IVT-5 showed least similarity of 0.39% in RAPD marker analysis. Where polymorphism percentage is 76.5%. The present results enable the selection of genetically distinct individuals such information may be useful to breeders willing to use genetically diverse introductions in soybean improvement process. The present investigation is concluded on Molecular characterization of soybean by using RAPD marker.

Keywords: Soybean, molecular marker, RAPD, PCR, DNA and genetic diversity

Introduction
Soybean (*Glycine max*) is one of the most valuable, versatile, and nutritionally important legumes globally. Soybean crop is a rich source of protein. Also, an economically important leguminous crop for feed, oil, and soy food products. It contains near about 40% protein and 20% oil. A potential source of protein and oil makes soybeans a large share in human nutrition therefore soybean is also an important crop for research. Molecular markers using RAPD technique is simple, fast, reliable and effective methods for detecting polymorphisms so it can be used to assess genetic diversity between genotypes. Polymorphic DNA markers can provide an ideal alternative method for evaluating genetic diversity in soybean genotypes. The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources. The present investigation is conducted on Molecular characterization of soybean by using RAPD marker.

Materials and Methods
A. Plant Materials: Nine genotypes of Soybean (*Glycine max* (L.) Merr.) Were collected from MPKV, Agricultural Research Station, Rahuri. Soybean genotypes used as experimental material such as 1] IVT-1, 2] IVT-2, 3] IVT-3, 4] IVT-4, 5] IVT-5, 6] AVT-23-89 7] AVT-23-90, 8] AVT-23-91, 9] AVT-23-93.

B. DNA Isolation: The isolation Plant genomic DNA from Seperatly used Nine genotypes of Soybean Crop leaves by using modified CTAB method. DNA extracted confirmation by Agarose Gel Electrophorosis method.

C. PCR Amplification for RAPD Analysis: Reaction mixture was prepared thin walled PCR tubes containing the following components as shown in (Table No.01) and Table No.02 shown Cyclic parameter of thermal cycler for RAPD. The RAPD Primers used for amplification such as OPA-07, OPA-08, OPA-09, OPA-10, OPA-12 and OPA-15.

Table 1: PCR components and stock solutions for RAPD

Sr. No.	Components	Stock	Require	Volume/µl Reaction
1.	D/W	---	---	18.5
2.	PCR buffer	10X	1X	2.5
3.	Primer	10 pm/ µl	10 pm	1.0
4.	dNTPs	25 mM	0.2 mM	0.2
5.	$MgCl_2$	25 Mm	1.5 mM	1.5
6.	*Taq* DNA polymerase	5 U/µl	1U/ µl	0.3
7.	DNA	50ng/µl	30ng	1.0
			Total	25 µl

Table 2: Cyclic parameter of thermal cycler for RAPD

Step	Temp (°C)	Duration	Cycles	Function
1.	94	2 min	1	Initial denaturation
2.	94	30 sec	40	Denaturation
3.	36	45 sec		Annealing
4.	72	2 min		Extension
5.	72	10 min	1	Final extension
6.	4	∞	1	Hold

Results and Discussion

Genetic analysis and RAPD polymorphism in soybean genotypes as shown in (Fig 01 to Fig 06) was carried out using 06 RAPD primers. In this study 09 genotypes of soybean were subjected to amplification by RAPD primers in PCR master cycler. The banding pattern thus obtained by RAPD marker clearly distinguished varieties into different clusters showing genetic diversity. 06 RAPD primers were selected for the genetic diversity. Out of them, OPA-07, OPA-10 and OPA-12, OPA-09 produced scorable bands with high degree of polymorphism. Total percent polymorphism by RAPD marker is 76.5%.

The similarity coefficient value ranged from 0.39 to 0.96 across Nine genotypes indicating high degree of genetic variation. This ultimately means high range of genetic diversity among the varieties studied. The highest genetic similarity to an extent of 0.96 was recorded between AVT-23-93 and AVT-23-89 varieties. Least genetic similarity 0.39 was observed in between AVT-23-89 and IVT -5.

The dendrogram RAPD analysis as shown in (Fig 07) that nine soybean varieties can be grouped into two major cluster *viz.* A and B. Cluster A Consist of single genotypes i.e. IVT-1. Cluster B divided into two sub cluster namely cluster B1 and Cluster B2. Sub cluster B1 shows six genotypes IVT-2, IVT-5, AVT-23-89, AVT-23-93, IVT-3.

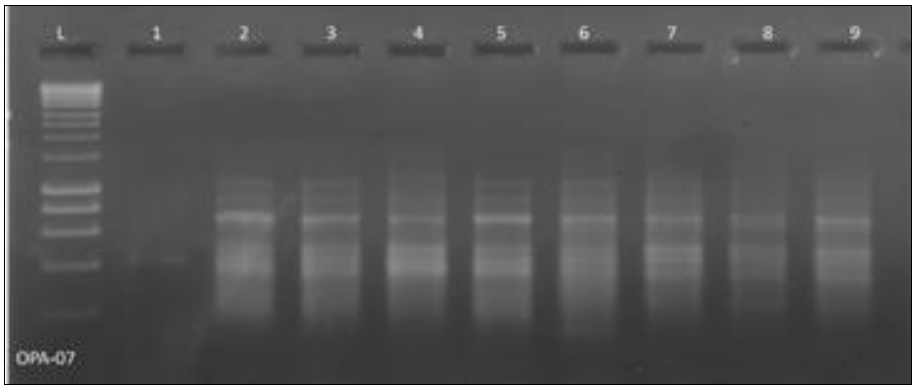

Fig 1: RAPD Profile of 09 Soybean genotypes Generated by RAPD Primer OPA-07

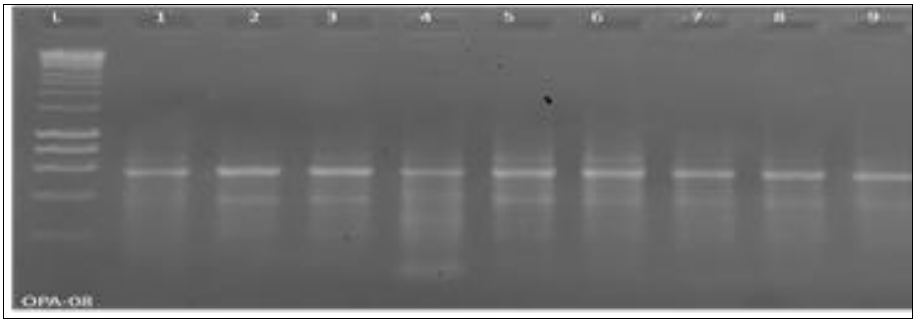

Fig 2: RAPD Profile of 09 Soybean genotypes Generated by RAPD Primer OPA-08

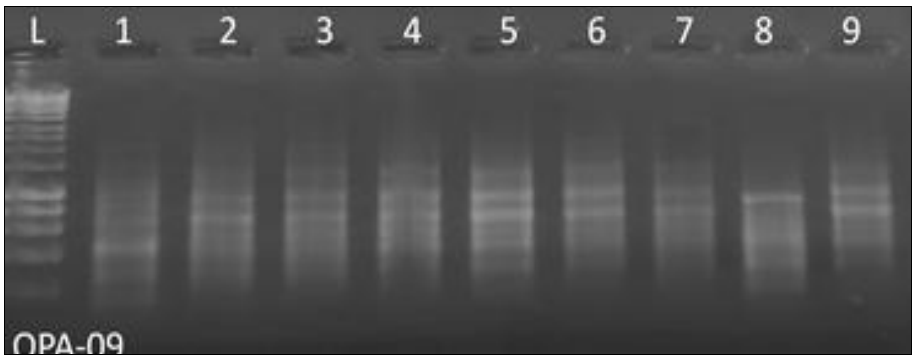

Fig 3: RAPD Profile of 09 Soybean genotypes Generated by RAPD Primer OPA-09

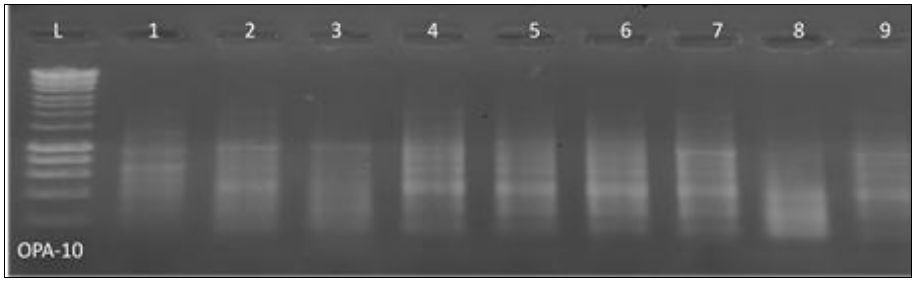

Fig 4: RAPD Profile of 09 Soybean genotypes Generated by RAPD Primer OPA-10

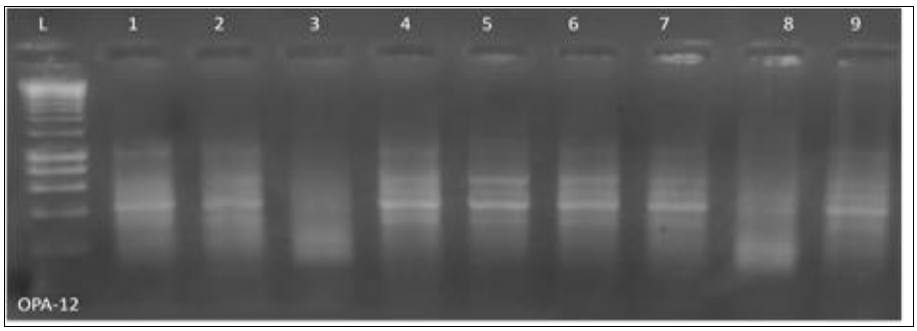

Fig 5: RAPD Profile of 09 Soybean genotypes Generated by RAPD Primer OPA-12

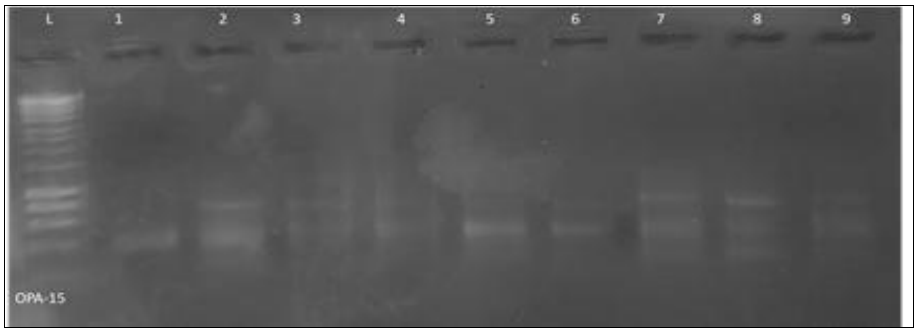

Fig 6: RAPD Profile of 09 Soybean genotypes Generated by RAPD Primer OPA-15

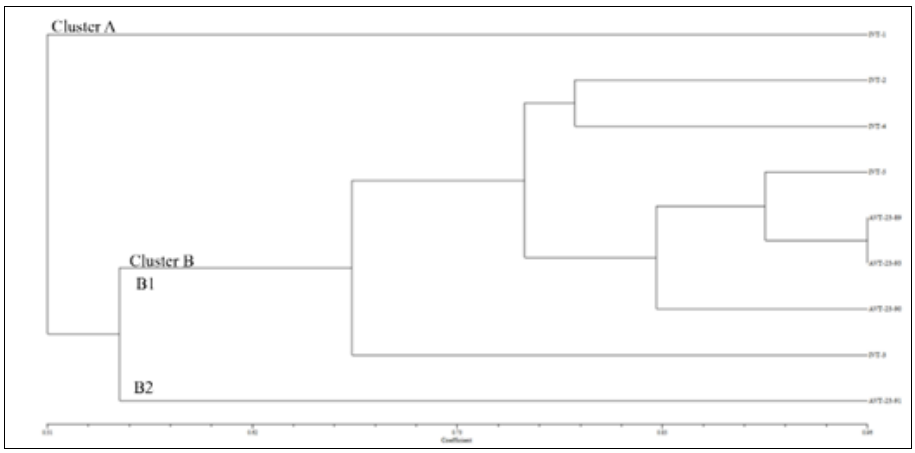

Fig 7: Dendrogram showing results of RAPD analysis of 09 Soybean genotypes

Summary and Conclusions

Highest similarity coefficient recorded in between AVT-23-93 and AVT-23-89 i.e.0.96. AVT-23-89 and IVT-5 showed least similarity of 0.39% in RAPD marker analysis. From this data it can be concluded that IVT-4 and IVT -5 were found to be closely related and AVT-23-89 and IVT-23-90 were found to be minimum similarity. The present results enable the selection of genetically distinct individuals. Such information may be useful to breeders willing to use genetically diverse introductions in soybean improvement process. From this data genetic diversity and cultivars identity was evaluated. RAPD marker should provide the more information on genetic variation of soybean. crop.

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