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Molecular profiling of soybean (*Glycine max* L.) genotypes using RAPD markers

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Abstract
DNA markers can provide an ideal al ternative method for evaluating genetic diversity in soybean germplasm. Soybean crop is nutritionally important legumes globally. The present investigation was carried out to study molecular and biochemical characterization of soybean (*Glycin Max* L) by using RAPD markers. PCR amplification was conducted using RAPD primers, specifically OPN-06, OPN-07, OPN-08, OPN-09 and OPN-10. The dendrogram revealed polymorphism at a high percentage. The findings indicated the highest genetic similarity (coefficient = 0.82) between AVT 23-89 and AVT23-92, while the lowest similarity 0.14 was observed between Phule Kimaya and Phule Kalyani in RAPD marker analysis. The genetic diversity uncovered in this study aligns with previous reports by Tidke*et al.* (2018), which recorded a polymorphism percentage of 86.95%.

Keywords: Soybean, RNase molecular marker, RAPD, PCR, DNA

Introduction
Soybean (*Glycine max*) is serves as the primary source for oil, meal, and feed production on a global scale, playing a substantial role in regional and national food initiatives. It possesses a diverse array of applications in various industries. This crop is aptly called as "Golden Bean" or "Miracle crop" of the 20th century, because of its multiple uses. Protein or enzyme variation can be used to study genetic diversity of crop germplasm. However, the limited number of isozymes of proteins and enzymes can limit their usefulness. Polymorphic DNA markers can provide an ideal al ternative method for evaluating genetic diversity in soybean germplasm. Genetic diversity evaluation among germplasms is an important and a prerequisite in any hybridization program and would promote the efficient use of genetic variations. The present investigation is conducted on Molecular characterization of soybean by using RAPD Primer.

Materials and Methods
A. Plant Materials: Twelve genotypes Soybean (*Glycine max* (L.)) Were collected from MPKV, Agricultural Research Station, Rahuri. Soybean genotypes used as experimental material such as 1) Phule kimaya, 2) Phule Agram, 3) Phule Kalyani, 4) AVT 23-89, 5) AVT 23-91, 6) AVT 23-92, 7) JS 335, 8) IVT-4, 9) IVT-15, 10) IVT-16, 11) IVT-25, 12) IVT-26.

B. DNA Isolation: The isolation Plant genomic DNA from Seperatly used Twelve 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 1) OPN-06, 2) OPN-07, 3) OPN-08, 4) OPN-9 and 5) OPN-10.

Results and Discussions
The present study entitled 'Molecular Profiling of Soybean (*Glycine max* L.) Genotypes Using RAPD Markers'. Genetic analysis and RAPD polymorphism in soybean genotypes was carried out using 05 RAPD Primer. In this study 12 varieties of soybean crop used to amplification by RAPD primers in PCR in PCR master cycler.

The banding pattern thus obtained by RAPD marker clearly distinguished varieties into different clusters showing genetic diversity as shown in (Fig 01 to Fig 03 Mention Primer Name). Genetic Similarity estimate based on RAPD banding pattern used for cluster analysis to present genetic relationship in the form of dendrogram Cluster (Fig.04).

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	50 ng/µl	30 ng	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

1-Phule Kinava, 2-Phule Agram, 5-Phule Kalyani, 4-AVT 23-89, 5-AVT 13-01, 6-AVT 23-92, 7-JS 335, 5-IVT-4, 9-IVT-15, 10-IVT-16, 11-IVT25, 12-1VT26
PCR amplification by RAPD markar

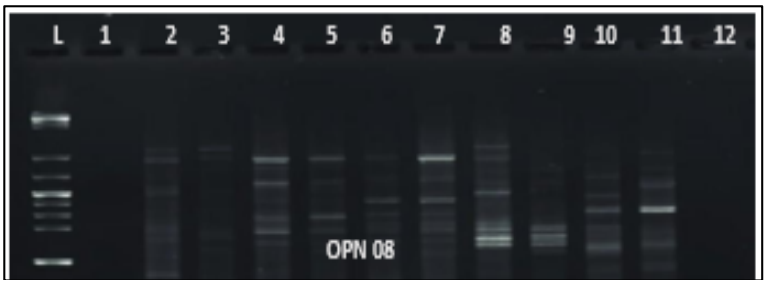

Fig 1: RAPD Profile of Soybean Varieties Generated by OPN-08

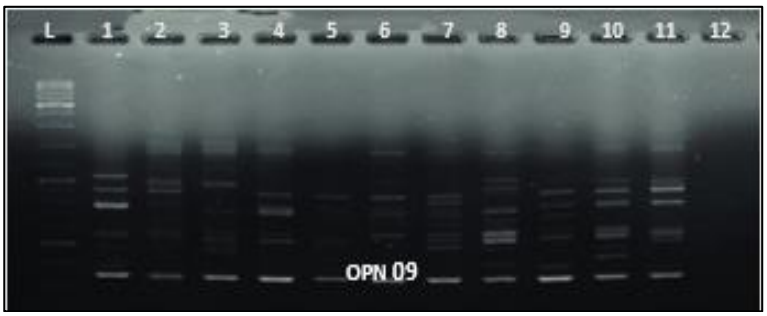

Fig 2: RAPD Profile of Soybean Varieties Generated by OPN-09

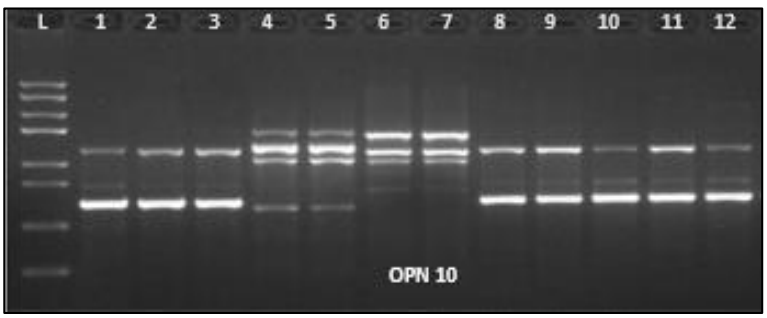

Fig 3: RAPD Profile of Soybean Varieties Generated by OPN-10

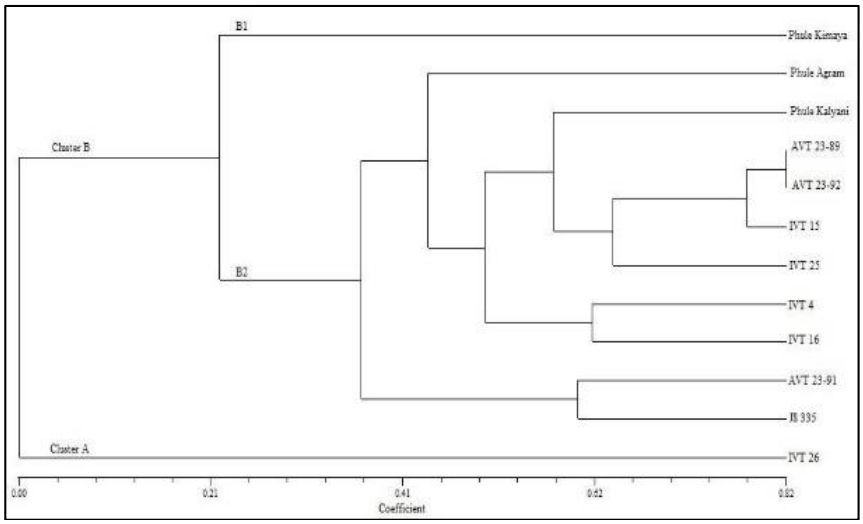

Fig 4: Dendrogram showing results of RAPD analysis of 12 Soybean genotypes.

Summary and Conclusions

After isolation of genomic DNA from 12 Soybean genotypes, they were subjected to PCR by using RAPD primers. Out of them, OPN-06, OPN-07, OPN-08, OPA-09 and OPN-10 produced scorable bands with high degree of polymorphism. Average number of amplicons per primer is 23. Average number of polymorphic amplicons per primer is 20. Total percent polymorphism by RAPD marker is 86.95. In present study, the similarity coefficient value ranged from 0.14 to 0.82 across twelve 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 0.82 was recorded between AVT 23-92 AVT-23-89 varieties. Least genetic similarity 0.14 was observed in between Phule Kalyani and Phule Kimaya.

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