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Genetic Variability and Diversity Studies in Soybean [Glycine max (L.) Merrill] using RAPD Marker

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Abstract- Soybean (Glycine max) is an important vegetable oilseed crop. It is considered to be a cash crop. It is a major source of edible vegetable oils and proteins which contains about 40% protein and 20% oil. The genetic diversity was estimated among 7 varieties of Soybean using 22 RAPD primers. The RAPD marker is useful tool for assessing genetic variation and resolving cultivars identities. Among the 22 primers, 11 primers showed polymorphism.

Index Terms- Soybean, RAPD, Polymorphism, Genetic diversity.

I. Introduction

Soybean (*Glycine max* (L.) Merr.)is a principle grain legume in developing countries where it meets the expanding needs for protein, edible oil and calories. It is a good source of cheap dietary protein in Africa. Soybean (*Glycine max* (L.) Merril) is a member of Papilionaceae family and believed to have originated in Northeastern China and distributed in Asia, USA, Brazil, Argentina etc. This crop is aptly called as "Golden Bean" or "Miracle crop" of the 20th century, because of its multiple uses. It is rich in lycine and vitamins A, B and D.

Soybean occupies 912.99 lakh ha of area with 2099 million tonnes of annual production and 22.99 q per ha productivity in the world. In India, it occupies 69 lakh ha of area with 66 million tonnes of production and 9.56 q per ha productivity.

Assessment of genetic diversity in available cultivars has important implications in understanding the progress made in any breeding programme **(**Chen L. F. O, Yun W. C, Kou H. Y And Chen M. H,1994**)**. Morphological markers are routinely used for estimating the genetic diversity, but recently many molecular marker techniques have developed into powerful tools to analyze genetic relationships **(**Bhandarkar S. 1999**)**. Molecular markers employed for the analysis of genetic diversity in soybean viz. random amplified polymorphic DNA (Das P. N, 2000).The objective of the present study was to investigate and compare genetic diversity using random amplified polymorphic DNA (RAPD) markers, for assessing the genetic base of released cultivars of Soybean **(**Bains K. S. and Sood K. C, (1984). The study also aims to generate molecular fingerprints for varietal identification.

II. Materials and Methods

Plant Materials and DNA isolation

The plant materials used for this study included seven genotype of Soybean popularly grown in different regions of Maharashtra. Seeds of these varieties were collected from Oil seed research centre , Latur (M.S.) India. Seeds of seven Soybean genotypes were germinated under field conditions. The DNA isolation was done as per CTAB method described by (Doyle and Doyle. 1990). One-week-old seedlings were ground in preheated CTAB buffer and incubated at 60°C for 1 h. The aqueous phase containing DNA was separated using chloroform : isoamyl alcohol (24:1). The DNA was precipitated with chilled isopropanol and the pellet was dissolved in 100 µl of T.E.buffer. The RNA was eliminated by adding 0.5 µl of RNase. The pellet was dissolved in appropriate amount ofT.E. (Tris10mM, EDTA 1mM) buffer. DNA samples wasquantified by UV spectrophotometry and finally diluted to a concentration of 25 ng/µl.

III. PCR Amplifications

RAPD markers were tested for their ability to detect polymorphisms using template DNA and 20 arbitrary 10 bp long oligonucleotides, as primers belonging to RPI series (GeNei, Banglore). PCR conditions were standardized using varying concentrations of primers and template DNA. After standardization, the reaction were carried out in 25 µl volume and contained 6.5 µl of 10× Taq buffer, 4µl of 2mM dNTP mix, 1µl primer,1µl of Taqpolymerase and 1µl MgCl2, 10.5µl Nuclease free water and 1µl template DNA. The thermal cycling program was carried out in a Thermal cycler. The PCR program had an initial denaturation step at 94°C for 5 min, followed by 44 cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min. A final extension step given at 72°C for 7 min. The amplified products were resolved by electrophoresis at 50V for 3 hours in 1.2% agarose gel in 1× TAE buffer. The DNA bands were visualized by staining gel in 1% ethidium bromide solution and photo- graphed under UV light using a Digidoc gel documentation system. A 100 bp DNA ladder was used as a molecular weight marker for determining the molecular weight of the amplified products.

IV. Scoring and Data Analysis

Digitized gel photograph of RAPD results were analyzed using NTSYS PC Ver.2.0 numerical software package. Data was recorded as 1 (presence) or 0 (absence), each of which were treated as an independent character. The bands which were very faint were not considered for scoring. For each primer,

PCR reactions were repeated two times and only reproducible bands were considered for analysis. The primers which did not produce amplification were repeated thrice before discarding them.The pair wise similarity between isolates and polymorphic bands were calculated using Jaccard's coefficient, a common estimator of genetic identity, or estimates interspecific relationships. The similarity co-efficients were used to construct a dendrogram for determining relationship using unweighted pair group method with arithmetic average (UPGMA). Robustness of clusters was evaluated by bootstrap analysis using NTSYS-pc version 2.0, Exeter software, New York.

V. RAPD Analysis

Universal Primers of RPI series were used to evaluate seven soybean genotypes. The PCR amplified products of each Primer were resolved on 2% agarose gel electrophoresis and the size of the amplified products was compared with DNA molecular weight marker. Random amplified polymorphic DNA (RAPD) markers have more polymorphism information content (PIC) value. These computations were performed using NTSYS-PC (ver. 2.02j; Exeter Software (N.Y., Rohlf, 1993).

Similarity matrix based on RAPD Profile

The RAPD patterns obtained from seven soybean accessions of *Glycine max*using primer RPI. The Jaccard estimate of simulator was used to construct a similarity matrix.

Cluster Analysis

Similarity coefficient matrices were used to generate a dendrogram of Soybean genotypes based on UPGMA analysis.

VI. Result and Discussion

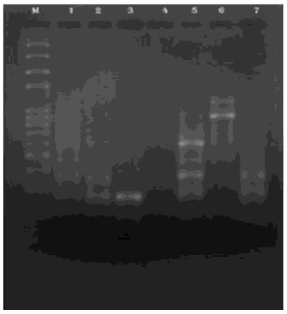
In the present investigation Random Amplified Polymorphic DNA (RAPD) markers were used to study the genetic diversity of seven soybean genotypes. DNA isolation was done successfully by the CTAB isolation method and it was also standardized for PCR amplification of DNA. In this study, 22 primers of RPI series were used. Among the 22 Primers,11Primers produced total 118 amplified bands. The typical banding patterns produced viz. shown in plate 1, in which 111 bands (94.06%) were polymorphic and 7 bands (5.94%) were found monomorphic viz. showed in table 1. Among 11 Primers used in this study, the percentage of polymorphic products ranged from 50% to 94.06%. In this RPI-4,RPI-5,RPI-6,RPI-8, RPI-9,RPI-10,RPI-11,RPI-12,RPI-15,RPI-19, and RPI-20 showed 100% of polymorphism and RPI-18 showed 50% of polymorphism. The similarity value viz. shown in table 2 ranged from 0.130 to 1.309 indicated substantial diversity present in the germplasm. Highest degree of similarity at 1.309 was observed between MAUS 71 and MAUS 32 and lowest similarity value at 0.130 was observed between MAUS 32 and MAUS 2. The cluster A is divided into two sub cluster i.e. A1, A2. The cluster A1 consist of two genotypes in which MAUS 61 and MAUS 1 were found closely related. The cluster A2 consist of three genotype in which MAUS 32 and MAUS 71 are closely related and genotype MAUS 158 is a solitary cluster. The cluster B also consist of two genotypes in which MAUS 81 AND MAUS 2 are closely related

Sr.No.	RAPD primers	Accession No.	Total no.of bands	Monomorphic bands	Polymorphic bands	Percentage of polymorphism	PIC value
1	RPI-4	AM773770	4	0	4	100%	-
2	RPI-5	AM773771	19	0	19	100%	0.79
3	RPI-6	AM773773	3	0	3	100%	-
4	RPI-8	AM773315	3	0	3	100%	-
5	RPI-9	AM750045	17	0	17	100%	0.76
6	RPI-10	AM773316	3	0	3	100%	0.67
7	RPI-12	AM773775	17	0	17	100%	0.72
8	RPI-15	AM765830	7	0	7	100%	0.77
9	RPI-18	AM773777	14	7	7	50%	0.42
10	RPI-19	AM773317	19	0	19	100%	0.37

11	RPI-20	AM765820	12	0	12	100%	0.75
12	Total		118	7(Avg-5.94)	111	94.06%	3.67

Table 1 :Details of RAPD band produced by 11 primers and polymorphism percentage

RAPD method was displayed appreciable inter population variation or molecular polymorphism between seven *Glycine max*varieties and phylogenetic tree was showing a relationship between seven, soybean varieties. This study has also confirmed, RAPD marker is potentially simple,rapid, reliable and effective method of detecting polymorphism for assessing genetic diversitybetween genotype and these help in the selection of parent for hybridization.RAPD technique is useful in areas of genetic diversity and DNA fingerprinting analysis. As the need to protect proprietary germplasm as it is likely to increase in the future, RAPD will have animportant role in securing a plant variety right by virtue of its unique efficiency in distinguishingclosely related germplasm. Future thrust will be directed towards the holistic use of RAPD primes for DNA fingerprinting, genetic analysis and linkage mapping in soybean.



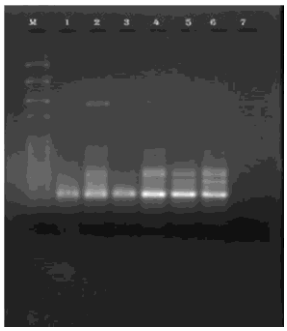


Plate 1&2. RAPD profiles for 7 Soybean cultivars produced using primer RPI-12&RPI-5.

Varieties-1)MAUS-81, 2)MAUS-6102, 3)MAUS-1, 4)MAUS-2, 5)MAUS-158, 6)MAUS-32, 7) MAUS-71.

Rows\Cols	MAUS81	MAUS6102	MAUS1	MAUS2	MAUS158	MAUS32	MAUS71
MAUS81	0.000						
MAUS6102	0.756	0.000					
MAUS1	0.399	0.824	0.000				
MAUS2	0.635	0.635	0.399	0.000			
MAUS158	0.441	0.532	0.693	0.360	0.000		
MAUS32	0.693	0.693	0.635	0.130	0.485	0.000	
MAUS71	0.693	0.824	0.532	0.824	0.982	1.309	0.000

Table 2. Similarity matrix for Jaccard's coefficient of seven Soybean genotypes obtained from RAPD Marker analysis.

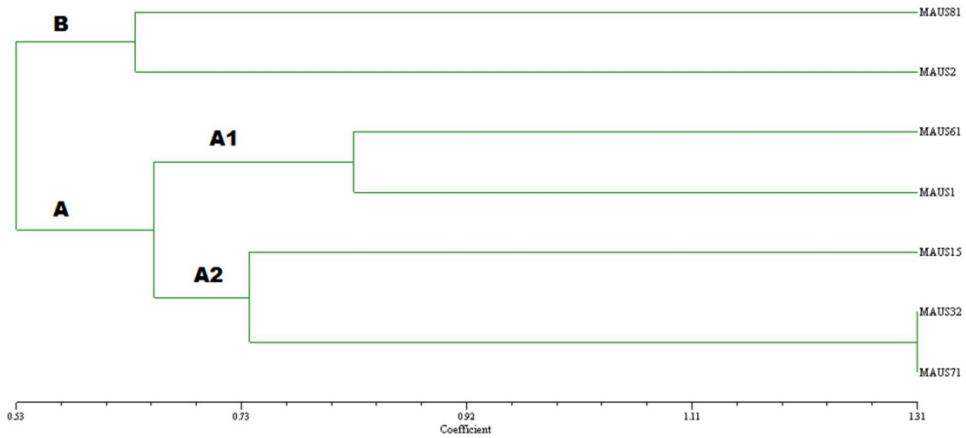


Fig.1 Dendrogram showing genetic diversity for RAPD markers in *Glycine max*.

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