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# Molecular Characterization of Different Soybean [Glycine max (L). Merril] genotypes by RAPD Markers

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**ABSTRACT**

The present investigation was carried out on molecular characterization of different soybean genotypes by using RAPD markers. Genetic diversity of 10 soybean genotypes was studied using Random Amplified polymorphic DNA (RAPD). 12 RAPD primers were used for screening 10 soybean genotypes from which total 334 fragments were amplified. It was observed that 41.10% bands were polymorphic and 58.88% were monomorphic. The percent of polymorphic amplicons in banding pattern was calculated and it was highest in OPA-01 (67.85%) followed by OPA-11 (66.66%). Polymorphism information content (PIC) was calculated and highest PIC value was observed in OPA-01 (0.26) and lowest was observed in OPA-08 and OPA-05 (0.03). Maximum amplicons were produced in OPA-01 (56) and minimum in OPA-08 (11). Genetic relationship between soybean genotypes was determined on the basis of Jaccard's pair wise similarity coefficient values and dendrogram was generated by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis using dice's similarity matrix through NT-SYS pc software. The dendrogram of RAPD analysis shows 8 clusters and sub clusters were revealed. The value of similarity coefficient ranged from 0.19 to 0.71. The maximum similarity percentage i.e. 71% was found between KDS-726 and KDS-922 genotypes. The minimum similarity i.e. 19% was found between IVT-33 and IVT-13. According to this information it is concluded that the KDS-726 and KDS-922 genotypes showed minimum genetic diversity as compare to the IVT-33 and IVT-13 genotypes. Therefore, understanding the genetic diversity of soybean genotypes is essential to broaden the genetic base and to further utilize in the MAS breeding program.

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**Keyword:** Genetic diversity, Glycine max, Molecular Markers, RAPD, Soybean

## Introduction

The soybean (*Glycine max* (L). Merril) is a species of legume, native to East Asia [1]. Soybean is the leading oil and protein crop of the world, which is used as a source of high quality edible oil, protein and livestock feed [2]. Soybean contains significant amounts of all the essential amino acids for humans, and so is a good source of protein and vegetable oils [1]. Soybean is the world's most important seed legume, which contributes to 25% of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding [3]. Soybean crop called as "Golden bean" or "miracle crop" of the 20th century because of its multiple uses. Soybean is one of the major foods commodities and can be consist a cheapest source of protein. The productivity of Soybean is high because 40% protein and 20% oil source. Soybean rank first among all oilseeds crop of world having 50% share in total oilseeds production.

Soybean contains significant amounts of all the essential amino acids need for humans, and so is a good source of protein and vegetable oils [1]. They are associated with a wide range of health benefits such as antimutagenic effect, anti-inflammatory properties and reduction in synthesis of low-density lipoprotein, antioxidant properties, and reduced effects of DNA damage (Ciabotti et al., 2016). However, the rate of growth in soybean yield has declined during the last 10 years, increasing by only 8% in this period. Among the states, Madhya Pradesh stood first with 55.84 lakh ha followed by Maharashtra (46.01 lakh ha), Rajasthan (10.62 lakh ha), Karnataka (3.82 lakh ha), Gujarat (2.24 lakh ha) and Telangana (1.51 lakh ha). 3,98,745 acres during 2021-22. It is therefore imperative to develop new technologies and resources that will allow the supply of soybean to meet the large growth in demand anticipated in the near future.

RAPD markers are a technique based on the PCR method to identify genetic variation. It has been also used as an assessment of intraspecific variation since 1990. Moreover, in recent years, RAPD was also used for genetic mapping, taxonomic study and

even used to detect genetic mutation in the treated plant. RAPD is well established genetic tool which provides a simple and fast approach to detect DNA Polymorphism for cultivars identification and diversity analysis [4,5]. RAPD is a dominant marker, RAPD-PCR requires very small quantity g/ml for assessment. Standard µ of genomic DNA i.e. 10-15 oligonucleotide (10 bp) long random sequence primer can be used to amplify the nanogram amount of total genomic DNA under low annealing temperature by PCR. Amplified product is generally separated on agarose gel electrophoresis [6].

RAPD is probably the easiest and cheapest methods for laboratory just beginning to use molecular markers. RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding. This is mainly due to the speed, cost and efficiency of the RAPD technique to generate large numbers of markers in a short period compared with previous methods. Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. Despite the reproducibility problem, the RAPD method will probably be important as long as other DNA-based techniques remain unavailable in terms of cost, time and labour [7]. Genetic diversity and variability tool for evaluating and determining cultivars identity. Generations of new and improved cultivars can be enhanced by new sources of genetic variation; therefore criteria for parental stock selection need to be considered not only by agronomic value, but also for genetic dissimilarity. Therefore, understanding the genetic diversity of soybean germplasm is essential to broaden the genetic base and to further utilize it in breeding program.

## Materials and Methods

### The Plant Materials

Soybean genotypes were collected from the MPKV-Agricultural Research Station, Kasbe Digraj, Sangli Maharashtra. Total 10 accessions were included in the study for analyzing genetic diversity of soybean (Table 1). All genotypes were sown in the College of Agricultural Biotechnology, Research Farm, Loni, Ahmednagar.

**Table 1: Genotypes and Source of Soybean Seeds**

| Sr. No. | Genotypes | Source | Sr. No. | Genotypes | Source |
|---|---|---|---|---|---|
| 1. | IVT-03 | MPKV-ARS, Kasbe Digraj, Sangli | 6. | MAC-2014 | MPKV-ARS, Kasbe Digraj, Sangli |
| 2. | IVT-35 | | 7. | IVT-13 | |
| 3. | MACS-450 | | 8. | RKS-18 | |
| 4. | KDS-922 | | 9. | IVT-33 | |
| 5. | KDS-726 | | 10. | IVT-21 | |

### DNA Extraction

Total genomic DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) protocol given by [8]. With some modifications. DNA was isolated from 0.5 g of fresh leaves of the 10-15 days seedlings. Tissue was crushed to a fine powder using liquid nitrogen and dispersed in 1 ml pre warmed (60oC) extraction buffer (2% CTAB, 1.4 M NaCl, 0.5 M EDTA, pH 8.0, 100 mM Tris-HCl, pH 8.0, PVP 2% and 0.2% beta-mercaptoethanol). After incubation for 1 hr at 60°C with intermittent swirling, the mixture was emulsified with an equal volume of chloroform: isoamyl alcohol (24:1). Following centrifugation, the supernatant was collected and mixed with 0.6 volume of isopropanol. The precipitated nucleic acid was spooled out, washed twice in 70% ethanol, dried under vacuum, dissolved in TE buffer (1 M Tris-HCl and 0.5 M EDTA pH 8.0) and treated with RNase and proteinase-K to remove RNA and protein respectively. DNA was tested for its quality and integrity on 1.5% agarose gel, quantified by spectrophotometer, diluted in TE buffer to a concentration of 25ng/µl and utilized for PCR analysis.

### Quantification of Genomic DNA

The quantity of DNA was checked using 1µl of DNA of each 10 soybean genotypes by using Nano Drop (ND-1000 Spectrophotometer) machine (Table 2). Stock DNA was diluted in TE buffer to make a working solution of 100ng/µl for PCR reaction. A part of DNA sample was diluted with appropriate quantity of TE buffer to yield a working concentration of 100ng/µl and stored at 4OC for further work until PCR amplification.

**Table 2: Genomic DNA Quantification**

| Sr. No. | Genotypes | Concentration of DNA | Optical Density (260/280) | Sr. No. | Genotypes | Concentration of DNA | Optical Density (260/280) |
|---|---|---|---|---|---|---|---|
| 1. | IVT-03 | 753.4 ng/µl | 1.8 | 6. | MAC-2014 | 729.4 ng/µl | 1.8 |
| 2. | IVT-35 | 603.8 ng/µl | 1.8 | 7. | IVT-13 | 652.6 ng/µl | 1.9 |
| 3. | MACS-450 | 810.1 ng/µl | 1.7 | 8. | RKS-18 | 744.9 ng/µl | 1.9 |
| 4. | KDS-922 | 589.3 ng/µl | 1.8 | 9. | IVT-33 | 422.5 ng/µl | 1.9 |
| 5. | KDS-726 | 683.1 ng/µl | 1.7 | 10. | IVT-21 | 759.4 ng/µl | 1.8 |

### PCR amplification for RAPD analysis

### Requirements

Template DNA, RAPD primers, 2X PCR master mix, sterile distilled water, el loading dye (6X), 50X TAE buffer, Ethidium bromide, DNA Ladder. The PCR amplification by using RAPD analysis with certain modifications [5]. The optimum specifications followed for DNA amplifications were as follows. RAPD primers were used for the PCR amplification. The amplification was carried out

in thermal cycler for 35 cycles under following PCR conditions. Reaction mixture was prepared in 0.2ml thin walled PCR tubes containing the following components. The total volume of each reaction mixture was 15µl (Table 5).

**Table 3: List of primers along with their sequences used for RAPD analysis**

| Sr. No. | Primers | Sequences (5' to 3') | Sr. No. | Primers | Sequences (5' to 3') |
|---|---|---|---|---|---|
| 1. | OPA- 01 | CAGGCCCTTC | 7. | OPA- 08 | GTGACGTAGG |
| 2. | OPA- 02 | TGCCGAGCTG | 8. | OPA- 09 | GGGTAACGCC |
| 3. | OPA- 03 | AGTCAGCCAC | 9. | OPA- 10 | GTGATCGCAG |
| 4. | OPA-05 | AGGGGTCTTG | 10. | OPA- 11 | CAATCGCCGT |
| 5. | OPA- 06 | GGTCCCTGAC | 11. | OPA- 12 | TCGGCGATAG |
| 6. | OPA- 07 | GAAACGGGTG | 12. | OPA- 17 | GACCGCTTGT |

**Table 4: Temperature Profile for PCR Amplification**

| Sr. No. | Steps | Temperature (°C) | Duration (Min) | Number of Cycle |
|---|---|---|---|---|
| 1. | Initial denaturation | 94 | 10 | 1 |
| 2. | Denaturation | 94 | 1 | 35 |
| 3. | Annealing | 28-34 | 1 | |
| 4. | Extension | 72 | 1 | |
| 5. | Final Extension | 72 | 10 | 1 |
| 6. | Hold | 4 | - | - |

**Table 5: Components, Stock and Volume of PCR reaction mixture**

| Components | Final Concentration | Volume for one tube (µl) |
|---|---|---|
| 2 X PCR Master Mix | 2X Master mix | 7.5 |
| Primer | 16 Pmol | 1.0 |
| Grade water | ---------- | 5.5 |
| Template DNA | 30ng/µl | 1 |
| Total | - | 15 µl |

**Data Analysis**

Submerged gel electrophoresis unit was used for fractionating RAPD markers on 1.5% agarose gel. 4µl loading dye was added to the amplified products in each tube and mixed well. 15µl of amplified products of each sample were loaded on 1.5% agarose gel containing 1x TAE buffer to separate the amplified fragments. The amplified products were resolved on 1.5% agarose gel at 50V for 2 hours. The gel was stained with ethidium bromide (0.5µl) after electrophoresis, the gel was carefully taken out of the casting tray and photograph was taken from the gel documentation. The Gene ruler 100bp DNA ladder plus was used as the standard to determine the size of the polymorphic fragments. The gel was visualized under UV- transilluminator (JASCO) and photographed using Gel-Doc system (UV-Tech Ltd).

The amplified fragment profiles were visually scored for the presence (1) or absence (0) of bands and entered in a scoring matrix. Pair-wise genetic similarities between soybean genotypes and polymorphic band were estimated by Jaccard"s similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and cluster obtained was based on unweighted pair group method for arithmetic mean (UPGMA) using sequential agglomerative hierarchical nested (SAHN) cluster analysis of NTSYS-PC version 2.02 [9,10]. The computer package NT SYS-pc version 2.1 was used to carry out cluster analysis. Molecular weights of the bands were estimated by using 100bp DNA ladder as standard. Percent polymorphism was calculated by using the formula,

$$\text{Polymorphic Percent (\%)} = \frac{\text{Polymorphic Amplicons}}{\text{Total Amplicons}} \times 100$$

**Results**

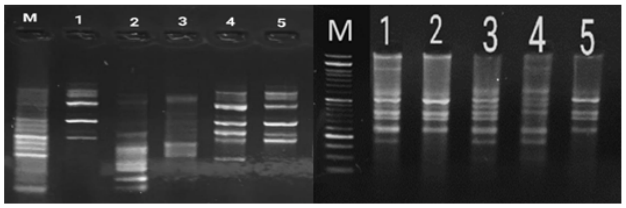
Molecular characterization of different genotypes of soybean (Glycine max) by using RAPD markers was carried out using 12 RAPD primers. The results obtained are presented under following points:

**Selection of suitable RAPD primers:** Universal primers of OPA series were used to evaluate polymorphism of 10 soybean genotypes. PCR amplified products of each primer were resolved on 1.5% agarose gel and the size of the amplified products was compared with marker DNA. 12 primers were screened (Table 3).

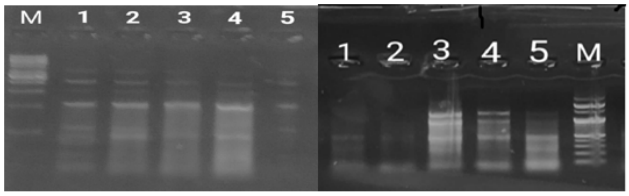
**Amplicons Statistics:** Universal random primers like OPA-01, OPA-02, OPA-03, OPA-05, OPA-06, OPA-07, OPA-08, OPA-09, OPA-10, OPA-11, OPA-12 and OPA-17 were used. Total 334 RAPD amplified amplicons were generated (Table 6). OPA-01 produced maximum number of amplicons (56 from all genotypes) followed by OPA-06 (36), OPA-09 (35) OPA-10 (35) while OPA-08 generated minimum number of amplicons (11) in the genomic pool. Higher numbers of polymorphic amplicons were observed in OPA-01 (38), OPA-11 (16), OPA-07 (14), OPA-02 (13) and OPA-17 (12) and lower in OPA-08 (4) and their percentage was calculated which was highest in OPA-01 (67.85%), OPA-11 (66.66%), OPA-07 (56%), OPA-02 (52%) and OPA-17 (46.15%) and lowest in OPA-09 (22.85%). Polymorphism information content (PIC) was calculated and highest PIC values were observed

in OPA-01 (0.26), OPA-11 (0.11) OPA-02 & OPA-07 (0.10) and lowest was observed in OPA-05 & OPA-08 (0.03). Higher numbers of monomorphic amplicons (27) were observed in OPA-09 and lower numbers of monomorphic amplicons were observed in OPA-11 (08) and OPA-08 (07). The percent of monomorphic amplicons in banding pattern was calculated which was highest in OPA-09 (77.14%), OPA-05 (76.19%) and OPA-12 (75&) while lowest was recorded OPA-1 (32.14%), OPA-11 (33.33%) and OPA-07 (44%).

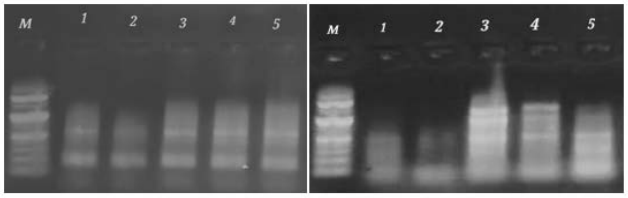
**Figure 2:** RAPD profile of 10 Soybean Genotypes



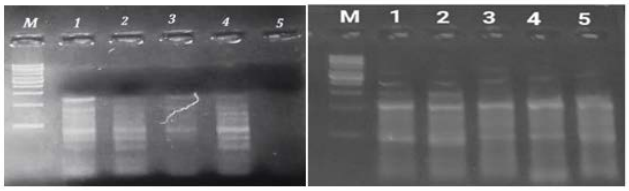

OPA-01




OPA-02

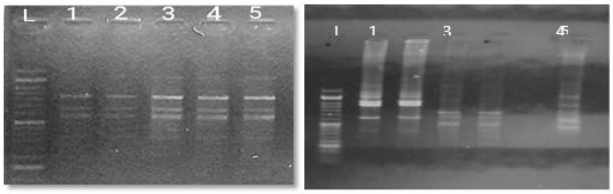



OPA-03

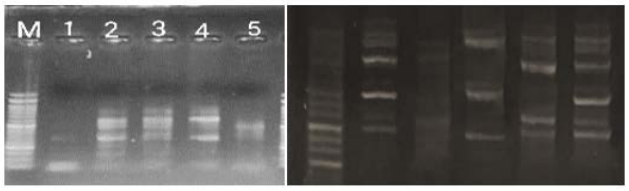



OPA-05

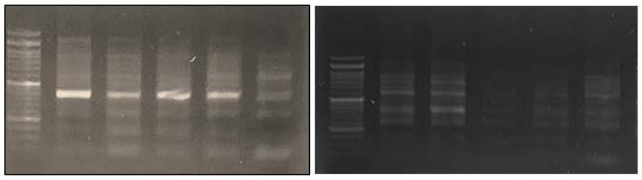
M- Ladder (100bp), 1) IVT-03, 2) IVT-35, 3) MACS-450, 4) KDS-922, 5) KDS-726,
1) MAC-2014, 2) IVT-13, 3) RKS-18, 4) IVT-33, 5) IVT-21



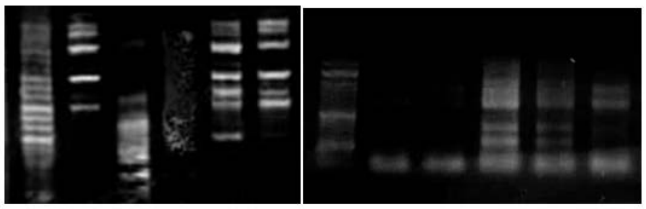

OPA-06




OPA-07

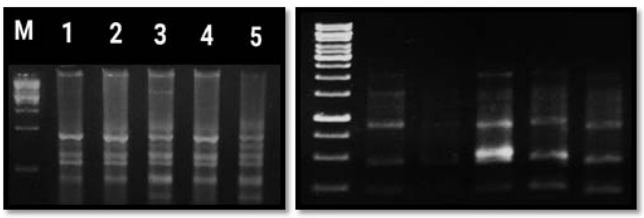


OPA-08

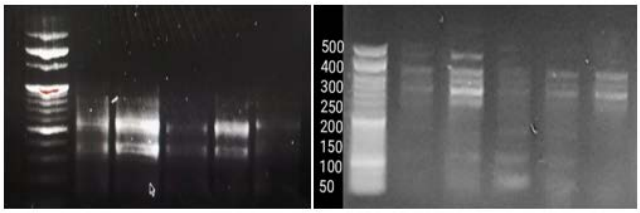


OPA-09

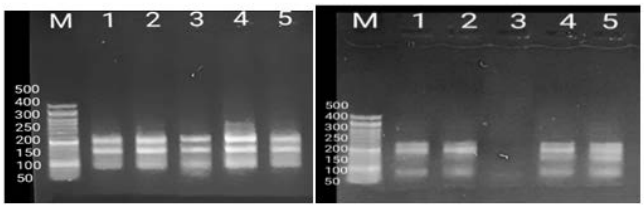
M- Ladder (100bp), 1) IVT-03, 2) IVT-35, 3) MACS-450, 4) KDS-922, 5) KDS-726,
1) MAC-2014, 2) IVT-13, 3) RKS-18, 4) IVT-33, 5) IVT-21



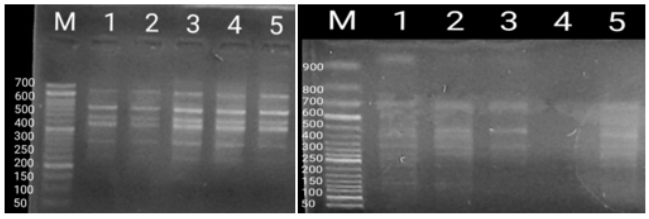

OPA-10




OPA-11




OPA-12




OPA-17

M- Ladder (100bp), 1) IVT-03, 2) IVT-35, 3) MACS-450, 4) KDS-922, 5) KDS-726,
1) MAC-2014, 2) IVT-13, 3) RKS-18, 4) IVT-33, 5) IVT-21

**Table 6: List of RAPD primers and polymorphic amplicons generated**

| Sr. No. | Primers (RAPD) | Monomorphic Amplicons | Monomorphic percent (%) | Polymorphic Amplicons | Polymorphism percent (%) | Total Amplicons | PIC Value |
|---|---|---|---|---|---|---|---|
| 1. | OPA-01 | 18 | 32.14 | 38 | 67.85 | 56 | 0.26 |
| 2. | OPA-02 | 12 | 48.00 | 13 | 52.00 | 25 | 0.10 |
| 3. | OPA-03 | 10 | 62.50 | 06 | 37.50 | 16 | 0.04 |
| 4. | OPA-05 | 16 | 76.19 | 05 | 23.80 | 21 | 0.03 |
| 5. | OPA-06 | 25 | 69.44 | 11 | 30.55 | 36 | 0.08 |
| 6. | OPA-07 | 11 | 44.00 | 14 | 56.00 | 25 | 0.10 |
| 7. | OPA-08 | 07 | 63.63 | 04 | 36.36 | 11 | 0.03 |
| 8. | OPA-09 | 27 | 77.14 | 08 | 22.85 | 35 | 0.06 |
| 9. | OPA-10 | 25 | 71.42 | 10 | 28.57 | 35 | 0.07 |
| 10. | OPA-11 | 08 | 33.33 | 16 | 66.66 | 24 | 0.11 |
| 11. | OPA-12 | 18 | 75.00 | 06 | 25.00 | 24 | 0.04 |
| 12. | OPA-17 | 14 | 53.84 | 12 | 46.15 | 26 | 0.08 |
| Total | | 191 | 58.88 | 143 | 41.10 | 334 | - |

## Cluster analysis

Amplified fragments were scored for presence (1) or absence (0) of band in each genotypes in Microsoft excel sheet and this scored data were used in NTSYS-pc software v.2.2 for cluster analysis. Dendrogram was generated by UPGMA cluster analysis based on Jaccard's similarity coefficients. Similarity coefficient values are mentioned in Table 7. The dendrogram RAPD analysis shows that 10 soybean genotypes were grouped into two major clusters viz. A and B. Cluster A divided into two sub clusters namely Cluster A1 and Cluster A2. Sub cluster A1divided into two sub clusters A1a and A1b .Cluster A1a show two genotypes IVT-03 and KDS-992. And A1b shows single genotypes i.e. KDS-922. Sub cluster A2 consist of single genotypes MACS-450. Cluster B having single genotypes IVT-35.

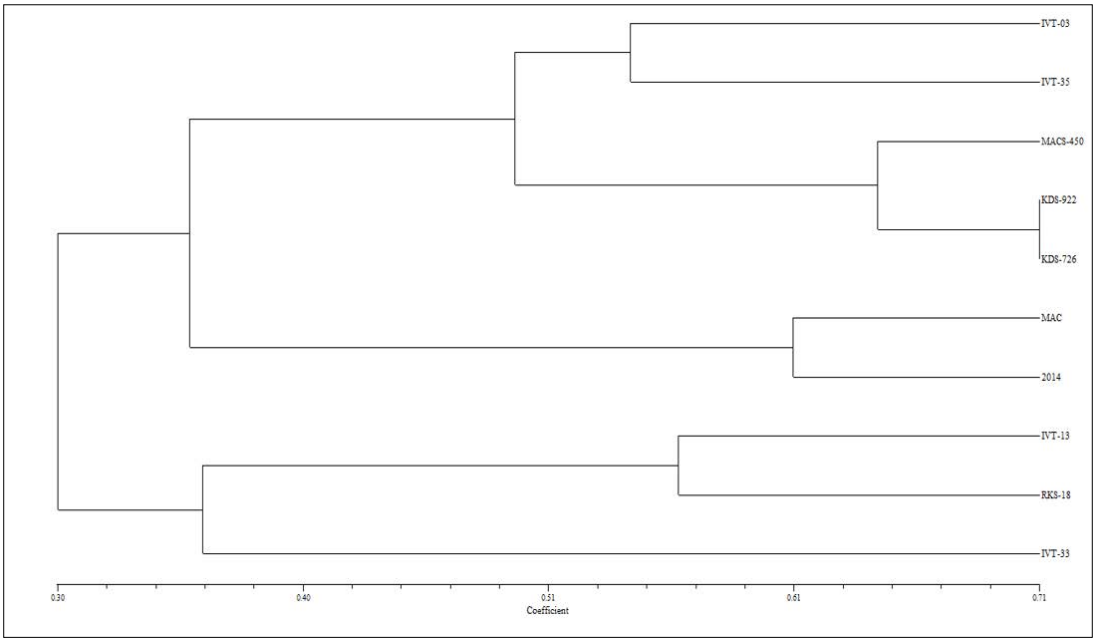



**Figure 1:** Dendrogram generated by UPGMA cluster analysis showing genetic diversity among the different soybean genotypes

## Jaccard's Binary similarity matrix based on Jaccard's coefficient for RAPD analysis

In present study ultimately means high range of genetic diversity among the soybean genotypes studied. A Jaccard's binary similarity matrix of combined data from 12 primers for the 10 soybean genotypes was prepared by scoring bands for presence or absence. DNA bands of same mobility (molecular weight) were assumed to be identical. Genetic relationship between soybean genotypes were determined on the basis of Jaccard's pair wise similarity coefficient values. The highest genetic similarity to an extent of 0.71 was recorded between KDS-726 and KDS-922 genotypes. Least genetic similarity 0.19 was observed in between IVT-33 and IVT-13. Genetic Similarity estimate (Jaccard's coefficient) based on RAPD banding pattern used for cluster analysis to present genetic relationship in the form of dendrogram (Fig.1) Jaccard's coefficient value for ten soybean genotypes are presented (Table 7).

**Table 7: Jaccard's binary similarity matrix for RAPD analysis**

| | IVT-03 | IVT-35 | MACS-450 | KDS-922 | KDS-726 | MAC-2014 | IVT-13 | RKS-18 | IVT-33 | IVT-21 |
|---|---|---|---|---|---|---|---|---|---|---|
| IVT-03 | 1.0 | | | | | | | | | |
| IVT-35 | 0.54 | 1.0 | | | | | | | | |
| MACS-450 | 0.40 | 0.43 | 1.0 | | | | | | | |
| KDS-922 | 0.66 | 0.55 | 0.64 | 1.0 | | | | | | |
| KDS-726 | 0.51 | 0.37 | 0.64 | 0.71 | 1.0 | | | | | |
| MAC-2014 | 0.38 | 0.31 | 0.39 | 0.47 | 0.45 | 1.0 | | | | |
| IVT-13 | 0.32 | 0.23 | 0.27 | 0.36 | 0.31 | 0.60 | 1.0 | | | |
| RKS-18 | 0.33 | 0.27 | 0.26 | 0.32 | 0.29 | 0.27 | 0.30 | 1.0 | | |
| IVT-33 | 0.33 | 0.29 | 0.25 | 0.29 | 0.31 | 0.20 | 0.19 | 0.56 | 1.0 | |
| IVT-21 | 0.30 | 0.28 | 0.33 | 0.33 | 0.36 | 0.34 | 0.31 | 0.30 | 0.41 | 1.0 |

## Discussion

In the present studies genetic diversity among 10 soybean genotypes was analyzed by 12 RAPD primers. The polymorphic RAPD could clear the distinguish soybean genotypes. Dendrogram were generated based on data recorded by the one marker systems. The result of cluster analysis indicated the separation of different Cluster A and Cluster B. Cluster A is having 7 genotypes within same species. KDS-726 and KDS-922 genotypes showed highest similarity of 0.71 and IVT-33 and IVT-13 genotypes showed least similarity of 0.19 and in RAPD marker analysis. Genetic diversity and variability tool for evaluating and determining cultivars identity. Generations of new and improved cultivars can be enhanced by new sources of genetic variation; therefore criteria for parental stock selection need to be considered not only by agronomic value, but also for genetic dissimilarity. Therefore, understanding the genetic diversity of soybean germplasm is essential to broaden the genetic base and to further utilize it in MAS breeding program.

## Acknowledgement

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