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Analysis of Genetic Diversity of 12 Genotypes of *Glycine max* by Using RAPD Marker

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A B S T R A C T

Soybean (*Glycine max*) is an essential legume crop in developing nations where it serves to meet the increasing demands for protein, edible oil and calories. In this study, we analysed genetic diversity of 12 soybean genotypes using Random Amplified Polymorphic DNA (RAPD) molecular markers. A total of 10 RAPD primers were used for the screening of 12germplasm line out of which 525 fragments were amplified with 10 random primers and 74.85 % were polymorphic. Genetic similarity matrix based on Jaccard Similarity Coefficients of soybean genotypes ranged from 0.30 to 0.77. These coefficients were used to construct a dendrogram using the unweighted pair group of arithmetic means (UPGMA). All 12 soybean genotypes were grouped into six clusters. The largest cluster consisted of 5 genotypes. The highest similarity among the soybean varieties were observed between JS-02-21 and DSB-61 (0.756). Present study indicated a great deal of germplasm diversity among these 12 genotypes. Our study revealed that RAPD technology is potentially simple, rapid, reliable and an effective method of detecting polymorphism for assessing genetic diversity between genotype and help in the selection of parent for hybridization.

Introduction

Soybean (*Glycine max*) is an annual crop and belongs to the family of Fabaceae. It is an important legume crop, known for its high quality of protein and oil content, and beneficiary secondary metabolites such as saponin, phenolic compound, Isoflavone, (Sakthivelu *et al*., 2008). According to USDA data base total production of soybean in India is 11.50 million tons in 2016 while the world production of Soybean is 345.96 million metric tons in 2016/17. Martin *et al.*, 2000 and Fu *et al.*, 2006 have reported that the slight genetic base is a main limitation in breeding programs, due to the deficiency of genetic variability. Introducing new germplasm sources in breeding programs may provide the required genetic variability for the robust growth and variation of cultivars to biotic and abiotic factors.

Therefore, plant germplasm is a usual source to increase the current soybean genetic base (Chung and Singh, 2008). The genetic makeup of soybean provides a wide variety of

uses, hence keeping it in high demand. Different kinds of markers were utilized for surveying hereditary variability of soybean genotypes–agronomic, morphological, biochemical attributes and molecular marker polymorphisms (Nelson, 2001; Giancola *et al*., 2002; Chowdhury *et al.*, 2002; Ude *et al*., 2003; Dong *et al.*, 2004; Bonato *et al*., 2006; Yamanaka *et al*., 2007; Malik *et al*., 2009; Goyal *et al*., 2012). All specified marker groups have constraints, however connected together they can give reliable data about analyzed germplasm (Sudaric *et al*., 2011). Smykal *et al.,* (2008) have reported the quantitative and qualitative characters between varieties and utilized to identify genera and species to evaluate systematic connection. In plant with a narrow genetic base such as soybean, Priolli *et al.,* (2002) have reported the morphological marker could not be sufficient for detection of differences between varieties.

In such case molecular marker can provide extra information of present diversity of germplasm. Subsequently they are extremely polymorphic and not affected by environmental conditions. Improvement has been made towards molecular markers progress in germplasm evaluation of diverse species. Li and Nelson (2001), Nikolic *et al*., (2004), Chen and Nelson (2005), Drinic *et al.*, (2008) have reported RAPD markers can be effectively used for evaluation of genetic diversity because they can produce large data set. This type of DNA maker has been widely used in determination of genetic diversity of soybean genotypes. The genetic diversity of soybean cultivar has been carried out by the use of morphological, biochemical and molecular markers. Previous study reported on the selection of parents in the development of soybean cultivar (Devendra *et al.*, 2015). In the present study we have conducted identification and analysis of genetic diversity of 12 soybean genotypes, based on polymorphic features and two and three dimensional Principal Component Analysis (PCA).

Materials and Methods

Genotypes

Twelve genotypes of soybean *viz.*, MAUS, MACS, Bregg, JS335, JS-02-21, CO-3, CO-2, MACS-C1, DSB, RKS, Hardee, and DSB-61were obtained from GKVK -UAS Bangalore.

Explants preparation

Seeds were surface disinfected with 70 % ethanol for 2 min, followed by 10 min in 5 % sodium hypochlorite (v/v) treatment and then rinsed with sterile water for four to six times to remove the traces of sterilants.

DNA Extraction and PCR analysis

Genomic DNA was extracted from seeds of 12 different soybean genotypes using the SDS method (Doyle and Doyle, 1987). DNA was quantified by using spectrophotometer at 260/280 nm. For quality assessment, DNA was electrophoresed on 0.8% agarose gel. The dilution of extracted DNA was verified again by spectrophotometery. A set of 10 Operon Technologies dreamer primer (Table 1) were used. PCR was performed in a thermal cycler with 20ng DNA as template, 25µl Amplification reaction contained 1µl of Taq DNA polymerase (Chromosome Biotech), 6.5 µl of 10X PCR buffer (Chromosome Biotech), 4 µl of dNTPs (Chromosome Biotech), 1µl of $MgCl_2$ (Qualigens), 1.0 µl of Primer (Genei), 10.5µl of Nuclease free water, (Chromosome Biotech), 1.0 µl of Template DNA. The DNA amplification protocol was 94°C for 3 min, followed by 40 cycles of 94°C for 1 min 28 °C for 1 min, 72°C for min 2 min and finally 72°C for 5

min. The amplification products were stored at 4ºC till electrophoresis was performed. At the time of electrophoresis and 3 µl of 6 X loading dye (GeNei, Bangalore) was added into PCR products. All amplification products were electrophoresed on 2% (w/v) agarose gels at 60 V for 3 hours, stained with Ethidium bromide, The sizes of amplified fragments were determined using standard 100 bp to >1000 bp DNA ladder mix (GeNei, Bangalore). Gel was photographed using a Gel documentation system (UVP MultiDoc-It)

Data scoring and analysis

Amplification products in the gel images were scored for presence (1) or absence (0) missing and doubtful case were scored. Homology of bands based on the distance of migration of amplified DNA fragments according to their molecular weights in the gel was determined. Molecular weights of the bands were estimated using 100bp DNA ladder (Genei, Banglore) as standard. Jackard IJ Similarity Coefficients (F) was calculated using the programme SIMQUAL.

The similarity matrix was subjected to UPGMA (Unweighted pair group method for arithmetic mean) for cluster analysis and a dendrogram was generated (Sokal and Michener, 1958). These computations were performed using the programme NTSYS-pc version 2.0, Exeter software, New York (Rohlf, 1993).

The polymorphic percentage of obtaining band was calculated of by using the following formula.

$$\text{Polymorphic Percents (\%)} = \frac{\text{Polymorphic Bands}}{\text{Total Bands}} \times 100$$

Result and Discussion

Universal random primers *viz*., OPA1, OPA2, OPA3, OPA4, OPA5, OPA6, OPA7, OPA8, OPA9 and OPA10 were used for the study and they generated 525 RAPD amplification products among all 12 Soybean genotypes (Fig. 1-3). Among RAPD markers, OPA-1 produced the maximum number of bands including monomorphic and polymorphic bands (78). While RAPD marker OPA-5 generated a minimum number of bands (26) in the genomic pool. Polymorphic bands in screened markers ranged from 19 to 68 and the maximum was observed in OPA-2 (68) followed by OPA-7 (61) Level of monomorphic bands in screened markers as it was observed in OPA-1 (48), OPA-3 (12), OPA-4 (12), OPA-5 (12), OPA-2 (0), and OPA-7 (0) is given in table 2. The percent amplified bands in banding pattern was calculated and it was highest in OPA-2 (100%) followed by OPA-7 (100%) while the minimum was recorded from OPA-1 (38.46%). (Table 2) Calculations for polymorphic information content (PIC) be completed using the formula of the expected heterozygosity (Smith *et al*., 2000) as: PIC=1-$\sum f^2 i$, where f is the percentage of genotypes in which the fragment is present. The PIC value is a sign of a high probability of obtaining polymorphism using that primer combination. Among Random amplified polymorphic DNA (RAPD) markers have more polymorphism information content (PIC) value among RAPD markers the highest PIC value was observed from OPA-9 (0.91) followed by OPA-1 (0.86). The minimum PIC value was observed from OPA-3 (0.69). (Table 2) The genetic relationship between soybean genotype was determined on the basis of Jackard IJ pair wise similarity coefficient values. The value of similarity coefficients ranged from 0.30 to 0.77.

Table.1 List of RAPD primers used in the diversity analysis of soybean

Sr.no.	Primer	Sequence (5'> 3')	TM (ºC)	GC Content (%)
1	OPA-01	CAGGCCCTTC	34.0	70
2	OPA-02	TGCCGAGCTG	34.0	70
3	OPA-03	AGTCAGCCAC	32.0	60
4	OPA-04	AATCGGGCTG	32.0	60
5	OPA-05	AGGGGTCTTG	32.0	60
6	OPA-06	GGTCCCTGAC	34.0	70
7	OPA-07	GAAACGGGTG	32.0	60
8	OPA-08	GTGACGTAGG	32.0	60
9	OPA-09	GGGTAACGCC	34.0	70
10	OPA-10	GTGATCGCAG	32.0	60

Source: GeNei Bangalore

Table.2 Characteristics of the amplification products obtained with 10 primers for RAPD

Sr.no.	Primer	Total Bands	Monomorphic Bands	Polymorphic Bands	Percent polymorphism	PIC value
1	OPA-01	78	48	30	38.46	0.86
2	OPA-02	68	0	68	100	0.83
3	OPA-03	31	12	19	61.29	0.69
4	OPA-04	56	12	44	78.57	0.85
5	OPA-05	26	12	14	53.84	0.74
6	OPA-06	41	12	29	70.73	0.77
7	OPA-07	61	0	61	100	0.86
8	OPA-08	58	12	46	79.31	0.85
9	OPA-09	51	12	39	76.47	0.91
10	OPA-10	55	12	43	78.18	0.85
		Total Bands= **525**	Total Monomorphic Bands=**132**	Total Polymorphic Bands=**393**		

Fig.1 RAPD banding pattern of twelve soybean genotypes using OPA-1 primers

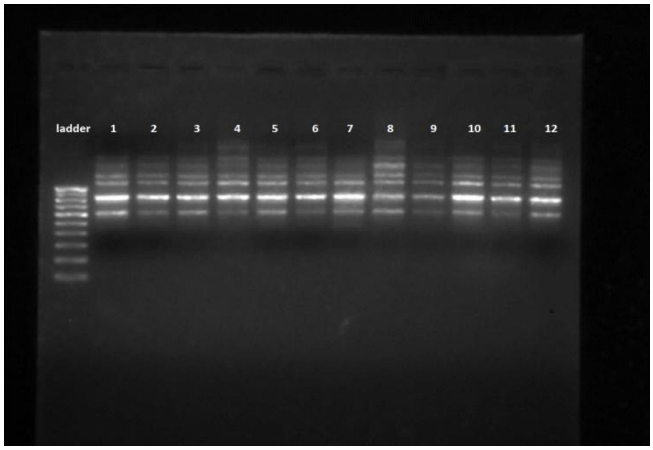


Fig.2 RAPD banding pattern of twelve soybean genotypes using OPA-4 primers

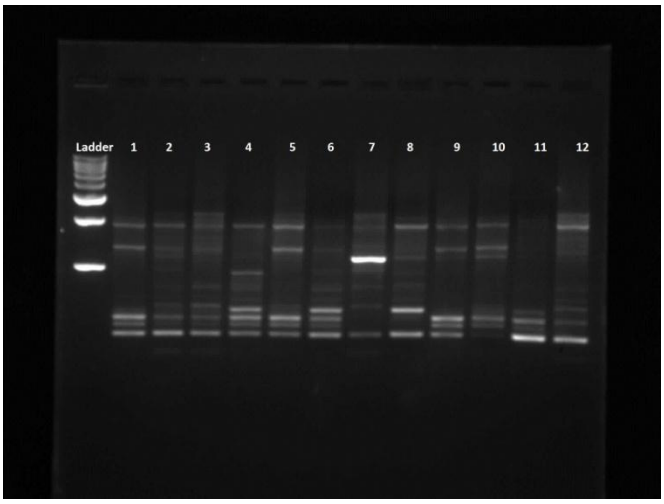


Fig.3 RAPD banding pattern of twelve soybean genotypes using OPA-2 primers

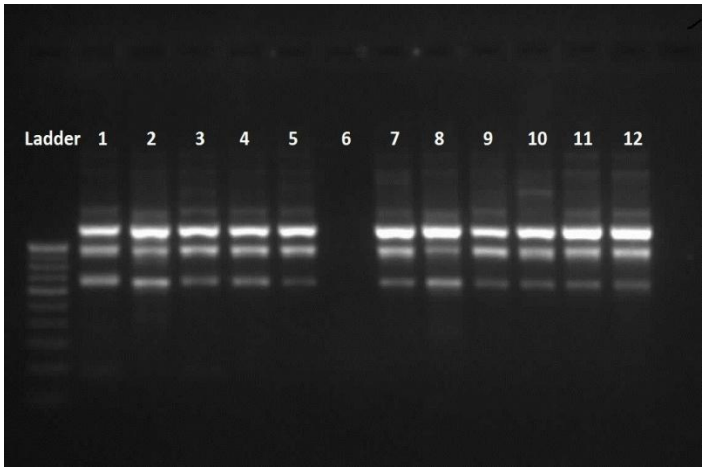


Fig.4 Dendrogram generated after RAPD analysis showing relationship among soybean genotypes used in this study

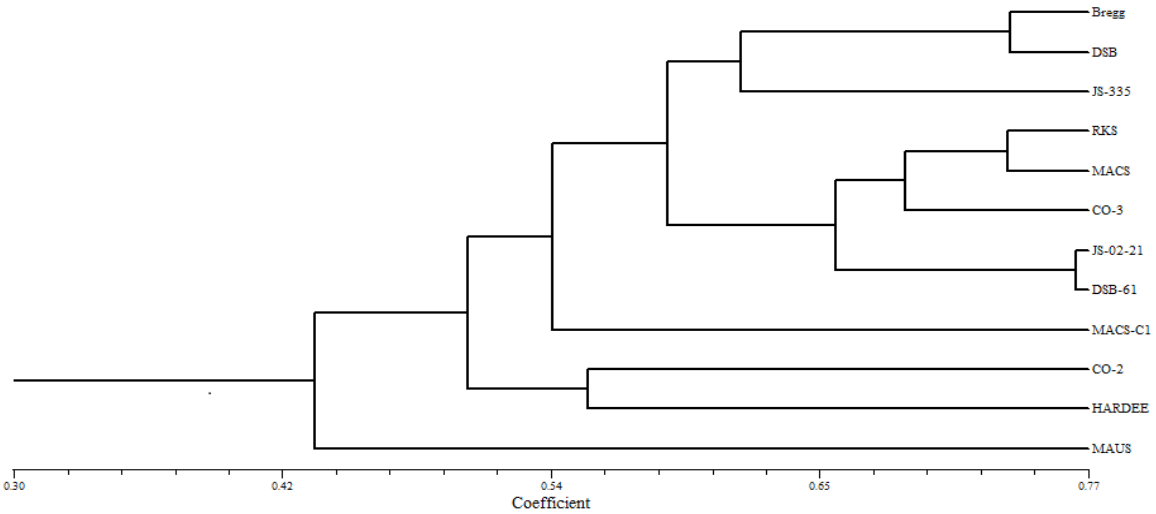


Fig.5 Two dimensional PCA scaling among soybean genotypes using RAPD data

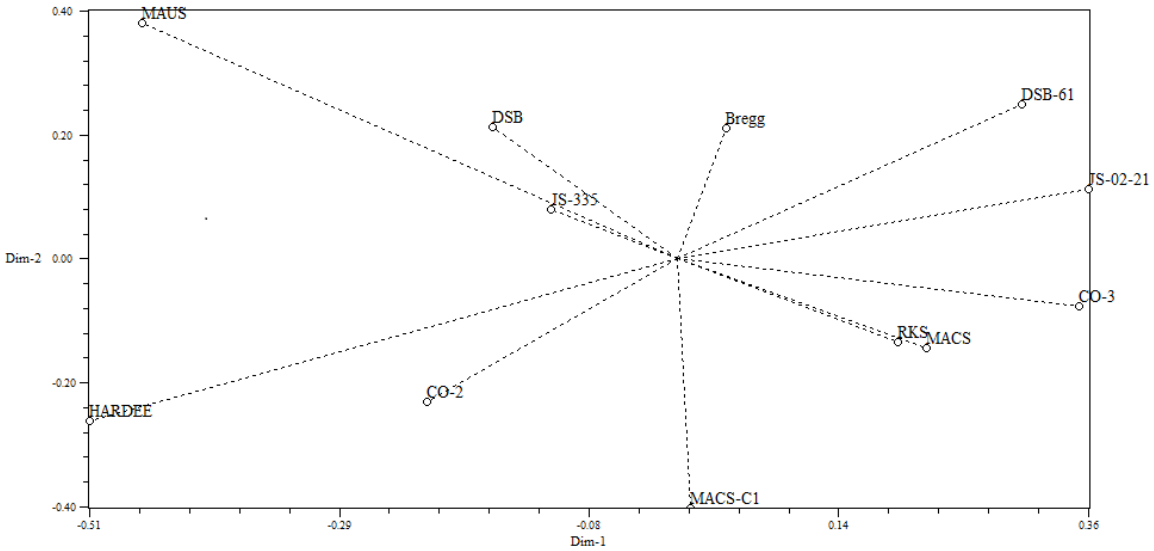
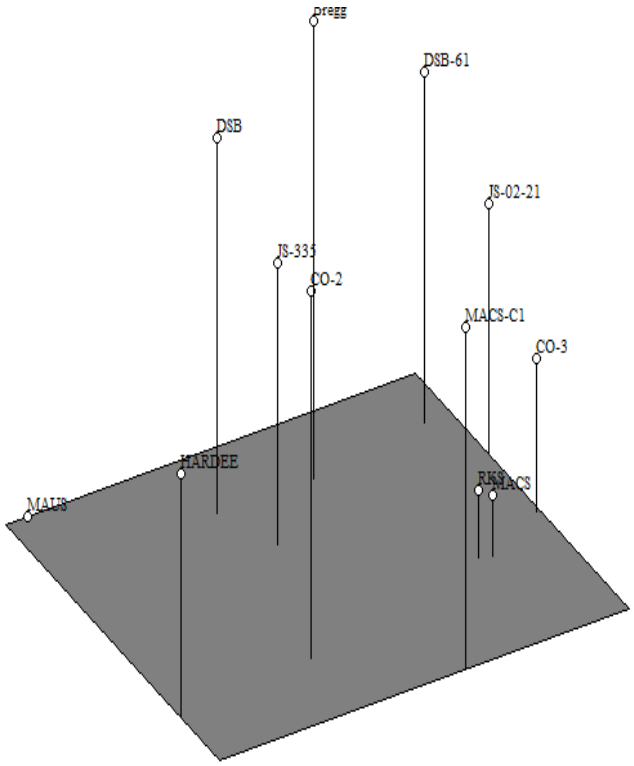


Fig.6 Three dimensional PCA among soybean genotypes using RAPD data



The Nei's similarity used to carry out the cluster analysis and to generate a dendrogram showing the relationships among the selected genotypes. All 12 genotypes were grouped into six clusters (Fig. 4). Second cluster is largest consist of 5 genotypes *i.e.* RKS, MACS, JS-02-21, CO-3 and DSB-61 The second largest cluster is the 1st cluster which

consists of 3 genotypes *i.e.* Bregg, DSB and JS-335 are closest. Then come 4th cluster in which MACS-C1 and CO-2 are close then come 3rd and 5thare solitary clusters. The highest similarity among the soybean varieties were observed between JS-02-21 and DSB-61 (0.756).In two dimensional PCA (Fig. 5) MAUS and MACS-C1 were not grouped together in two dimensional and three dimensional PCA clustering (Fig. 6) while both these genotypes formed separate sub cluster in the dendrogram.

Devendra *et al.,* (2015) studied the molecular characterization and genetic diversity assessment in soybean verities using SSR makers they have conclude that genetic similarity coefficient for the largely numbers of pairs was in the range 0.1-0.4 signifying high diversity between the chosen genotypes. All the 48 verities could be easily differentiate from each other's base on the selected markers. Shashank *et al.,* (2014) studied the analysis of genetic diversity in 20 cotton germplasm line using RAPD markers. They have concluded that genetic similarity coefficient base on Jaccard similarity coefficient of cotton genotypes were ranging from 0.14-0.19. All the 20 varieties could be easily distinguish from each other's. Dhirendra *et al.,* (2013) reported the genetic diversity in soybean germplasm identified by RAPD markers. In this study they have used 30 RAPD primers for amplification and Out of these 259 loci, 253 loci were found to be polymorphic (97.68%), concluded that RAPD methods could be used to investigate the genetic diversity of soybean germplasm. Present results depicted efficient use of RAPD technique to determine genetic distance among genotypes. It is therefore concluded that RAPD marker is potentially simple, rapid, reliable and effective method of detecting polymorphism for assessing genetic diversity between genotype and these help in the selection of parent for hybridization.

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