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Molecular Marker based Genetic Diversity Analysis in Soybean [*Glycine max* (L.) Merrill] Genotypes

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A B S T R A C T

Soybean is an important legume and oilseed crop with high protein (40%) and oil (20%). RAPD markers were used to access the genetic diversity among twenty four soybean genotypes. A total of Twenty primers were used out of which 18 got amplified which produced 164 bands and all were found polymorphic i.e. 100% polymorphism. The total number of amplified bands varied between 2 (OPF-19) and 16 (OPA-01) with an average of 9.1 bands per primer. The overall size of the amplified fragments ranged between 100 and 2500 bp. The Polymorphic Information Content (PIC) values ranged from 0.126 (OPP-01) to 0.399 (OPF-19) with an average of 0.295. Jaccard's similarity coefficient values ranged from 0.12 to 0.70 with an average of 0.41. Cluster analysis based on Jaccard's similarity coefficient using Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) grouped all the 24genotypes into three major groups at a similarity coefficient of 0.53. A total of four primers detected in the study produced four unique bands in four genotypes. The results showed that the level of genetic variation was high among the soybean genotypes.

Introduction

Soybean (*Glycine max* (L.) Merrill) is a diploidized, allotetraploid (2n=40), autogamous plant belongs to legume family. It has originated in the eastern half of North China in the 11th century B.C. or perhaps a bit earlier (Fukuda, 1933 and Singh, 2010). This crop is aptly called as "Golden Bean" or "Miracle crop" of the 20th century, because of its multiple uses. It is a principle grain legume in developing countries where it meets the expanding needs for protein, edible oil and calories. It contains 40-42% protein, 18-22% oil comprising of 85% unsaturated fatty acids and 15% saturated fatty acids, 28% carbohydrate and good amount of other nutrients like phosphorus, calcium, vitamins, iron etc. (Antalina, 1999) and rich in lycine and vitamin A, B and D. It also consist many therapeutic components and has increased its importance in industrial, agricultural and medicinal sectors.

Genetic diversity evaluation among germplasms is an important and a prerequisite in any hybridization program and would promote the efficient use of genetic variations

(Paterson *et al.,* 1991; Chen *et al.,* 1994; Dwivedi *et al.,* 2001). The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources (Tahir and Karim, 2011). Marker systems have been successfully used over the last several decades to construct genetic maps, assess genetic diversity and locate genes of interest in a number of agriculturally important crops for the desired traits (Garcia *et al.,* 2005). Different methodological approaches such as morphological, protein, Isozyme and molecular markers have been employed to assess genetic diversity in crop plants. Among them, the DNA based molecular marker approach has been found to be superior, because of its capability to reveal more polymorphism (Mignouna *et al.,* 1998). Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and population.

RAPD markers offer many advantages such as higher frequency of polymorphism, rapidity, technical simplicity, requirement of a few nanograms of DNA, no requirement of prior information of any DNA sequence and feasibility of automation (Fahima *et al.,* 1999; Subudhi and Huang, 1999; Chowdhury *et al.,* 2001; Zenglu and Nelson, 2002; Yu *et al.,* 2005; Kumari *et al.,* 2009). The RAPD technique which was developed by Williams *et al.,* (1990) has been widely applied in either identification of cultivars (Hu and Quiros, 1991) or estimating genetic relationship and diversity among crop germplasm (Jain *et al.,* 1994).

RAPD markers have been used for genetic diversity analysis in soybean by many workers (Thompson and Nelson, 1998; Thompson *et al.,* 1998, Brown-Guidera *et al.,* 2000; Li *et al.,* 2001; Li and Nelson, 2001; Singh *et al.,* 2006; Ojo *et al.,* 2012; Khare *et al.,* 2013; Bharose *et al.,* 2017). In the present work, we have applied RAPD markers to characterize and assess the genetic variability in selected 24 soybean genotypes and to determine the phylogenetic relationship among them.

Materials and Methods

Twenty four genotypes of soybean were procured and investigated in the present study (Table 1). Young fresh and healthy leaves were collected and DNA extraction was done following the cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990).The extracted DNA was analysed on 0.8% agarose gel and was diluted to an optimum concentration using TE for polymerase chain reaction (PCR). A total of 20 arbitrary decamer primers were initially used, out of which 18 primers showed clear, scorable and highly polymorphic bands (Table 2).

Different parameters were tested to determine optimal concentrations of template DNA, $MgCl_2$, dNTPs, Taq DNA polymerase, primer and different temperatures and time intervals during denaturation, annealing and elongation steps which affect amplification, banding pattern and reproducibility. For this, varying concentrations of template DNA (50 ng, 100 ng, 200 ng), primers (0.10 µM, 0.20 µM, 0.30 µM, 0.40 µM, 0.50 µM), dNTPs (0.5 mM, 1 mM, 1.5 mM, 2.0 mM) and $MgCl_2$ (0.5mM, 1.0 mM, 1.5 mM and 2.0 mM) were used in a reaction volume of 20 µl in different combinations at different annealing temperatures (38ºC, 40ºC, 43ºC, 45ºC, and 48ºC). In brief, reproducible and clear banding patterns were obtained in a reaction mixture of 20 ml containing 1x reaction buffer, 1 unit of Taq DNA polymerase, 200 mM each of dNTPs mix, 0.5 µM/reaction of primer's and 50 ng of template DNA.

The Polymerase Chain Reaction was performed in a programmable thermo-cycler DNA Engine (Biorad, Germany) using the following cycling parameters: an initial denaturation (94ºC) for 5 minutes, Denaturation (94ºC) for 2 minutes, Primer annealing (36ºC) for 1 minute, Primer Extension (72ºC) for 2 minutes (37 cycles), followed by Final Primer Extension (72ºC) for 10 minutes and a hold temperature of 4ºC.

The amplified products, after PCR reaction, were separated on 1.2% agarose gel in 1x TAE buffer using ethidium bromide(EtBr) staining dye. The size of the amplified DNA fragments was determined using 100 bp and 1 kb DNA ladders (Bangalore Genie, India) as standard markers. DNA fragments were visualized under UV-trans-illuminator and photographed using gel documentation system. Scoring of amplicons obtained from different RAPD markers was done on the basis of presence (used as 1) or absence (used as 0) of bands for each primer. For banding pattern only clear and unambiguous bands were scored for each primer. Comparison of band position was done with molecular weight of standard DNA ladders. Accordingly, a rectangular binary matrix was obtained and statistical analysis was performed using the NTSYS-pc version 2.02e (Rohlf, 1998). A pair wise similarity matrix was generated and the cluster analysis was performed via Unweighted Pair Group Method with Arithmetic averages (UPGMA) to develop a dendrogram. A two dimensional and three dimensional principal component analysis (PCA) was constructed to provide another means of testing the relationship among the genotypes.

Results and Discussion

Among the 20 RAPD primers used for initial screening, 18 markers produced polymorphic, reproducible and scorable bands. A total of 164 amplified bands were obtained of which all were polymorphic and showed 100% polymorphism (Table 2). The total number of amplified bands varied between 2 (primer OPF-19) and 16 (primer OPA-01) with an average of 9.1 bands per primer. The overall size of PCR amplified products ranged between 100 bp to 2500 bp. The percent polymorphism was 100% for all the genotypes. The average Polymorphic Information Content (PIC) was 0.295 ranging from 0.126 (OPP-01) to 0.399 (OPF-19). Figure 1 showing the amplification pattern obtained from primer OPP-01 and OPP-04 produced 10 and 14 polymorphic band respectively.

Four unique bands (band which is present in a particular genotype but absent in rest of the genotypes) were detected in four genotypes viz., JS-20-79, PS-1543, Himso-1685 and NRC-98 with 4 RAPD primers (OPJ-04, OPP-05, OPP-06 and OPD-05). All four genotypes gave single distinct bands. The size of these unique bands ranged from 200-2100 bp (Table 3).

The data obtained by using RAPD were further used to construct similarity matrix using ‘Simqual’ sub-programme of software NTSYS-pc. Based on RAPD similarity matrix data, the values of similarity coefficient ranged from 0.12 to 0.70 i.e.12-70 % or genetic diversity ranged from 30 to 88% (Table 4). The average similarity across all the genotypes was found out to be 0.41 showing that the genotypes were highly diverse from each other. The maximum similarity coefficient 0.70 was observed between SL-983 and DS-2961 and RVS 2002-22 and RKS-111 showing minimum diversity followed by PS-1543 and Himso-1685 and KDS-722 and MAUS-609 with a similarity coefficient value of 0.69 and 0.68 respectively. The minimum similarity coefficient 0.12 was observed between PS-

1539 and MACS-1419 indicating maximum diversity followed by PS-1539 and NRC-98, PS-1539 and MACS-1410 and MACS-1419 and BAUS-27 with a similarity coefficient of 0.14.

The RAPD cluster tree analysis of 24 *G. max* L. genotypes showed that they could be mainly divided into 3 major clusters at a similarity coefficient of 0.29(Fig. 2). Cluster I included 12 genotypes viz., KDS-726, DS-3050, SL-983, DS-2961, AMS-1001, JS-20-79, RKS-109, DS-3047, RVS-2002-4, KDS-722, MAUS-609 and PS-1539 at a similarity coefficient of 0.31. It could be divided into 2 sub-clusters. The sub-cluster first contained two genotypes SL-983 and DS-2961 similar to each other at a very close to 0.70 similarity coefficient while the second sub-cluster consisted two genotypes KDS-722 and MAUS-609 that are related to each other at 0.68 similarity coefficient. Cluster II included 8 genotypes at a similarity coefficient of 0.42. These genotypes are MACS-1410, PS-1543, Himso-1685, RVS-2002-22, RKS-111, JS-20-53, RSC-10-17 and BAUS-27. This cluster could be further divided into two sub-clusters.

Table.1 Pedigree and source of 24 genotypes of *Glycine max* L. Merrill

S.No.	Genotypes	Pedigree	Source
1.	KDS-726	JS-93-05 X EC-241780	SANGLI (MH)
2.	PS-1539	PS-1024 X JS-335	PANTNAGAR
3.	DS-3050	DT-23 X DT-227	DELHI
4.	SL-983	SL-525 X PK-1368	LUDHIANA
5.	DS-2961	MO-74 X JS-335	DELHI
6.	RKS-109	RKS-224 X PK-1024	KOTA
7.	SL-955	SL-599 X PK-1283	LUDHIANA
8.	DS-3047	DT-23 X DT-27	DELHI
9.	AMS-1001	Mutant of JS-93-05	AMARAWATI
10.	JS-20-79	JS-97-52 X JS-(15) 90-5-12-1	JABALPUR
11.	MACS-1419	EC-391343 X MACS-450	PUNE
12.	NRC-98	Ankur X PK-1024	INDORE
13.	RVS-2002-4	JP-120 X JS-335	SIHORE
14.	KDS-722	AMS-99 X EC-241780	SANGLI (MH)
15.	MAUS-609	Himso-1563 X MAUS-71	PARBANI
16.	NRC-107	Mutant of NRC-37	INDORE
17.	MACS-1410	MAUS-144 X MACS-450	PUNE
18.	JS-20-53	JS-97-52 X JS-20-02	JABALPUR
19.	PS-1543	PS-1029 X JS-335 X PS-1241	PANTNAGAR
20.	Himso-1685	H-330 X HARDEE	PALAMPUR
21.	RVS-2002-22	NRC-37 X JS-39-05	SIHORE
22.	RKS-111	RKS-45 X RKS-24	KOTA
23.	BAUS-27	PK-472 X L-119	RANCHI
24.	RSC-10-17	MAUS-144 X RAUS-5	RAIPUR

Table.2 DNA amplification profile and polymorphism generated in *Glycine max* L. Merrill by 18 RAPD primers

S. No.	Primer Code	Molecular weight range (bp)	Total no. of bands amplified (x)	Polymorphic bands		PIC*
				Number	Frequency (%)	
1.	OPA-01	300-2300	**16**	**16**	100	0.257
2.	OPC-08	350-2100	13	13	100	0.322
3.	OPD-05	300-2000	11	11	100	0.295
4.	OPD-12	250-1200	9	9	100	0.183
5.	OPE-03	200-1300	10	10	100	0.359
6.	OPF-17	200-500	3	3	100	0.218
7.	OPF-19	300-700	**2**	**2**	100	**0.399**
8.	OPJ-04	250-2100	14	14	100	0.269
9.	OPP-01	350-1800	10	10	100	**0.126**
10.	OPP-02	200-2100	13	13	100	0.392
11.	OPP-04	100-2500	14	14	100	0.379
12.	OPP-05	300-1800	12	12	100	0.178
13.	OPP-06	200-2200	10	10	100	0.312
14.	OPP-07	300-1200	7	7	100	0.386
15.	OPP-08	300-1300	7	7	100	0.297
16.	OPP-09	300-800	3	3	100	0.343
17.	OPP-12	1000-1600	3	3	100	0.303
18.	OPP-16	200-1800	7	7	100	0.298
	Total		**164**	**164**	**100**	**0.295**

*Polymorphic Information Content

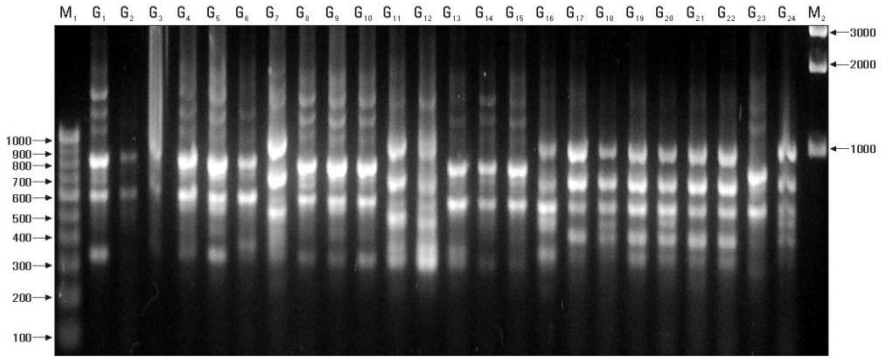
Table.3 Genotype specific unique bands as detected by RAPD primers in *Glycine max* L. Merrill

S. No.	Primer code	Total no. of unique bands	Genotype	Size of band (bp)
1.	OPJ-04	1	JS-20-79	2100
2.	OPP-05	1	PS-1543	800
3.	OPD-05	1	NRC-98	400
4.	OPP-06	1	Himso-1685	200
Total	**4**			

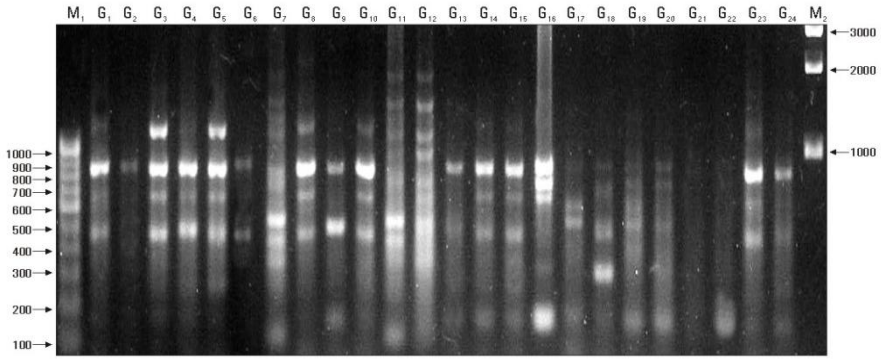
Table.4 Jaccards similarity coefficient for RAPD profile of *Glycine max* L. Merrill genotypes

Genotypes	KDS-726	PS- 1539	DS-3050	SL-983	DS-2961	RKS-109	SL-955	DS-3047	AMS-1001	JS20-79	MACS-1419	NRC-98	RVS2002-4	KDS-722	MAUS-609	NRC-107	MACS-1410	JS20-53	PS-1543	HIMO-1685	RVS2002-22	RKS-111	BAUS-27	RSC10-17
KDS-726	1.00																							
PS-1539	0.27	1.00																						
DS-3050	0.41	0.35	1.00																					
SL-983	0.44	0.31	0.58	1.00																				
DS-2961	0.44	0.35	0.61	**0.70**	1.00																			
RKS-109	0.39	0.38	0.52	0.60	0.57	1.00																		
SL-955	0.30	0.15	0.32	0.43	0.44	0.36	1.00																	
DS-3047	0.42	0.18	0.49	0.49	0.47	0.46	0.39	1.00																
AMS-1001	0.36	0.32	0.51	0.59	0.65	0.55	0.43	0.52	1.00															
JS20-79	0.39	0.27	0.44	0.65	0.54	0.48	0.39	0.53	0.56	1.00														
MACS-1419	0.26	**0.12**	0.24	0.35	0.34	0.29	0.58	0.36	0.37	0.33	1.00													
NRC-98	0.26	0.14	0.23	0.36	0.33	0.30	0.49	0.27	0.32	0.27	0.44	1.00												
RVS2002-4	0.37	0.32	0.43	0.41	0.37	0.35	0.19	0.36	0.39	0.33	0.18	0.24	1.00											
KDS-722	0.39	0.28	0.36	0.42	0.44	0.43	0.25	0.39	0.42	0.38	0.20	0.30	0.46	1.00										
MAUS-609	0.44	0.35	0.45	0.49	0.53	0.48	0.29	0.40	0.49	0.39	0.24	0.31	0.49	0.68	1.00									
NRC-107	0.27	0.17	0.29	0.33	0.31	0.33	0.50	0.32	0.34	0.27	0.56	0.34	0.20	0.23	0.33	1.00								
MACS-1410	0.20	0.14	0.26	0.37	0.32	0.33	0.31	0.29	0.34	0.27	0.25	0.29	0.22	0.20	0.29	0.27	1.00							
JS20-53	0.27	0.22	0.33	0.36	0.35	0.40	0.23	0.24	0.30	0.25	0.17	0.23	0.30	0.28	0.32	0.27	0.47	1.00						
PS-1543	0.27	0.19	0.33	0.46	0.39	0.41	0.36	0.37	0.40	0.38	0.31	0.32	0.26	0.25	0.35	0.36	0.57	0.51	1.00					
HIMSO-1685	0.23	0.17	0.33	0.40	0.37	0.36	0.35	0.29	0.35	0.30	0.27	0.29	0.27	0.27	0.31	0.34	0.53	0.60	0.69	1.00				
RVS2002-22	0.22	0.18	0.29	0.38	0.35	0.36	0.32	0.34	0.38	0.32	0.29	0.27	0.22	0.22	0.31	0.30	0.62	0.51	0.65	0.65	1.00			
RKS-111	0.21	0.17	0.29	0.36	0.34	0.37	0.30	0.32	0.34	0.28	0.23	0.28	0.26	0.24	0.30	0.33	0.56	0.58	0.65	0.61	**0.70**	1.00		
BAUS-27	0.29	0.23	0.24	0.30	0.31	0.25	0.20	0.20	0.27	0.26	0.14	0.18	0.27	0.25	0.33	0.19	0.34	0.42	0.38	0.37	0.34	0.36	1.00	
RSC10-17	0.26	0.17	0.30	0.36	0.30	0.35	0.26	0.27	0.29	0.28	0.21	0.21	0.22	0.27	0.25	0.29	0.31	0.50	0.47	0.47	0.39	0.47	0.41	1.00

Fig.1 RAPD profile of *Glycine max* L. Merrill generated through OPP-01 and OPP-04 primer respectively



RAPD profile generated through primer - OPP-01



RAPD profile generated through primer - OPP-04

M_1 = 100 bp DNA Ladder **M_2**= 1000 bp DNA Ladder

G_1 - G_{24} represent following *Glycine max* genotypes :

G_1 - KDS-726	G_2 - PS-1539	G_3 - DS-3050	G_4 - SL-983	G_5 - DS2961
G_6 - RKS-109	G_7 - SL-955	G_8 - DS-3047	G_9 - AMS-1001	G_{10} - JS-20-79
G_{11} - MACS-1419	G_{12} - NRC-98	G_{13} - RVS-2002-4	G_{14} - KDS-722	G_{15} - MAUS-609
G_{16} - NRC-107	G_{17} - MACS-1410	G_{18} - JS-20-53	G_{19} - PS-1543	G_{20} - Himso-1685
G_{21} - RVS-2002-22	G_{22} - RKS-111	G_{23} - BAUS-27	G_{24} - RSC-10-17	

Fig.2 Dendogram constructed with UPGMA clustering method of 24 *Glycine max* L. Merrill genotypes using RAPD primers

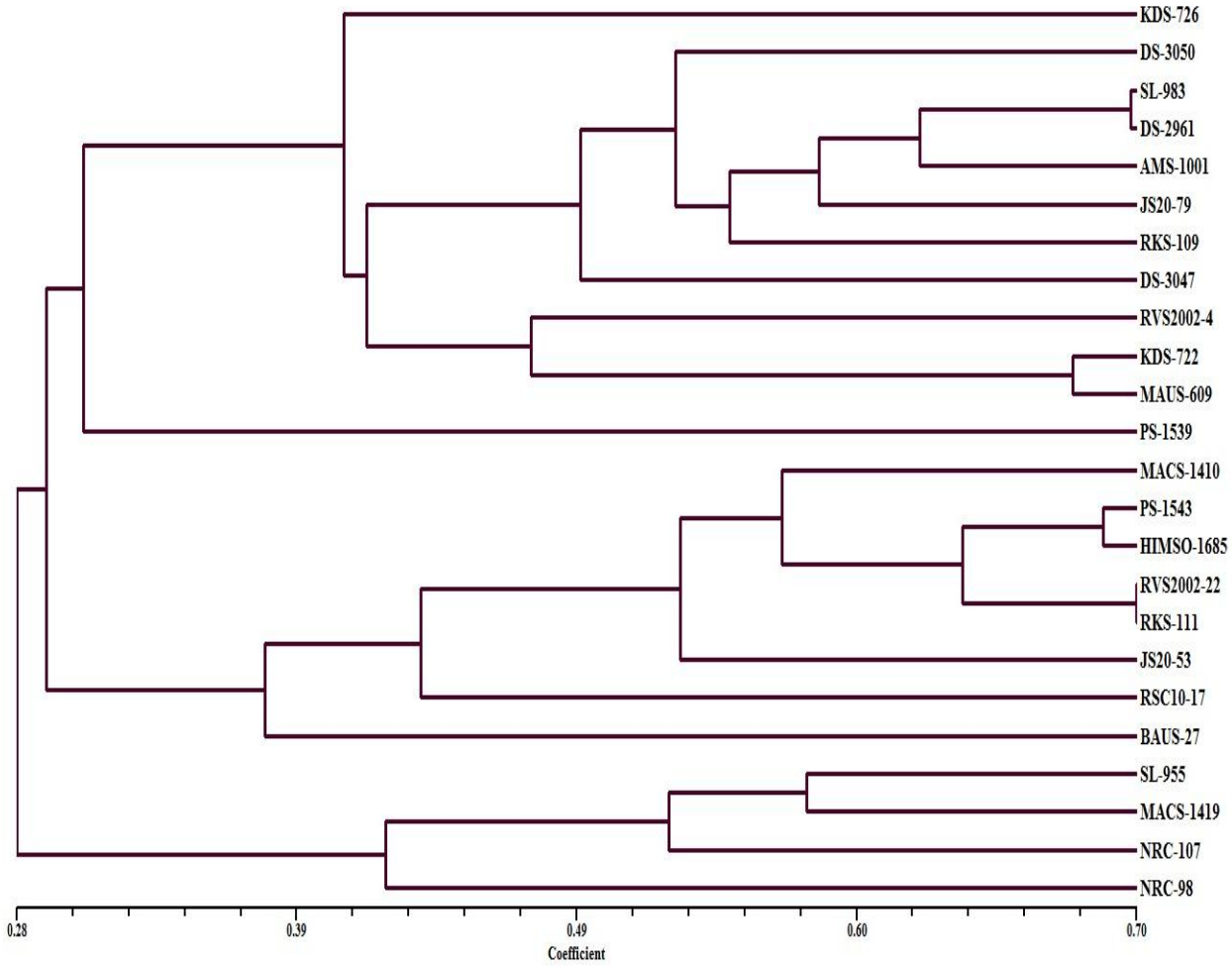


Fig.3 Two dimensional PCA (Principle Component Analysis) scaling of 24 genotypes of *Glycine max* L. Merrill using RAPD markers

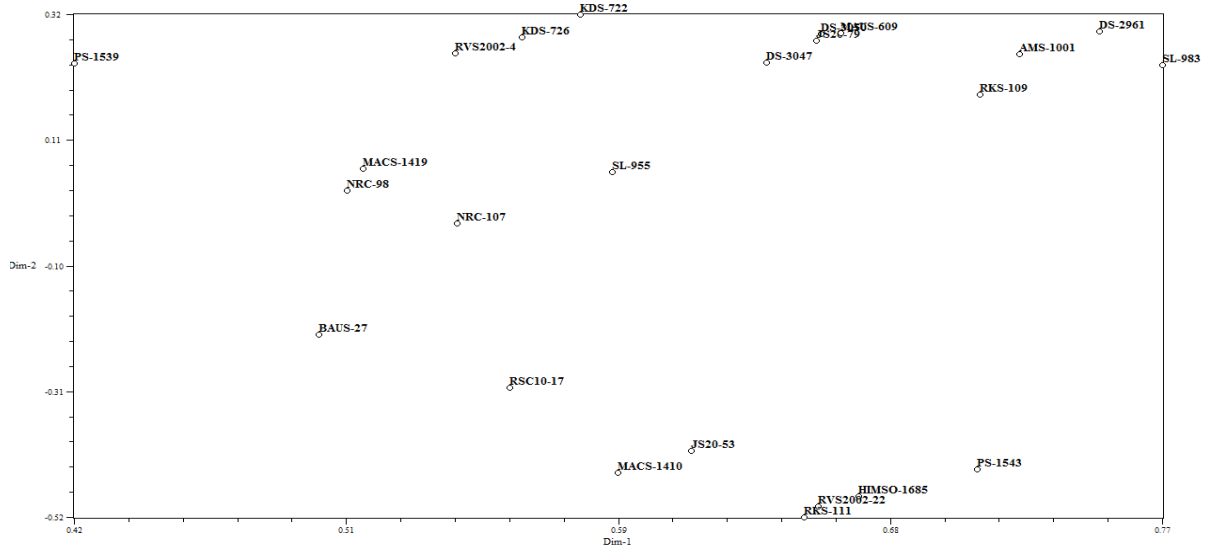
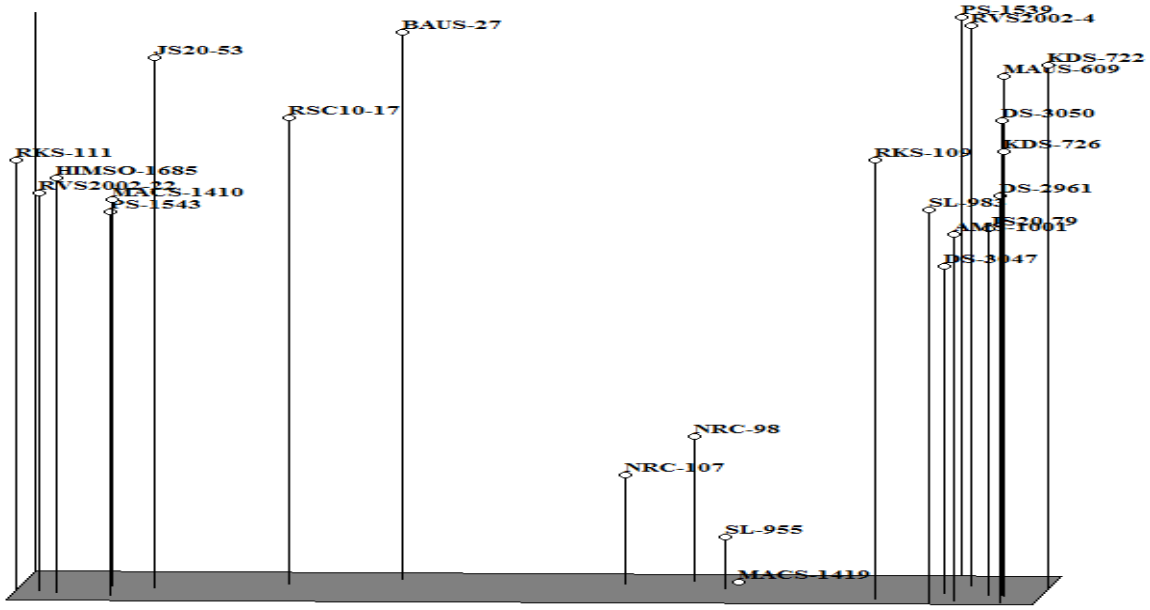


Fig.4 Three dimensional PCA (Principle Component Analysis) scaling of 24 genotypes of *Glycine max* L. Merrill using RAPD markers



First sub-cluster consisted of two genotypes viz., PS-1543 and Himso-1685 which were similar to each other at a similar coefficient of 0.69. The second sub-cluster contained two genotypes named RVS-2002-22 and RKS-111. These were related to each other at a similarity coefficient of 0.70. The cluster III included 4 genotypes viz., SL-955, MACS-1419, NRC-107 and NRC-98 at a similarity coefficient of 0.42. It could be divided into one sub-cluster. This sub-cluster included genotypes SL-955 and MACS-1419 which were similar to each other at similarity coefficient of 0.58.

Two and three dimension principal component analysis based on RAPD data (Figs. 3 and 4, respectively) showed similar clustering of 24 genotypes as evident from cluster tree analysis. Dice similarity coefficients ranged from 0.42 to 0.77. Most of the genotypes tended to cluster mainly into three clusters. Cluster I included 12 genotypes (KDS-726, DS-3050, SL-983, DS-2961, AMS-1001, JS-20-79, RKS-109, DS3047, RVS2002-4, KDS-722, MAUS-609 and PS-1539) second included 8 genotypes MACS-1410, PS-1543, HIMSO-1685, RVS2002-22, RKS-111, JS20-53, RSC10-17 and BAUS-27) and Cluster III included 4 genotypes viz., (SL-955, MACS-1419, NRC-107 and NRC-98).

In present study, we found that all the primers studied produced 100% polymorphism, relatively high proportion compared to previous reports such as Khare *et al.*, (2013) (97.68%), Mundewadikar and Deshmukh (2014) (94.06%) and Singh *et al.*, (2008) (89.9%).

The RAPD methods displayed genetic variation among 24 soybean genotypes and phylogenetic tree was showing a relationship among them. This study has confirmed, RAPD marker is potentially simple, rapid, reliable and effective method of detecting polymorphism for assessing genetic diversity among genotypes. The banding pattern obtained from RAPD

markers can be used to characterize soybean genotypes. It is observed that there is a wide range of genetic diversity among selected genotypes, thus they can be used for further crop improvement programmes.

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