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ISSR Based Molecular Characterization of Soybean [*Glycine max* (L.) Merrill] Genotypes

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ABSTRACT
Assessment of genetic diversity among twenty four soybean genotypes was done using ISSR markers. Twenty ISSR primers were used of which 19 gave amplification. 182 bands were produced of which 177 were found polymorphic (97.25% polymorphism). Number of amplified bands varied from 3 (UBC-872 and UBC-878) to 17 (UBC-814) with an average of 9.57 per primer. The overall size of the amplified fragments ranged between 200 and 2500 bp. The average Polymorphic Information Content (PIC) was 0.344 ranging from 0.192 (UBC-818) to 0.445 (UBC-817). Jaccard's similarity coefficient values ranged from 0.27 to 0.89 with an average of 0.58. 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 0.50. A total of three primers detected in the study produced three unique bands in three genotypes. The results showed that the level of genetic variation was high among the soybean genotypes.
Keywords: Soybean, ISSR, Genetic diversity, Polymorphism, Similarity coefficient.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a diploidized, allotetraploid (2n=40), autogamous plant belongs to legume family which is one of the oldest cultivated crops. It was cultivated in the eastern half of Northern China as early as the 11th century, but domestication probably occurred even earlier in the same region [25]. It is known as "Golden Bean" and "Miracle Crop" of the 21st century because of its multiple uses. 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. [3] and rich in lysine and vitamin A, B and D. It also consist many therapeutic components and has increased its importance in industrial, agricultural and medicinal sectors. Recent studies revealed that, even though soy proteins are of plant sources and typically lower in certain amino acids compared to animal proteins such as those found in eggs or cow's milk; it receives similar protein quality rating as egg or cow's milk protein [2].

Genetic diversity evaluation among germplasms is an important and a prerequisite in any hybridization program and would promote the efficient use of genetic variations [10, 16]. Knowledge of genetic diversity and relationships among soybean may play a significant role inbreeding programs to improve grain yield, oil and protein content and provide valuable information that can be used by plant breeders as a parental line selection tool. . Different methods have been used to evaluate genetic diversity and pattern of variation in soybean such as assessing morphological traits [13, 15], biochemical markers [8], and more recently DNA markers [6, 14, 20, 24]. The polymerase chain reaction (PCR) based on molecular markers such as random-amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSRs) are being extensively used to study the genetic diversity in a number of plant species at the species as well as cultivar level [21]. ISSR (inter-simple sequence repeat) is a type of molecular marker, proposed by Zietkiewicz *et al*. [26], for fingerprinting. The ISSR applies the principle of simple sequence repeat (SSR) and anchored polymerase chain reaction (PCR) amplification by designed primers that can randomly

amplify DNA fragments between inversely inverted and closely spaced microsatellite, and the ISSR is simply inherited as dominant and reliable marker system for many organisms. ISSR markers have a high degree of polymorphism, high reproducibility, when compared with other markers, and can achieve results in a timely and cost-effectively manner and prior knowledge on the genome is not required [7, 12]. Therefore, ISSR markers are very useful in studies that aim at determining the genetic distance between parents, as well as the performance of the hybrids for the construction of genetic linkage maps, and for the characterization of accessions and cultivars of several species [19, 22].
ISSR markers have been used for genetic diversity analysis in soybean by many workers [17, 4, 1, 5].
In the present work, we have applied ISSR markers for characterization and assessment of genetic variability in selected 24 soybean genotypes and to determine the phylogenetic relationship among them.

MATERIAL AND METHODS

In present study, twenty four genotypes of soybean were procured and investigated (Table-1). Young fresh and healthy leaves from different genotypes were used as a source material for DNA extraxtion.DNA extraction was done following the cetyl trimethyl ammonium bromide (CTAB) method [9].The extracted DNA was analysed on 0.8% agarose gel and was diluted to an optimum concentration for polymerase chain reaction (PCR) analysis. A total of 20 ISSR primers were screened, out of which 19 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 time intervals during denaturation, annealing and elongation steps which affect amplification, banding pattern and reproducibility. Reproducible and clear banding patterns were obtained in a reaction mixture of 20 µl containing 1x reaction buffer, 1 unit of *Taq* DNA polymerase, 200 µM 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 (94^0C) for 5 minutes, Denaturation (95^0C) for 1 minutes, Primer annealing (28.9^0C-55.5^0C) for 1 minute, Primer Extension (72^0C) for 2 minutes (40 cycles), followed by Final Primer Extension (72^0C) for 5 minutes and a hold temperature of 4ºC.
After PCR reaction, the amplified products were separated on 1.2% agarose gel in 1x TAE buffer using ethidium bromide (EtBr) staining dye. Standard markers of 100 bp and 1 kb DNA ladders (Bangalore Genie, India) were used to determine the size of the amplified DNA fragments. DNA fragments were visualized under UV-trans-illuminator and photographed using gel documentation system. On the basis of presence (used as 1) or absence (used as 0) of bands for each primer, scoring of amplicons obtained was done. Only clear and unambiguous bands were scored for banding pattern 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 [18]. A pair wise similarity matrix was generated and the cluster analysis was performed via Un-weighted 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.

RESULT AND DISCUSSION

Twenty ISSR primers were used in the present investigation, out of which ninteen showed amplification. A total of 182 bands were generated of which 177 were found polymorphic *i.e.* 97.25% polymorphism. The total number of amplified bands ranged from 3 (UBC-872 and UBC-878) to 17 (UBC-814) with an average of 9.57 per primer (Table-2).
The polymorphism percentage ranged from as low as 77.78% (UBC-840) to as high as 100% (UBC-810, UBC-811, UBC-813, UBC-815, UBC-817, UBC-818, UBC-822, UBC-824, UBC-826, UBC-834, UBC-836, UBC-845, UBC-848, UBC-854, UBC-872, UBC-873 and UBC-878.) and the average polymorphism was 97.25%. DNA banding profile of individual plant DNA samples from 24 genotypes of *G. max* L. after amplification with ISSR primers are depicted in Fig.1.
Three unique bands were detected in three genotypes *viz.,* PS-1539, SL-955 and NRC-107 with 3 ISSR primers (UBC-814, UBC-818 and UBC-834). All the genotypes gave single number of distinct bands. The size of these unique bands ranged from 200-1800 bp. (Table 3).
The data obtained by using ISSR markers were further used to construct similarity matrix using 'Simqual' sub-programme of software NTSYS-pc. Based on ISSR similarity matrix data, the values of similarity coefficient ranged from 0.27 to 0.89 *i.e.,* 27-89% or genetic diversity ranged from 11 to 73% (Table 4). The average similarity across all the genotypes was found out to be 0.58 showing that the genotypes were highly diverse from each other. Maximum similarity value of 0.89 was observed between genotypes

BAUS-27 and RSC-10-17 followed by KDS-722 and MAUS-609 and RKS-111 and BAUS-27 with a similarity coefficient of 0.86 and 0.85 respectively. Genotypes SL-983 and RVS-2002-22 were found to be genetically diverse with a minimum similarity value of 0.27 followed by KDS-726 and PS-1543, SL-983 and MACS-1410, SL-983 and PS-1543 and RVS-2002-4 and RVS-2002-22 having similarity values of 0.30. Similarily, Yan *et al*. [24] reported large genetic diversity among a wild soybean population from 15 selected ISSR primers, with the similarity coefficient varying from 0.17 to 0.89.

The ISSR cluster tree analysis showed that they could be divided into 3 major clusters at a similarity coefficient of 0.50 (Fig. 2). The cluster I included 12 genotypes *viz.,* KDS-726, DS-3050, PS-1539, DS-2961, JS20-79, KDS-722, MAUS-609, RKS-109, SL-983, AMS-1001, DS-3047 and RVS2002-4 at a similarity coefficient of 0.59. Cluster I could be divided into 4 sub-clusters. In sub-cluster I, KDS-726 and DS-3050 were related to each other at 0.72 similarity coefficient. In sub-cluster II, genotypes DS-2961 and JS-20-79 were related to each other at 0.78 similarity coefficient. In sub-cluster III, genotypes KDS-722 and MAUS-609 were related to each other at 0.86 similarity coefficient. In sub-cluster IV, genotypes SL-983 and AMS-1001 were related to each other at 0.74 similarity coefficient. The cluster II included 4 genotypes *viz*., SL-955, MACS -1419, NRC-107 and NRC-98 at a similarity coefficient of 0.50. Cluster II could be divided into 1 sub-clusters. In this sub-cluster, there were two genotypes *viz.,* MACS-1419 and NRC-107. Both the genotypes were related to each other at 0.60 similarity coefficient. The cluster III included 8 genotypes *viz*., MACS -1410, JS20-53, PS-1543, HIMSO-1685, RVS2002-22, RKS-111, BAUS-27 and RSC10-17 at a similarity coefficient of 0.59**.** Cluster III could be divided into 2 sub-clusters. In first sub-cluster, there were two genotypes *viz.,* HIMSO-1685 and RVS2002-22. Both the genotypes were related to each other at 0.82 similarity coefficient. In second sub-cluster, two genotypes BAUS-27 and RSC-10-17 were related to each other at 0.89 similarity coefficient.

Two and three dimension principal component analysis based on ISSR data (Fig. 3 and4, respectively) showed similar clustering of genotypes as evident from cluster tree analysis. Dice similarity coefficients ranged from 0.59 to 0.82. Cluster I included twelve genotypes *viz.,* KDS-726, DS-3050, PS-1539, DS-2961, JS20-79, KDS-722, MAUS-609, RKS-109, SL-983, AMS-1001, DS-3047 and RVS2002-4. Cluster II included four genotypes *viz*., SL-955, MACS -1419, NRC-107 and NRC-98. Cluster III included eight genotypes *viz*., MACS -1410, JS20-53, PS-1543, HIMSO-1685, RVS2002-22, RKS-111, BAUS-27 and RSC10-17.

In present study, we found that 19 out of 20 primers studied produced 97.25% polymorphism, relatively high proportion compared to previous reports such as Yan *et al.,* [23] (89.23%), Sepehri *et al.,* [20] (81.91%) and Agarwal [1] (77.89%).

The study has confirmed, ISSR markers are rapid, reliable, simple and effective method of detecting polymorphism for assessment of genetic diversity among genotypes. Assessment of genetic variability in soybean genotypes through ISSR markers can be done better as compared to morphological as well as isozyme and RAPD markers. Characterization of soybean genotypes can be done using the banding pattern obtained from ISSR markers. The studied genotypes can be used for further crop improvement programme because of a wide range of genetic diversity was present among selected genotypes.

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

S.No.	Genotypes	Pedigree	Source
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. by 19 ISSR primers.

S. No.	Primer Code	Ta* (°C)	Molecular weight range (bp)	Total no. of bands amplified	Polymorphic bands		PIC**
					Number	Frequency (%)	
1.	UBC-810	42.9	300-1400	10	10	100	0.336
2.	UBC-811	43.3	500-1800	9	9	100	0.312
3.	UBC-813	43.5	400-1600	10	10	100	0.292
4.	UBC-814	41.3	350-2500	**17**	**14**	82.35	0.294
5.	UBC-815	44.9	500-2100	9	9	100	0.362
6.	UBC-817	52.7	300-1600	13	13	100	**0.445**
7.	UBC-818	52.1	300-1600	12	12	100	**0.192**
8.	UBC-822	45.8	300-1800	13	13	100	0.293
9.	UBC-824	49.0	600-1800	8	8	100	0.449
10.	UBC-826	53.3	400-1300	11	11	100	0.261
11.	UBC-834	51.9	200-1100	10	10	100	0.444
12.	UBC-836	43.3	250-1000	7	7	100	0.375
13.	UBC-840	45.8	300-1500	9	7	77.78	0.342
14.	UBC-845	47.7	250-1500	5	5	100	0.375
15.	UBC-848	55.5	250-1500	13	13	100	0.313
16.	UBC-854	48.0	400-1800	10	10	100	0.413
17.	UBC-872	28.9	600-1100	**3**	**3**	100	0.334
18.	UBC-873	45.0	500-1900	10	10	100	0.35
19.	UBC-878	55.9	400-700	**3**	**3**	100	0.359
	Total			**182**	**177**	**97.25**	**0.344**

Ta*=Annealing temperature, **Polymorphic Information Content

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

S. No.	Primer code	Total no. of unique bands	Genotype	No. of unique bands	Size of band (bp)
1.	UBC-814	1	SL_955	1	1800
2.	UBC-818	1	NRC-107	1	1600
3.	UBC-834	1	PS-1539	1	200
Total	**3**				

Table 4: Jaccards similarity coefficient for ISSR profile

	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																							
ASSAAASPS-1539	0.64	1.00																						

												1.00
											1.00	0.86
										1.00	0.68	0.63
									1.00	0.37	0.43	0.45
								1.00	0.58	0.37	0.44	0.43
							1.00	0.41	0.42	0.59	0.79	0.74
						1.00	0.76	0.38	0.39	0.55	0.72	0.71
					1.00	0.65	0.70	0.36	0.44	0.62	0.69	0.66
				1.00	0.49	0.44	0.47	0.52	0.47	0.46	0.43	0.46
			1.00	0.41	0.63	0.63	0.66	0.44	0.42	0.62	0.76	0.73
		1.00	0.75	0.46	0.65	0.76	0.78	0.39	0.40	0.59	0.76	0.76
	1.00	0.77	0.59	0.39	0.57	0.74	0.70	0.36	0.39	0.53	0.63	0.63
1.00	0.57	0.66	0.64	0.49	0.65	0.62	0.63	0.35	0.40	0.61	0.61	0.64
0.64	0.61	0.70	0.67	0.43	0.61	0.67	0.70	0.40	0.40	0.59	0.72	0.74
0.72	0.53	0.57	0.58	0.41	0.52	0.54	0.55	0.35	0.32	0.53	0.56	0.57
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**

								1.00
							1.00	**0.89**
						1.00	0.85	0.79
					1.00	0.70	0.72	0.71
				1.00	0.82	0.73	0.78	0.80
			1.00	0.77	0.74	0.71	0.71	0.72
		1.00	0.69	0.68	0.67	0.68	0.70	0.68
	1.00	0.59	0.61	0.59	0.66	0.55	0.57	0.54
1.00	0.43	0.42	0.35	0.33	0.34	0.36	0.37	0.35
0.43	0.39	0.40	0.40	0.41	0.37	0.43	0.43	0.45
0.46	0.39	0.41	0.38	0.40	0.37	0.45	0.44	0.44
0.39	0.31	0.36	0.33	0.35	0.30	0.43	0.37	0.37
0.45	0.38	0.40	0.36	0.38	0.39	0.37	0.43	0.40
0.61	0.42	0.41	0.34	0.35	0.40	0.34	0.39	0.36
0.47	0.39	0.39	0.35	0.35	0.34	0.41	0.41	0.40
0.41	0.40	0.36	0.39	0.39	0.34	0.37	0.37	0.40
0.37	0.34	0.35	0.33	0.33	0.31	0.40	0.39	0.40
0.55	0.40	0.37	0.38	0.35	0.36	0.40	0.40	0.36
0.41	0.36	0.38	0.36	0.38	0.36	0.39	0.41	0.42
0.42	0.36	0.37	0.35	0.33	0.32	0.39	0.38	0.39
0.35	0.30	0.34	0.30	0.31	**0.27**	0.35	0.33	0.34
0.36	0.37	0.37	0.38	0.37	0.35	0.39	0.39	0.40
0.38	0.44	0.36	0.36	0.37	0.36	0.38	0.39	0.40
0.35	0.36	0.33	0.30	0.31	0.32	0.33	0.31	0.32
NRC-107	**MACS-1410**	**JS20-53**	**PS-1543**	**HIMSO-1685**	**RVS2002-22**	**RKS-111**	**BAUS-27**	**RSC10-17**

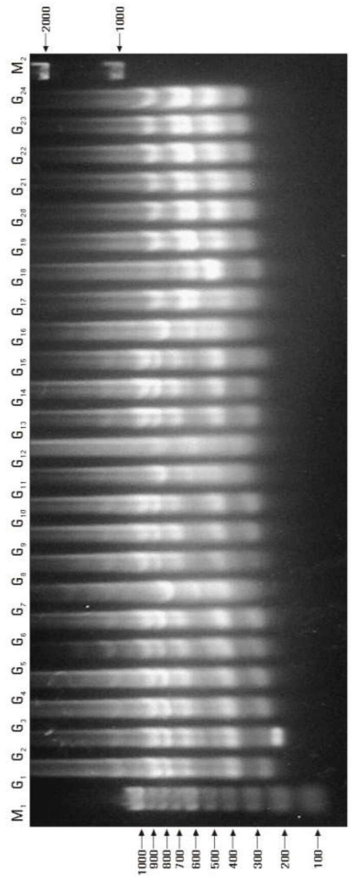

Plate-1 : ISSR profile generated through primer - UBC-834

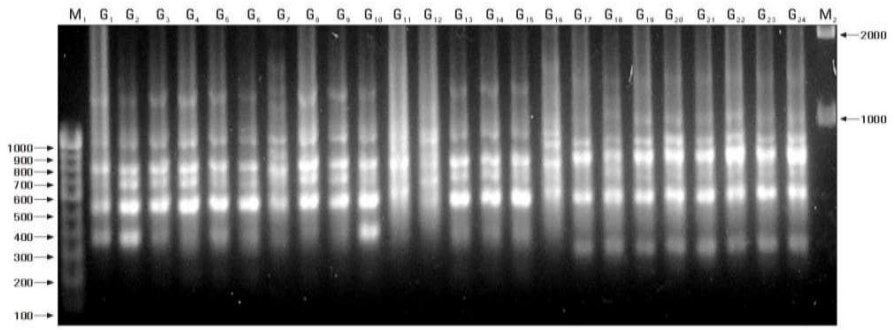

Plate-2 : ISSR profile generated through primer - UBC-840

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. 1 ISSR profile of *Glycine max* L. Merrill generated through UBC-834 and UBC-840 primer respectively

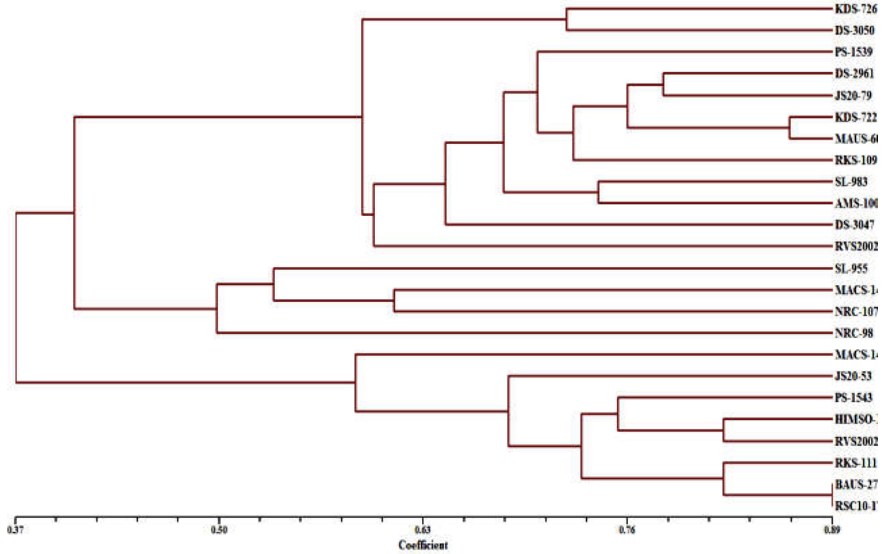

Fig.2: Dendogram constructed with UPGMA clustering method of 24 *Glycine max* L. genotypes using ISSR prmers

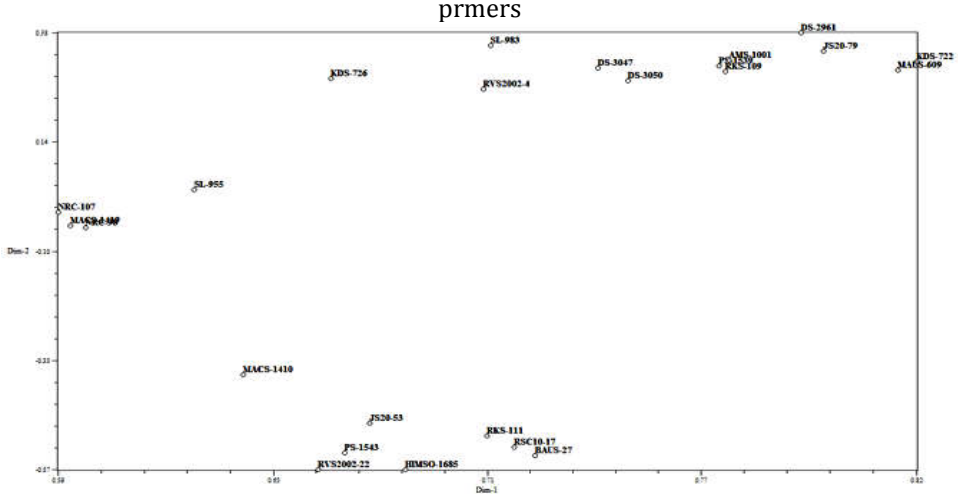

Fig.3: Two dimensional PCA (Principle Component Analysis) scaling of 24 genotypes of *Glycine max* L. using ISSR markers

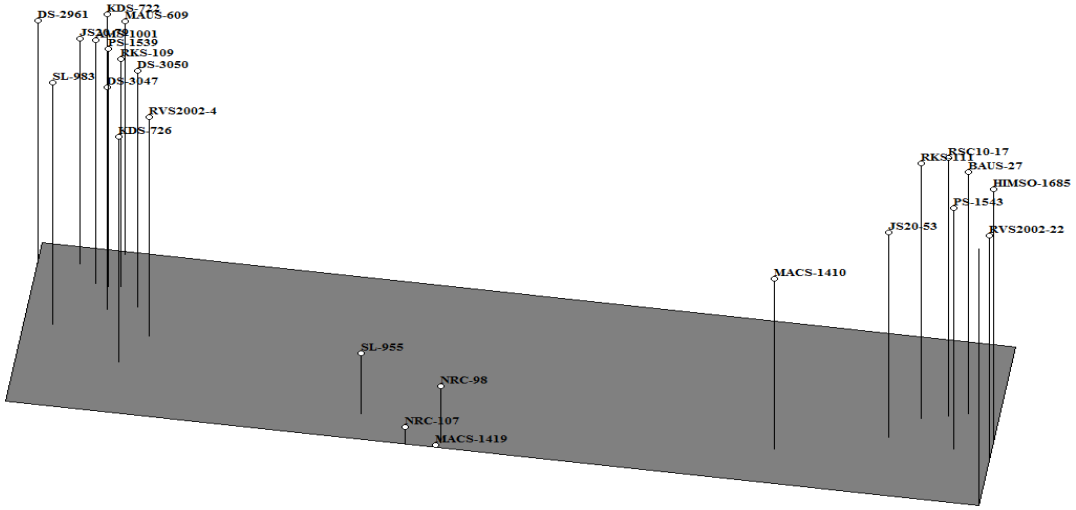

Fig.4: Three dimensional PCA (Principle Component Analysis) scaling of 24 genotypes of *Glycine max* L. using ISSR markers

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