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USE OF PROMOTER-ANCHORED RAPD ANALYSIS FOR DIVERGENCE ASSESSMENT IN SOYBEAN AS AGAINST CONVENTIONALLY USED ISSR ASSAY

Y.S. Mokate[1*], V.P. Chimote[1*], M.P. Deshmukh[2], K. Gawai[3], A.A. Kale[1] and A.S. Jadhav[1]

[1]State level Biotechnology Centre, [3]Department of Agricultural Botany, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722, Maharashtra (India)
[2]Soybean Breeder, Agricultural Research Station, Kasbe Digraj - 416 305, Sangli, Maharashtra (India)
*e-mail: *vivekchimote@rediffmail.com; ysmokate@rediffmail.com*

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ABSTRACT

The present study was aimed to compare the genetic diversity among 24 soybean genotypes as revealed by Promoter-anchored amplified polymorphism based on RAPD (PAAP-RAPD) primer-combinations analysis and Inter Simple Sequence Repeat (ISSR) analysis. All the 16 PAAP-RAPD primer-combinations, derived from 4 Promoter-based primers (TA1/G1/CA1/GC1) and 4 RAPD primers (OPE 1/5/7/9) showed amplification, of which 8 were polymorphic. They produced 63 loci, of which 52 were polymorphic. All 20 ISSR primers used were polymorphic generating 115 alleles of which 95 were polymorphic. The similarity coefficient based on ISSR and PAAP-RAPD analysis ranged from 0.67 to 0.93 and 0.41 to 1.00, respectively. The PAAP-RAPD consensus tree revealed 17 clusters; while ISSR revealed all genotypes as separate clusters. In 2D scatter plot, these were broadly classified into 5 major groups. Group-Ia had lowest divergence (MAUS71, KDS 344, KDS387, NRC94, KDS722, Bragg and KDS743) while Group-Ib had low divergence (Birsa Soya1, KDS749, KDS726, KDS739 and JS335). Moderate divergence was observed in Group II (JS9305 and RKS 18) and Group III (Himso 1684, Dsb22 and MACS450). Group IV (KDS741, VLS65, Rpp3 PI462312 variant, EC241778 and PI462312) had highest divergence. Only two genotypes (KDS753 and DS228) were placed separately in both the analyses. The study revealed that PAAP-RAPD has an advantage that it may specifically target regulatory regions of various candidate genes during molecular tagging for a specific trait.

Key words: Diversity, ISSR, PAAP-RAPD markers, soybean,

INTRODUCTION

Soybean [*Glycine max* (L.) Merill] is the largest oilseed crop grown across the globe. It is a rich source of protein and oil. During plant evolution under domestication, yield of soybean has shown increase but it reduced the genetic base of crops. It is generally presumed that intensive artificial selection has reduced the genetic diversity present in foundation stock. Using only a limited number of introductions from the center of origin would be expected to impose introduction bottleneck, thereby restrict the availability of genetic variation for subsequent creation of elite cultivars. Therefore, it is essential to develop programmes which will increase the genetic base of a crop. Genetic diversity is considered base for biodiversity as well as essential for effective conservation

and utilization of genetic resources of targeted species and population, and is vital to widen the genetic base for crop improvement (Gizlice *et al.*, 1994). Traditional methods of estimating genetic diversity and relationship among plant groups use morphological characters, agronomic information and biochemical variations (Nelson, 1987). Although morphological information is essential in breeding soybean but collection of such information is laborious and time consuming task.

The parents with high diversity give highly variable progenies as reflected through use of markers including molecular markers (Hill *et al.*, 1998). DNA markers are unlimited in numbers and selectively neutral so can be organized in linkage maps (Thormann and Osborn, 1992). Molecular markers have become a novel powerful tool for breeders to search new source of variation and investigate genetic factors controlling quantitatively inherited traits. Molecular markers like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), inter simple sequence repeat (ISSR), etc. have become important in cultivar identification, genetic relationship, distinctiveness and diversity analysis (Patterson *et al.*, 1996; Tara Satyavathi, 2006). The information on diversity pattern allows breeders to understand evolutionary relationship amongst the accessions while genetic relationship and distinctiveness of a cultivar facilitates selection of less related germplasm for inter-crossing which ensures sustainable breeding programme.

Presently many alternative molecular marker techniques have been developed with an inclination towards gene-targeted functional markers (Gupta *et al.*, 2004). Targeted marker techniques involve incorporation of gene or promoter elements in primers (Poczai *et al.*, 2013). They are of five major groups i.e. conserved DNA and gene family based markers; transposable element based markers; resistance-gene based markers; RNA-based markers; and targeted fingerprinting markers (Poczai *et al.*, 2013). Targeted fingerprinting markers (TFMs) take advantage of increasing knowledge of genomics to improve their sensitivity and resolution. Anchoring elements (e.g. gene promoters or start codons) are added to various parts of primers to ensure directed amplification of gene-related regions or sites flanking the targeted region. They include DALP-direct amplification of length polymorphism (Desmarais *et al.*, 1998), PAAP-Promoter anchored amplified polymorphism (Pang *et al.*, 2009), SRAP-sequence-related amplified polymorphism (Li and Quiros, 2001), TRAP-targeted region amplified polymorphism (Hu and Vick, 2003), CoRAP-conserved region amplification polymorphism (Wang *et al.*, 2008) and SCoT-start codon targeted polymorphism (Collard and Mackill, 2009). Pang *et al.* (2009) developed PAAP-RAPD technique that targeted the regulatory regions by designing degenerate promoter primers based on conserved core promoter sequences in combination with RAPD primers. The PCR fragments are generated from three different sources i.e. RAPD primers, promoter primers and their combination. Those amplified by latter two types are interesting because of the possibility to find polymorphisms from potential promoter regions in the genome.

Characterization of crop by markers reveals similarities and diversity among cultivars of a crop which also helps in identifying gene pool or origin of a cultivar. For development of elite cultivars, the genetic base has to be enlarged. The species whose gene pool is identified through markers are used for developing elite cultivars by exchanging germplasm. The use of markers characterizes and develops a DNA profile. These DNA profiles of crops are used in management of genetic resources of a crop in gene bank. DNA profiles help in selection of distant parent for obtaining higher genetic variation. Conventional arbitrary primers based markers have proved to be of little use in tagging and revealing molecular mechanism governing a particular trait. PAAP-RAPD markers are highly abundant and moderately polymorphic; and can be easily visualized using normal agarose gel unlike other targeted fingerprinting markers that require high resolution PAGE gel electrophoresis. PAAP-RAPD being more targeted could be more suited for molecular tagging of particular trait. In present study efforts were made to compare the conventional ISSR marker with more targeted PAAP-RAPD markers as well as to assess the diversity in soybean genotypes studied.

MATERIALS AND METHODS

Materials

The soybean bean genotypes were obtained from Soybean Breeder, Agricultural Research Station, Kasbe Digraj, Dist-Sangli, Maharashtra (India). The pedigree of these 24 genotypes is as under:

S. No.	Genotypes	Pedigree
1	MAUS-71	JS71-05 × JS87-38
2	KDS-344	JS-335 × EC-241780
3	KDS-378	JS71-05 × JS87 -38
4	NRC-94	Ankur × PK-1024
5	KDS-722	AMS-9933 × EC-241780
6	KDS-726	JS-9305 × EC-241780
7	Birsa Soya	Mutant of Sepaya black
8	KDS-743	JS-9305 × AMS1
9	KDS-749	NRC-80 × JS-9305
10	KDS-754	JS-9305 × AMS1
11	KDS-739	JS-9305 × EC-241780(Pubescent)
12	JS-335	JS78-77 × JS71-05
13	JS-9305	Selection from PS 73-22
14	DS-228	JS-335 × PI-462312 (Ankur)
15	KDS-753	JS-9305 × EC-241780
16	RKS-18	MACS 450 × Monetta
17	HimSo-1684	SL284 × Bragg
18	Dsb-22	JS-335 × EC-241780
19	MACS-450	Bragg × MACS111
20	KDS-741	JS-9305 × EC-241780
21	VLS-65	JS-9305 × EC-241780
22	Rpp3 PI 462312 variant	Rpp3 resistance gene donor from Indian variant
23	EC 241778	Introduction from USA
24	PI 462312.1	*Rpp3* resistance gene donor from India

For genomic DNA isolation, the seeds of each genotype were planted in plastic pots and healthy leaves collected from individual plants 15 days after sowing.

Isolation of genomic DNA from leaves

The genomic DNA was extracted from 100 mg leaves as per modified CTAB protocol (Keim *et al*., 1988). DNA pellets were dissolved in 100 µL TE elution buffer and their concentration measured by UV visible spectrophotometer (Nanodrop, USA) at 260 and 280 nm and by 0.8% (w/v) agarose gel electrophoresis along with known amount of λphage DNA. Based on this, all DNA samples were diluted to 20 ng μL^{-1} with TE buffer.

Primers used for PCR amplification

Twenty ISSR primers and 16 degenerate promoter-RAPD decamer primer-combinations were used for PCR amplification (Table 2 and 3). Gradient PCR amplification for different gene-specific primers was carried out to optimize annealing temperature of each primer.

DNA amplification by ISSR primers

The amplification of 20 ISSR primers was conducted in 20 µL reaction mixture having 200 µM of each dNTP, 1 unit Genei *Taq* polymerase, corresponding to 1X buffer A (with 1.5 mM $MgCl_2$), 20 picomole ISSR primer and 40 ng template DNA. The reaction mixture was subjected to PCR profile of 40 cycles (at 94°C for 30 sec., annealing temperature as per primer for 30 sec. and 72°C for 90 sec.) and final extension at 72°C for 10 min. in a thermal cycler (Eppendorf, Germany). The amplified fragments were electrophoresed on 1% (w/v) agarose gel and visualized in gel documentation system (FlorChem™ Alpha Innotech, USA).

PAAP-RAPD analysis with degenerate promoter-RAPD primer combinations

PCR amplification with 16 degenerate promoter-RAPD decamer primer combinations was conducted in 20 µL reaction mixture having 200 µM of each dNTP, 1 unit Genei *Taq polymerase*, corresponding 1X buffer A (with 1.5 mM $MgCl_2$), 20 picomoles of degenerate promoter primer, 20 picomoles of decamer RAPD primer, and 40 ng template DNA. The reaction mixture was subjected to PCR profile of 40 cycles (at 94°C for 30 sec., annealing temperature as per primer for 30 sec. and 72°C for 90 sec.) and final extension at 72°C for 10 min. in a thermal cycler. The amplified fragments were electrophoresed on 2.5% (w/v) agarose gel and visualized in gel documentation system.

Data analysis

The clearly resolved PCR amplified bands were scored manually as binary matrix. The polymorphism information content (PIC) value was calculated as $PIC = \Sigma(1-P^2_i)/ n$ where n is the

number of band positions analyzed in the set of accessions and *Pi* is the frequency of i^{th} pattern. The resolving power (Rp) of primers to distinguish accessions was calculated as $Rp = \Sigma Ib$, where Ib is band informativeness, $Ib = 1–[2 \times (0.5–pi)]$ and *pi* is the proportion of accessions containing band *i* (Prevost and Wilkinson, 1999). Diversity analyses were carried out using computer packages NTSYSpc (Rohlf, 1998). The dice similarity coefficients were estimated and used for clustering analysis based on unweighted pair group method using arithmetic averages (UPGMA) to get dendrogram constructed. Principal coordinate analysis (PCO) involved transforming similarity values to scalar products to get Eigenvectors and finally getting its 2-D scatter-plot graph.

RESULTS AND DISCUSSION

Present study was conducted to assay divergence in soybean genotypes using ISSR markers and PAAP-RAPD markers.

ISSR and PAAP-RAPD PCR amplification analysis

On ISSR analysis, all the 20 primers amplified a total of 115 alleles within size of 0.10-0.96 kb. Of these 95 ISSR bands were polymorphic (82.6%) of which 1 was unique. Maximum bands were observed with primer ISSR-805 (9 bands) and least with primer ISSR-885 (3 bands). Seven ISSR primers showed 100% polymorphism with PIC values varying from 0.26 to 0.69. Ten ISSR primers were highly informative with PIC values $\geq$0.50; while other ten ISSR primers were moderately informative with PIC values of 0.26-0.50. The number of ISSR bands/genotype ranged from 69 (BirsaSoya-1) to 89 (DS-228) (Table 1). Similar observations on ISSR analysis in soybean have been previously reported (Saxena *et al*., 2009; Baloch *et al*., 2010; He *et al*., 2011).

On PAAP-RAPD analysis, amplification was observed in all the 16 RAPD-degenerate promoters based primer combinations (though some of them did not amplify in all the samples studied) with 14 combinations showing polymorphism. A total of 63 bands in the size of 0.101-0.78

Table 1: Sequences and fixed optimum annealing temperature for ISSR primers

ISSR primers	Sequences of primers (5'-3')	T_{ann} (°C)	No. of bands	Polymorphic bands	PIC value	Rp value	Size of loci (kb)
P805	CACACACACACAGC	50	9	9	0.51	12.08	0.11-0.40
P808	AGAGAGAGAGAGAGAGC	50	6	4	0.45	8.58	0.15-0.90
P812	GAGAGAGAGAGAGAGAA	48	7	7	0.69	6.32	0.10-0.41
P816	GCAGAGAGAGAGAGA	45	4	4	0.69	3.82	0.12-0.37
P817	CACACACACACACACAA	48	5	4	0.58	5.16	0.13-0.50
P825	ACACACACACACACACACT	48	6	5	0.54	10.00	0.13-0.47
P827	ACACACACACACACACACG	50	6	4	0.43	8.32	0.33-0.96
P829	TGTGTGTGTGTGTGTGC	50	7	5	0.43	9.82	0.31-0.91
P834	AGAGAGAGAGAGAGAGYT	50	7	5	0.33	10.74	0.10-0.41
P840	GAGAGAGAGAGAGAGAYT	50	5	3	0.27	8.32	0.10-0.43
P841	GAGAGAGAGAGAGAGAYC	52	4	3 (1 unique)	0.40	5.4	0.10-0.28
P842	GAGAGAGAGAGAGAGAYG	52	7	7	0.64	6.82	0.10-0.45
P856	ACACACACACACACACYG	52	5	4	0.62	4.5	0.13-0.54
P857	ACACACACACACACYC	45	5	5	0.44	6.16	0.10-0.58
P868	GAAGAAGAAGAAGAAGAA	45	6	5	0.26	10.16	0.14-0.48
P880	GGAGAGGAGAGGAGA	48	6	5	0.34	9.5	0.10-0.48
P885	BHBGAGAGAGAGAGAGA	50	3	3	0.60	3.66	0.10-0.23
P888	BDBCACACACACACACA	50	5	5	0.59	5.74	0.10-0.35
P889	DBDACACACACACACAC	49	8	5	0.26	13.32	0.12-0.59
P891	HVHTGTGTGTGTGTG	49	4	4	0.53	5.4	0.10-0.45

IUPAC code for mixed bases for degenerate primers: Y = C/T; H = A/C/T; V = A/C/G; D = A/G/T; B = C/G/T

Table 2: Sequences of primers and fixed optimum annealing temperature for PAAP-RAPD primers

RAPD primers (5'-3')	Degenerate promoter primers (5'-3')	T_{ann} (°C)	Total bands	Polymorphic loci	PIC value	Rp value	Fragment size (kb)
OPE 9 (CTTCACC CGA)	G1 (GCCACSTGTC)	28	5	3	0.66	4.8	0.11-0.46
	GC1 (NNNGGGCGGN)		2	1	0.62	1.82	0.13-0.28
	CA1 (YRRCCAATWSR)		4	4	0.88	2.08	0.20-0.61
	TA1 (CTATAWAWASM)		5	5	0.93	2	0.31-0.58
OPE 7 (AGATGCA GCC)	G1	46.6	6	5	0.68	5.8	0.11-0.35
	GC1		2	2	0.62	1.9	0.12-0.29
	CA1		4	2	0.97	1	0.28-0.45
	TA1		1	0	0.91	0.58	0.29-0.35
OPE5 (TCAGGGA GGT)	G1	30.4	3	3	0.30	4.58	0.12-0.48
	GC1		5	5	0.81	3.58	0.15-0.69
	CA1		7	7	0.94	3.16	0.20-0.49
	TA1		6	6	0.91	3.08	0.21-0.67
OPE1 (CCCAAGG TCC)	G1	17	4	3	0.82	3	0.13-0.39
	GC1		4	4	0.92	2.25	0.29-0.78
	CA1		2	0	0.93	0.32	0.25-0.49
	TA1		3	2	0.95	0.4	0.50-0.70

IUPAC code for mixed bases for degenerate primers: M = A/C; R = A/G; W = A/T; Y = C/T; S = C/G; N = A/C/G/T

kb were generated, out of which 52 loci were polymorphic (81.96% polymorphism) and 3 were unique (Table 2). Each primer thus produced on average 3.12 polymorphic bands. Among the PAAP-RAPD primers, OPE-5 + CA1 produced maximum number of 7 loci followed by 6 bands with two primer combinations (OPE-7 + G1; OPE-5 + TA1) and 5 bands with 3 primer combinations (OPE-9 + TA1; OPE-5 + GC1; OPE-9 + G1). However, least number of loci was amplified by OPE-7 + TA1 primer (1 loci). Eight primer combinations showed 100% polymorphism. The identified polymorphic primer combinations could be used for molecular tagging of economic trait of interest. The number of bands observed/ genotype ranged from 41 (VLS-65) to 49 (Rpp3 PI-462312 variant).

Genetic diversity analysis by ISSR markers

Low divergence was observed in soybean genotypes based on their genetic dissimilarities in ISSR analysis with pair-wise similarity values ranging from 0.67 to 0.91 and mean similarity coefficient of 0.80. Minimum similarity value of 0.67 was observed between two genotype combinations *i.e.* NRC-94 and EC-241778; and KDS-722 and VLS-65, followed by BirsaSoya1 and HimSO-1684 (0.70) and KDS-378 and HimSO-1684(0.71). Maximum similarity value was noticed between varieties Bragg and KDS-739(0.93); KDS-749 and Bragg (0.92); Phule Kalyani (DS-228) and KDS-753 (0.91), followed by KDS-743 and KDS-749 (0.90) and KDS-726 and KDS-743 (0.90). The study revealed high genetic diversity and high discrimination capacity of ISSR markers to distinguish individual genotypes. It appears that the crossing between soybean varieties having low similarity coefficient and falling in different clusters may result in heterosis.

The ISSR analysis of 24 soybean varieties generated two major and two minor cluster groups as presented in dendrogram (Fig. 1). The first major group I consists of 5 subgroups, Ia (4 genotypes); Ib (5 genotypes); Ic (Birsa Soya-1); Id (JS-335) and Ie (NRC-94). Second major group consisted of 4 subgroups i.e. IIa (3 genotypes); IIb (3 genotypes); IIc (KDS-741) and IId (RKS-18). Minor group III consisted of 3 exotic genotypes (Rpp3 PI-462312 variant, PI-462312 and EC-241778); while Minor group IV had distinctly placed hill genotype VLS-65. The grouping observed in dendrogram was also reflected in their 2D scatter plot placement (Fig. 2). The entire group I genotypes with little divergence were distinguished from rest 3 groups by first component (first axis). Even clustering pattern in dendrogram in groups II, III and IV was reflected in 2D scatter plot. Group IIa/b were placed on different components2nd axis, with group IIc (KDS741) and group IId (RKS18) sharing another quarter component with minor group III (most distinct exotic genotypes)

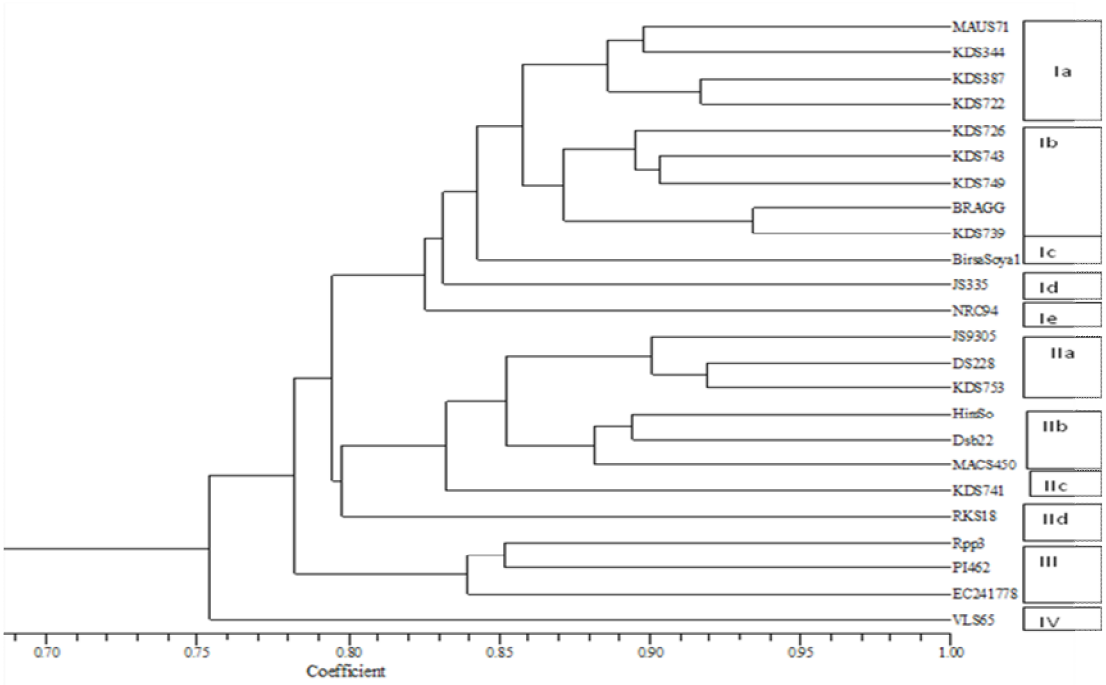

Fig. 1: Consensus tree showing clustering of 24 soybean genotypes using ISSR markers

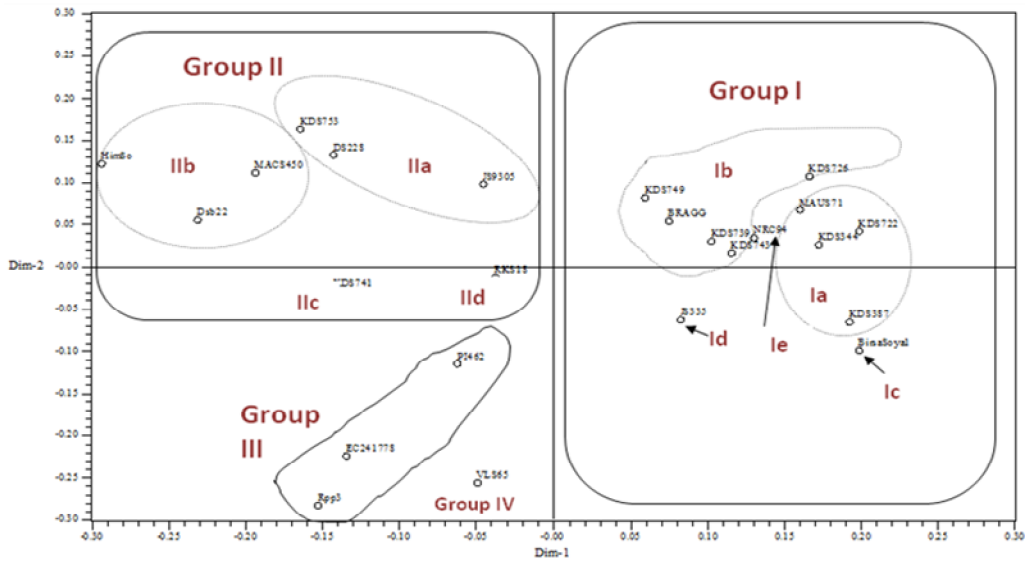

Fig. 2: ISSR analysis derived 2D Scatter plot

and minor group IV (VLS-65). Nine out of 11 local genotypes developed by ARS Kasbe Digraj were placed on same half of 2nd component Y-axis (-0.30); however, if we consider 1st axis 4 and 7 local genotypes were distinguished from each other.

Xie and Yoshihito (2005) reported that ISSR makers could efficiently classify the soybean cultivars from different geographical places. Saxena *et al.* (2009) reported that primer 50 SS was useful to find out the gene(s) resistant to YMV in soybean. Baloch *et al.* (2010) reported narrow germplasm base among the 21 soybean genotypes from Turkey on ISSR analysis. He *et al.* (2011) found similarity coefficient ranging from 0.45 to 0.89 among the 25 Chinese soybean varieties on ISSR analysis. Alamri (2014) reported moderate to low divergence in 108 soybean accessions from 11 different gene pools with both ISSR and RAPD markers.

Genetic diversity analysis by PAAP-RAPD markers

On PAAP-RAPD analysis, the similarity coefficient values ranged from 0.41 to 0.94. Most divergent genotype combinations with minimum similarity value of 0.41 were KDS-743 and VLS-65; and KDS-743 and Dsb-22, followed by KDS-749 and PI-462312(0.51) and NRC-94 and VLS-65(0.55). Maximum similarity value was noticed between genotype KDS-743, Birsa Soya1, KDS-726 and RKS-18 (1.00); KDS-722 and KDS-749 (0.94), followed by MAUS-71 and Birsa Soya1 (0.93) and KDS-726 and DS 228 Phule Kalyani (0.92). The UPGMA based dendrogram of 24 soybean varieties generated 17 clusters in two major groups (Fig. 3). 1st major group consisted of 2 subgroups, Ia (14 genotypes in 7 clusters i to vii) and distinct Ib (JS-9305 and RKS-18). Second major group consisted of four subgroups, IIa(HimSo-1684, Dsb-22 and MACS-450), IIb (KDS-741), IIc (VLS-65) and IId (three genotypes as in ISSR analysis, Rpp3 PI-462312 variant, EC-241780 and PI-462312).

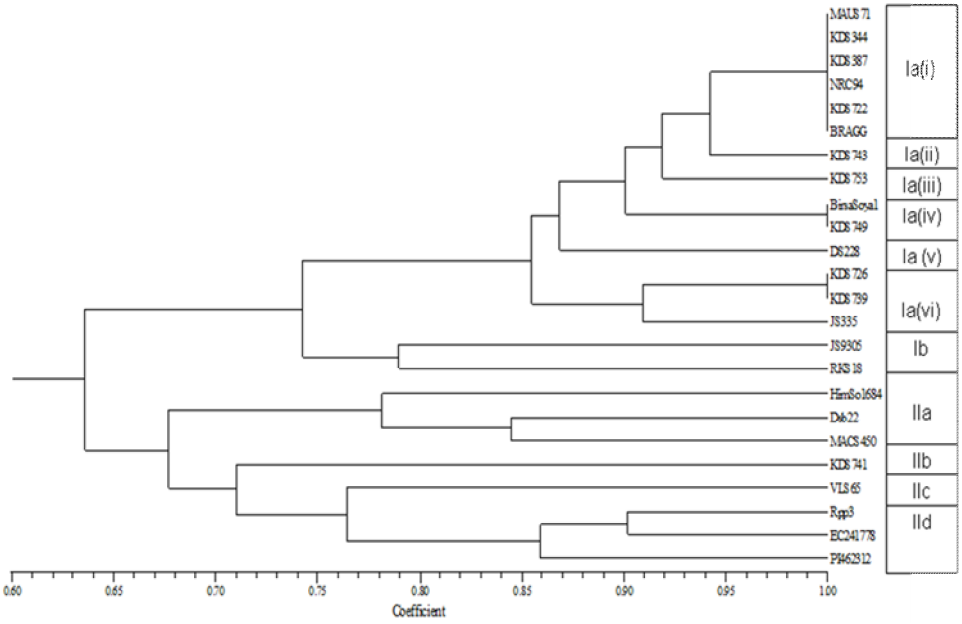

Fig. 3: Consensus tree showing clustering of 24 soybean genotypes using PAAP-RAPD markers

The genotypes clustering in dendrogram is reflected in their 2 D scatter plot replacement (Fig. 4). Almost all the group I genotypes (except KDS726 and RKS18) and 2nd group were placed on either side on 1st component (1st axis). Only 6 undifferentiated Ia(i) subgroup genotypes were present in 1st quarter (+/+ for both components); differentiated from rest of subgroup Ia genotypes (except KDS-726) placed in another quarter defined by 1st (0.0 to +0.4) and 2nd axis (0 to -0.4). Subgroup Ib (RKS18 and JS-9305) was placed around centre of 1st axis along with KDS-726 from subgroup Ia(vi). Overall 2nd group was more divergent than 1st group both in dendrogram and 2D scatter plot analysis. The 3rd quarter component comprised of 5 genotypes of subgroups IIb; IIc and IId. In 4th quarter component 3 subgroup IIa genotypes (MACS-450, Dsb-22, HimSo-1684) were observed along with Ib.

Comparative analysis of ISSR and PAAP-RAPD revealed that most divergent genotypes could be further split into separate groups as X (PI-462312, EC-241778, Rpp3 PI-462312 variant, VLS-65), Y (MACS-450, Dsb-22, HimSo-1684) and intermediate group Z (KDS-741 and RKS18) from the rest of group by 1st component. Another divergent group consisted of JS9305; DS228 and KDS-753 went along with them in ISSR analysis but showed placement closer to other genotypes in PAAP-RAPD. Rest of genotypes showed very little genetic divergence in both analysis with slightly divergent genotypes among them being Birsa Soya-1; JS335, KDS-726 and KDS-749. From both these studies, it may be concluded that crossing between soybean varieties having wide divergence

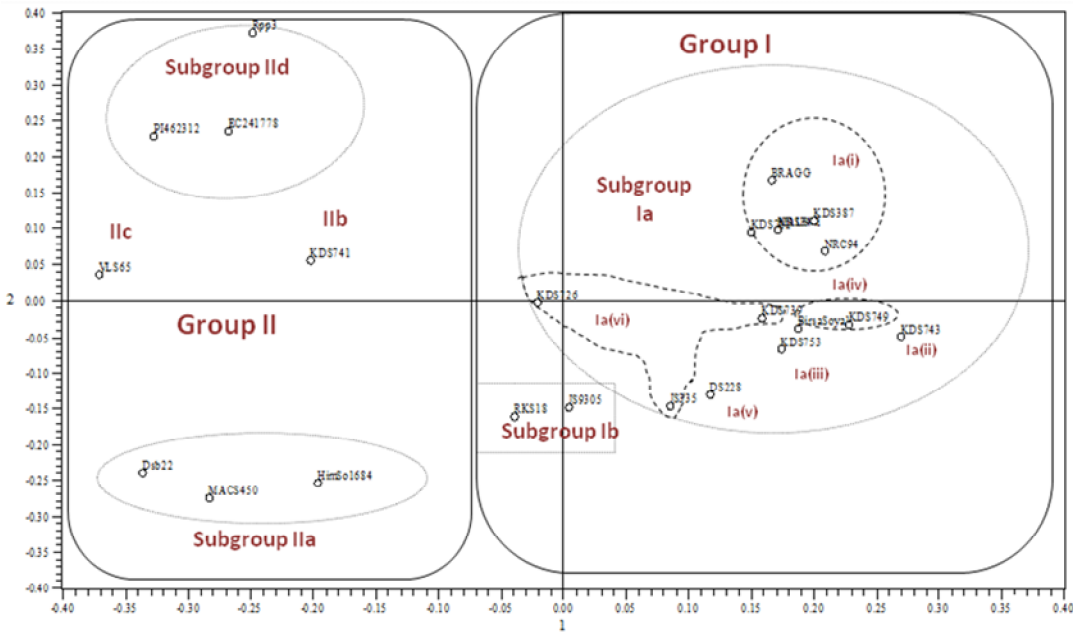

Fig. 4: Promoter-RAPD analysis derived 2 D scatter plot

divergence and that fall in different groups and subgroups on crossing may result in obtaining heterosis. Pang *et al*., (2009) used PAAP-RAPD markers to identify polymorphisms in regulatory regions of the cotton genome. They observed that the PAAP-RAPD derived groupings of forty cotton genotypes were consistent with their genetic and geographical origins. PAAP-RAPD fragments were cloned and their sequence analysis confirmed that they contained an array of *cis*-regulatory sequences in addition to the core promoter sequences (Pang *et al*., 2009).

Conclusion: Molecular analysis of soybean genotypes in relation to diversity assessment using ISSR and PAAP-RAPD analysis revealed high discrimination; with former method being more efficient. All the ISSR primers amplified 95 polymorphic bands with 82% polymorphic loci; with 100% polymorphism in 7 primers. Ten ISSR primers used were informative based on PIC and Rp values. In PAAP-RAPD analysis, all 16 PAAP-RAPD primer combinations yielded polymorphic bands (79.3% polymorphism) with 2 primers (OPE 7+CA1 and OPE 1+TA1) giving unique loci. Together PAAP-RAPD analysis could distinguish only 17 clusters in 24 genotypes studied. Almost similar clustering pattern was observed with both ISSR and PAAP-RAPD primers. The similarity coefficient values for ISSR analysis and PAAP-RAPD indicated large diversity among soybean genotypes. Unique loci produced by ISSR and PAAP-RAPD primers may be variety specific. The ISSR and PAAP-RAPD analysis depicted that soybean genotypes used in present study were moderately divergent. Divergent genotypes identified may be used in future breeding programmes.

REFERENCES

AOAC. 1986. *Official Methods of Analysis.* Association of Official Analytical Chemists. Washington, USA.

Alamri, S. 2014. *Comparative Analysis of Soybean (Glycine max) Accessions Using ISSR and RAPD Markers.* M.Sc. (Biology) thesis. Laurentian University, Sudbury, Ontario, Canada.

Anonymous 2012. FAO STAT data base. Rome, Italy (http//www.faostat.fao.org).

Baloch, F.S., Kurt, C. and Arioglu, H. 2010. Assaying of diversity among soybean and peanut genotypes at DNA level. *Turkish Journal of Agriculture and Forestry*, **34**: 285-301.

Collard, B.C.Y. and Mackill, D.J. 2009 Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter*, **27**: 86-93.

Cox, T.S. 1985. Relationship between coefficient of parentage and similarity indices in the soybean. *Crop Science*, **25**: 529-532.

Desmarais, E., Lanneluc, I. and Lagnel, J. 1998. Direct amplification of length polymorphisms (DALP), or how to get and characterize new genetic markers in many species. *Nucleic Acids Research*, **26**: 1458-1465.

Gizlice, Z., Carter Jr. T.E., and Burton, J.W. 1994. Genetic base for North American public soybean cultivars released between 1947 and 1988. *Crop Science*, **34**: 1143-1151.

Gupta, P.K., and Rustgi, S. 2004. Molecular markers from the transcribed/expressed region of the genome in higher plants. Functional and Integrative, *Genomics*, **4**: 139-162.

He, H., Wei, S. and Zhang, Y. 2011. Genetic diversity analysis of soybean germplasm in Heilongjiang Province by ISSR marker, *Soybean Science*, **1**: 37-40.

Hu J. and Vick B.B.A. 2003. Target region amplification polymorphism: a novel marker technique for plant genotyping. *Plant Molecular Biology Reporter*, **21(3)**: 289-294.

Keim, P., Olson, T.C. and Shoemaker, R.C. 1988. A rapid protocol for isolating soybean DNA. *Soybean Genetic Newsletter*, **15**: 150-152.

Li, G. and Quiros, C.F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theoretical and Applied Genetics*, **103**: 455-461.

Nelson, R.L. 1987. Evaluation of USDA Soybean germplasm collection of maturity groups 000 to IV (PI 273-483 to PI 472-107). *USDA Technical Bulletin*, 17-18.

Pang, M., Percy, R. G., Hughs, E. and Zhang, J. 2009. Promoter anchored amplified polymorphism based on random amplified polymorphic DNA (PAAP-RAPD) in cotton. *Euphytica*, **167**: 281-291.

Patterson, A.H.C. 1996. *Genome Mapping in Plants.* R.H. Lands Co., Austin, USA.

Poczai, P., Varga, I., Laos, M., Cseh, A., Bell, N., Valkonen, J.P. and Hyvönen, J., 20. Advances in plant gene-targeted and functional markers: A review. *Plant Methods*, **9:** 6.

Prevost, A. and Wilkinson, M.J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and Applied Genetics*, **98**: 107-112.

Rohlf, F.J. 1998. NTSYS-PC: Numerical taxonomy and multivariate analysis system, version 2.0 (Exeter Software, New York)

Saxena, P., Singh, K., Bhatt, U. and Khanna, V. 2009. Identification of ISSR marker for the resisance to yellow mosaic virus in soybean (*Glycine max* (L.) Merrill). *Pantnagar Journal of Research*, **7(2)**: 166-170

Tara Satyavathi, C., Bhat, K.V., Bharadwaj, C, Tiwari S.P., Chaudhary V.K. 2006. AFLP based DNA profiling and genetic diversity assessment of Indian soybean [*Glycine max* (L.) Merrill] varieties. *Genetic Resources and Crop Evolution*, **53**: 1059-1069.

Thormann, C.E. and Osborn, T.C.1992. Use of RAPD and RFLP markers for germplasm evaluation of application of RAPD technology to plant breeding. *Plant Breeding*, **1**: 470-490.

Wang, Q., Zhang, B. and Lu Q., 2008. Conserved region amplification polymorphism (CoRAP), a novel marker technique for plant genotyping in *Salvia miltiorrhiza*. *Plant Molecular Biology Reporter*, **27**: 139-143.

Xie, F. and Yoshihito T. 2005. Phylogenetic analysis of soybean (*Glycine max* (L.) Merr.) cultivars from different regions through ISSR marker. *Soybean Science*, **3**: 161-165.