Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (8):3825-3837
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Comparative study of genetic diversity in Indian soybean (*Glycine max* L. Merr.) by AP-PCR and AFLP

Prema Kondetti[1], Narendra Jawali[2], S. K. Apte[2] and Mahadev Ganpat Shitole*[1]

[1]Department of Botany, University of Pune, Ganeshkhind, Pune, India
[2]Molecular Biology Division, Bhabha Atomic Research Centre, Trombay, Mumbai, India

---

ABSTRACT

A total of six AP-PCR and AFLP primer combinations were used to study the genetic diversity in 55 Indian soybean varieties. The average numbers of amplified bands per assay unit were significantly ($p<0.1$) high for AFLP (121) than AP-PCR (82). The average number of polymorphic bands for AFLP (27.1) was significantly ($p<0.1$) higher than AP-PCR (17.6). The average polymorphism percentages for AP-PCR and AFLP were comparable. However, the average marker indexes of AFLP (8.5) primer combinations were significantly ($p<0.1$) higher as compared to AP-PCR (5.8), the high index was mainly attributed to high average number of bands, while the PIC values per assay unit of AFLP and AP-PCR were comparable. The study revealed that AFLPs are more efficient at revealing polymorphic loci than AP-PCR technique. Mantel's tests for correlation using Jaccard's similarity matrices between AP-PCR with combined AP-PCR+AFLP data and AFLP with combined AP-PCR+AFLP data was carried out and r = 0.8 and 0.9 were obtained respectively indicating strong significant correlation. The clustering of varieties in dendrogram generated by UPGMA analysis indicated no trend with respect to pedigree and common parentage analysis indicating that, two varieties derived from same cross might be diverse if the selection pressure operating is different. Over all genetic diversity in Indian soybean varieties was moderate, as revealed by AP-PCR, AFLP and AP-PCR+AFLP data.

Key words: Genetic diversity, Indian soybean, AP-PCR and AFLP

INTRODUCTION

The soybean (*Glycine max* (L.) Merr.), rich in seed protein (range 30-48%, average 40) and oil (range13-22% average 20%), is an economically important crop for feed, oil and soybean products [1]. Soybean is ranked number one in world oil production (48%) in the international trade market. Soybean is a world crop cultivated widely in the United States, Brazil, Argentina, China and India. The United States is the leader in soybean production. In India, soybean is important oilseed crop after groundnut, rapeseed and mustard. At present, it occupies 6 million hectare with a production of about 6 million tonnes. From a total of 7.28 million tonnes of edible oil per year soybean accounts for about 0.6 million tonnes.

Genetic diversity in the germplasm is an important component of plant improvement programme. Accurate estimation of level and pattern of diversity can be invaluable in crop breeding for diverse applications for example, in selection of diverse parents for genetic crosses [2] and introgressing of desirable genes from diverse germplasm into available genetic base [3]. The genetic diversity has been traditionally assessed by analysis of morphology or

biochemical traits. The assessment of phenotype may not be reliable measure of genetic difference since, the environment influences the phenotypic traits. Hence, molecular methods for measuring genetic diversity are nowadays preferred over traditional methods. Presently a large number of molecular marker methods are available.
In our study we have used AP-PCR and AFLP techniques to assess the genetic diversity. AP-PCR involves the use of single long primer of 10-50 bp, there by generating discrete amplification pattern. In comparison to RAPD, AP-PCR often produces reproducible band since amplification occurs at more stringent conditions. AP-PCR has been used to analyze genetic diversity in many plants, for example in *Populus* [4], olive [5], rice [6], mungbean [7], and also in cultivated soybean [8]. AFLP markers are genomic restriction fragments detected after selective PCR amplification [9]. The major advantage of AFLP marker is the generation of multiple marker bands in single assay. In addition AFLP markers were shown to be highly reproducible [10]. AFLP has been used in many plant species for the study of genetic diversity. For example in *Hordeum* [11], *Lens* [12], *Vitis vinifera* [13], *Camellia* [14], *Lactuna* [15], and *Miscanthus* [16]. In soybean Powell et al. [17], Maughan et al. [18], Ude et al. [19] and Satyavathi et al. [20] used AFLPs to study genetic diversity.

In India, there are over 72 soybean varieties released from different breeding stations since 1960 and they are adapted to grow in wide range of geographical zones. The low genetic diversity in soybean is one of the reasons why soybean varieties have made only modest advances in yield for last 20 years [1]. However, Satyavathi et al. [20] reported a very high genetic diversity. Hence, to confirm the extent of diversity among 55 varieties of soybean grown under diverse geographical zones, analysis was carried out by AP-PCR and AFLP markers. The results of genetic diversity analysis by the two methods were compared.

MATERIALS AND METHODS

Plant material

The 55 varieties of soybean used for genetic diversity analysis were obtained from National Research Centre for Soybean (NRCS) Indore, India and Agharkar Research Institute (ARI), Pune, India. The list of varieties used, their parentage, area of adaptation and maturity period are given in Table 1.

Isolation and quantification of DNA

The seedlings were allowed to grow and the tender leaves from 5-7 days old seedlings were used for DNA extraction. Genomic DNA was isolated from leaf tissue (~ 0.5g) by the method described by Nalini et al. [21]. The quantity and quality of DNA was estimated by electrophoresis on agarose gel and comparing the relative intensities of ethidium bromide staining [22] of sample and of standard molecular size marker (Hind III digested λ DNA).

AP-PCR amplification

A total of 30 AP-PCR primers (size/length> 17bp) were screened using two varieties (out of 55) on 2% agarose gel for consistent polymorphic profile, of which 12 that showed good profile were furthest checked on PAGE gel using 20 varieties. Out of these 12, six primers (listed in Table 2) showing 14 or more polymorphic bands were used for genotyping all the varieties (listed in Table 1.). The PCR reaction was carried out similar to Saini et al. [7], except few modifications of 15mM Tris-HCl, pH 9.0; 100 µM each of dNTP, (Banglore genei Pvt Ltd.), and 1.0 unit of *Taq* DNA polymerase (Banglore genei Pvt Ltd.). Amplifications were performed in an Eppendorf Master Cycler gradient (Eppendorf Germany). The PCR cycling conditions were as follows, one cycle at 94^0C, 55^0C and 72^0C for 5min, followed by 35cycles at 94^0C, 45^0C and 72^0C for 1 min and a final extension at 72^0C for 10 min. Amplification products were separated by electrophoresis at a constant power of 50 watts, for approximately 3 hours on 4 or 5 % polyacryamide gel depending on the size of product (molecular marker φX174 *Hae* III) and detected by silver staining.

Table 1. List of Indian soybean varieties analyzed, their parentage, area of adaptation and maturity period.

S No.	Varieties of soybean	Pedigree	Area of adaptability	Duration (Days)
1	ADT-1			
2	Ankur	Single plant selection from a composite of 22 cross	NP	115-120
3	BirsaSoy	Mutant of Sepaya black	NE	106-110
4	Bragg	Jackson×D49-2491	CZ, NP, NH	112-115
5	Co-1	Selection from EC 39821		85-90
6	DS-40			
7	Durga	EC 14437×Bragg		102-105
8	GujratSoy-1	Secetion form Punjab-1 variety		90-95
9	GujratSoy-2	Selection from Geduld variety		105-110
10	HaraSoy	Himso-1520 × Bragg	CZ, SZ	108-130
11	Improved Pelican	Tanloxi ×P.I.60406	SZ	112-115
12	IndiraSoy-9	Selection from JS 80-21	CZ,NE	106
13	JS-2	Selection from Tehri-Garhwal material.	CZ	90-95
14	JS-335	JS-78-77 ×JS 75-1	CZ	95-100
15	JS-39-05			
16	JS-71-05	Selection from Lectype exotic material	CZ	90-95
17	JS-75-46	Improved Pelican× Semmes	CZ	100-106
18	JS-76-205	Bragg × Kalitur	CZ	105-110
19	JS-79-81	Bragg × Harasoy	CZ	102-105
20	JS-80-21	JS75-1×PK 73-49	CZ,NE	90-109
21	JS-90-41	P73-7 × Hark	CZ	90-95
22	KB-79	Hardee ×Monetta	SZ	85-93
23	Lee	S-100 × CNS	NH	105-115
24	Lsb-1	Selection form MACS-330	SZ	70-75
25	MACS-124	JS-2× I.P	SZ	95-105
26	MACS-13	Hampton× EC7034	CZ	90-100
27	MACS-57	JS-2× I.P	SZ	85-100
28	MACS-58	JS-2× I.P	CZ	90-100
29	MAUS-1	Mutant from DS 87-14	CZ,SZ	90-95
30	MAUS-2	Selection from SH 84-14	SZ	105-110
31	MAUS-32	Selection from JS-80-21	CZ,SZ	100-105
32	MAUS-47	PS 73-7 × Hardee	CZ,SZ	90-95
33	MAUS-61-2			
34	MAUS-71			
35	Monetta	An exotic variety EC 2587	CZ,SZ	80-85
36	NRC-12	Bragg mutant (Mutant 95-10)	CZ	96-99
37	NRC-2	Induced mutant of Bragg	NH, CZ	103-106
38	NRC-37	Punjab-1× Gaurav	CZ	96-100
39	NRC-7	Selection from S-69-96	CZ	90-99
40	PalamSoy	JS72-45-1 × Punjab-1	NH	100-115
41	PB-1			
42	PK-1092			
43	PK-308	T-31× Hardee	NH, NP	110-115
44	PK-327	UPSM-82 × Semmes	NH, NP	100-105
45	PK-472	Hardee× Pb.1	CZ	100-105
46	PS-1029	PK-327 × PK-416	SZ	90-95
47	PUSA-16	CNS× Lee	NP, NH, NE	105-115
48	PUSA-22	Punjab × Clark 63	NP, NH, CZ	105-110
49	PUSA-24	Shelby× Bragg	NP, NH, NE	110-115
50	PUSA-37	Bragg Java-16	NP, CZ, SZ	105-115
51	PUSA-40	8-3 × Lee	SZ	110-115
52	RAUS-5			
53	Shilageet	Selection from EC9309	NP, NH	100-105
54	VLS-1	Mutant of Bragg	NH	110-113
55	VLS-21	Selection from VHC 3055	NH	120-122

CZ- central Zone, NE- North Eastern zone, NP-Northern Plain zone, NH-Northern Hill zone and SZ-southern zone.

Table 2. Primers used for AP-PCR analysis			
Sr. No.	**Primer name**	**Sequence**	**No. of base pair**
1	SS9L	5'-TTAATATCACCACCACAC-3'	18
2	SS11L	5'-TGGTATTGTGCGTGTTGA-3'	18
3	SS11R	5'-TCTTCAGCCTCATTGTGC-3'	18
4	SS19R	5'-TGAGACACAGACACAACTCT-3'	20
5	SS24L	5'-TTTAATATCACCACCACACC-3'	20
6	SS26R	5'-CAGGCATAGTGTCACTCTT-3'	19

AFLP amplifications

A total of 35 primer combinations of the nature E_{A+2} and M_{C+2} were used on a set of 20 varieties to select primers yielding high number of polymorphic bands. Among these combinations six that yielded 16 or more bands were used for analysis. AFLP analysis was performed as described by Vos et al. [9]. The preselective and selective amplification were performed according to Vos and Kuiper [23]. The primers for preselective and selective amplifications are given in Table 3. The PCR products were denatured by incubating at 94°C for 3 min, and immediately transferred on chilled ice, which were then separated by electrophoresis at a constant power of 50 watts, for approximately 2 hours, on 4% polyacrylamide gels and detected by silver staining.

Table 3. Details of the adapter and preselective and selective amplification primers		
Primer/Adapter	**Sequence**	**Length (in bp)**
	Double stranded Adapters	
Mse Ia	5'-GACGATGAGTCCTGAG-3'	16
EcoR Ia	5'-CTCGTAGACTGCGTACC-3'	17
	Preselective amplification primers	
E_A	5'-GACTGCGTACCAATTCA-3'	17
Mc	5'-GATGAGTCCTGAGTAAC-3'	17
	Selective amplification primers	**No. of base pair**
$E_{ACA}+M_{CTT}$	5'-GACTGCGTACCAATTCACA-3' + 5'-GATGAGTCCTGAGTAACTT-3'	19 19
$E_{AGT}+M_{CAT}$	5'-GACTGCGTACCAATTCAGT-3'+ 5'-GATGAGTCCTGAGTAACAT-3'	19 19
$E_{ACT}+M_{CAA}$	5'-GACTGCGTACCAATTCACT-3'+ 5'-GATGAGTCCTGAGTAACAA-3'	19 19
$E_{ACC}+M_{CAA}$	5'-GACTGCGTACCAATTCACC-3'+ 5'-GATGAGTCCTGAGTAACAA-3'	19 19
$E_{AGG}+M_{CTG}$	5'-GACTGCGTACCAATTCAGG-3'+ 5'-GATGAGTCCTGAGTAACTG-3'	19 19
$E_{AGG}+M_{CAC}$	5'-GACTGCGTACCAATTCAGG-3'+ 5'-GATGAGTCCTGAGTAACAC-3'	19 19

Data analysis

The AP-PCR and AFLP bands were scored as present (1) and absent (0), each of which was treated as an independent characteristic regardless of its intensity. Polymorphic information content for each marker was calculated using the formula; PIC=1- $\sum Pij^2$ were *Pij* is the frequency of *j*th pattern for marker *i* summed over n pattern. The marker index was calculated as the product of PIC and the number of polymorphic bands per assay unit. All the data analysis was performed using NTSYS-pc (Numerical Taxonomy System, version 2, Rohlf 1990). The SIMQUAL programme was used to calculate the Jaccard's coefficient .The Jaccard's coefficient was calculated using the formula; J= Nab/ (Nab+Na+Nb) where Nab is the number of bands shared by samples, Na is the number of bands in sample a and Nb is number of bands in sample b. Similarity matrix based on these indices were calculated. Similarity matrix was utilized to construct dendrogram by UPGMA (Un weighted Pair Group Method with Arithmetic Average). Correlation between the Jaccard's similarity coefficient matrices obtained with the AP-PCR, AFLP and combined AP-PCR+AFLP was compared by Mantel's test [24]. This test measures the correlation of the matrices and a correlation value (*r*) greater than 0.5 is considered statistically significant at a probability of 0.01, if the number of OUT (Operational Taxonomic Units) exceeds 15 [25]. Principle coordinate analysis was performed in order to highlight the resolving power of the ordination.

RESULTS

AP-PCR analysis

The analysis using six primers yielded a total of 492 scorable fragments (size range 270 to 2000 bp), of which 21.8% were found to be polymorphic (Table 4).

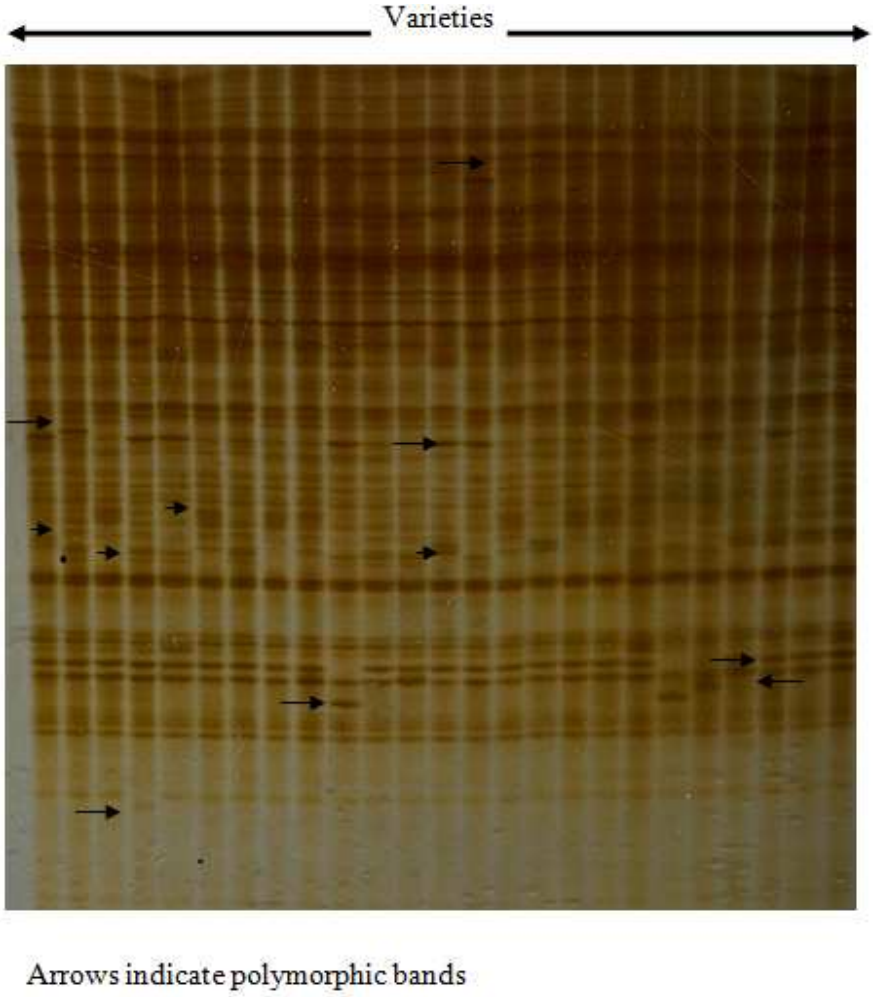
Table 4. Total number of bands, number of polymorphic bands, percentage polymorphism, polymorphic information content and marker index, for AP-PCR and AFLP primers.

Sr. No.	Primer		Amplified bands		Polymorphic band		Percentage polymorphism		PIC		Marker index	
	1	2	1	2	1	2	1	2	1	2	1	2
1	SS9.1	$E_{ACC}+M_{CAA}$	81	148	21	28	25.9	18.9	0.38	0.31	7.95	8.62
2	SS11.1	$E_{ACT}+M_{CAA}$	100	100	14	38	14.0	38.0	0.27	0.30	3.74	11.41
3	SS11.2	$E_{AGG}+M_{CAC}$	73	124	21	21	28.8	16.9	0.31	0.37	6.49	7.68
4	SS19.2	$E_{AGG}+M_{CAC}$	64	112	12	16	18.8	14.3	0.32	0.31	3.89	5.04
5	SS24.1	$E_{ACA}+M_{CTT}$	92	135	23	25	25.0	18.5	0.32	0.28	7.34	7.06
6	SS26.2	$E_{AGT}+M_{CAT}$	82	107	15	38	18.3	35.5	0.37	0.30	5.49	11.21
Total			492	726	106	166						
Average			82	121	17.67	27.17*	21.8	23.7	0.33	0.31	5.82	8.5*

*- Significant at p<0.1, 1-Data for AP-PCR, 2-Data for AFLP.

The number of fragments obtained per primer ranged from 64 to 100 with an average of 82. The number of polymorphic fragments per primer ranged from 12 to 23 with an average of 17.6. The polymorphic information content (PIC) for primers ranged from 0.27 to 0.38 with an average of 0.33. The marker index ranged from 3.7 to 7.3 with an average of 5.8. Fig. 1 shows the fragment profile of some varieties on 4% polyacrylamide gel using primer 24.1.

Fig. 1: Denaturing (4%) polyacrylamide gel showing AP-PCR profiles using primer 24.1



AFLP analysis

AFLP analysis using six primers yielded a total of 726 scorable fragments (size range 190 2000 bp) of which 23.7% were polymorphic. The number of fragments obtained per primer ranged from 107 to 148 with an average of 121. The number of polymorphic fragments per primer ranged from 16 to 38 with an average of 27.1. The polymorphic information content (PIC) ranged from 0.28 to 0.37 with an average of 0.31. The marker index ranged from 5.0 to 11.4 with an average of 8.5.

AP-PCR and AFLP data

The average number of total bands scored by AFLP (121) technique was significantly ($t = 4.2$, $p<0.05$) higher than AP-PCR (82). The average number of polymorphic bands amplified by AFLP (27.1) was significantly ($t = 2.43$, $p<0.05$) higher than AP-PCR (17.6). The average value of percentage polymorphism and PIC for the assay units for both techniques were comparable, whereas the average marker index of AFLP (8.5) was significantly ($t = 2.16$, $p<0.1$) high as compared to AP-PCR (5.8).

Matrix correlation

The correlation between the distance matrices for the varieties obtained by AP-PCR and combined matrix of AP-PCR+AFLP was significant with $r = 0.79$, $p< 0.01$. The correlation between distance matrices of AFLP and combined AP-PCR+AFLP was also significant with $r =0.9$, $p< 0.01$. The correlation between the distance matrices of AP-PCR and AFLP techniques was analyzed and $r = 0.45$, $p< 0.01$ was obtained.

Clustering of varieties

A dendrogram generated by UPGMA analysis using distance matrix obtained by AP-PCR, grouped the 55 varieties into major three clusters at a J value of 0.48 (Fig. 2a). The Jaccard's similarity coefficient ranged from 0.41 to 0.94. Cluster I comprises of 18 varieties. The cluster can be further subdivided into two subclusters Ia and Ib with nine varieties each. Cluster II comprises of 12 and cluster III of 24 varieties respectively. Only one variety Lsb-1 was separate from all other varieties.

The results of PCoA analysis were comparable to the cluster analysis (Fig. 3a). The first three most informative co-ordinates explained 59.5% of total variation. The variety Lsb-1 appears to be distinct from all the other varieties.

A dendrogram based on UPGMA analysis using distance matrix obtained by AFLP, grouped the 55 varieties into five clusters at a J value of 0.56 (Fig. 2b). The Jaccard's similarity coefficient ranged from 0.44 to 0.93. Cluster I comprises of seven varieties. Cluster II is the largest and subdivided into two subclusters IIa, and IIb comprising of 13 and 14 varieties respectively. Cluster III comprises of ten varieties. Cluster IV and V comprises of four and three varieties respectively, four varieties (Ankur, PK-1092, MACS-124 and MACS-58) appear as separate OUTs (Operational Taxonomic Units).

The results of PCoA analysis were comparable to the cluster analysis (Fig. 3b). The first three most informative co-ordinates explained 62.9 % of total variation.

A dendrogram based on UPGMA analysis using distance matrices obtained by AFLP+AP-PCR combined data, grouped the 55 varieties into major three clusters at a J value of 0.50 (Fig. 2c.). The Jaccard's similarity coefficient ranged from 0.46 to 0.94. Cluster I comprises of 24 varieties, which were subdivided into three subclusters of 4, 13 and 7 varieties each respectively. Cluster II is largest with 26 varieties and is subdivided into subcluster IIa and IIb with 4 and 22 varieties each respectively. Cluster III comprises of three varieties and two varieties (Lsb-1and MACS-58) was separate from all other varieties.

The results of PCoA analysis were comparable to the cluster analysis (Fig. 3c). The first three most informative co-ordinates explained 60.7 % of total variation.

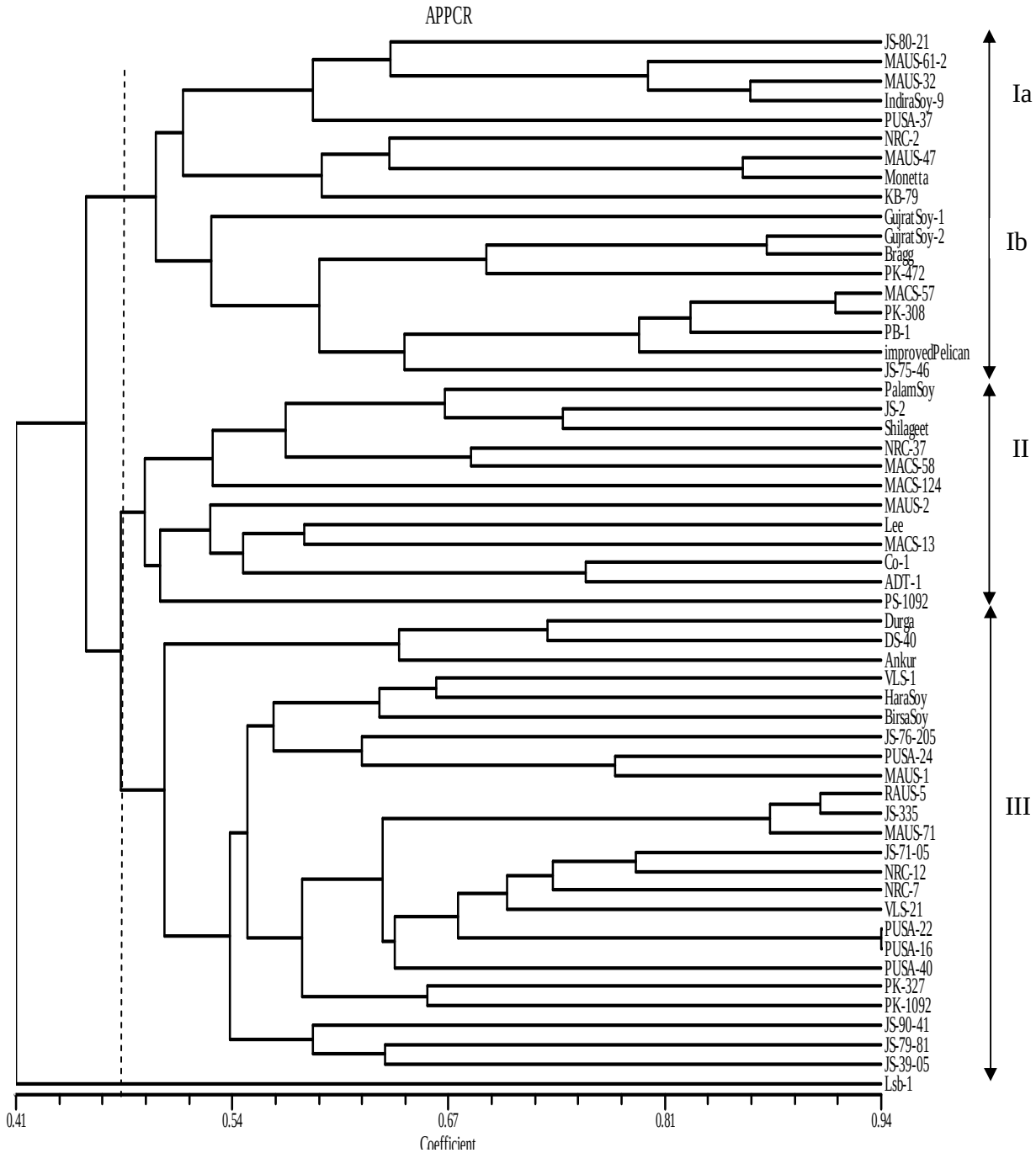


Fig. 2a. Dendrogram generated using UPGMA analysis, showing relationship between varieties, using AP-PCR data.

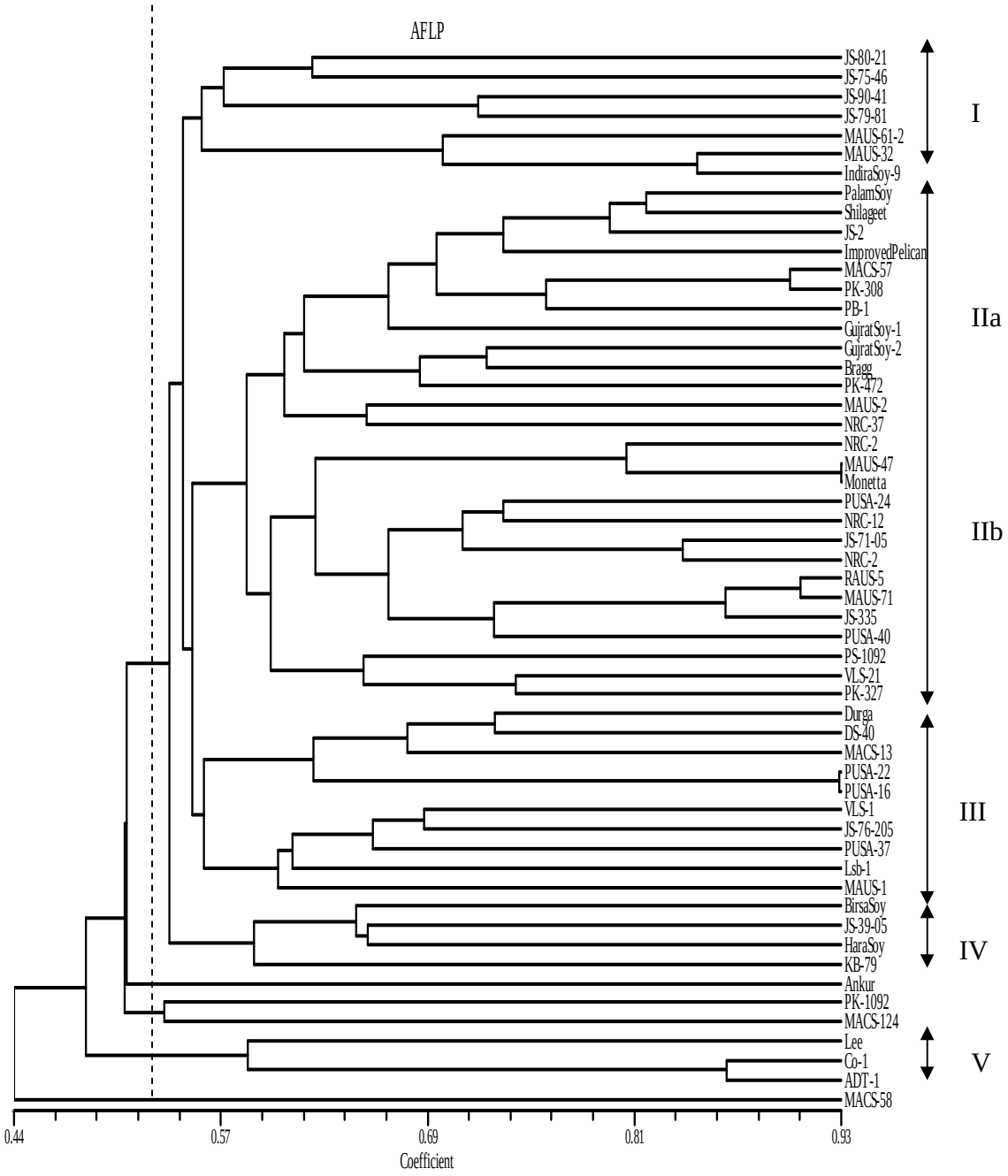


Fig. 2b. Dendrogram generated using UPGMA analysis, showing relationship between varieties, using AFLP data.

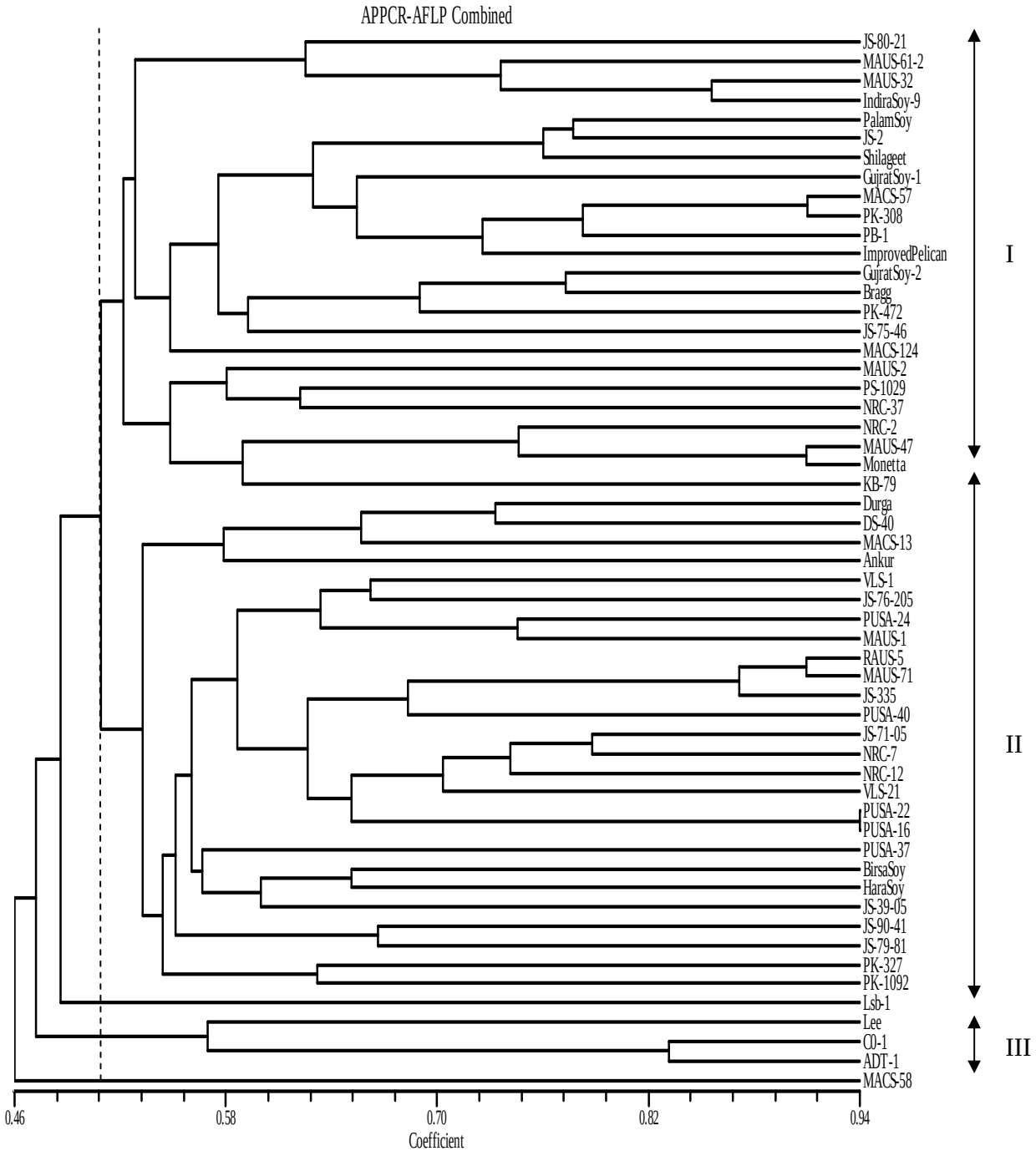


Fig. 2c. Dendrogram generated using UPGMA analysis, showing relationship between varieties, using AP-PCR+AFLP data.

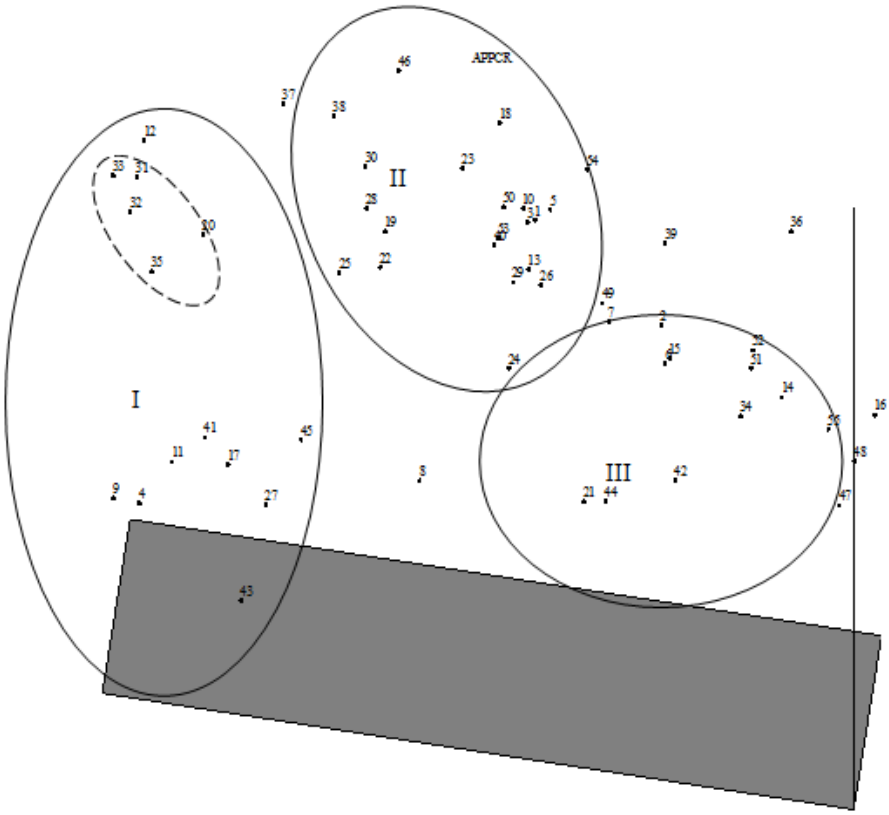


Fig. 3a

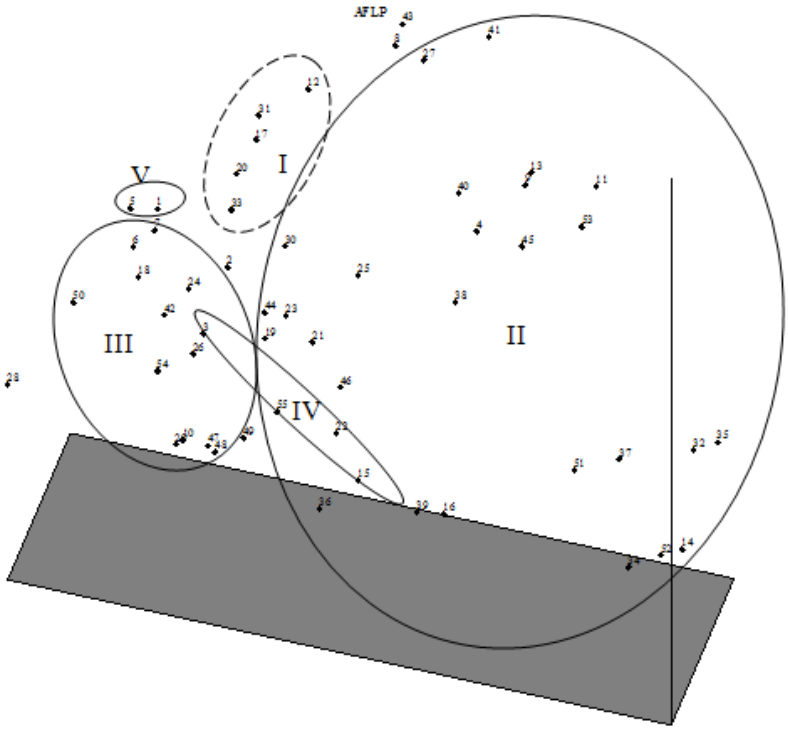


Fig. 3b

DISCUSSION

Polymorphism and marker efficiency

AFLP has been used for diversity analysis by Maughan et al. [18] and Ude et al. [19] among soybean accessions from different countries and they reported an average of 8.5 and 18 polymorphic bands with percentage polymorphism of 17 and 27 respectively. These values are comparable to our results that is, average number of polymorphic bands 27.1 and percentage polymorphism of 23.7 (Table 4). In contrast, Satyavathi et al. [20] reported a range of 51 to 128 fragments per primer with an average of 104 and a percentage polymorphism ranging from 83 to 100 per primer with an average of 95%. The very high percentage polymorphism (95%) reported by Satyavathi et al. [20] could be due to, the method $\pm$ 1 bp tolerance limit set during data scoring using fluorescent primers, analyzed in a sequencer that gives numerous peak corresponding to amplified fragments.

There are two distinct concepts of genetic variation at population level first, richness which corresponds to total number of genotypes or alleles present in the population second, evenness or frequency of different types of alleles in the population [26]. The overall utility of a given marker system is a balance between, the level of polymorphism detected and the extent to which an assay can identify multiple polymorphism [27]. Not much difference in average percent polymorphism between AP-PCR and AFLP was observed, but AFLP detected more number of polymorphic loci with an average of 27.1 in comparison with 17.6 of AP-PCR. The average marker index for AFLP primer combinations was high (8.5) as compared to AP-PCR (5.8), the high index was mainly attributed to high average number of bands, while the PIC values per AFLP and AP-PCR bands were similar. Similar results were reported for barley [28] and wheat [29].

Matrix correlation

The correlation between the matrices of AP-PCR and AFLP is moderate (50%), whereas correlation between the matrices of AP-PCR and AFLP with the combined matrix of AP-PCR+ AFLP was high (r=0.8 for AP-PCR and r=0.9 for AFLP at $p<0.01$). This suggests that both the techniques are essentially leading to consensus, as the data from both the techniques (AP-PCR and AFLP) match with combined data (AP-PCR+ AFLP). Comparable results with respect to genetic similarity estimates by different marker system have been reported in soybean [17] and also in other crops [28, 29]. The range of Jaccard's similarity coefficient by AP-PCR, AFLP and combined AP-PCR+AFLP were comparable that is 0.41 to 0.94, 0.44 to 0.93 and 0.46 to 0.94 respectively.

Clustering of varieties

The pattern of diversity in both AP-PCR and AFLP dendrogram were similar to combined AP-PCR+AFLP dendrogram (reflected in high correlation values). Even though there are some differences among the clustering pattern in dendrogram from AP-PCR and AFLP, it can be seen that among the clusters the grouping of varieties is very similar. Similar grouping of varieties was observed in AP-PCR and AFLP dendrograms (Fig. 2a and 2b) which were as follows; 1) MAUS 61-2, MAUS-32 and Indira Soy-2; 2) PalamSoy, Shilageet and JS-2; 3) MACS57, PK308 and PB-1; 4) Gujrat Soy-2, Bragg and PK-472; 5) NRC-2, MAUS-2 and Monetta; 6) NRC-12, NRC-7 and JS71-05; 7) RAUS-5, JS-335 and MAUS-71, 8) Durga, DS-40 and MACS13; 9) Birsa Soy, and Hara Soy; 10) C0-1 and ADT-1.

According to Loarce et al. [30], larger the number of parameters used to compare individuals genetically the more accurate the estimate of diversity between them. The factor affecting the genetic diversity estimate by different marker techniques is the number of markers used in an analysis [31, 32]. Generally, precision improves as more marker loci are detected in the analysis [33]. Dendrogram constructed using combined data of AP-PCR-AFLP will hence provide better estimate of diversity, as compared to individual dendrogram of AP-PCR and AFLP.

The dendrogram generated by UPGMA analysis using the AFLP+AP-PCR combined data, grouped the 55 varieties into three major clusters at a J value of 0.50 (Fig. 2c). The clustering of theses varieties indicated no trend with respect to pedigree and common parentage analysis indicating that, two varieties derived from same cross might be diverse if the selection pressure operating is different. However, IndiraSoy-9 and MAUS-32 are placed in same sub cluster as that of JS-80-21 and these two varieties are selection from JS-80-21. These three varieties grouped together in all the three dendrograms (AP-PCR, AFLP and AP-PCR+AFLP) and also in all three PCoA plots (Fig. 2a, 2b, 2c, 3a 3b and 3c). The mutant varieties of Bragg that are; VLS-1, NRC-2 and NRC-12 were placed in different clusters indicating changes in genome during mutagenesis. The pattern of clustering obtained in our study carried out by AP-PCR, AFLP and AP-PCR+AFLP amongst Indian varieties is different than reported by Satyavathi

et al. [20]. This can be explained due to difference in, the total number of varieties, techniques used, protocol for AFLP amplifications, primers used and the method of detection. In our method comparative analysis of all the varieties was carried out in a single gel and presence and absence of bands is scored at the same time, in comparison to automated sequencers were samples are loaded sequentially and require stringent conditions to exclude error in each run. In comparison, their detection method using fluorescent primers, is highly sensitive and numerous peaks corresponding to amplified fragments would be detected. To identify real polymorphism and exclude non-significant background the restrictive parameters of $\pm$ 1 bp may not be sufficient for analysis. In this type of analysis fragments that may be significant, but are not very frequent in the genome, are not considered for calculation. Therefore, can carry frequent mistake, similar reasoning is also given by Maluf et al. [34] in study of genetic diversity of *Coffea Arabica*, using fluorescent primers and detection by ABI 377 automated sequencer.

The diversity among soybean varieties was studied by karmakar et al. [35] using phenotypic characters and clustered 41 varieties into six clusters. Seven varieties (Pusa 40, VLS-1, PUSA-16, Ankur, BirsaSoy, PUSA-37 and PK-327) which group in cluster II (Fig. 2c) in our analysis are common to cluster II in analysis by Karmakar et al. [35]. The varieties Lee and Co-1 grouped in cluster III and MACS-57, JS-80-21, GujratSoy-1 and GujratSoy-2 varieties group in cluster I in our analysis similar grouping was reported by Karmakar et al. [35]. The genetic distance for 40 genotypes of soybean collected from different states of India and abroad was estimated using D^2 statistics by Tyagi and Sethi [36]. These genotypes were grouped into six clusters. The overall clustering of varieties in their analysis was different than our analysis, as around 50% of the varieties included in their study were different. However, PUSA-24, PUSA-37 and VLS-21 which grouped in cluster II (Fig 2c) in our analysis are similarly grouped by them. The range of Jaccard's similarity coefficient by AP-PCR, AFLP and combined AP-PCR-AFLP are comparable which were 0.41 to 0.94, 0.44 to 0.93 and 0.46 to 0.94 respectively. The soybean varieties in India were developed from both the introduced varieties and the native local land races [37]. Indian soybean varieties can be grouped into four, first comprising of varieties Bragg, lee, Improved Pelican, Hardee, Monetta and Shilajeet which are direct introductions. Second, comprising of Co-1, Gujrart Soy-1, GujratSoy-2, VLS-2 and JS 71-5, which are direct selections from exotic or indigenous material. Third, comprising of varieties developed by hybridization. Fourth, comprising of varieties developed through mutation breeding [38]. The recent use of local varieties in breeding program is useful in broadening genetic base. Thus, the extent of diversity among the soybean varieties is not very low as reported earlier, but moderate, this could be because of the genetic improvement programmes in India which apart from the introduced varieties (from USA), includes varieties developed by direct selections from exotic or indigenous material [37], hybridization and mutation breeding [38]

Our study indicated that, clustering of these varieties indicated no trend with respect to pedigree and common parentage analysis. Diversity among soybean varieties is not very high as reported recently [20] and not very low as reported earlier [1] but moderate, as revealed by AP-PCR, AFLP and AP-PCR+AFLP data. AFLPs are more efficient at revealing polymorphic loci than other marker techniques, similar to other reports by Sharma et al. [12] in lens, Maguire et al. [27] in mangrove, Russell et al. [28] in barley, Bohn et al. [29] in wheat.

REFERENCES

[1] R. J. Singh, T. Hymowitz, *Genome*, **1999,** 42, 605-616.
[2] B. A. Barrett, K. K. Kidwell, *Crop Sci.*, **1998,** 38, 1261—1271.
[3] J.A. Thompson, R.L. Nelson, L.O. Vodkin, *Crop Sci.*, **1998,** 38, 1348-1355.
[4] O. Torjek, E. Kiss, J. Kiss, M. Kondrak, G. Gyulai, J. Gergacz, L. Heszky, *Acta Biol Hung.*, **2001,** 52, 2-3, 345-54.
[5] F. Kockar, R. Ilikci, *Z Naturforsch [C]*, **2003**, 58, 11-12, 837-42.
[6] Q.M. Yi, W.G. Deng, Z.P. Xia, H.H. Pang, *Hereditas*, **1995**, 122, 2, 135-41.
[7] Saini, K.S. Reddy and N. Jawali, *Indian J Biotech.*, **2004,** 3, 511-518.
[8] Brik, A.F. and Sivolap, IuM. Tsitol, *Genet.*, **2001**, 35, 5, 3-9.
[9] P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. Vandeee, M. Homes, A. Frijers, J. Pot, J. Peleman, M. Kuiper, M. Zabeau, *Nucl Acids Res.*, **1995**, 23, 4407-4414.
[10] C.J. Wyn-Jones, K.J. Edwards, S. Castaglione, M.O. Winfield, F. Sala, C. van de Wiel, G. **B**redemeijer, B. Vosman, E. Matthes, A. Malcevschi, , N. Marmiroli, , R. Aert, G. Volckaert, J. Rueda, R. Linacero, A. Vazquez, A. Karp, *Mol Breed*., **1997**, 3, 381-390.
[11] X. Qi, P. Lindhout, *Mol. Gen. Genet*., **1997,** 254, 330-336.
[12] S.K. Sharma, M.R. Knox, T.H.N. Ellis, *Theor. Appl. Genet*., **1996**, 93, 751-758.

[13] E. Sensi, R. Vignani, W. Rohde, S. Biricolti, *Vitis*, **1996,** 35, 183-188.
[14] S. Paul, F.N. Wachiro, W. Powell, R. Waugh, *Theor. Appl. Genet*., **1997**, 94, 255-263.
[15] M. Hill, H. Witsenboer, M. Zabeau, P. Vos, R. Kesseli, R. Michelomore, *Theor. Appl. Genet.*, **1996,** 93, 1202–1210.
[16] J.M. Greef, M. Deuter, C. Jung, J. Schondelmaier, *Genet. Res. Crop Evol.*, **1997,** 44, 185-195.
[17] W. Powell, M. Morgante, C. Andra, M. Hanafey, J. Vogel, S. Tingey, A. Rafalski, *J. Mol. Breed.*, **1996,** 2, 225-238.
[18] P.J. Maughan, M.A. Saghai Maroof, G.R. Buss, G.N. Huestis, *Theor. Appl. Genet.*, **1996,** 93, 392-401.
[19] G.N. Ude, W.J. Kenworthy, J.M. Costa, P.B. Cregan, J. Alvernaz, *Crop Sci*., **2003,** 43,1858-1867.
[20] C.T. Satyavathi, K.V. Bhat, C. Bharadwaj, S.P. Tiwari, V.K. Chaudhury, *Gene. Res. Crop Evol.*, **2006**, 53, 1069-1079.
[21] E. Nalini, S.G. Bhagwat, N. Jawali, *BARC Newsletter*, **2004,** 249, 208-214.
[22] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular cloning: A laboratory manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, New York **1989,** 2nd edition,
[23] P. Vos, M. Kuiper, In: G. Caetano-Anollés and P.M. Gresshoff, (Ed.). DNA markers: Protocols, Applications and Overviews. (J. Wiley & Sons, inc., New York.) **1997,** pp. 115-131.
[24] N. Mantel, *Cancer Research*, **1967,** 27, 209-220.
[25] F.J. Lapointe, P. Legendre, Sys. *Biol.*, **1992,** 41, 378–384.
[26] O.H. Frankel, A.H.D. Brown, J.J. Burdon, *Crop Sci*., **1995,** 40, 783–791.
[27] T.L. Maguire, R. Peakall, P. Saenger, *Theoretical and Applied Genetics*, **2002,** 104, 388-398.
[28] J.R. Russell, J.D. Fuller, M. Macaulay, B.G. Hatz, A. Jahoor, W. Powell, R. Waugh, *Theor. Appl. Genet.*, **1997,** 95, 714–722.
[29] M. Bohn, F. Utz, A.E. Melchinger, *Crop Scienc,*. **1999,** 39, 228–237.
[30] Y. Loarce, R. Gallego, E. Ferrer, *Euphytica*, **1996,** 88, 107-115.
[31] M.M. Messmer, A.E. Melchinger, W.L. Woodman, E.A. Lee, K.R. Lamkey, *Theor Appl Genet.*, **1991,** 83, 97–107.
[32] O.S. Smith, J.S.C. Smith, S.L. Bowen, R.A. Tenborg, *Maize Genet Coop News Lett*., **1992,** 66, 66.
[33] J.G. Tivang, J. Nienhuis, O.S. Smith, *Theor. Applied Genet.*, **1994,** 89, 259-264.
[34] M.P. Maluf1, M. Silvestrini, L. M. de-Campos Ruggiero, M. Oliveiro, G. Filho, C.A. Colombo, *Sci. Agric.*, **2005,** 62,4, 66-373.
[35] P.G. Karmakar, S.M. Hosiah, P.S. Bhatnagar. *Soybean Genetics Newsletter*, **1998,** 25, 86-87.
[36] S D Tyagi, J. Sethi, *Research Journal of Agricultural Sciences*, **2011,** 2, 2, 288-290.
[37] S.P. Tiwari, O.P Joshi, A.N. Sharma, The advent and renaissance of soybean: a landmark in Indian agriculture. NRCS, Indore, **1999**, 1-58.
[38] P.G. Karmakar, P.S Bhatnagar, *Euphytica*, **1996,** 90, 95-103.