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Molecular characterization and identification of candidate markers for seed longevity in soybean [*Glycine max* (L.) Merill]

Jagadish Hosamani, M. B. Arun Kumar, A. Talukdar[1], S. K. Lal[1] and M. Dadlani*

Division of Seed Science and Technology, IARI, New Delhi 110 012; [1]Div. of Genetics, IARI, New Delhi 110 012

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Abstract

Soybean [*Glycine max* (L.) Merill] genotype varies in storability period during ambient storage. In this study, 33 genotypes of soybean varying in storability (good and poor) and seed coat colour (black and yellow) were characterized with 53 SSR and 51 RAPD markers. Polymorphisms detected by SSR and RAPD markers were 62.26 and 68.62%, respectively. Genotypes with black seed coat colour showed better storability (89.85%) than the yellow seed coated genotypes (71.15%) indicating possible association of black seed coat colour with good storability. Genetic similarity coefficients obtained through SSR data analysis grouped the genotypes into two major clusters representing black and yellow seeded genotypes. SSR markers Satt371, Satt453 and Satt618 produced specific allelic bands making them candidate markers for linkage with seed storability and testa colour. RAPD markers data grouped the genotypes broadly into two major clusters that had no congruence with seed longevity and testa colour.

Key words: Soybean, seed longevity, candidate marker, SSR, RAPD and testa colour

Introduction

Seeds are uniquely equipped to survive as viable regenerative organisms until the time and place are right for the beginning of a new generation; however, like any other form of life, they cannot retain their viability indefinitely and eventually deteriorate and die. Therefore maintenance of seed viability and vigour from harvest till next sowing, which may be for one planting season or more, is crucial for the success of agriculture and crop production. Soybean [*Glycine max* (L.) Merill] seed reaches its maximum potential for germination and vigour at physiological maturity [1], which then gradually declines till harvest, followed by a more rapid decline thereafter. Soybean seed is classified as “poor storer” [2], when compared to other grain crops. Germination is often reduced below the minimum standards prior to planting time under warm and humid or hot climate [3]. Loss of germination potential is more acute in tropical and sub-tropical regions compared to temperate environments [4]. A number of seed characters such as seed size, percent hard seededness, seed coat thickness and permeability, hull percentage, oil content *etc*., are associated with seed quality in soybean and were shown to be under genetic control [5, 6]. These traits are being utilized in breeding programs to improve seed quality and seed longevity in soybean. Verma and Ram [7] reported that two to four genes probably governs seed longevity in soybean.

Significant progress has been made in soybean genomics to target important genes, which has provided a deeper insight into the soybean genome structure and organization. Many reports about the construction of soybean genetic linkage maps using various markers have been published [8, 9]. Based on the construction of soybean genetic maps, quantitative trait loci (QTL) for a number of agronomic traits in soybean have been mapped [10]. Singh *et al*. [11] reported that the four SSR markers (Satt434, Satt538, Satt281 and Satt598) are associated with seed coat permeability and electrolyte leaching in soybean. Another set of four SSR markers (Satt538, Satt285, Satt600 and Satt434) has been reported to be significantly associated with seed longevity in an $F_{2:3}$ soybean population in a cross involving good and poor storer genotypes [12]. A number of RFLP and SSR markers for hard seededness, seed oil concentration,

*Corresponding author’s e-mail: jd_research@iari.res.in
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seed protein and seed sizes having positive associations with seed longevity have been reported in soybean [8, 10]. However, applicability of these markers is yet to be ascertained in the Indian soybean genotypes. Therefore, an attempt was made to characterise a set of good and poor storer soybean genotypes with SSR and RAPD markers. Possible association of testa colour with seed longevity was also studied.

Material and methods

Plant material

The experimental material consisted of 33 soybean genotypes collected from the Division of Genetics, IARI, New Delhi (Table 1). Among these, 14 were black seeded and 19 were yellow seeded genotypes, and varied in their responses to storability.

Seed storage

About 150 grams of untreated soybean seeds were packed in cloth bags and stored under ambient conditions of laboratory with average relative humidity (RH) of 65±5% and temperature of 25±2°C. Samples were drawn after 12 months. The germination and molecular studies were carried as per standard procedures.

Germination test

Germination was tested at 25°C in three replications of 100 seeds each, following the between-paper (BP) method [14]. The germinated seeds were evaluated into normal seedlings, abnormal seedlings, hard seeds and dead seeds. Germination percentage was recorded on the basis of normal seedlings.

Accelerated ageing

The accelerated ageing test (AAT) was conducted under recommended conditions [15, 16] with slight modification of ISTA [14]. Thirty grams of soybean seeds were subjected to an accelerated ageing at 100 per cent RH and 41.3°C temperature in an incubator. The samples were drawn after 96 h and the germination was tested on three replications of 100 seeds for each variety as described above.

Molecular characterization

The genomic DNA was isolated from the overnight hydrated seeds of 33 genotypes using CTAB method as described by Gupta *et al*. [17]. Polymorphism survey was carried out with 53 SSR markers distributed across the soybean genome [9], and had already been reported to be highly polymorphic [12, 18, 19]. On average, 2-3 markers per chromosome covering telomere through centromere were selected. A total of 51 randomly chosen RAPD markers were also used for molecular characterization of soybean genotypes for seed longevity.

Table 1. List of 33 soybean genotypes used in the present investigation

S.No.	Genotypes	Testa colour	S.No.	Genotypes	Testa colour
1	AMSS-34	Black	18	P-250	Yellow
2	DS-74	Black	19	P-732	Yellow
3	DS-MM-64	Black	20	P-871	Yellow
4	G-2253	Black	21	P-876	Yellow
5	G-2265	Black	22	P-884	Yellow
6	G-2601	Black	23	P-898	Yellow
7	G-2603	Black	24	PK-262	Yellow
8	G-2614	Black	25	PK-416	Yellow
9	G-2651	Black	26	PK-472	Yellow
10	M-253	Black	27	TAMS-38	Yellow
11	M-1090	Black	28	EC-13969	Yellow
12	M-11913	Black	29	EC-1023	Yellow
13	TGx444-422	Black	30	EC-34141	Yellow
14	P-761	Black	31	EC-93751	Yellow
15	P-218	Yellow	32	EC-18761	Yellow
16	P-222	Yellow	33	MACS-681	Yellow
17	P-241	Yellow			

SSR amplification was carried out in a 25 µl reaction mixture consisting of 25 ng of template DNA, 0.2 µM each of forward and reverse primers, 400 µM dNTP mix, 10X PCR assay buffer (Banglore Genei), and one unit of *Taq* DNA polymerase (Banglore Genei). The PCR reactions were performed with initial denaturation at 94°C for five min. followed by 35 cycles of denaturation at 94°C for one min, annealing for 30 sec at 55°C, and extension for 30 sec at 72°C with a final extension for seven min. The amplified products were separated on 2.5% agarose gel. Gels were run for 3 h at 75 V in 1X TBE buffer. DNA fragments were visualized under UV light and photographed using gel documentation system.

Polymorphism Information Content (PIC) for each SSR marker was calculated using the following formula of Botstein *et al*. [20].

$$PIC = 1 - \sum_{i=1}^{n} p_{ij}^2$$

Where, 'n' is the number of the marker alleles for marker i, and P_{ij} is the frequency of the j^{th} allele for marker i.

The RAPD amplification was performed as described by Williams *et al.* [21] in a 25 µl containing 25 ng of template DNA, 0.2 µM primer, 400 µM dNTP mix, 10X PCR assay buffer (Banglore Genei, India), and one unit of *Taq* DNA polymerase (Banglore Genei). The PCR reaction was carried out with the initial denaturation at 94°C for five min. followed by 45 cycles of denaturation at 94°C for one min, annealing for one min at 35°C, and extension for one min. at 72°C. The final extension at 72°C was performed for five min. Based on banding pattern obtained, the polymorphism percentages were calculated for different RAPD markers as per Blair *et al*. [22].

Data analysis

The data was analysed by using SAS software package version 9.2 for calculation of Minimum Significant Difference (MSD). All the replicated data was subjected to Tukey's Studentized Range (HSD) Test. DNA fragment profiles representing a consensus of two independent replicates were scored in a binary form with'0' indicating the absence and '1' indicating the presence of the band. Using the binary data, a similarity matrix was constructed using the Jaccard's coefficients, which was further subjected to clustering unweighted pair group method analysis (UPGMA), and a dendrogram was generated. These analyses were performed using the NTSYSPc software package version 2.1.

Results and discussion

Percent germination of freshly harvested seeds was comparable in good and poor storer genotypes. The initial germination percentage and the first count of all the varieties were above 90% (Table 2). In case of good storer genotypes it was 98.5% and 97.29% while in poorer storers it was 96.21% and 95.63%, respectively. After 12 months of ambient storage (25±2°C and 65±5% RH), however, seed germination declined significantly in all genotypes. The reduction was more pronounced in the poor storers than the good storers. In case of good storer genotypes, the mean germination and the first count percentage recorded was 89.85% and 84%, whereas in poor storers it was 71.15% and 64.95%, respectively. Under high temperature and RH conditions of Accelerated ageing (AA), the germination and first count percentage of good storers were significantly higher (81.33% and 56.10%) than poor storer genotypes (56.97% and 18.56%) (Table 2).

It was confirmed in this study that seed longevity data under accelerated ageing (100% RH and 41.3 °C temperature) resembles to that obtained after 12 months of storage. A high and significant correlation (r = 0.7284**) between germination final count after ambient storage, and germination first count after accelerated ageing reaffirmed the validity of the latter as a test [14] to assess the seed quality (vigour). A similar high correlation (r = 0.6521**) was reported by Usha [16]. It was evident that the genotypes differed significantly with respect to storability (Table 2). The black seeded varieties, in general showed better storability. This is in conformity with the reports published earlier [23-25].

SSR analysis

Among the 53 SSR markers used, 33 markers were polymorphic (62.26% polymorphism). The polymorphic markers produced 77 alleles, ranging from two to four alleles per locus, with an average of 2.33. Satt193 and Satt281 had the highest number of alleles (four), and the rests had two to three alleles. PIC values for the markers ranged from 0.0432 (Satt431) to 0.6997 (Satt193) with an average of 0.3709 (Table 3). Polymorphism in Indian soybean in general, is low. However, a higher level of polymorphism was observed in the study. It might be due to the diverse genotypes used in the study. The genotypes varied not only for testa colour and longevity, but for other morphological traits as well. The number of alleles per marker locus as well as their PIC values also reflected the variability present in the genotypes. Such variability offers scope of selection for genotype with good storability and other important traits.

Candidate markers for seed storability

A few SSR markers clearly discriminated the two groups of genotypes *i.e*. good storer and poor storers (Table 3). Satt371 produced two alleles *viz*., $Satt371_{160}$ and $Satt371_{180}$. Allele $Satt371_{180}$ was present only in the good storer genotypes while $Satt371_{160}$ was present in 18 of the 19 poor storers. Thus the allele $Satt371_{180}$ can be said to have close linkage with good storability as well as black seed coat colour. Similarly, a few other alleles like $Satt565_{200}$, $Satt184_{200}$ and $Satt619_{200}$ etc. are specific to good storer genotypes (Table 3). Therefore, these markers may be good candidate for identifying marker linked with good storability and black seed coat colour. On the other hand, marker allele $Satt618_{130}$ (Fig. 1) was present only in poor storer genotypes making it to be candidate marker for linkage with poor storability trait. Similarly, marker alleles $Satt371_{160}$, $Satt175_{160}$, $Satt598_{160}$, $Satt281_{220}$ and

Table 2: Germination percentage of soybean genotypes before and after ambient storage and accelerated ageing

S.No.	Genotypes	Fresh seeds		Stored seeds		Accelerated aged seeds	
		First count	Final count	First count	Final count	First count	Final count
	Good storers						
1	AMSS-34	98(83.44)^{ba}	98(83.44)^{bac}	80(63.43)^{dec}	88(69.73)^{dc}	56.00(48.45)^{cb}	83.33(66.44)^{bdec}
2	DS-74	99(85.37)^{a}	99(85.37)^{ba}	80(63.43)^{dec}	90(71.62)^{bdc}	49.33(44.61)^{cbd}	80.33(63.79)^{fbdecg}
3	DS-MM-64	98(81.87)^{bac}	99(85.37)^{ba}	90(71.62)^{a}	94(75.95)^{ba}	33.33(35.14)^{fcehdg}	74.00(59.54)^{fhdecg}
4	G-2253	96(78.71)^{bdac}	97(81.95)^{bac}	86(68.06)^{bac}	90(71.62)^{bdc}	52.67(46.54)^{cbd}	74.66(60.07)^{fhdecg}
5	G-2265	96(78.46)^{bdac}	98(81.87)^{bac}	84(66.52)^{bdac}	88(69.90)^{dc}	50.00(45.00)^{cbd}	73.33(59.17)^{fhdecg}
6	G-2601	99(85.37)^{a}	99(85.37)^{ba}	86(68.06)^{bac}	90(71.62)^{bdc}	92.00(73.57)^{a}	98.66(86.15)^{a}
7	G-2603	96(78.46)^{bdac}	98(81.87)^{bac}	88(69.73)^{ba}	96(78.46)^{a}	84.00(66.52)^{a}	93.33(75.55)^{bac}
8	G-2614	98(81.87)^{bac}	98(81.87)^{bac}	82(64.91)^{bdc}	90(71.62)^{bdc}	48.00(43.85)^{cbd}	65.33(54.21)^{fhdeijg}
9	G-2651	99(85.37)^{a}	99(85.37)^{ba}	88(69.73)^{ba}	96(78.46)^{a}	40.00(39.19)^{fcebd}	66.66(54.79)^{fhdeijg}
10	M-253	95(77.12)^{bdc}	100(90.00)^{a}	78(62.04)^{fde}	88(69.73)^{dc}	42.00(40.37)^{cebd}	72.00(58.43)^{fhdeijcg}
11	M-1090	98(81.87)^{bac}	99(85.37)^{ba}	84(66.42)^{bdac}	86(68.06)^{d}	82.67(65.87)^{a}	96.00(78.46)^{ba}
12	M-11913	95(77.12)^{bdc}	99(85.37)^{ba}	82(64.91)^{bdc}	86(68.06)^{d}	42.00(40.37)^{cebd}	88.00(69.90)^{bdac}
13	TG x 444-422	98(81.87)^{bac}	99(85.37)^{ba}	88(69.73)^{ba}	92(73.57)^{bac}	60.00(50.84)^{b}	90.66(72.29)^{bdac}
14	P-761	97(80.12)^{bdac}	97(80.12)^{bc}	80(63.43)^{dec}	84(66.42)^{ed}	53.33(46.92)^{cbd}	82.33(65.72)^{fbdec}
	Mean of good storers	97.29(81.21)	98.5(84.19)	84.00(66.57)	89.85(71.77)	56.10(49.08)	81.33(66.03)
	Poor storers						
15	P-218	96(78.46)^{bdac}	96(78.46)^{bc}	70(56.79)^{fhg}	74(59.35)^{gf}	49.33(44.61)^{cbd}	86.66(69.62)^{bdac}
16	P-222	97(80.12)^{bdac}	97(80.12)^{bc}	68(55.55)^{ihg}	74(59.35)^{gf}	18.67(24.82)^{fikhjg}	58.66(50.01)^{fheijg}
17	P-241	96(78.46)^{bdac}	99(85.37)^{ba}	74(59.35)^{feg}	78(62.04)^{ef}	13.33(20.73)^{ikhj}	54.66(47.69)^{fhijg}
18	P-250	98(81.87)^{bac}	98(81.87)^{bac}	64(53.15)^{ijhk}	70(56.79)^{gifh}	22.67(28.28)^{fiehjg}	74.66(59.79)^{fhdecg}
19	P-732	97(80.12)^{bdac}	99(85.37)^{ba}	62(51.94)^{lijhk}	66(54.33)^{gijh}	33.33(35.14)^{fcehdg}	68.00(55.66)^{fhdeig}
20	P-871	97(80.12)^{bdac}	97(80.12)^{bc}	60(50.78)^{lijk}	64(53.15)^{ijh}	14.67(22.19)^{ikhj}	42.66(40.75)^{ij}
21	P-876	98(81.87)^{bac}	98(81.87)^{bac}	66(54.33)^{ijhg}	72(58.09)^{gfh}	14.67(21.19)^{ikhj}	68.00(55.66)^{fhdeig}
22	P-884	96(78.46)^{bdac}	97(80.12)^{bc}	60(50.78)^{lijk}	74(59.35)^{gf}	14.67(22.19)^{ikhj}	52.00(46.21)^{hijg}
23	P-898	97(80.12)^{bdac}	97(80.12)^{bc}	70(56.79)^{fhg}	74(59.35)^{gf}	29.33(32.71)^{fiehdg}	69.33(56.75)^{fhdeig}
24	PK-262	93(74.68)^{d}	94(75.95)^{bc}	48(43.85)^{m}	62(51.94)^{ij}	13.33(21.09)^{ikhj}	48.00(43.85)^{hij}
25	PK-416	94(75.95)^{dc}	95(77.12)^{bc}	58(49.42)^{lik}	64(53.15)^{ijh}	12.00(20.09)^{ikj}	36.00(36.80)^{j}
26	PK-472	94(75.82)^{dc}	94(75.82)^{bc}	54(47.30)^{lm}	60(50.78)^{j}	10.67(18.99)^{ikj}	54.66(47.73)^{fhijg}
27	TAMS-38	93(74.68)^{d}	94(75.82)^{bc}	64(53.15)^{ijhk}	72(58.09)^{gfh}	20.00(26.57)^{fiehjg}	57.33(49.24)^{fheijg}
28	EC-13969	93(74.89)^{dc}	93(74.89)^{c}	74(59.35)^{feg}	78(62.04)^{ef}	12.00(20.09)^{ikj}	45.33(42.29)^{hij}
29	EC-1023	98(81.87)^{bac}	98(81.87)^{bac}	74(59.35)^{feg}	78(62.04)^{ef}	10.67(18.81)^{ikj}	52.00(46.15)^{hijg}
30	EC-34141	93(74.89)^{dc}	93(74.89)^{c}	56(48.45)^{lmk}	66(54.33)^{gijh}	3.33(10.40)^{k}	42.66(40.75)^{ij}
31	EC-93751	96(78.46)^{bdac}	96(78.46)^{bc}	74(59.35)^{feg}	78(62.04)^{ef}	8.00(15.55)^{kj}	49.33(44.61)^{hij}
32	EC-18761	96(78.71)^{bdac}	96(78.71)^{bc}	60(50.78)^{lijk}	70(56.79)^{gifh}	16.00(23.47)^{ikhjg}	52.00(46.15)^{hijg}
33	MACS-681	95(77.12)^{bdc}	97(80.12)^{bc}	78(62.04)^{fde}	78(62.04)^{ef}	37.33(37.62)^{fcebdg}	70.66(57.53)^{fhdeicg}
	Mean of poor storers	95.63(78.24)	96.21(79.32)	64.95(53.82)	71.15(57.63)	18.56(24.44)	56.97(49.32)
	Total mean	96.33(79.50)	97.18(81.38)	73.03(59.23)	79.09(63.63)	34.48(34.90)	67.31(56.41)
	MSD at 5%	7.096	9.833	5.420	5.439	14.95	18.37
	SE (d)	1.207	1.427	1.055	1.057	1.753	1.943

Note: Values in the parenthesis are arc sin transformed. Similar alphabets indicate non-significant differences between the values, following Tukey's Studentized Range (HSD) Test.

Table 3: Status of SSR alleles in good and poor storer genotypes of soybean

SSR locus	Allele size(bp)	No. of genotypes		Efficiency of allele (%)	PIC	SSR locus	Allele size(bp)	No. of genotypes		Efficiency of allele (%)	PIC
		GS(14)	PS(19)					GS(14)	PS(19)		
Satt534	200	-	9	47.36	0.4224	Satt538	200	-	1	5.260	0.4949
	180	3	-	21.42			130	-	11	57.89	
Satt565	200	14	-	100	0.1653		110	7	-	50.00	
	180	-	3	15.78		Satt194	240	13	-	92.85	0.0588
Satt371	180	14	-	100	0.4959		220	-	0	0	
	160	-	18	94.73		Satt600	220	5	-	35.71	0.4040
Satt184	200	14	-	100	0.3673		180	-	15	78.94	
	160	-	8	42.1			160	-	2	10.52	
Satt619	200	14	-	100	0.4628	Satt193	260	4	-	28.57	0.6997
	180	-	12	63.15			240	9	-	64.28	
Satt481	200	-	8	42.1	0.3967		220	-	10	52.63	
	160	13	-	92.85			200	-	7	36.84	
Satt463	200	-	6	31.57	0.4444	Satt554	260	-	14	73.68	0.4527
	180	14	-	100			240	8	-	57.14	
Satt022	200	14	-	100	0.2130	Satt354	240	8	-	57.14	0.5583
	180	-	4	21.05			220	-	12	63.15	
Satt302	200	14		100	0.4775		180	-	5	26.31	
	160	-	13	68.42		Satt285	240	-	1	5.26	0.1139
Satt453	220	-	17	89.47	0.4995		200	13	-	92.85	
	200	14	-	100		Satt431	220	12	-	85.71	0.0432
Satt460	220	13	-	92.85	0.5987		180	-	4	21.05	
	200	-	14	73.68		Satt513	160	4	-	28.57	0.4821
	160	-	4	21.05			140	-	14	73.68	
Sat_299	200	-	16	84.21	0.4995		120	-	6	31.57	
	180	14	-	100		Satt175	200	1	-	7.14	0.1644
Sat_394	220	5	-	35.71	0.4233		180	2	-	14.28	
	200	-	15	78.94			160	-	18	94.73	
	180	-	4	21.05		Satt233	180	6	-	42.85	0.4995
Satt196	220	-	5	26.31	0.2975		160	-	9	47.36	
	200	14	-	100		Satt598	170	3	-	21.42	0.2130
Satt618	130	-	19	100	0.4775		160	-	18	94.73	
	110	13	-	92.85		Satt281	240	-	1	5.26	0.1240
Satt549	240	14	-	100	0.1653		220	-	18	94.73	
	220	-	3	15.78			180	5	-	35.71	
Satt592	200	-	7	36.84	0.3343		160	-	2	10.52	
	180	14	-	100		Satt389	220	5	-	35.71	0.2975
Satt545	200	11	-	78.57	0.4959		200	-	18	94.73	
	160	-	15	78.94		Satt197	200	13	-	92.85	0.3967
								160	-	8	42.1

Note: GS denotes good storer genotypes, PS denotes poor storer genotypes, PIC denotes polymorphism information content

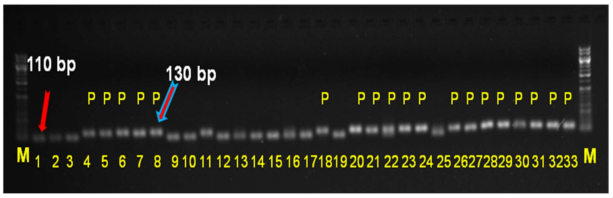

Fig. 1. Amplification pattern of the soybean genotypes obtained using the SSR marker Satt618. Lane M- 20 bp ladder. Lane 1-3, 9-17, 19, 25-good storer genotypes, 4-8, 18, 20-24, 26-33-poor storer genotypes (Table 1). P: Poor storer

$Satt389_{200}$ were present in 18 of the 19 poor storer genotypes indicating their possible association with the trait. Of these markers, Satt598 and Satt281 have already been reported to be linked with storability trait [11, 12]. Therefore, rest of the markers may be tested in segregating population to confirm their association with storability in soybean.

Markers Satt371, Satt453 and Satt618 produced specific allelic bands with respect to storability and testa colour. This was in agreement with the earlier reports stating that black seeded landraces of tropical and subtropical regions such as T-49, Birsa-1 and Kalitur had better storability than yellow seeded temperate varieties such as Clark-63, Lee and Bragg [18, 23, 25]. The markers, Satt371 and Satt453 are from telomeric regions of the chromosome, known to influence seed ageing and longevity.

The similarity coefficients were used as input data for the cluster analysis using NTSYSPc2.1 programme and the resulting dendrogram was prepared (Fig. 2). It showed two major clusters *viz*., A and B at similarity level of 35%. Cluster A contained 14 genotypes which were of good storer (black seeded), while the cluster B contained 19 poor storer (yellow seeded) genotypes. The cluster A was further divided into two sub clusters *viz*., A_1 with 13 genotypes and A_2 with only one genotype (G-2601) at similarity level of 44%. Cluster B was further divided into two sub clusters *viz*., B_1 with 18 genotypes and B_2 with only one genotype (MACS-681) at similarity level of 37%. G-2614, TGX 444-422 and P-222, P-241 genotypes present in sub cluster A_1 and Cluster B_1 showed maximum genetic similarity (88%) and minimum similarity (63%), respectively.

RAPD analysis

Among the 51 RAPD markers used, 35 markers were polymorphic (68.62% polymorphism). Among the polymorphic markers, marker OPD-11 showed

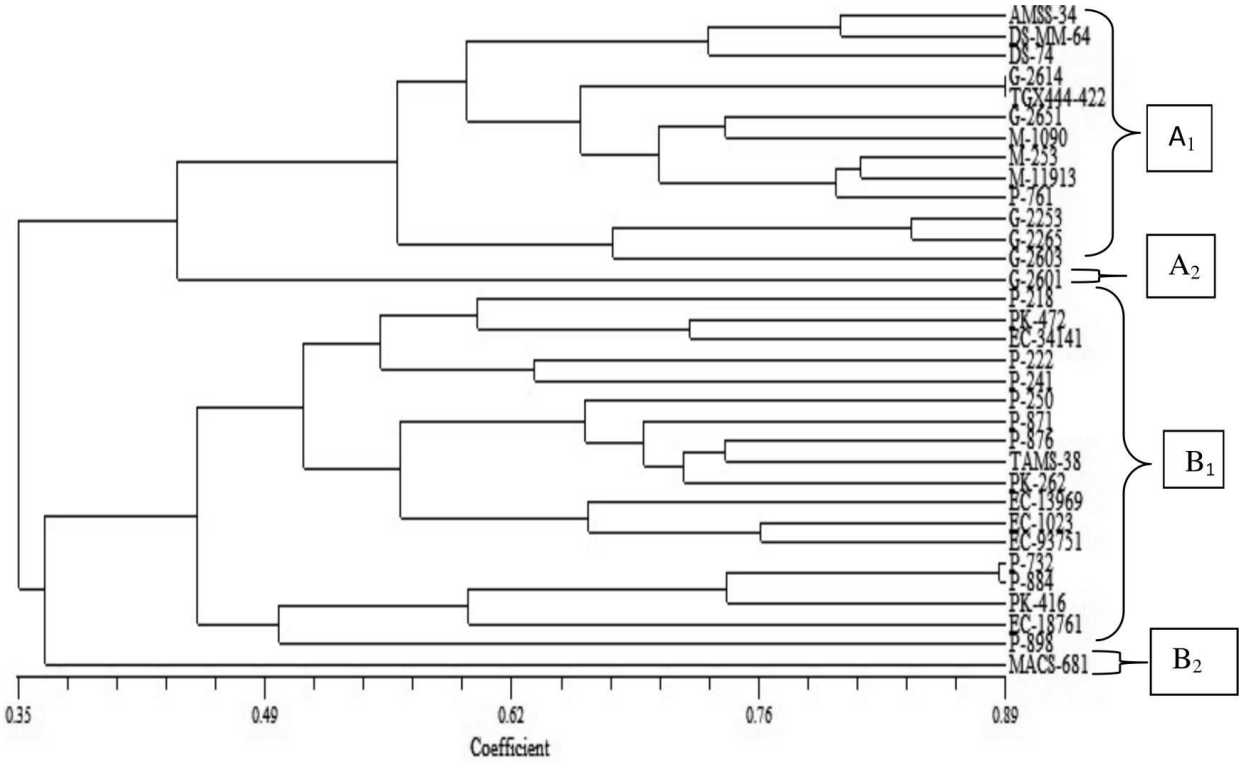

Fig. 2. Dendrogram of 33 soybean genotypes constructed using 33 SSR markers. The bar below the dendrogram represents similarity matrix based on Jaccard's coefficients

maximum number (14) of bands. Marker SSTR-12 showed maximum polymorphism (72.73%) with 11 bands. Over the genotypes and markers, a total of 305 bands were produced, with an average of 8.71 bands per genotype and marker. However, when only the numbers of polymorphic bands were considered, it was 99 which indicated that 32.45% of the total bands generated were polymorphic. While considering the number of polymorphic bands per marker over all the markers, the average found to be 2.83. Fig. 3 displays the amplification pattern of the soybean genotypes obtained using the RAPD marker SSTR 2.

The similarity coefficients were used as input data for the cluster analysis using NTSYSPc2.1 programme and the resulting dendrogram was prepared (Fig. 4). The dendrogram construct showed two major clusters *viz*., A and B at similarity level of 88%. Cluster A contained maximum number of genotypes (32) while the cluster B was comprised of only one genotype (P-898). Cluster A was further divided into two sub clusters *viz*., A_1 contained 31 genotypes and A_2 comprised of only one genotype (EC-34141) at similarity level of 88.5%. AMSS-34, DS-74 and P-871, PK-472 genotypes present in sub cluster A_1 showed maximum genetic similarity (98%) and minimum similarity (94%), respectively.

RAPD being a random marker produced large number of bands, however level of polymorphism was

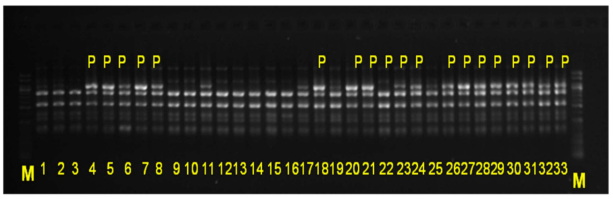

Fig. 3. Amplification pattern of the soybean genotypes obtained using the RAPD marker SSTR 2. Lane M-20 bp ladder. Lane 1-3, 9-17, 19, 25-good storer genotypes, 4-8, 18, 20-24, 26-33-poor storer genotypes (Table 1). P: Poor storer

comparable with that of SSR. Further, the grouping of genotypes based on RAPD data did not follow any pattern, nor were specific to any particular phenotype like seed coat colour or longevity. Therefore, SSR markers were found to be more polymorphic and usable than RAPD.

The study thus could identify a set of good storer soybean genotype along with candidate SSR markers for identifying markers linked to seed storability traits.

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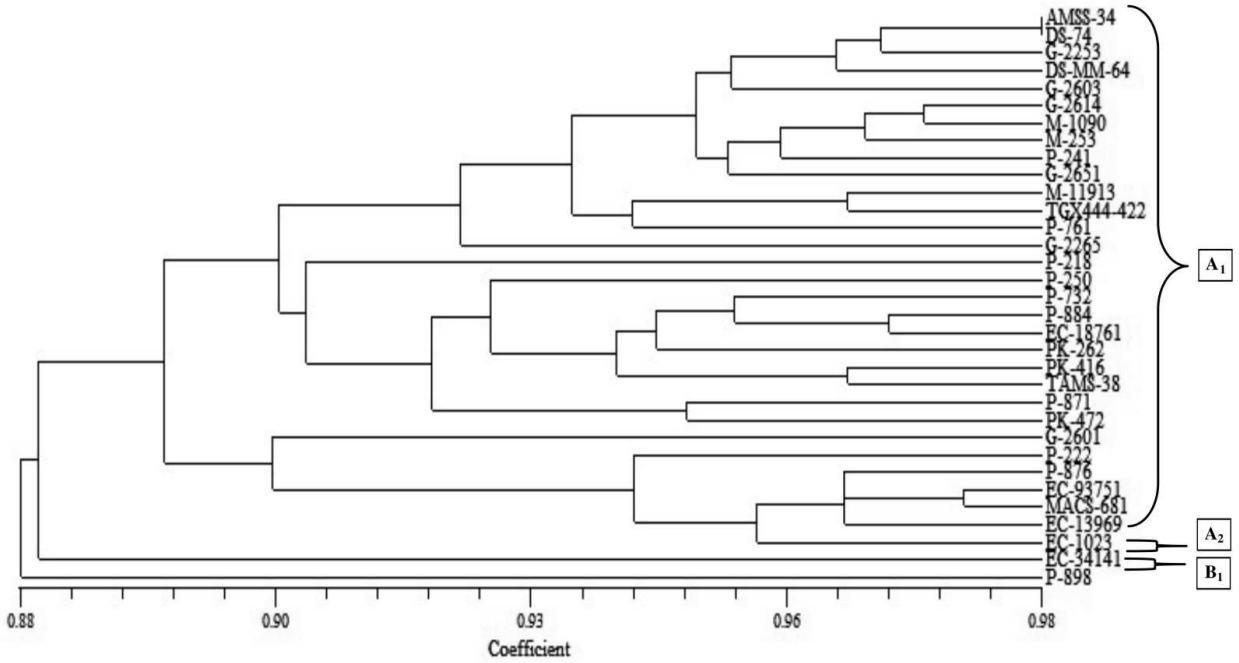

Fig. 4. Dendrogram of 33 soybean genotypes constructed using 35 RAPD markers. The bar below the dendrogram represents similarity matrix based on Jaccard's coefficients

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