



## Characterization of soybean (*Glycine max*) genotypes for seed longevity using SSR markers

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Received: 28 November 2015; Accepted: 18 December 2015

### ABSTRACT

Soybean [*Glycine max* (L.) Merr.] genotypes exhibit varied seed longevity and in general are poor storers. Selected 20 genotypes of soybean exhibiting contrasting longevity were characterized using 46 SSR markers. Among these, good storer genotypes registered better storability (Germination > 90 %; electrical conductivity 30  $\mu$ S/cm/g seed and germination on accelerated ageing up to 73%), whereas poor storer genotypes registered (Germination up to 67 %; electrical conductivity 57  $\mu$ S/cm/g seed and germination on accelerated ageing up to 57%) during eight months of laboratory ambient storage (average 25 $\pm$ 2 °C and 65 $\pm$ 5% RH). Of the used 46 SSR markers, only 50% markers resolved polymorphism. Polymorphism Information Content (PIC) values ranged between 0.163 to 0.553 with an average of 0.379. Polymorphic markers produced 52 alleles, ranging from two to three alleles per locus, with an average of 2.26 alleles per locus. Genetic similarity coefficient data grouped soybean genotypes into four major clusters. SSR marker Satt423 having contrasting allelic combination segregated distinctively good and poor storer genotypes, making it putative candidate marker linked to seed storability and may be used for screening large number of genotypes. Good storer genotypes identified in this study may be utilized in soybean breeding programme.

**Key words:** Cluster analysis, PIC, Polymorphic marker, Seed longevity, Soybean, SSR

Soybean [*Glycine max* (L.) Merrill] an important oilseed crop, is gaining significance in eradicating malnutrition and maintaining soil fertility. Seed deterioration is a serious problem in developing countries like India, whereby seeds are often stored with poor control of humidity and temperature. Maintenance of soybean seed viability and vigour soon after harvest until next planting is crucial for the successful crop production. A number of seed characters such as seed size, hard seededness, permeability, etc. are reported to be associated with seed quality and were under genetic control in soybean (Kilen and Hartwig 1978, Verma and Ram 1987, Karmakar *et al.* 1999). Verma and Ram (1986) reported that two to four genes probably governed seed longevity in soybean.

Molecular markers, having inherent ability to produce unique DNA profiles, are widely adapted in various crop improvement programmes. Available molecular marker

technology has made possible the genetic dissection and characterization of many quantitatively inherited seed quality traits in soybean. A few microsatellites or Simple Sequence Repeat (SSR) markers are highly polymorphic, abundant and distributed throughout the genome (Cregan *et al.* 1999). Molecular markers tightly linked to desired genes are valuable tool to detect genotypes of interest. Marker assisted selection (MAS) using DNA markers instead of phenotypic assay increases the precision and efficiency of subsequent selection pressures in breeding. In agriculture, seed quality in terms of seed longevity is rarely considered by breeders in any crop improvement programme. However, there is an emerging trend in contemporary breeding programme to identify the factors that affect seed quality traits and to tailor cultivar development to specific end uses.

A set of four SSR markers (Satt538, Satt285, Satt600 and Satt434) has been reported to be associated with seed longevity in a F<sub>2:3</sub> soybean population [a cross of Birsa Soya 1 (Good storer, black seeded)  $\times$  JS 71-05 (Poor storer, yellow seeded)] (Singh *et al.* 2008). Similarly, a number of RFLP and SSR markers (for hard seededness, seed oil concentration, seed protein and seed size) have been reported to show positive association to seed longevity in soybean (Keim *et al.* 1990, Hyten *et al.* 2004).

Seed longevity in soybean is a complex trait; information on genetic basis of seed storability is limited. Thus, characterization of soybean genotypes for seed longevity

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using SSR markers would be an appropriate approach. However, the applicability of markers is yet to be ascertained in soybean genotypes. Therefore, an attempt was made to characterize a set of good and poor storer soybean genotypes with SSR markers.

#### MATERIALS AND METHODS

The experimental material consists of 59 soybean genotypes, collected from the Division of Genetics and Division of Seed Science and Technology, ICAR-IARI, New Delhi, which were screened for storability. 20 genotypes, 10 each from good and poor storers, were selected for molecular characterization based on the seed storability. The details are presented in Table 1.

About 150 g of untreated fresh (*kharif* 2013 harvest) soybean seeds were packed in muslin cloth bags and stored under laboratory ambient conditions of Delhi with an average relative humidity of 65±5% and temperature of 25±2 °C. Samples were drawn randomly for evaluation of germination (%) prior to and at eight months from start of seed storage. Germination was tested in three replications of 100 seeds each, following between-paper method at 25°C (ISTA 2011). The accelerated ageing test (AAT) was conducted as per ISTA (2011). About 30 g of soybean seeds were stored in nylon net bag at 100% RH in sealed desiccators maintained at 41.3 °C. The desiccator was equilibrated to the given RH and temperature for 24 h before keeping the sample. The samples were drawn at 96 h from start of AAT. The electrical conductivity (EC) was measured following the standard procedure (ISTA 2011). Three replications of 50 seeds each

were soaked in 250 ml of double distilled water at 20 °C for 24 h and electrical conductance was measured with a conductivity meter (Eutech-Model 215 R) and calculated as µS/cm/g seed.

Genomic DNA of soybean genotypes was extracted following the method of Doyle and Doyle (1990). Polymorphism survey was carried out using 46 SSR markers (Table 2) distributed across the soybean genome (Cregan *et al.* 1999) and were reported to be highly polymorphic (Singh *et al.* 2008, Hamidreza *et al.* 2014). leaf samples (0.5 g) were collected from 10-days-old seedlings and were homogenized to fine powder in liquid nitrogen. The fine powder was transferred to 2 ml centrifuge tubes and 1.2 ml CTAB extraction buffer was added. The extraction mixture was thoroughly mixed by inverting tubes and incubated in water bath at 60°C for one h. During incubation, the contents of tubes were mixed by inverting tubes many times. Equal volume of chloroform:Isoamylalcohol (24:1) was added; and mixed thoroughly by gently inverting tubes several times. Tubes were centrifuged at 12 000 rpm at laboratory temperature for 20 min. Upper aqueous phase from centrifuge tubes was transferred to fresh tubes and to this 0.6 volume of Isopropanol was added and incubated at -20° C for one h. The DNA was pelleted down by centrifugation at 12 000 rpm for 10 min at 4° C. The pellet was washed with 70% ethanol, air-dried and dissolved in TE buffer. For purification of crude DNA, RNAase was added @ 50 mg/g tissue and tubes were re-incubated at 37° C for one h. SSR amplification was carried out using BIOER XP thermal cycler in a 12.5 µl PCR reaction mixture consisting of 25 ng of template DNA, 0.1 µM each of forward and reverse primers, 0.2 mM dNTP mix, 1X PCR assay buffer, and one unit of *Taq* DNA polymerase. The PCR reactions were performed with initial denaturation at 94°C for six min, followed by 35 cycles of denaturation at 94°C for one min, annealing for 30 sec at 48°-57°C with an extension for 30 sec at 72°C, and with a final extension for 10 min. The amplified products were separated on 3% agarose gel. Gel was run for two and half sp at 130 V in 1X TBE buffer (100 mM Tris, 100 mM boric acid, 2 mM EDTA, pH 8.3). DNA fragments were visualized under UV light and photographed using gel documentation system. PIC for each SSR marker was calculated using the Botstein *et al.* (1980) formula.

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

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 data was analyzed using SAS software (version 9.4) 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, respectively. Using the binary data, a similarity matrix was constructed using

Table 1 List of 20 selected soybean genotypes exhibiting contrasting longevity

Genotype	Testa colour
<i>Good storer</i>	
AMSS-34	Black
DS-74	Black
EC-13969	Yellow
G-2253	Black
G-2603	Black
G-2614	Black
G-2651	Black
M-253	Black
M-11913	Black
MACS-1311	Yellow
<i>Poor storer</i>	
P-250(129)	Yellow
P-876(146)	Yellow
PS-1480	Yellow
JS(SH)-93-37	Black
DSB-19	Yellow
VLS-81	Yellow
AMS-56	Yellow
MAUS 608	Yellow
KBS-2011	Yellow
DS-2708	Yellow

Table 2 List of 46 SSR primers used for molecular characterization

SSR locus	Linkage group	Forward sequence (5'→3')	Reverse sequence (5→3')
Satt545	A1	CAATGCCATTCCATATTTGTT	CAATTGCCCTAGTTTGTATAG
Satt538	A2	GCAGGCTTATCTTAAGACAAGT	GGGGCGATAAACTAGAACAGGA
Satt194	C1	GGGCCCAACTGATATTTAATTGTAA	GCGCTTTGTGTTCCGATTTTGAT
Satt600	D1b	GCGCAGGAAAAAAAAACGCTTTTATT	GCGCAATCCACTAGGTGTTAAT
Satt193	F	GCGTTTCGATAAAAATGTTACACCTC	TGTTTCGCAATTATTGATCAAAAAT
Satt554	F	GCGATATGCTTTGTAAGAAAATTA	GCGCAAGCCCAATATTACAAAT
Satt399	C1	AAGCCAACCTTATAATTCTTTTCAT	ATATGGGCTTACTTACCCATCATAGA
Satt002	D2	TGTGGGTAAAATAGATAAAAAT	TCATTTTGAATCGTTGAA
Satt354	I	GCGAAAATGGACACCAAAAGTAGTTA	GCGATGCACATCAATTAGAATATACAA
Satt285	J	GCGACATATTGCATTA AAAACATACTT	GCGGACTAATTCTATTTTACACCAACAAC
Satt431	J	GCGTGGCACCTTGATAAATAA	GCGCACGAAAAGTTTTTCTGTAACA
Satt513	L	GCGCATCACAAGTTTTATAGATGCTGA	GAGGTCTAGTGCTTTGGTAAGGTT
Satt175	M	GACCTCGCTCTCTGTTTCTCAT	GGTGACCACCCCTATTCCCTTAT
Satt233	A2	AAGCATACTCGTCGTAAC	GCGGTGCAAAGATATTAGAAA
Satt598	E	CGATTTGAATATACTTACCGTCTATA	CACAATACCTGTGGCTGTTATACTAT
Satt281	C2	AAGCTCCACATGCAGTTCAAAAC	TGCATGGCACGAGAAAAGAAGTA
Satt389	D2	GCGGCTGGTGTATGGTGAAATCA	GCGCCAAAACCAAAAGTTATATC
Satt534	B2	CTCCTCCTGCGCAACAACAATA	GGGGGATCTAGGCCATGAC
Satt434	H	GCGTTCCGATATACTATATAATCCTAAT	GCGGGGTAGTCTTTTTATTTAACTTAA
Satt371	C2	TGCAAACCTAAGTGGATCACTCA	GAGATCCCAGAAATTTTAGTGTAACA
Satt184	D1a	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT
Satt619	A1	GGCAGAAGTAGTACGCTTCTGATT	GCGGTAAAGCATAATAGATCAGCCT
Satt481	L	GGGTAAACCGTCCACACATCTATT	GACGGTTTTAAACGGTAAGAAAAT
Satt463	M	TTGGATCTCATATTCAAACCTTCAAG	CTGCAAATTTGATGCACATGTGTCTA
Satt022	N	GGGGGATCTGATTGTATTTTACCT	CGGGTTTCAAAAAACCATCCTTAC
Satt302	H	GCGAACTGTAGTTTACTAAAAATAAGTG	GCGGACTGAATTAATATTGGTGTGGAATT
Satt453	B1	GCGGAAAAAAAAACAATAACAACA	TAGTGGGGAAGGGAAGTTACC
Satt460	C2	GCGCGATGGGCTGTTGGTTTTTAT	GCGCATAACGATTTGGCATTCTTCTATTG
Satt154	D2	AGATACTAACAAGAGGCATAAAACT	AAAGAAACGGAACCTAATACTACATT
Satt196	K	TTGGGAAATAGTGATTGAGGTA AAA	AAATCCCATTGAATGAGAATAAG
Satt618	M	GCGGTGATATTACCCCAAAAAATGAA	GCGCTAGTTTCTAGTGGAAGATGAGT
Satt549	N	GCGGCAAACTTTGGAGTATTGCAA	GCGCGCAACAATCACTAGTACG
Satt592	O	GCGAAGATTGGTCTTTTATGTCAAATG	GCGGAGGAATACAAGTCTCTATTCAA
Sat_299	I	GCGACAAGGCACTCACATCTCTTCTC	GCGCTACCCATAACAAAAAGTTCAAATC
Sat_394	J	GCGGACAGTGTGCTCCTCATATAATAG	GCGTGACTCGGACTTGAAGATAATAATG
Satt197	B1	CACTGCTTTTTCCCTCTCT	AAGATACCCCAACATTATTGTGAA
Satt045	E	TGGTTTCTACTTTCTATAATTATTT	ATGCCTCTCCCTCTCT
Satt269	F	GCGTGCCAGGTAGAAAAATATTAG	GCGGTTTTTCACTTTTCAA AATC
Satt423	F	TTCGCTTGGGTTCCAGTTACTT	GTTGGGGAATTA AAAAATG
Satt160	F	TCCCACACAGTTTTCATATAATATA	CATCAAAAGTTTATAACGTGTAGAT
Satt380	J	GCGAGTAACGGTCTTCTAACAAGGAAAG	GCGTGCCCTTACTCTCAAAAAA
Satt414	J	GCGTATTCCTAGTCACATGCTATTTCA	GCGTCATAATAATGCCTAGAACATAAA
Satt313	L	GCGGTAAGTCATGGCTTTTTAATCTT	GCGCGAGGTATGGAACCTA ACTCACA
Satt523	L	GCGATTTCTTCTTGAAGAATTTTCTG	GCGCTTTTTCGGCTGTTATTTTAACT
Satt143	L	GTGCCACAAATTTAAAATTACTCA	TCCCTCCCTTTTGATTTACAC
Satt565	C1	GCGCCCGAACTTGTAATAACCTAAT	GCGCTCTCTTATGATGTTTATAATAA

the Jaccard's coefficients, which was further subjected to clustering with Unweighted Pair Group Method Analysis (UPGMA). The dendrogram was generated. These analyses were performed using the NTSYS-PC software package version 2.1 (Rohlf 1993).

## RESULTS AND DISCUSSION

Per cent germination of freshly harvested seeds was comparable in good and poor storer genotypes. The germination percentage of all genotypes at start of seed storage was  $\geq 95\%$ ; good storer genotypes registered 98%,

whereas poor storers registered 96%. Seed germination declined significantly in all genotypes during eight months of laboratory ambient storage (average  $25\pm 2^\circ\text{C}$  and  $65\pm 5\%$  RH). The reduction was more in poor storers than those of good storers. In good storer genotypes, mean germination percentage registered was up to 91%, whereas in poor storers it was up to 67% (Table 3). Under high temperature and RH conditions of accelerated ageing (AAT), germination percentage of good storers was significantly higher (73%) than those of poor storer genotypes (57%). It was in the line of seed longevity results obtained during eight months of ambient storage. A high and significant correlation ( $r = 0.723^{**}$ ) between germination during ambient storage and germination on AAT reaffirmed the validity of the latter as a test to assess the seed quality (vigour). A similar high correlation ( $r = 0.6521^{**}$ ,  $r = 0.7284^{**}$ ) was reported by Usha (2009) and Hosamani *et al.* (2013) in soybean, respectively. It was evident that the genotypes differed significantly with respect to storability. Good storers have shown lower electrical conductivity than the poor storers, at the beginning of seed storage mean electrical conductivity was  $17 \mu\text{S/cm/g}$  seed and  $28 \mu\text{S/cm/g}$  seed for good and poor storers, respectively. Whereas, during eight months

storage it was  $30 \mu\text{S/cm/g}$  seed and  $57 \mu\text{S/cm/g}$  seed in good and poor storers, respectively (Table 3). A significant negative correlation was observed between germination during eight months storage and electrical conductivity ( $r = -0.930^{**}$ ). These findings were in agreement with Hosamani *et al.* (2013) in soybean.

#### *Molecular characterization of soybean genotypes for seed longevity*

*Polymorphic information content:* Among 46 SSR markers used, 23 markers were polymorphic. The polymorphic markers recorded 52 alleles, ranging from two to three alleles per locus, with an average of 2.26 alleles per locus. Satt434, Satt545, Satt600, Satt197, Satt513 and Satt175 had highest number of alleles (three alleles per locus). In the present investigation, PIC values for 23 SSR primers ranged between 0.163 to 0.553 with an average value of 0.379 (Table 4). The primer Satt513 showed highest PIC value of 0.553 followed by Satt545, Satt233, Satt434 and Satt600 with 0.528, 0.510, 0.482 and 0.455, respectively. Primer Satt281 showed the lowest PIC value (0.163). Thus, the primers with higher PIC value were more helpful for molecular characterization of genotypes. This was in

Table 3 Germination (%), electrical conductivity in soybean genotypes

Genotype	Testa colour	Germination (%)			EC ( $\mu\text{S/cm/g}$ seed)	
		at the start of seed storage	8 months stored seeds	on AAT	at the start of seed storage	8 months stored seeds
<i>Good storer</i>						
AMSS-34	Black	100 (89.96) <sup>a</sup>	95 (77.05) <sup>a</sup>	94 (75.79) <sup>a</sup>	14 <sup>h</sup>	26 <sup>e</sup>
DS-74	Black	99 (84.23) <sup>ab</sup>	93 (74.63) <sup>b</sup>	88 (69.70) <sup>bc</sup>	16 <sup>fgh</sup>	31 <sup>de</sup>
EC-13969	Yellow	98 (81.84) <sup>bcde</sup>	92 (73.54) <sup>bc</sup>	66 (54.31) <sup>de</sup>	21 <sup>ef</sup>	39 <sup>d</sup>
G-2253	Black	99 (84.23) <sup>abc</sup>	91 (72.51) <sup>bcd</sup>	72 (58.03) <sup>b</sup>	14 <sup>h</sup>	26 <sup>e</sup>
G-2603	Black	97 (79.99) <sup>cde</sup>	92 (73.54) <sup>bc</sup>	67 (54.92) <sup>cd</sup>	15 <sup>gh</sup>	28 <sup>e</sup>
G-2614	Black	99 (84.23) <sup>abc</sup>	90 (71.54) <sup>bed</sup>	65 (53.71) <sup>de</sup>	18 <sup>fgh</sup>	32 <sup>de</sup>
G-2651	Black	97 (79.99) <sup>cde</sup>	91 (72.51) <sup>bed</sup>	70 (56.77) <sup>bc</sup>	14 <sup>h</sup>	24 <sup>e</sup>
M-253	Black	98 (81.84) <sup>abcde</sup>	90 (71.54) <sup>bed</sup>	65 (53.71) <sup>de</sup>	17 <sup>fgh</sup>	31 <sup>de</sup>
M-11913	Black	100 (89.96) <sup>a</sup>	90 (71.54) <sup>cd</sup>	65 (53.71) <sup>de</sup>	18 <sup>fgh</sup>	28 <sup>e</sup>
MACS-1311	Yellow	98 (81.84) <sup>abcd</sup>	89 (70.60) <sup>d</sup>	80 (63.41) <sup>e</sup>	20 <sup>efg</sup>	33 <sup>de</sup>
	Mean value	98 (84.23)	91 (72.51)	73 (58.67)	17	30
<i>Poor storer</i>						
P-250(129)	Yellow	96 (78.43) <sup>cde</sup>	70 (56.77) <sup>e</sup>	54 (47.28) <sup>gh</sup>	25 <sup>de</sup>	58 <sup>abc</sup>
P-876(146)	Yellow	97 (79.99) <sup>cde</sup>	68 (55.53) <sup>efgh</sup>	52 (46.13) <sup>h</sup>	30 <sup>abc</sup>	56 <sup>bc</sup>
PS-1480	Yellow	98 (81.84) <sup>abcde</sup>	66 (54.31) <sup>fghi</sup>	55 (47.85) <sup>gh</sup>	27 <sup>cd</sup>	55 <sup>bc</sup>
JS(SH)-93-37	Black	95 (77.05) <sup>de</sup>	70 (56.77) <sup>ef</sup>	61 (51.33) <sup>f</sup>	18 <sup>fgh</sup>	39 <sup>d</sup>
DSB-19	Yellow	97 (79.99) <sup>cde</sup>	65 (53.71) <sup>ghi</sup>	59 (50.16) <sup>fg</sup>	32 <sup>ab</sup>	59 <sup>abc</sup>
VLS-81	Yellow	95 (77.05) <sup>de</sup>	64 (53.11) <sup>i</sup>	52 (46.13) <sup>h</sup>	28 <sup>bcd</sup>	61 <sup>abc</sup>
AMS-56	Yellow	95 (77.05) <sup>de</sup>	65 (53.71) <sup>hi</sup>	64 (53.11) <sup>f</sup>	29 <sup>abcd</sup>	62 <sup>ab</sup>
MAUS 608	Yellow	98 (81.84) <sup>abcde</sup>	66 (54.31) <sup>fghi</sup>	52 (46.13) <sup>h</sup>	29 <sup>abcd</sup>	52 <sup>c</sup>
KBS-2011	Yellow	95 (77.05) <sup>de</sup>	68 (55.53) <sup>efgh</sup>	60 (50.75) <sup>fg</sup>	31 <sup>abc</sup>	58 <sup>abc</sup>
DS-2708	Yellow	98 (81) <sup>bcde</sup>	69 (56.23) <sup>efg</sup>	61 (51.33) <sup>f</sup>	33 <sup>a</sup>	66 <sup>a</sup>
	Mean value	96 (78.43)	67 (54.92)	57 (49.00)	28	57
	MSD at 5 %	10.16	4.24	3.52	5.36	9.14

AAT: Accelerated ageing test, EC: electrical conductivity, 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 4 Status of SSR alleles in good and poor storer genotypes of soybean

SSR locus	Allele size (bp)	No. of genotype		PIC
		GS (10)	PS (10)	
Satt 434	200	2	0	0.482
	195	3	8	
	190	5	2	
Satt 371	290	4	4	0.374
	260	5	5	
Satt 523	190	6	8	0.331
	180	4	2	
Satt 302	240	5	6	0.372
	200	5	4	
Satt 453	250	6	9	0.304
	240	4	1	
Satt 423	250	0	10	0.375
	220	10	0	
Satt 481	150	4	2	0.352
	140	6	5	
Satt 196	208	4	6	0.371
	200	6	2	
Satt 549	230	9	10	0.358
	220	1	0	
Satt 545	200	1	3	0.528
	195	6	5	
	190	3	2	
Satt 285	210	0	4	0.268
	200	10	6	
Satt 600	210	0	5	0.455
	200	9	3	
	180	2	1	
Satt 233	200	10	6	0.371
	190	0	4	
Satt 598	290	7	1	0.375
	280	1	7	
Satt 281	230	9	9	0.163
	180	1	1	
Satt 184	190	5	5	0.163
	175	3	5	
Satt 022	220	9	6	0.375
	200	2	5	
Satt 592	260	2	6	0.368
	235	8	3	
Satt 197	190	6	7	0.442
	180	0	2	
Satt 269	155	4	1	0.374
	350	0	4	
Satt 513	260	9	7	0.553
	220	0	4	
	190	9	2	
Satt 431	175	2	4	0.360
	240	0	8	
	200	10	3	
Satt 175	200	0	2	0.246
	185	10	7	
	170	0	1	

SSR: Simple sequence repeat, GS: good storer, PS: poor storer, PIC: polymorphism information content.

agreement with previous findings of Hosamani *et al.* (2013), where PIC values ranged between 0.0432 (Satt431) to 0.6997 (Satt193) with an average of 0.3709.

A dendrogram developed based on UPGMA analysis using 23 SSR markers, grouped 20 soybean genotypes into four major clusters at 63% simple matching similarity level (Fig 1). Among four clusters, Cluster II emerged the largest and comprised of seven genotypes which was further subdivided into two sub-clusters, IIA with three (MACS1311, JSSH9337, and KBS2011) and IIB with four (P876, MAUS608, DS2708, PS1480) genotypes, respectively. Cluster III was second largest, having five genotypes (DS74, G2603, G2614, M253 and P250) followed by cluster I and IV consisting of four (AMSS34, G2651, EC13969 and G2253) and three (Dsb19, AMS56 and VLS81) genotypes, respectively. There was maximum level of similarity (0.90) between EC13969 and G2253 both of which were good storers and grouped as cluster I.

In the present investigation, SSR marker Satt423 depicted distinct polymorphism between good and poor storer. UPGMA cluster analysis done separately for Satt423 depicted clear clustering of good and poor genotypes into two major clusters (Fig 2). Cluster I included good storers, whereas cluster II comprised poor storers only.

*Candidate markers for seed longevity* : Hosamani *et al.* (2013) reported that SSR markers Satt371, Satt453 and Satt618 produced specific allelic bands with respect to their seed quality and testa colour. However, in present study SSR marker, Satt423 was found to discriminate clearly two groups, i.e. good storer and poor storers. Satt423 produced two alleles of 254 and 225 base pair size, wherein allele Satt423<sub>225</sub> was linked only to good storer genotypes, and Satt423<sub>254</sub> allele was associated with poor storers. Thus allele Satt423<sub>225</sub> is clearly depicted to be closely linked with better storability. Therefore, Satt423 proved to be a good candidate marker linked with soybean

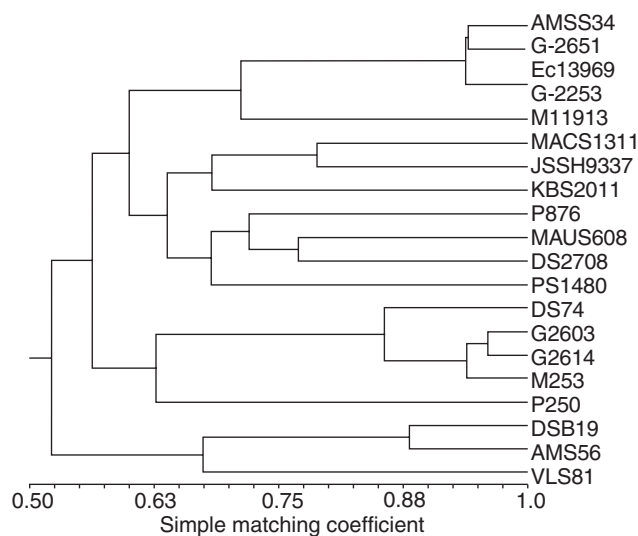


Fig 1 Dendrogram developed for 20 soybean genotypes using 23 SSR markers, where the bar below the dendrogram represents simple matching coefficient

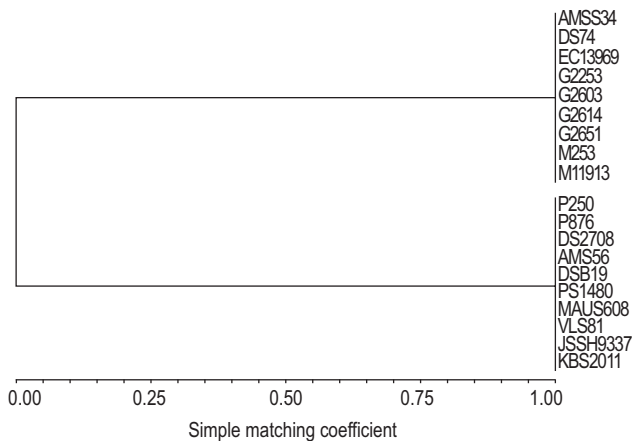


Fig 2 Dendrogram developed for 20 soybean genotypes using Satt423 marker, where the bar below the dendrogram represents simple matching coefficient

seed storability, illustrating its usefulness in marker assisted selection of genotypes for breeding cultivars with good seed longevity.

The present study demonstrated the usefulness of molecular markers to study complex quantitative traits like seed longevity in soybean, an important oilseed crop. Limited number of polymorphic markers used in the present study, did not allow the precise characterization of the genotypes. Therefore, there is a need to apply more polymorphic markers well-distributed on different linkage groups. Numbers of good storer soybean genotypes were identified under the present study which can be directly utilized in soybean crop improvement programme. Identification of candidate marker (Satt423) linked with storability will help in screening the storability of large number of soybean genotypes in limited time.

#### ACKNOWLEDGEMENT

The first author is thankful to the Indian Council of Agricultural Research (ICAR) and ICAR-IARI, New Delhi, India for the grant of Senior Research Fellowship and facilities during Ph D programme.

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