

## Characterization of seed longevity traits by using molecular markers in soybean [*Glycine max* (L.) Merrill]

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**ABSTRACT** Soybean [*Glycine max* (L.) Merrill] genotype varies in storability period during ambient storage. In this study, six genotypes of soybean, varying in storability, were characterized with 14 SSR markers. Out of 14 primers screened for seed longevity, the primer Satt 285, Satt 316, Satt 434 and Satt 538 gave distinct banding pattern. They produced an amplicon size range of 184 bp for those genotypes which are having poor seed longevity amongst the studied genotypes and the same loci produced amplicon size range up to 235 bp for those genotypes having good seed longevity.

**Key words:** Soybean, Seed longevity, SSR markers

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 seed production programme. Molecular markers tightly linked to desired genes are a valuable tool to detect the soybean genotypes of interest, thereby saving time and resources. Recent advances in molecular genetics, particularly the availability of number of molecular markers and high resolution genetic linkage maps is known to provide beneficial tool for genetic dissection and characterization of quantitatively inherited seed quality traits in soybean. Longevity of soybean seeds is said to be influenced by a number of traits. These traits include seed size, seed hardness, seed coat thickness, permeability, hull percentage, oil content *etc.* which are said to be under genetic control. These traits have been used in the breeding programme to improve seed quality and longevity in soybean [1]. Microsatellites or Simple Sequence Repeat (SSR) markers are highly polymorphic, abundant and distributed throughout the genome. Marker assisted selection (MAS) using DNA markers instead of phenotypic assays reduce the cost and increases the precision in subsequent selection steps applied in plant breeding. In agriculture, seed quality in terms of seed longevity is rarely considered by the breeders.

Presently, there is an emerging trend in the breeding programmes to identify such factors that influence the seed quality traits and to develop the cultivar for particular end uses.

### MATERIAL AND METHODS

For screening of the genotypes with SSR markers, six genotypes *viz.*, Kalitur, Birsa soya, JS-9305, JS-335, JS-71-05 and Co-1 were selected and sown in pots. Disease free tender leaves were taken from 2 to 3 weeks old seedlings of each genotype and used for DNA extraction. DNA was extracted based on cetyl-trimethyl ammonium bromide (CTAB) method [2]. The purified diluted genomic DNA extracts (50-100 ng) of soybean genotype was used as template DNA for PCR. Commercial kit of DNA primers were obtained from Imperial Life Sciences (P) Limited, Haryana, India. The sequence details of the primers are presented in the Table 1. The markers were selected based on the earlier studies where they were reported to be linked to the seed quality traits. The four individual dNTPs such as dATP, dGTP, dCTP and dTTP were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore. *Taq* DNA polymerase (5 units/ml) and 10x *Taq* buffer were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore.

### RESULT AND DISCUSSION

Wider diversity in any crop provides the first step to breeder for its improvement. Genetic variation is a pre-

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Table 1. List of oligonucleotide primers used for molecular analysis

Sl. No.	SSR loci	Sequence
1	Satt 285	GCGACATATTGCATTA AAAACATACTT GCGGACTAATTCTATTTTACACCAACAAC
2	Satt 434	GCGTTCCGATATACTATATAATCCTAAT GCGGGGTTAGTCTTTTTATTTAACTTAA
3	Satt 538	GCAGGCTTATCTTAAGACAAGT GGGGCGATAAACTAGAACAGGA
4	Satt 600	GCGCAGGAAAAAAAAACGCTTTTATT GCGCAATCCACTAGGTGTTAAT
5	Satt 598	CGATTTGAATATACTTACCGTCTATA CACAACTCCTGTGGCTGTTATACTAT
6	Satt 281	AAGCTCCACATGCAGTTCAAAAC TGCATGGCACGAGAAAGAAGTA
7	Satt 658	GCGTTGAGTGGTAAAATTTATAATTTAA ACTTGGCCCGGAAGTGCTCAATTG
8	Satt 187	GCGTTTTAATTTATGATATAACCAA GCGTTTTATCTCTTTTCCACAAC
9	Satt 310	GCGAGTTTTTATCTCATGACTTTT GCGGGGTATGGACCTAAAGAAAC
10	Satt 472	GCGAATACATAAACTCAAATTCAAATCATA GCGTCTATAAATTTCAATTCATAGTTTCAAT
11	Satt 459	TCGTGTTAGATTTTTACTGTACATT AACTGCATACCCTTTGTTTGAA
12	Satt 489	GCGTGTGCTTGCTTCTCTTAGACTGACT GCGTACTACTTACCCTGTTTGTCTAAAA
13	Satt 316	GTGAGAACTAGCCAAGAATAGA CAATTGTTTCAAATGACACT
14	Satt 288	GCGGGGTGATTTAGTGTGTTGACACCT GCGCTTATAATTAAGAGCAAAAGAAG

requisite for any crop improvement programme to be successful. DNA based molecular markers act as versatile tools to study variability and diversity in different plant species. Though a range of plant characters are currently available for distinguishing between closely related individuals, their sensitivity to environment and less genome coverage hinders their usage. DNA based molecular markers clearly allow the comparison of genetic material of two individual plants avoiding any environmental influence on gene expression.

Molecular genetic markers have brought phenomenal changes in the area of plant biotechnology by their ability to produce unique DNA profiles in various crops. DNA markers have become fundamental tools for research involving soybean improvement programs. Availability of molecular

marker technology has made possible the genetic dissection and characterization of many quantitatively inherited seed quality traits in soybean. Presently, many kinds of DNA based molecular markers such as RFLP, RAPD, SSR and AFLP *etc.* are available which detect polymorphism at the DNA level.

The present study employed microsatellites or simple sequence repeat (SSR) markers to assess genetic polymorphism. They are highly polymorphic, abundant and distributed throughout the genome [3].

#### SSR analysis

The 14 SSR primers used in the present study produced scorable, unambiguous markers. A total of 24 alleles were detected, out of which 20 were polymorphic. Polymorphism percentage was 85.71. The number of

**Table 2. Molecular diversity for seed longevity in soybean genotypes**

Primers	Number of alleles	Number of polymorphic alleles	Polymorphism
Sat 285	2	2	100
Sat 489	1	0	50
Sat 316	2	2	100
Sat 658	2	2	100
Sat 434	2	2	100
Sat 472	2	2	100
Sat 288	2	2	100
Sat 459	1	0	50
Sat 598	2	2	100
Sat 538	2	2	100
Sat 600	2	2	100
Sat 310	2	2	100
Sat 187	1	0	50
Sat 281	1	0	50
Total	24	20	85.71

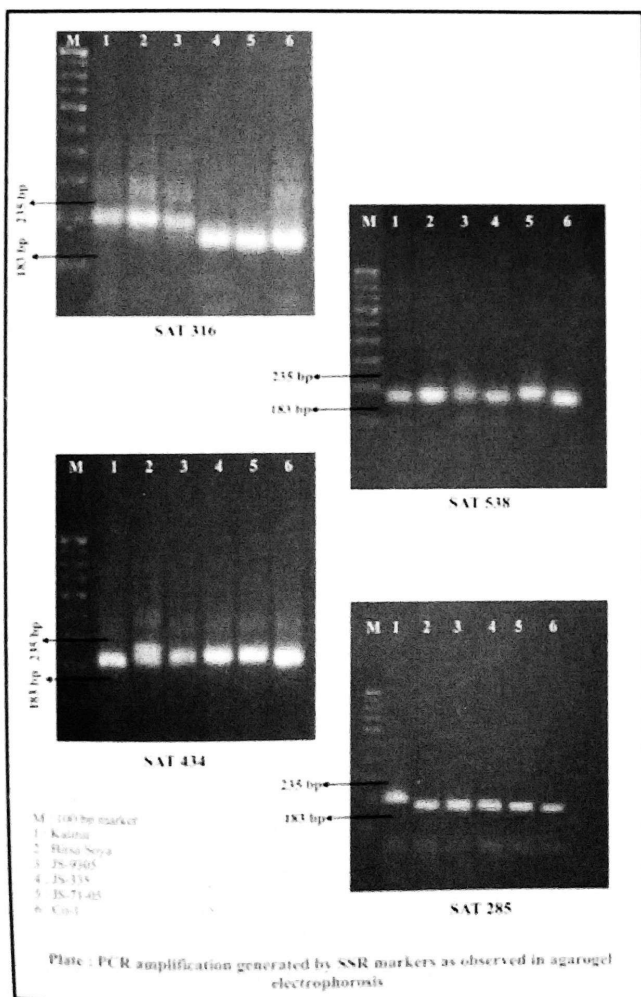


Fig.1. SSR profile of six soybean genotypes

alleles detected per primer pair ranged from 1 to 2 with an average of 1.5. Maximum number of amplified products (2) was observed in the profiles of the primers Satt 285, Satt 316, Satt 658, Satt 434, Satt 472, Satt 288, Satt 598, Satt 538, Satt600 and Satt 310. Minimum number of amplified product (1) was observed in the profiles of primers Satt 489, Satt 187 and Satt 281. The primers Satt 489, Satt 459, Satt 187 and Satt 281 were found to be 50 per cent polymorphic and rest were 100% polymorphic (Table 2). The SSR products size ranged from 180 to 250 bp. The SSR marker profiles of six genotypes generated by the primers Satt 316, Satt 538, Satt 434 and Satt 285 are given in Fig. 1.

This distinct banding pattern of the good and poor seed longevity genotypes gave a clear cut differentiation between the two extremes of seed longevity at the molecular level. These SSR markers are already reported to be linked to the seed longevity traits [4-6]. This result is in conformation with the earlier studies. However, other SSR primers did not show distinct banding pattern, even though they are linked to seed longevity. Hence there is need for further validation of these markers with large number of genotypes.

#### CLUSTER ANALYSIS

##### Similarity index

The binary data from the polymorphic primers were used for computing Jaccard's similarity indices which

were then applied for pair wise comparison among the six genotypes. The similarity coefficients based on 24 alleles of 14 SSR markers ranged from 0.30 to 0.85. Among the six genotypes, the highest similarity index (0.85) was observed between Birsa soya & Kalitur and JS-9305 & Birsa soya. The lowest similarity index (0.30) was observed between JS-7105 & Kalitur. Based on the similarity coefficients clusters were developed and are presented in Table 3.

**Table 3. Similarity coefficients of six soybean genotypes based on molecular markers**

Genotypes	Kalitur	Birsa soya	JS-9305	JS-335	JS-71-05	Co-1
<b>Kalitur</b>	1.00					
<b>Birsa soya</b>	0.85	1.00				
<b>JS-9305</b>	0.69	0.85	1.00			
<b>JS-335</b>	0.54	0.69	0.58	1.00		
<b>JS-71-05</b>	0.30	0.44	0.48	0.72	1.00	
<b>Co-1</b>	0.57	0.57	0.38	0.77	0.59	1.00

#### Clusters based on dendrogram

The similarity values obtained for pair wise comparison of SSR markers among the six soybean genotypes were used to construct dendrogram based on Jaccard's coefficient and the results are presented in Table 4. The six genotypes formed three clusters at nearly 68 per cent similarity levels. A dendrogram based on UPGMA analysis grouped the six genotypes into three clusters, There was clear clustering of the six genotypes in 3 major Clusters, which were grouped as good and poor seed longevity based on molecular data, where Cluster I included the genotypes with good seed longevity and Cluster III included genotypes with poor seed longevity.

**Table 4. Cluster analysis of six soybean genotypes based on molecular markers**

Cluster Number	Number of genotypes	Name of genotype
I	3	Kalitur, Birsa soya and JS-9305
II	2	JS-335, Co-1
III	1	JS-71-05

## CONCLUSION

Six soybean genotypes were subjected to SSR marker analysis to screen the genotypes for seed longevity traits, out of which primers Satt 285, Satt 316, Satt 434 and Satt 538 gave distinct banding pattern for identification of storage potential of soybean genotypes. From the result it was concluded that Kalitur and Birsa soya recorded good storability

potential compared to other genotypes whereas, JS-71-05 has poor storability.

## REFERENCES

1. KARMARKAR PG, PS BHATNAGAR AND SM HUSAIN (1998). Identification of bold seeded soybean genotypes with good longevity in segregation generation of crosses. *Soybean Genet. Newslett.*, **25**: 76.
2. SAGHAI-MAROOF MA, KM SOLIMAN, RA JORGENSEN AND RW ALLARD (1984). Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Nat. Academy Sci., USA*, **81**: 8014-8018.
3. CREGAN PB, T JARVIK, ALBUSH, RC SHOEMAKER, KG LARK, AL KAYA, TT VAN-TOAI, DG LOHNES, J CHUNG AND JE SPECHT (1999). An integrated genetic linkage map of the soybean genome. *Crop Sci.*, **39**: 1464-1490.
4. SINGH RK, RK RAIPURIA, VS BHATIA, R ANITA, PUSHPENDRA AND MS HUSAIN (2008). Identification of SSR markers associated with seed coat permeability and electrolyte leaching in soybean. *Physiol. Mol. Biol. Pl.*, **14**: 173-77.
5. KHARE D, P SAHU, N SAINI, AN SHRIVASTAVA AND M BHALE (2011). Soybean seed longevity. *Paper presented in National Seed Congress*, pp, 217-24.
6. JAGADISH H, MB ARUN KUMAR, A TALUKDAR, SK LAL AND MD ADLANI (2013). Molecular characterization and identification of candidate markers for seed longevity in soybean [*Glycine max* (L.) Merrill]. *Indian J. Genet.*, **73**: 64-71.