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Characterization of Contrasting Soybean Genotypes for Seed Longevity Using SSR Markers

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ABSTRACT: In Soybean [*Glycine max* (L.) Merrill], seed storability is a major problem and identifying the genotypes with good storability as well as the DNA marker associated with seed storability will help in Markers Assisted Selection (MAS) programme targeted for breeding soybean genotype with better seed storability. In this study, six genotypes of soybean, varying in storability, were characterized using 14 SSR markers that were reported to be linked or associated with seed longevity. Out of 14 primers screened, the primers Satt 285, Satt 316, Satt 434 and Satt 538 gave distinct banding pattern among the good and poor storers. They produced the allele of size range from 184 bp in genotypes having poor seed longevity and the allele of size 235 bp for those genotypes having good seed longevity.

Key words: Soybean, Seed longevity, Genotype, Primer

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 to till next sowing, which may be for one planting season or more, is crucial for the success of seed production programme. In soybean, seed storability is the major problem that needs to be addressed for the success of seed production programme as well as national soybean production.

Addressing the seed storability problem in soybean through conventional breeding programme is a challenging task not because of lack of diversity in soybean germplasm for seed storability but because of the time involved in phenotyping of the breeding lines for seed storability during the varietal development. Marker assisted selection (MAS) using DNA markers can ease, to certain extent, the breeding of soybean genotypes with better seed storability. For successful implementation of MAS, the first and foremost thing is to identify the DNA markers that are linked or associated with the character of interest for which the breeding programme is developed [1]. In this endeavour, few SSR markers have been reported to be linked or associated with seed longevity [2, 3, 4] using limited number of available genotypes. However, for the precise utilization of these SSR markers in MAS programme, we need to validate them across the soybean germplasm with genotypes expressing contrasting seed storability traits. Hence, in the present investigation, the reported SSR markers were used to charcterize the soybean genotypes with contrasting seed storability and assess the possibility of using the reported SSR markers in MAS programme of soybean, targeting the seed storability trait.

MATERIALS AND METHODS

Six genotypes *viz.*, Kalitur, Birsa soya, JS-9305, JS-335, JS-71-05, Co-1 were used for the present study. The natural storage of these genotypes in

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HDPE bag were carried out under ambient storage conditions at seed quality and research laboratory, seed unit, UAS, Dharwad, for 12 months. Among these genotypes, highest germination was observed in Kalitur (63.5%) and Birsa soya (62.3 %) whereas lowest in JS-335(59.3%), JS-9305(58.5%), JS-71-05(53.8 %) and Co-1(57.8 %) at the end of twelve month of storage period. Based upon natural storage, the genotypes are grouped as good storers and poor storers in this investigation.

Disease free seedlings of each genotype were raised in pots. Two to three weeks old seedlings (one seedling in each genotype) were used for DNA extraction [5]. The purified diluted genomic DNA (100 ng) of soybean genotype was used as template DNA for SSR marker analysis. 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 that are reported to be linked to the seed quality traits [4, 6 7]. The four individual dNTPs and TaqDNA polymerase (5 units/ml) and 10x Taq buffer were obtained from M/s Biogene, Bangalore

PCR Conditions: Master Mix was prepared for the six genotypes in tubes for individual primer by mixing different components in the proportion as shown in Table 2. The master mix was distributed to all the tubes (23 mL/tube) and 2 mL of template DNA from the respective genotypes were added making the final volume of 25 mL.

Agarose gel electrophoresis: Amplification products were subjected to electrophoresis in 2.5 per cent agarose gel. Agarose gel (2.5%) was prepared using electrophoresis grade agarose (Himedia) in a volume of 200 ml electrophoresis buffer (1xTAE) sufficient for a gel. Ethidium bromide was added at the concentration of 9 ml per 100 ml. The gel was allowed to set fully before removing the comb. Entire PCR products were thoroughly mixed with loading dye (bromophenol blue) before loading into the well. And then subjected to 100 volts for one and half hours for separation of PCR fragments. A 100 bp ladder (Biogene, Bangalore) was used as a known standard size marker. After the run, the gel was viewed under ultraviolet light and DNA banding pattern was photographed.

Scoring the amplified fragments: The products that were amplified were scored visually by comparing with the standard marker of size 100 bp. The fragments obtained from amplification was scored by denoting '1' for the presence and '0' for the absence, each of which was treated as an independent character regardless of its intensity, thus producing a binary data matrix. The binary data were used as an input for further calculation.

Statistical analysis of the profile of the amplified fragments: Pair wise genetic similarities between genotypes were estimated by DICE similarity coefficient. Clustering was done using the symmetric matrix of similarity coefficient and clusters obtained based on Unweighted Pair Group Arithmetic Mean (UPGMA) using SHAN module of NTSYS-PC version 2.0 [8].

RESULTS AND DISCUSSION

The DNA based molecular markers act as versatile tools to study genetic variability and diversity in different plant species. These molecular markers clearly allow the comparison of genetic material of two individual plants avoiding any environmental influence on gene expression. 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 simple sequence repeat (SSR) markers to assess genetic polymorphism. They are highly polymorphic, abundant and distributed throughout the genome [2]. Molecular markers tightly linked to desired genes are a valuable tool to detect genotypes of interest, saving time and resources. Marker assisted selection (MAS) using DNA markers instead of phenotypic assays reduces cost and increases the precision and efficiency of

Table 1. List of oligonucleotide primers used for molecular analysis

S.No.	SSR loci	Sequence
1	Satt285	GCGACATATTGCATTAAAAACATACTTGCGGACTAATTCTATTTTACACCAACAAC
2	Satt434	GCGTTCCGATATACTATATAATCCTAATGCGGGGTTAGTCTTTTTATTTAACTTAA
3	Satt538	GCAGGCTTATCTTAAGACAAGTGGGGCGATAAACTAGAACAGGA
4	Satt600	GCGCAGGAAAAAAAAACGCTTTTATTGCGCAATCCACTAGGTGTTAAT
5	Satt598	CGATTTGAATATACTTACCGTCTATACACAATACCTGTGGCTGTTATACTAT
6	Satt281	AAGCTCCACATGCAGTTCAAAACTGCATGGCACGAGAAAGAAGTA
7	Satt658	GCGTTGAGTGGTAAAATTTATAATTAAAACTTGGCCCGCGAAGTGCTCAATTG
8	Satt187	GCGTTTTAATTTATGATATAACCAAGCGTTTTATCTCTTTTTCCACAAC
9	Satt310	GCGAGTTTTTATCTCATGACTTTTGCGGGGGTATGGGACCTAAAGAAAC
10	Satt472	GCGAATACATAAAACTCAAATTCAAATCATAGCGTTCTATAAATTTCATTCATAG TTTCAAT
11	Satt459	TCGTGTTAGATTTTTACTGTCACATTAACTGCATACCCTTTGTTTGAA
12	Satt489	GCGTGTGCTTGCTTCTCTTAGACTGACTGCGTACTACTTACCCTGTTTGTCTAAAA
13	Satt316	GTGAGAAACTAGCCAAGAATAGACAATTGTTTCCAAATGACACT
14	Satt288	GCGGGGTGATTTAGTGTTTGACACCTGCGCTTATAATTAAGAGCAAAAGAAG

subsequent selection steps applied in breeding.

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 alleles detected per primer pair ranged from 1 to 2 with an average of 1.5. Primers *viz.*, Satt 285, Satt 316, Satt 658, Satt 434, Satt 472, Satt 288, Satt 598, Satt538, Satt600, Satt 310 have produced two alleles and primer Satt489 , Satt 187 and Satt 281 produce one allele. The primers Satt 489, Satt 459, Satt 187 and Satt 281 were proved to be 50 per cent polymorphic and rest were 100% polymorphic are presented in the Table 3. 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 Plate.

This distinct banding pattern for the good and poor seed longevity genotypes gave a clear cut differentiation between the two extremes of seed longevity at the molecular level. These particular SSR markers are already reported to be linked to the seed longevity traits [3, 4, 6, 7]. This result

Table 2. Components of master mix (25 µL)

Components	Quantity (µL/tube)
10X Assay bufferd	2
NTPs (2 mM)	1
Forward Primer (3 pM)	1
Reverse Primer (3 pM)	1
Taq DNA polymerase (5 U/µL)	0.2
Nano pure water	17.8
Template DNA (40 ng)	2

Table 3. Molecular diversity for seed longevity in soybean genotypes

Primers	No. of alleles	No. of polymorphic allele	Polymorphism
Sat285	2	2	100
Sat489	1	0	50
Sat316	2	2	100
Sat658	2	2	100
Sat434	2	2	100
Sat472	2	2	100
Sat288	2	2	100
Sat459	1	0	50
Sat598	2	2	100
Sat538	2	2	100
Sat600	2	2	100
Sat310	2	2	100
Sat 187	1	0	50
Sat281	1	0	50
Total	24	20	85.71

is on par with the earlier studies. However, other SSR primers did not show clear distinct banding pattern, even though they are linked to the seed longevity, and hence, there is further need for 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. The similarity index values obtained for each pair wise comparison among the six genotypes (Table 4). 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 and Kalitur and JS-9305 & Birsa soya. The lowest similarity index (0.30) was observed between JS-7105 and Kalitur.

Table 4. Similarity coefficients of six soybean genotypes based on molecular markers

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

Table 5. Cluster analysis of six soybean genotypes based on molecular markers

Cluster No.	No. of genotypes	Name of genotype
I	3	Kalitur Birsa soya JS-9305
II	2	JS-335 Co-1
III	1	JS-71-05

Clusters based on dendrogram: The similarity values obtained for each 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 5. 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. Cluster I included three genotypes viz., Kalitur, Birsa soya, JS-9305, having good seed longevity while cluster III comprised of one genotype which is having poor seed longevity. Cluster II comprised of JS-335 and Co-1, which were of relatively

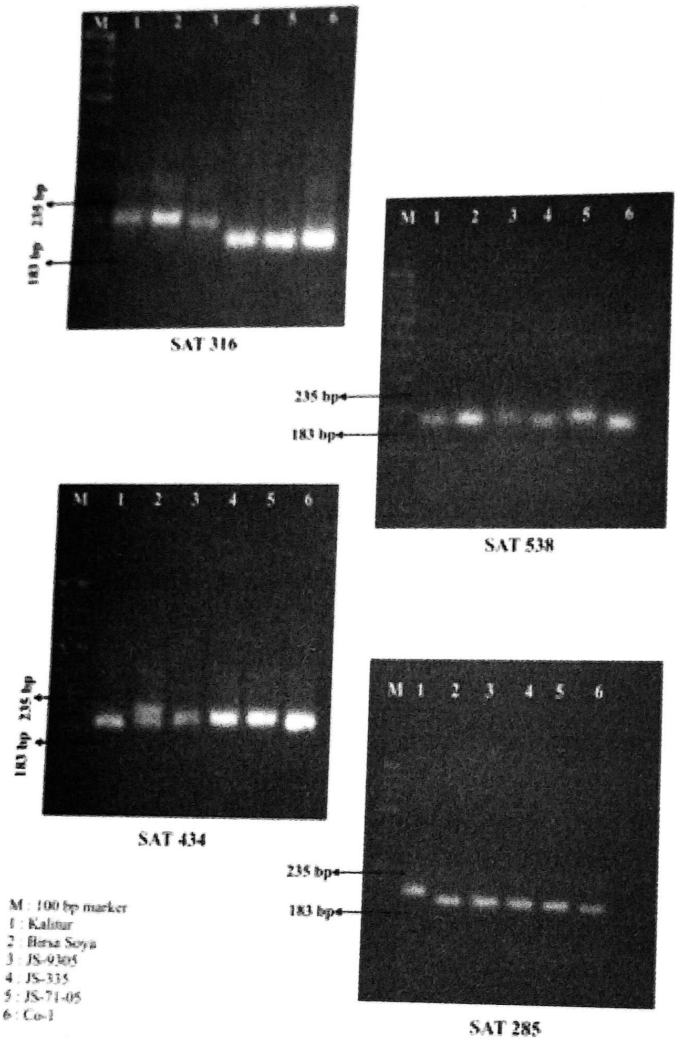

Fig. 1. PCR amplification generated by SSR markers as observed in agarogel electrophorosis

intermediate seed longevity.

Six soybean genotypes were subjected to SSR marker analysis to screen the genotypes for seed longevity traits out of which primer Satt 285, Satt 316, Satt 434 and Satt 538 gave distinct banding pattern for identification of storage potential of soybean genotypes. From the analysis, it was determined that Kalitur and Birsa soya recorded good storability whereas JS-335 and Co-1 showed medium storability and poor seed longevity was noticed in JS-71-05.

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