Validation of Drought Tolerance Gene-linked Microsatellite Markers and Their Efficiency for Diversity Assessment in a Set of Soybean Genotypes

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Soybean is well-thought-out to be a major crop owing to its significant involvement as vegetable oil and protein in human diet. However, inopportune, its production has been melodramatically declined attributable to the commonness of drought related stress.

Study Design: During the present study a total of 53 soybean genotypes were selected. For molecular diversity analysis as well as validation total 12 SSR markers were used. Molecular screening of soybean genotypes was done to determine the efficiency of available markers in genetic diversity analysis as well as their validation on the basis of their association with drought tolerance gene.

Place and Duration of the Study: The present study was conducted at Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Gwalior, Rajmata Vijayraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India during the year 2018 - 2019.
**Methodology:** Template DNA of all 53 selected soybean genotypes extracted for molecular screening. The current investigation has been accomplished to validate the available SSR markers with their efficiency in genetic diversity analysis in a set of soybean genotypes.

**Results:** Among applied drought tolerance gene-linked 12 SSR molecular markers, the highest genetic diversity (0.6629) was noticed in Satt520 while lowest (0.0370) was in Satt557 with an average of 0. 3746. While, the highest PIC value was 0.5887 prearranged by Satt520 and lowest 0.0363 by Satt557 with the mean worth of 0.3063.

**Conclusion:** Dendrogram constructed on the basis of banding profile of employed markers was able to discriminate some putative drought tolerant genotypes i.e., JS97-52, JS95-60 from rest of the genotypes. The results of the present examination may donate towards enhancement of soybean genotypes to bread drought tolerant varieties.

**Keywords:** Climate change; molecular diversity; drought; microsatellites; sustainable agriculture; water stress.

1. **INTRODUCTION**

Soybean is among the important crops because of its use as a source of vegetable oil in addition to proteins throughout the world [1,2]. Drought is an abiotic stress and envisaged to be increased in future [3]. It is a serious issue because of its role in reduction of production of important crops including soybean. Obtainability of adequate water supports in growth as well as development of plants. But, amendment in weather is a foremost reason of drought situations in several parts of the world. Drought stress may easily damage to the susceptible crop varieties. So, it is needed to identify drought tolerant varieties among the accessible varietal resources or advance a new variety with tolerant mechanism against drought.

Recognition or selection of a drought tolerant genotype is conceivable through morpho-physiological traits [4-6] under field conditions, biochemical [7-9] and biotechnological tools [10-12] with varying degree of success. However, numerous issues may affect the recital of a genotype/variety throughout field trials and may mislead the accurate identification. By reason of these confine an array of molecular markers has been applied to be acquainted with drought tolerant genotype/variety as they are commonly free from ecological influences. Numerous studies have been conducted to categorize genotypes/varieties of crop plants including soybean have been employing different classes of dominant as well as co-dominant molecular markers viz., Random Amplified Polymorphic DNA, Inter Simple Sequence Repeats, Amplified Fragment Length Polymorphism and Simple Sequence Repeat to study genetic diversity in soybean [13-16]. Among all the cited markers, SSRs have been extensively used in crop plants because of their higher level of polymorphisms, higher polymorphic information content (PIC), co-dominant inheritance and dispersal in the whole genome [17-22]. The present study was accomplished to screen putative drought tolerant soybean genotypes based on SSR markers.

2. **MATERIALS AND METHODS**

The current investigation was entailed of 53 *Glycine max* (L.) Merrill genotypes (Table 1) with diverse reactions to drought viz: susceptible and tolerant as investigated during previous studies [10-12]. The seeds were acquired from College of Agriculture, JNKVV, Jabalpur, RAK, College, Sehore and Zonal Agricultural Research Station, Morena, RVSKVV, Gwalior, Madhya Pradesh, India. The laboratory work was conducted at Molecular Biology Laboratory, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, India. Leaf samples of each of the genotype collected after 20 days after sowing for genomic DNA extraction.

2.1 **SSR Molecular Marker Analysis**

Genomic DNA from collected young leaves was carried out using CTAB method [23] with required modifications as adopted during our previous study [15]. Extracted DNA was evaluated qualitatively and quantitatively with the use of Nano spectrophotometer. Quantified DNA samples were diluted up to 15ng/μl for further analysis. Initially, a total of 20 SSR markers (Table 2) were selected on the basis of published literature for screening of drought tolerant and susceptible genotypes and procured from
3. RESULTS AND DISCUSSION

2.2 Data Analysis

SSR markers were amplified across all the genotypes. All twelve SSR markers (Table 3) were efficaciously genotyped initially (Table 2) but out of them only 53 soybean genotypes were attempted to amplify. A total of twenty drought tolerance linked SSR markers as seed related traits, YMV, molecular markers employed in this investigation offered valuable evidence about genetic diversity present in soybean genotypes as they were linked with other crops like pearl millet [5].

A total of twenty drought tolerance linked SSR markers were attempted to amplify 53 soybean genotypes initially (Table 2) but out of them only twelve SSR markers (Table 3) were efficaciously amplified across all the genotypes. All twelve SSR markers were found to be polymorphic.

Similar to this, Bisen et al. [15] reported less than 50% (23 out of 50 SSR markers) amplification and polymorphism efficiency of SSR markers in Indian soybean. The mean polymorphic alleles were 2.25. Out of twelve, three SSR markers viz., Satt226, Satt500 and Satt520 amplified maximum three alleles each and the rest of the markers were found to be able to amplify only two alleles each. The highest major allele frequency (0.9811) was observed in Satt557 tracked by 0.8868 in Satt174 while lowest (0.3585) in Satt520. The average major allele frequency was 0.7123. The highest genetic diversity (0.6629) was demonstrated by Satt520 while lowest (0.0370) was in Satt557. The average genetic diversity was 0.3746. Among all twelve SSR molecular markers the highest PIC value was 0.5887 given by Satt520 (Fig. 1) and lowest 0.0363 by Satt557 with an average PIC value of 0.3063. Similar to the present finding, the polymorphism of SSR loci perceived in this study match with the earlier data of Bisen et al. [15] and PIC values were in agreement with previous result of Sahu et al. [26]. Hipparagi et al. [27] found an average PIC value of 0.36 with SSR markers in soybean. According to various other researchers, PIC values were ranged from 0.199 to 0.87 [28,15]. Higher value of PIC indicates the presence of various alleles in every locus, and is also important in the identification of molecular markers-based analysis of variability [29,15].

Owing to high level of reproducibility and co-dominant inheritance, SSR markers have been practiced for distinguishing genotypes and investigating genetic relationships among 53 soybean genotypes. Microsatellites have been employed for genetic diversity analysis among soybean genotypes by various research groups [30,31,26]. The present study with 53 genotypes including a variety of imperative cultivars from India is the important study so far, to characterize the variation at molecular level. The twelve SSR markers employed in this investigation offered valuable evidence about genetic diversity present in soybean genotypes as they were linked with genotypes. For impressive genetic diversity analysis, number of alleles, polymorphic alleles, polymorphism percentage, and effective number of alleles, allele frequency, genetic diversity and polymorphism information content for each SSR locus were computed. The PIC values were generally good for all the SSR loci tested with an average of 0.266. One SSR loci revealed PIC values higher than 0.5 and, Satt510 was notable owing to its relatively higher polymorphism (four

Imperial Life Sciences Pvt. Ltd, Gurgaon, Haryana, India. Diluted DNA was amplified by PCR in a total volume of 10 μl comprising 25 ng template DNA, 1×buffer (75 mM Tris.HCl; pH 9.0), 50 mM KCl, 20 mM (NH₄)₂SO₄, 2 mM MgCl₂, 200 μM of each dNTP, 5 pmol procured SSR primers and 1-unit Taq DNA polymerase (Fermentas). PCR reactions were performed in a Bio-Rad thermocycler. Cycling parameters were initial denaturation step at 94 °C for 5 min, tracked by 94 °C, 30 s, 52–58 °C, 30 s and 72 °C, 30 s. This cycle was repeated 35 times, trailed by 5 min final extension at 72 °C. The amplified artifacts were separated on 3.5 % agarose gels and detected by ethidium bromide staining. Allele sizes were estimated in comparison with 100 bp DNA ladder (Fermentas).

2.2 Data Analysis

The PCR products generated by SSR were investigated by scoring qualitatively for presence or absence of bands. A genetic similarity between the genotypes was quantified by the similarity coefficient. In instance of SSRs, Polymorphism Information Content (PIC) was computed conferring to Anderson et al. [24] perusing the equation: 

$$PIC_j = 1 - \sum_{i=1}^{n} P_i^2$$

Where, $i$ = the $i^{th}$ allele of the $j^{th}$ marker, $n$ = the number of alleles at the $j^{th}$ marker and $p$ = allele frequency.

3. RESULTS AND DISCUSSION

Drought stress affects plant growth and development at every stage of life [25]. Molecular characterization and discrimination of drought tolerant and susceptible genotypes/varieties of soybean are important for further development of tolerant varieties with higher yield potential. Discrimination based on molecular data confirms the real diversity and genetic distance among/between genotypes. Earlier, various studies have been conducted to screen soybean genotypes for specific traits with the use of molecular markers as seed related traits [18], YMV [6], charcoal rot and Rhizoctonia root rot [16], drought [10] and in other crops like pearl millet [5].
alleles). The average numbers of alleles per locus in our analysis was lesser than the past study conducted by Kaewwongwal et al. [32] where it was 9.05. However, Bisen et al. [15] detected an average of 1.97 alleles per locus across 38 soybean genotypes. This high rate of SSR polymorphism may be attributed to the selected set of SSR markers which were previously tested for polymorphism among a set of genotypes. Nevertheless, the lower allele number and PIC values designates low allelic diversity in present set of soybean accessions. The SSR allelic diversity distinguished among soybean genotypes in this experimentation was low comparison to previous experimentation [33].

The UPGMA cluster analysis was accomplished employing SSR data. The clustering was done based on genetic similarity between and among studied soybean genotypes. Initially 53 soybean cultivars were divided into two clusters one minor and one major (Fig. 1). Minor cluster contained six genotypes, namely: JS97-52, JS95-60, JS93-05, RVS-14, MACS-58 and NRC-2. Among these six genotypes JS97-52 was alone and rest of the five genotypes showed similarity with each other. The clustering of bulky numeral of soybean germplasm lines in a single cluster indicates that soybean germplasms assemblage is having high genetic affiliation among genotypes. Among all 53 genotypes, JS97-52 formed a separate sub cluster and in a previous study it has been reported as drought tolerant genotype during field experiment as well as gene expression analysis [4]. In his experiment, genotype JS95-60 was also found as drought tolerant variety. Similarly in present study, genotype JS95-60 has shown similar banding pattern as in genotype RVS-14 with Satt174 and Sat_205 markers. These markers have been reported drought tolerant gene linked markers in soybean by researchers in their studies [34,35]. The similar banding pattern indicates drought tolerant nature of genotype RVS-14. Genotypes JS95-60 and JS93-05 share common parent.

Major cluster contained forty-seven genotypes and this cluster was further divided into two sub clusters, one major and one minor. Minor group had ten genotypes including MACS-15-20, RSC10-70, SKF-SPS-11, RVS-76, KDS980, KDS992, RSC-10-71, JS335, RVS2011-35 and RVS2007-6. Among these ten genotypes KDS992 and KDS980 shared common parent i.e., JS93-05. The major sub cluster contained 37 genotypes and it was later splinted into two sub groups. Major sub group contained 21 genotypes viz., MACSNRC-1575, NRC-147, AGS111, AMS100-39, MACS1520, JS20-94, NRC86, EC457286, NRC125, PS-1613, VLS94, SL-1068, RSC10-52, RSC130, G-29, JS20-34, JS20-84, MACS575, NRC SL-1, PS-1092 and NRC127 while minor cluster had genotypes namely, SP37, SL-1123, NRC76, AMSMBC-18, NRC131, NRC134, RVS18, RVS24, AMS2014-1, RVS2001-4, JS20-98, JS20-69, JS20-71, JS20-116, NRC-132 and JS20-29. Similar clustering was found in previous studies conducted on microsatellite-based diversity analysis among Indian soybean genotypes [36,26].

![Fig. 1. Electrophoretic banding pattern of template DNA samples of soybean genotypes amplified with SSR markers](image-url)
| S. No. | Genotypes     | Source/Pedigree                          | S. No. | Genotypes     | Source/Pedigree                          |
|--------|---------------|------------------------------------------|--------|---------------|------------------------------------------|
| 1.     | JS 20-29      | JS 97-52 x JS 95-56                      | 28.    | RSC-10-52     | NRC 37X JS335                            |
| 2.     | JS 20-69      | JS 97-52 x SL 710                        | 29.    | SL-1123       | Selection from AGS751                    |
| 3.     | JS 335        | JS 78-77 x JS 71-05                      | 30.    | SL-1068       | SL75XSL525                               |
| 4.     | JS 20-98      | JS 97-52x JS SL710                       | 31.    | AGS 111       | Germplasm accession                      |
| 5.     | JS 20-94      | JS 97-52 x JS 20-02                      | 32.    | EC457286      | Germplasm accession                      |
| 6.     | JS 93-05      | Selection from PS 73-22                  | 33.    | MACS725       | JS93-05X MAUS71                          |
| 7.     | JS 20-116     | JS 97-52 x JSM 120 A                     | 34.    | SP 37         | Not known selection                      |
| 8.     | JS 95-60      | Selection from PS 73-22                  | 35.    | NRC-125       | EC54688xps1044                           |
| 9.     | JS 97-52      | PK 327 x L 129                           | 36.    | NRC-132       | JS97-52X PI086023                        |
| 10.    | JS 20-84      | JS 98-63 x PK 768                        | 37.    | NRC-134       | NRC7XAGS191                             |
| 11.    | JS 20-34      | JS 98-63 x PK 768                        | 38.    | NRC SL-1      | JS33XSLS52                               |
| 12.    | JS 20-71      | JS 97-52 x JS 90-5-12-1                  | 39.    | PS 1092       | PS1042 x MACS 450                        |
| 13.    | RVS 2007-6    | JS 20-10 x MAUS162                       | 40.    | PS 1613       | PS1225XPS1042                            |
| 14.    | RVS 2011-35   | JS 335 X PK 1042                         | 41.    | AMS 2014-1    | AMS99-33XH6P5                            |
| 15.    | RVS 2001-4    | JS 93-01x EC 390981                      | 42.    | KDS 992       | JS93-05XEC241780                         |
| 16.    | RVS -14       | JS 93-05x EC 390981                      | 43.    | VLS -94       | VL Soya59X VS2005-1                      |
| 17.    | RVS -24       | J.P 120 x JS 335                         | 44.    | SKF-SPS -11   | Not known selection                      |
| 18.    | RVS -18       | JSM110XJSM66                             | 45.    | RVS 76        | MAUS-162XJSM-66                          |
| 19.    | NRC- 76       | NRC-37XL-27                              | 46.    | NRC127        | JS97-52XPI542044                         |
| 20.    | NRC -86       | RK515XEC481309                           | 47.    | KDS980        | JS93-05XAMS1                             |
| 21.    | NRC -130      | EC390977xEC538828                        | 48.    | G-29          | Germplasm                                |
| 22.    | NRC -131      | EC390977xEC538828                        | 49.    | RSC-10-70     | JS335X Bragg                             |
| 23.    | NRC -147      | Germplasm accessions C210                | 50.    | RSC-10-71     | Bragg XJS335                             |
| 24.    | AMSMBC -18    | Mutant of Bragg                          | 51.    | NRC-2         | Induced mutant of Bragg                  |
| 25.    | AMS-100-39    | Mutant of JS39-05                        | 52.    | MACS-15-20    | NRC37XMohetta                            |
| 26.    | MACS – 1520   | EC241780XMACS330                         | 53.    | MACS-58       | JS2 x Improve pelican                    |
| 27.    | MACSNRC-1575  | PI542044XJS9305                          |        |               |                                          |
Table 2. List of SSR markers used for screening of soybean genotypes

| S.No. | Primers | Forward 5’-3’ | Reverse 3’-5’ | References |
|-------|---------|---------------|---------------|------------|
| 1     | Satt383 | CGATCTAACACGC ATATTTCCCTCTG | CTTCCTAATATTGGCA ACCTCTATG | [37] |
| 2     | Satt557 | GCGGGATCCACCA TGTAATATGTG | GGCACAACCCCTTTAT TGAA | Zhang et al. (2012) |
| 3     | Satt577 | CAAGCTTAAGTCT TGGTCTTTCTCT | GGCCTGACCCAAAACCTA AGGGAGTC | Li et al. (2017) |
| 4     | Sat_171 | GCGCTCCTCTTTTT TTTCACCTTC | GGCCTGGGGATTTTG TATTTTT | [34] |
| 5     | Satt321 | CACCGTGCTAAA ACTGTGCTGT | GCGTGCAAGAGATTTT AGACATC | [34] |
| 6     | Satt244 | GCGCCCCATATGT | TTAAATTTATAGGAG TTTATTCACAG | [34] |
| 7     | Satt393 | CAAGCCCATAAAC GAAATAAA | GCTCGGCTTGGCTTT TGCTACA | [37] |
| 8     | Satt520 | GCCGTGGCAAGA GTGACA | GCGATTTGGACTTTCT A | [34] |
| 9     | Satt540 | CTGCGGAATCAAG CTGGTAGTTAC | CCGTGATTGCGAAGAG GATATT | [34] |
| 10    | Satt547 | GCGCTATCCGATC CATATGCTG | TGATTTCCGCTAGTTAAT ATCA | [34] |
| 11    | Satt551 | GAATATCACGCGA GAATTTTAC | TATATGCCAACCCCTTACAAT | [34] |
| 12    | Satt286 | GCGGCGTTAATTT ATGCCCGGAAA | GCGTTTGGCTAGAAT TTCAAGTTCA | [34] |
| 13    | Sat_312 | GCCGCTCCCATATA | GCGAAGCGAACAATAA TCAACATC | [38] |
| 14    | Sat_044 | AAATAATTTTATA GGTACATGTT | TTAACACTAAGATTAG GTCTAA | [38] |
| 15    | Satt226 | GCCGAAACACTCA CTATAGAACATAC | GCGTCTCCTACTTTTCAATTC | [34] |
| 16    | Sat_375 | GGCTGTATTAATGAT TGACATTAGGTTCA | GCGTGCAAAGAAGAACT | [34] |
| 17    | Satt174 | TTTGTCTTCTTTGCTTCT | TTTGTCTTCTTTAC | [34] |
| 18    | Sat_205 | GCCGCTTTTGC TGGTCTGTTC | GCGAGCTTTTTAATT TAGAAATCAAT | [35] |
| 19    | Satt489 | GCGTGCTTGTGCT | GCGTACTACCTACCTG | [35] |
| 20    | Satt500 | GCCGAACGACCATG ATATACACA | GCGTCTTTGAAAGCA TTGTTATA | [39] |
Fig. 2. Dendrogram representing SSR markers-based relationship among genotypes of soybean
Table 3. Different parameters analyzed with drought linked SSR markers in soybean

| Marker  | Major Allele Frequency | Allele No. | Gene Diversity | PIC Value |
|---------|------------------------|------------|----------------|-----------|
| Sat_044 | 0.6604                 | 2          | 0.4486         | 0.3480    |
| Sat_171 | 0.5660                 | 2          | 0.4913         | 0.3706    |
| Sat_205 | 0.8302                 | 2          | 0.2820         | 0.2422    |
| Sat_375 | 0.8679                 | 2          | 0.2293         | 0.2030    |
| Satt174 | 0.8302                 | 2          | 0.2008         | 0.1806    |
| Satt226 | 0.5849                 | 3          | 0.5005         | 0.3928    |
| Satt244 | 0.6038                 | 2          | 0.4785         | 0.3640    |
| Satt500 | 0.7547                 | 3          | 0.3788         | 0.3199    |
| Satt520 | 0.3585                 | 3          | 0.6629         | 0.5887    |
| Satt540 | 0.6792                 | 2          | 0.4357         | 0.3408    |
| Satt551 | 0.7736                 | 2          | 0.3503         | 0.2889    |
| Satt557 | 0.9811                 | 2          | 0.0370         | 0.0363    |
| Mean    | 0.7123                 | 2.25       | 0.3746         | 0.3063    |

4. CONCLUSIONS

The clusters formed during the present study based on SSR markers data were able to differentiate few drought tolerant genotypes from rest of the susceptible genotypes. The grouping of the genotypes also indicates clustering of most of the genotypes according to their centers of development. Some of the genotypes showing higher similarity were also developed with the use of common parents during hybridization programme. These results confirm the efficiency of SSR markers to discriminate the genotypes according to their genetic makeup as well as targeted traits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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