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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, inopportunely, 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 Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India during the year 2018 - 2019.

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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

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 $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 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].

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

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, NRC130, 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].

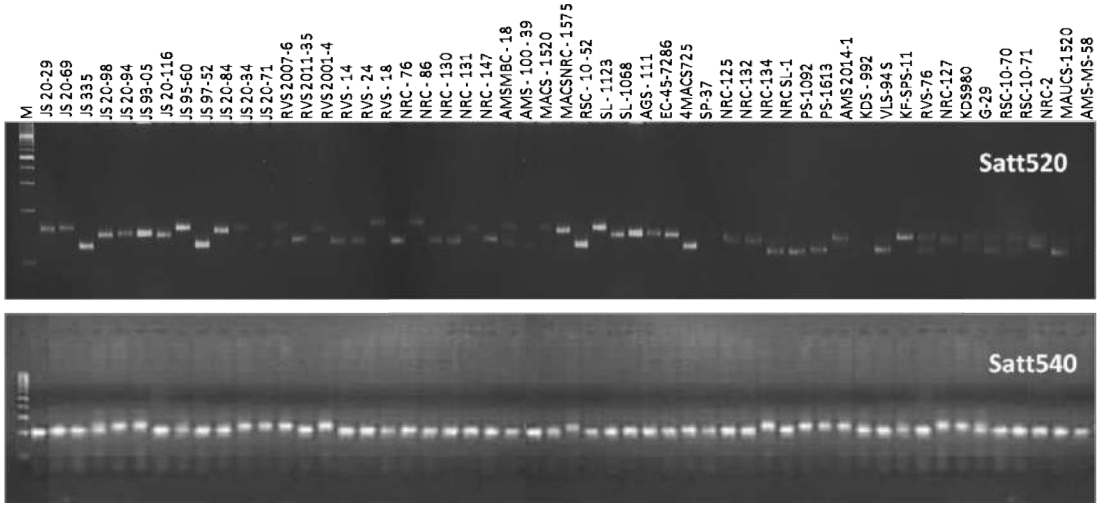

Fig. 1. Electrophoretic banding pattern of template DNA samples of soybean genotypes amplified with SSR markers

Table 1. List of soybean genotypes with their parentage

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	SL755XSL525
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	JS335XSL525
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	RKS15XEC481309	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 JS93-05	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 ATATTTCCTCTG	CTTCCCTAATATTGGCA ACCTCTATG	[37]
2	Satt557	GCGGGATCCACCA TGTAATATGTG	GCGCACTAACCCTTTAT TGAA	Zhang et al. (2012)
3	Satt577	CAAGCTTAAGTCT TGGTCTTCTCT	GGCCTGACCCAAAACTA AGGGAAGTG	Li et al. (2017)
4	Sat_171	GCGCTCCTCTTTT TTTCACTTTC	GCGCGTGGGATTTTGG TATTTTT	[34]
5	Satt321	CACCGTCGTAAAA ACTGTGTCGT	GCGTGTCAAAGAGTTTT AGACATC	[34]
6	Satt244	GCGCCCCATATGT TTAAATTATATGGAG	GCGATGGGGATATTTTC TTTATTATCAG	[34]
7	Satt393	CAAGCCCATAAAC GAAATAA	GCTCGGCTTGGCTTGTT TACTA	[37]
8	Satt520	GCGGTGTGCAAGA GTGACA	GCGCATTTGGACTTTCT A	[34]
9	Satt540	CTGGCGAATCAAG CTTTGTAAC	CCGTGATTGCGAAGAG GATATT	[34]
10	Satt547	GCGCTATCCGATC CATATGTG	TGATTTCGCTAGGTAAA ATCA	[34]
11	Satt551	GAATATCACGCGA GAATTTTAC	TATATGCGAACCCTCTTACAAT	[34]
12	Satt286	GCGGCGTTAATTT ATGCCGGAAA	GCGTTTGGTCTAGAATA GTTCTCA	[34]
13	Sat_312	GCGCCTCCCATTA CTTCGGATTAGTTA	GCGAACGCAACAAATAA TCAAACATC	[38]
14	Sat_044	AAAAAATATTTATA GGTTACATGTG	TTACCACTAAGAATTAG GTCTAA	[38]
15	Satt226	GCGAAACAACTCA CTTAAGCAATACAT	GCGTCCTCCTACCTTTC TTATC	[34]
16	Sat_375	GCGTGTTAATGAT TGCATAAGGTTCG	GCGTGTCAAAAGAAACT CAATAAAGAAAAAT	[34]
17	Satt174	TTTCATTTCTTTGC CTTCT	TTCGTAGTCCGTCTTTCAT	[34]
18	Sat_205	GCGCCTTTTCGTC TGTTCTGTTC	GCGAGCTTTTAAAAATT TAGAAATCAAT	[35]
19	Satt489	GCGTGTGCTTGCT TCTCTTAGACTGACT	GCGTACTACTTACCCTG TTTGTCTAAAA	[35]
20	Satt500	GCGAACGACCATG ATAATCACA	GCGCTCATTTGAAAGCA TTGTTATA	[39]

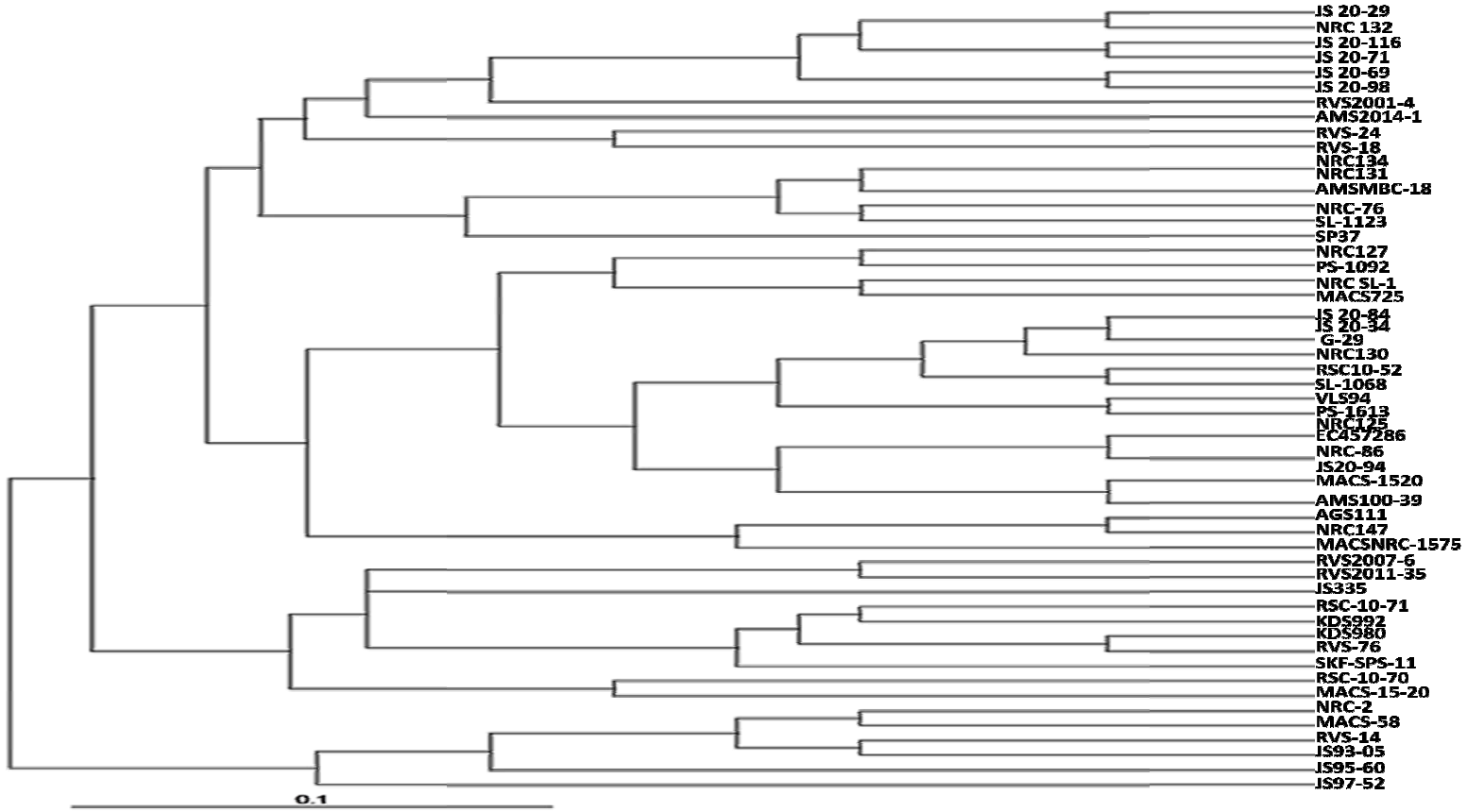

Fig. 2. Dendrogram representing SSR markers-based relationship among genotypes of soybean

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	0.8868	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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