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Research Article

Identification of unique alleles and assessment of genetic diversity of soybean genotypes using SSR markers and seed traits

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Abstract
Thirty polymorphic SSR markers and seven seed traits were used to determine the genetic diversity among twenty five soybean genotypes that differ with respect to seed coat color. A total of 133 alleles were detected and the number of alleles for each SSR locus varied from two to seven with an average of 4.0. Polymorphic information content varied from 0.81 to 0.07. Jaccard's similarity coefficient grouped the genotypes into two major clusters. To test the goodness of fit of clusters to SSR markers, cophenetic correlation was estimated. Cophenetic value of 0.98 indicated a very good fit. A combination of SSR and seed traits data grouped the genotypes into two major clusters with grouping of all the brown seeded genotypes into one subcluster. A total of 21 unique alleles were identified in twenty five soybean genotypes which is a valuable resource for DNA fingerprinting studies. A set of nine SSR markers (Satt 600, Satt 463, Satt 371, Satt 193, Satt 538, Satt 126, Satt 286, Satt 281 and Satt 656) could differentiate all the soybean genotypes.

Key Words
Genetic diversity, SSR markers, Soybean, unique alleles, seed traits

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an important legume crop grown for protein and oil. The high nutritional value of 40 per cent protein and 20 per cent oil makes it a miracle crop of the twentieth century. Around 55 per cent of total vegetable oil in the world is contributed by soybean crop. India ranks fifth in soybean production (10.98 m.t.) in the world with area of 10.47 m.ha and productivity of 1049 kg/ha. A prerequisite for crop improvement is the study of existing genetic variability in the germplasm for utilization in breeding program. Studying genetic variability of soybean germplasm differing for seed coat colour helps in the selection of parents for development of mapping populations and to derive transgressive segregants with respect to seed longevity and widening the genetic base (Kumawat *et al.,* 2015). Cultivars suitable for different agro climatic conditions were developed by soybean researchers through introduction, selection, mutation, hybridization of elite cultivars and germplasm followed by systematic breeding and evaluation programmes (Chauhan *et al.,* 2015). The use of DNA markers for germplasm characterization in addition to morphological traits provides additional information which is highly useful for germplasm registration and protection. SSR markers have been widely used in the genetic diversity

studies of the soybean germplasm collections worldwide and high levels of polymorphism at SSR loci have been reported for both the number of alleles per locus and gene diversity (Wang *et al.,* 2006a, 2010; Fu *et al.,* 2007; Wang and Takahata, 2007; Li *et al*., 2008; Singh *et al.,* 2010; Tantasawat *et al.,* 2011). With advancement in science, DNA markers associated with specific traits are identified and utilized in germplasm characterization and marker assisted selection. The present investigation on molecular characterization of soybean germplasm has been carried out to assess the existing genetic diversity and identification of genotype specific markers.

MATERIALS AND METHODS

Twenty-five soybean genotypes having variable seed coat colour were collected from Agricultural Research Station, Adilabad, Prof. Jayashankar Telangana State Agricultural University (PJTSAU), Telangana (**Table 1**) as seed coat colour plays a prominent role in seed longevity. The germplasm in this study included released cultivars and advanced breeding lines. Seed traits such as seed coat colour, 100 seed weight, seed length, seed width, seed thickness, seedling length and seedling dry weight were recorded. The seed traits excluding seed coat colour were classified as small, medium, large and extra-large. Data was scored as 0 or 1 and analysed along with SSR data using NTSYS-pc ver.2.02.

Genomic DNA was isolated from young leaves following the CTAB (cetyl trimethyl ammonium bromide) procedure as described by Saghai-Maroof *et al.* (1984). Quantification was accomplished by analyzing the DNA on 0.8% agarose gel stained with ethidium bromide using diluted lambda DNA as standard. The genomic DNA was diluted to 50 ng/µl for PCR. A total of 34 SSR markers distributed across the integrated linkage map of soybean (Cregan *et al.,* 1999) were used. The primer sequences of SSR makers along with the annealing temperatures are presented in **Table 2**. DNA was amplified in a total reaction volume of 10µl with the following cycling conditions: Initial denaturation at 94°C for 5 min. followed by denaturation at 94°C for 1 min. annealing at 48-58°C (based on primer Tm) for 30 sec. and extension at 72°C for 45 sec. This cycle was repeated 35 times, followed by 7 min. final extension at 72°C. The amplified products were separated on 3% MetaPhor agarose gel and detected by ethidium bromide staining. The gel was visualized in UV transilluminator and photographed using gel documentation system.

The PCR products were analyzed by scoring qualitatively for the presence (1) or absence (0). Polymorphism Information Content (PIC) was calculated according to Anderson *et al.* (1993) using the following equation:

$$PIC_{j=}1-\sum^{n}_{i=1} P_i^2$$

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.
The similarity matrix was analysed using NTSYS-pc ver.2.02 to produce an agglomerative hierarchical classification by employing UPGMA with average linkage. Genetic similarity matrices were generated based on polymorphic SSR markers and seed traits (NTSYS-pc2.02).

RESULTS AND DISCUSSION

Seed storability is the major constraint in soybean seed production. The present investigation deals with the study of genetic diversity among twenty five coloured soybean genotypes based on the seed traits and SSR markers

Table 1. List of soybean genotypes used in the present study

Genotype	Seed Coat Colour	Genotype	Seed Coat Colour
CAT 195 BR4	Brown	DS91-3	Yellow
CAT 192 BR15	Brown	EC 12503	Yellow green
CAT 1622 TG X 302-2A	Brown	CAT 971-GP2	Yellow green
CAT 194 BR3	Brown	G828	Yellow
NG 1142 CHICO	Brown	NRC-130	Yellow
NRC 2755	Brown	DS 24110	Yellow
CAT 243 DE 201	Black	EC 113416	Yellow green
UPSL 387	Black	KARUNE	Green
KALITUR	Black	PSPB-23	Green
G1922	Black	CAT 2059 GC 84058-18-4	Green
TG X 849D-13-4	Black	AGS 12 CAT25-A	Yellow
IC 16572	Black	NRC 105	Green
CAT 1852 TG X 854-25D	Black		

as seed coat colour is a predominant trait affecting seed storability. The information is highly useful for plant breeders in selection of parental lines for hybridization program aimed at improving seed storability in soybean.

Among all the available DNA markers, SSRs have been used successfully in estimation of genetic diversity and studying the relationships among soybean genotypes in different populations (Wang *et al.,* 2006a,b; Guan *et al.,* 2010; Wang *et al.,* 2010; Jain *et al.,* 2017). A total of thirty-four SSR markers distributed on 18 of 20 linkage groups of soybean were used for studying genetic diversity among which twenty five soybean genotypes having varying seed coat colour. Among 34 SSR markers studied, 30 were polymorphic and produced 133 alleles. The number of alleles for each SSR locus varied from two (Satt 598, Satt 162, Satt 619 and Satt 285) to seven (Satt 534 and Satt 133) with an average of 4.0 (**Table 3**). The size of the alleles ranged from 78 to 346 bp (**Fig. 1**). Polymorphic information content is a measure of the allelic differentiation. The highest PIC value was observed for the marker Satt 126 (0.81) and the lowest for Satt 285 (0.07) (**Table 3**). A combination of nine SSR markers (Satt 600, Satt 463, Satt 371, Satt 193, Satt 538, Satt 126, Satt 286, Satt 281 and Satt 656) was able to differentiate all the 25 soybean genotypes. In addition, 21 rare alleles (allele having frequency of less than 5%) from 30 SSR loci were identified. A maximum of two rare alleles/SSR locus were identified (Satt 463, Satt 565, Satt 631, Satt 686 and Satt 133). It was also observed that the frequency of rare alleles was much higher at SSR loci which have large number of alleles (Jain *et al.,* 2004; Gupta and Manjaya, 2017).

Table 2. List of SSR primers used in the present study

Primer name	Primer sequence		Annealing temp^{0}C
	Forward primer	Reverse primer	
Satt 286	GCGGCGTTAATTTATGCCGGAAA	GCGTTTGGTCTAGAATAGTTCTCA	55
Satt 534	CTCCTCCTGCGCAACAACAATA	GGGGGATCTAGGCCATGAC	60
Satt 565	GCGCCCGGAACTTGTAATAACCTAAT	GCGCTCTCTTATGATGTTCATAATAA	55
Satt 371	TGCAAACTAACTGGATTCACTCA	GAGATCCCGAAATTTTAGTGTAACA	55
Satt 184	GCGCTATGTAGATTATCCAAATTACGC	GCCACTTACTGTTACTCAT	51
Satt 619	GGCAGAACTAGTACGCTTCTGATT	GCGGTTAAGCATAATAGATCAGCCT	53
Satt 481	GGGTTAACCGTCCACACATCTATT	GACGGTTTTAAACGGTAAGAAAAT	48,50
Satt 463	TTGGATCTCATATTCAAACTTTCAAG	CTGCAAATTTGATGCACATGTGTCTA	52
Satt 193	GCGTTTCGATAAAAATGTTACACCTC	TGTTCGCATTATTGATCAAAAAT	48
Satt 175	GACCTCGCTCTCTGTTTCTCAT	GGTGACCACCCCTATTCCTTAT	53
Satt 598	CGATTTGAATATACTTACCGTCTATA	CACAATACCTGTGGCTGTTATACTAT	48,50
Satt 281	AAGCTCCACATGCAGTTCAAAAC	TGCATGGCACGAGAAAGAAGTA	50
Satt 389	GCGGCTGGTGTATGGTGAAATCA	GCGCCAAAACCAAAAGTTATATC	55
Satt 538	GCAGGCTTATCTTAAGACAAGT	GGGGCGATAAACTAGAACAGGA	51
Satt 600	GCGCAGGAAAAAAAAAACGCTTTTATT	GCGCAATCCACTAGGTGTTAAT	52
Satt 285	GCGACATATTGCATTAAAAACATACTT	GCGGACTAATTCTATTTTACACCAACAAC	50
Satt 434	GCGTTCCGATATACTATATAATCCTAAT	GCGGGGTTAGTCTTTTTATTTAACTTAA	52
Satt 162	GGGAAGAAGTATATGCTACATCAA	GGGTTAATTTTTATCTTCTAATAGTTT	48
Satt 523	GCGATTTCTTCCTTGAAGAATTTTCTG	GCGCTTTTTCGGCTGTTATTTTTAACT	53
SOYGPATR	GGAAGAAAGTATTGGTCTGT	AGGAGAGAGTGGAGAGATTA	54
Satt 631	GGTAGATCCAGGAGCTTGAGTCAG	GCGCATCTCACTGCACTTGATTTT	55
Satt 126	GCTTGGTAGCTGTAGGAA	ATAAAACAAATTCGCTGATAT	55
Satt 129	TTCAGTACAAGTCGGGTGAATAATAATA	TCACATGTTCGGGACTTAAGGTAT	56
Satt 168	CGCTTGCCCAAAAATTAATAGTA	CCATTCTCCAACCTCAATCTTATAT	56
Satt 656	GCGTACTAAAAATGGCAATTATTTGTTG	GCGTGTTTCAGTATTTGGATAATAGAAT	55
Satt 686	ACGGAAAATAAATGAAACTAAGA	GCGCTATCAGATAGAGAAGCAGAAGAAT	55
Satt 289	GCGCCCAGGTTTAAAAGT	CTGCCCCATCACTAGCCCTTCTT	55
Satt 133	GCAAATGAAGAAAAGATGGATT	TAAAGCGATGGTTGAAGAAAG	56
Satt 390	AGTGGCTGATAAAAAAAATACTCA	ATAATCGCGGCACAATAATTC	55
Satt 038	GGGAATCTTTTTTTCTTTCTATTAAGTT	GGGCATTGAAATGGTTTTAGTCA	55

Table 3. List of SSR primers for genotyping of 25 soybean genotypes

SSR locus	Allele size range (bp) approximate	Number of alleles	PIC value	Rare alleles
Satt 600	150-210	4	0.71	0
Satt 463	110-200	5	0.65	2
Satt 286	188-238	4	0.68	1
Satt 371	250-278	5	0.75	0
Satt 481	78-110	4	0.64	0
Satt 281	190-240	5	0.75	1
Satt 285	195-200	2	0.07	1
Satt 619	112-125	2	0.43	0
Satt 175	150-165	4	0.57	1
Satt 523	110-135	5	0.72	0
Satt 162	300-320	2	0.58	0
Satt 193	215-250	6	0.80	0
Satt 538	110-135	4	0.59	1
Satt 038	164-200	4	0.56	0
Satt 434	325-346	6	0.75	1
Satt 598	165-175	2	0.14	0
Satt 534	148-188	7	0.81	1
Satt 565	158-200	6	0.70	2
Satt 184	148-183	5	0.70	1
Satt 129	117-150	4	0.60	1
Satt 168	154-206	4	0.65	0
Satt 631	116-172	6	0.76	2
Satt 126	120-164	6	0.81	0
Satt 656	135-153	5	0.75	0
Satt 686	261-293	4	0.56	2
Satt 289	202-217	4	0.67	1
Satt 389	226-250	4	0.69	0
Satt 133	213-293	7	0.80	2
SOYGPATR	116-124	4	0.64	1
Satt 390	243-252	3	0.55	0

Genetic similarity matrices of soybean genotypes were generated using polymorphic SSR markers. The genetic similarity coefficient between individuals based on SSR data ranged from 0.07 [CAT-2059-GC-84058-18-4 (green) and AGS12-CAT25A (yellow)] to 0.51 [UPSL-387 (black) and NRC-130 (yellow)]. The dendrogram based on UPGMA clustering clearly classified the twenty-five soybean genotypes into two distinct clusters indicating the diverse nature of the germplasm (**Fig. 2**). Cluster I divided into two subclusters IA comprising of three genotypes - CAT 195 BR4& NG1142 CHICO (brown), DS91-3 (yellow); IB having ten genotypes of which five are black seeded-IC 16572, G1922, CAT 243 DE201, TGX849D-13-4& UPSL 387 (black), CAT 971 GP2, AGS12 CAT25A& NRC 130 (yellow), EC 12503 (yellow green), KARUNE (green). Cluster I is further classified into two subclusters IIA & IIB. Cluster IIA having five genotypes [DS24110 (yellow), EC 113416 (yellow green), CAT 2059GC84058-18-4 (green), G828 (yellow), NRC 2755 (brown)] and IIB with seven genotypes [PSPB 23, NRC 105 (green), CAT 194 BR3, CAT 1622 TGX302-2A, CAT 192 BR15 (brown), KALITUR, CAT 1852 TGX854-25D (all black)]. To test the goodness of fit of clustering to a set of data cophenetic correlation or cophenetic value was estimated using the COPH and MXCOM options in NTSYS-pc2.02 program. The cophenetic value of 0.98 obtained using the SSR data indicated a very good fit of clustering to SSR data. Although thirty polymorphic SSR markers have been used, however as few as nine SSR markers (Satt 600, Satt 463, Satt 371, Satt 193, Satt 538, Satt 126, Satt 286, Satt 281 and Satt 656) could differentiate the 25 soybean genotypes.

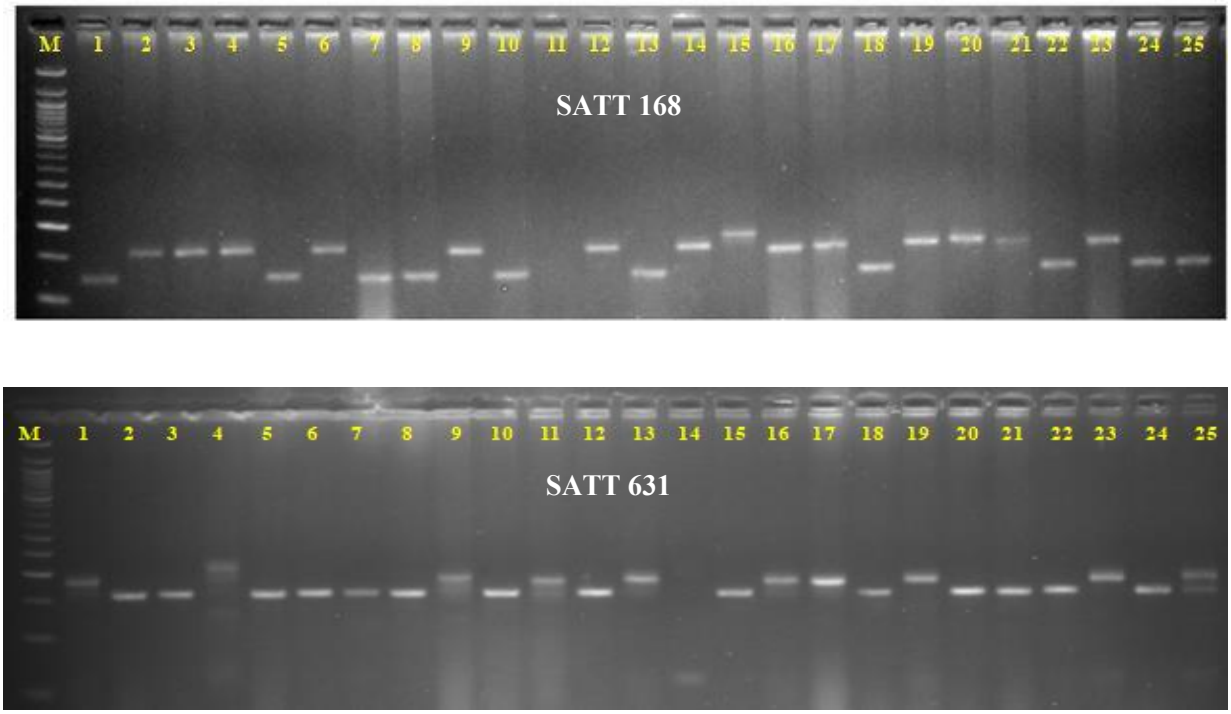

Fig.1. PCR amplification profile generated with SSR markers (Satt 631& 168) in 25 soybean genotypes

M-50 bp Ladder	7. KARUNE	14. DS 24110	21. KALITUR
1. CAT 195 BR4	8.AGS 12 CAT 25A	15. PSPB-23	22. CAT 194 BR3
2. IC 16572	9. TG X 249D-13-4	16. EC 113416	23.CAT 1852 TG X 854-25D
3. NG 1142 CHICO	10. NRC 130	17. CAT 2059 GC 84058-18-4	24. NRC 105
4. DS 91-3	11. EC 12503	18. CAT 192 BR15	25. CAT 1622 TG X 302-2A
5. CAT 243 DE 201	12. UPSL 387	19. G828	
6. G1922	13. CAT 971-GP2	20. NRC 2755	

Maintenance of seed longevity from harvest to till the subsequent sowing is the biggest challenge that needs to be addressed in soybean. In general, the brown and black seeded genotypes have good storability compared to yellow or green seeded types (Hosamani *et al.,* 2013). Molecular diversity existing among the good and poor storer genotypes may be exploited by including them in crossing program for improving seed storability in soybean. Jaccard's similarity coefficient of 0.22 between the good storer genotypes PSPB-23 (green seeded), CAT-1852-TGX854-25D (black seeded) and the poor storer NRC 130 (yellow) (based on accelerated ageing test) indicated diverse nature of these genotypes. Hence the molecular diversity existing among the good and poor storer genotypes may be exploited by including them in crossing program for improving seed storability in soybean.

Genetic diversity among the soybean genotypes using SSR markers was also assessed in combination with seed traits that determine seed longevity and seed vigour such as seed coat colour, 100 seed weight, seed length, seed width, seed thickness, seedling length and seedling dry weight. The genetic similarity coefficient ranged from 0.04 [TGX849D-13-4 (black) and NRC 2755 (brown)] to 0.57 [CAT-1622-TGX302-2A (brown) and CAT-194-BR3 (brown). Cluster analysis grouped the genotypes into two major clusters (**Fig. 3**). Cluster I is divided into two sub clusters (IA & IB) and all the brown seeded genotypes were grouped in 1A indicating more similarity among the genotypes. Cluster IB is composed of seventeen genotypes. Cluster II is the smallest with only two yellow seeded genotypes (KARUNE& NRC 105). Seed traits had dominant role in the clustering of the genotypes. Majority of the clusters had genotypes of single coat colour. Five clusters were common in both SSR and SSR + seed traits-based grouping. Several studies to assess genetic diversity based on yield/ yield contributing traits and seedling traits have been carried out to identify the promising parental lines for hybridization-based crop improvement program in soybean (Kachhadia *et al.,* 2014; Manav and Arora, 2018).

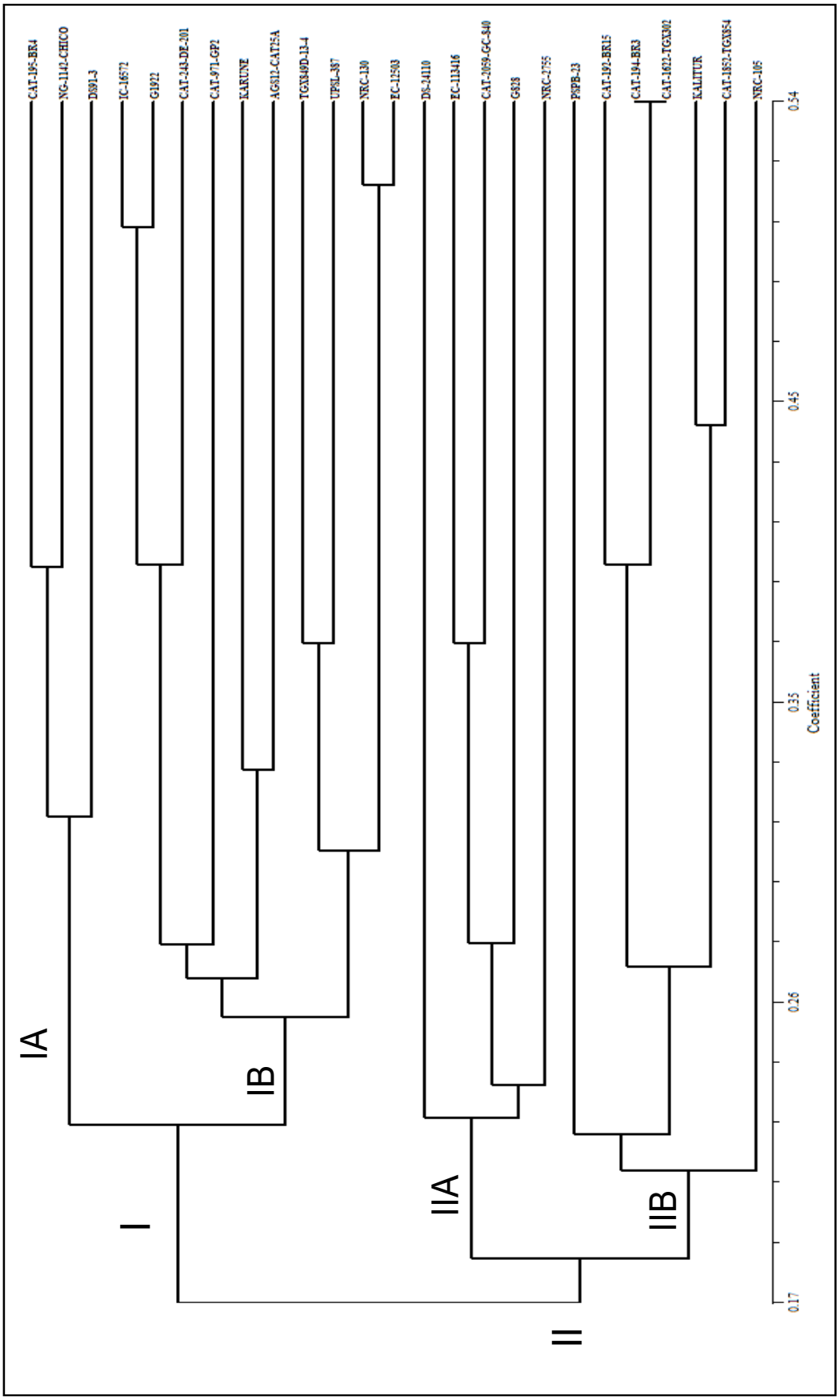

Fig. 2. Phylogram of twenty five soybean genotypes based on Jaccard similarity coefficient of thirty polymorphic SSR loci

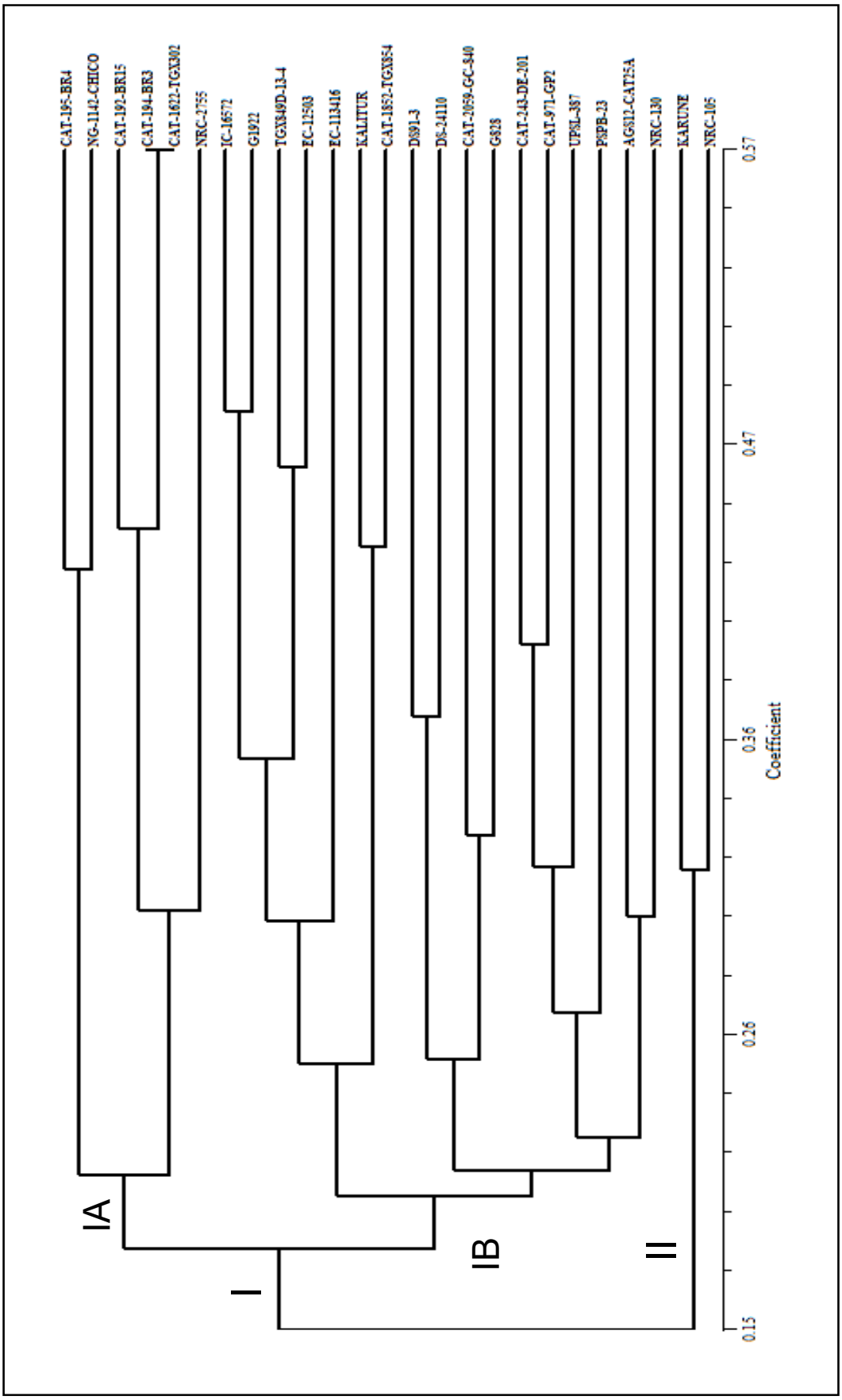

Fig. 3. Dendrogram of twenty-five soybean genotypes based on Jaccard's similarity coefficient of thirty polymorphic SSR data and seed traits

Table 4. Unique alleles for soybean genotypes

S.No.	Primer	Allele size (bp) approximate	Genotypes with unique allele
1	Satt 463	135	NRC 2755
2	Satt 463	200	CAT2059 GC 84058-18-4
3	Satt 286	188	PSPB-23
4	Satt 281	198	NRC 2755
5	Satt 285	195	KARUNE
6	Satt 175	165	NRC 105
7	Satt 538	110	PSPB-23
8	Satt 434	330	NRC 2755
9	Satt 534	158	NG 1142 CHICO
10	Satt 565	163	NRC 2755
11	Satt 565	190	NG 1142 CHICO
12	Satt 184	153	NRC 105
13	Satt 129	150	G828
14	Satt 631	165	NRC 2755
15	Satt 631	172	PSPB-23
16	Satt 686	261	DS91-3
17	Satt 686	271	AGS12 CAT 25A
18	Satt 289	217	PSPB-23
19	Satt 133	276	NRC 2755
20	Satt 133	293	NRC 105
21	SOYGPATR	124	NRC 2755

SSR marker information pertaining to unique and rare alleles have a high discriminatory power and can serve as diagnostic markers for unambiguous soybean varietal identification/DNA fingerprinting (Gupta and Manjaya, 2017). A total of 21 unique alleles were amplified by sixteen SSR loci in twenty five soybean genotypes in a size range of 110-330bp (Satt 463, Satt 286, Satt 281, Satt 285, Satt 175, Satt 538, Satt 434, Satt 534, Satt 565, Satt 184, Satt 129, Satt 631, Satt 686, Satt 289, Satt 133 and SOYGPATR (**Table 4**). The markers Satt 463, Satt 565, Satt 631, Satt 686 & Satt 133 amplified two specific amplicons each in seven different genotypes. SSRs have been shown to produce the highest polymorphism compared to other marker systems such as RFLPs, AFLPs and RAPDs, and much greater ability to identify unique alleles in elite and PI (plant introduction) soybean germplasm when compared to other marker systems (Narvel *et al.,* 2000; Wang *et al.,* 2006a). Earlier studies involving ninety soybean cultivars resulted in the identification of eight SSR markers for DNA fingerprinting. The study also revealed the presence of fifty four rare alleles including nineteen genotype specific or unique alleles (Gupta and Manjaya, 2017).

The study revealed diverse nature of soybean genotypes with respect to seed traits and SSR markers which may be utilized in soybean varietal improvement program to enhance seed longevity. The rare alleles/unique alleles of SSR markers are important genomic resources for soybean germplasm characterization/DNA fingerprinting.

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REFERENCES

Chauhan, D.K., Bhat, J.A., Thakur, A.K., Kumari, S., Hussain, Z. and Satyawathi, C.T. 2015. Molecular characterization and genetic diversity assessment in soybean (*Glycine max* (L.) Merr.) varieties using SSR markers. *Indian Journal of Biotechnology*, **14**: 504-510.

Cregan, P,B., Jarvik, T., Bush, A.L., Shoemaker, R.C., Lark, K.G., Kahler, A.L., Kaya, N., vanToai, T.T., Lohnes, D.G., Chung, J. and Specht, J.E. 1999. An integrated genetic linkage map of the soybean genome. *Crop Science,* **39**: 1464-1490. [Cross Ref]

Fu, Y.B., Peterson, G.W. and Morrison, M.J. 2007. Genetic diversity of Canadian soybean cultivars and exotic germplasm revealed by simple sequence repeat markers. *Crop Science,* **47**: 1947-1954.[Cross Ref]

Guan, R., Chang, R., Li, Y., Wang, L., Liu, Z. and Qiu, L. 2010. Genetic diversity comparison between Chinese and Japanese soybeans (*Glycine max* (L.) Merr.) revealed by nuclear SSRs. *Genetic Resources and Crop Evolution*, **57**: 229-242. [Cross Ref]

Gupta, S.K. and Manjaya, J.G. 2017. Genetic diversity and population structure of Indian soybean [*Glycine max* (L.) Merr.] revealed by Simple Sequence Repeat markers. *Journal of Crop Science and Biotechnology,* **20** (3): 221-231. [Cross Ref]

Hosamani, J., Dadlani, M., Santha, I.M., Arun Kumar, M.B. and Sherry, R.J. 2013. Biochemical phenotyping of soybean [*Glycine max* (L.) Merill] genotypes to establish the role of lipid peroxidation and antioxidant enzymes in seed longevity. *Agricultural Research Journal,* **2** (2): 119-126. [Cross Ref]

Jain, S., Jain, R. and Mccouch, S. 2004. Genetic analysis of Indian aromatic and qualityrice (*Oryza sativa* L.) germplasm using panels of Xuorescently-labled microsatellite markers. *Theor Appl Genet.,* **109**:965–977. [Cross Ref]

Jain, R.K., Joshi, A., Jain, D., Rajpurohit, D. and Priyanka, J. 2017. ISSR based molecular characterization of soybean [*Glycine max* (L.) Merrill] genotypes. *Bulletin of Environment, Pharmacology* and *Life Sciences,* **7**(1): 46-54.

Kachhadia, V.H., Baraskar, V.V., Vachhani, J.H., Patel, B.M. and Barad, H.R. 2014. Genetic divergence in soybean [Glycine max (L.) Merrill.]. *Electronic Journal of Plant Breeding*, **5**(3): 563-566.

Koutu, G.K., Arpita, S., Yogendra, S. and Tiwari, S. 2019. Molecular Characterization and Genetic Diversity Assessment of Soybean Varieties using SSR Markers. *International Journal of Current Microbiology and Applied Sciences*, **8** (04): 173-182. [Cross Ref]

Kumawat, G., Singh, G., Gireesh, C., Shivakumar, M., Arya Mamta, Agarwal, D.K. and Husain, S.M. 2015. Molecular characterization and genetic diversity analysis of soybean (*Glycine max* (L.) Merr.) germplasm accessions in India. *Physiology Molecular Biology Plants,* **21**(1): 101-107. [Cross Ref]

Li, W., Han, Y., Zhang, D., Yang, M., Teng, W., Jiang, Z., Qiu, L. and Sun, G. 2008. Genetic diversity in soybean genotypes from north-eastern China and identification of candidate markers associated with maturity rating. *Plant Breed.,* **127**: 494-500. [Cross Ref]

Manav and Arora, R.N. 2018. Assessment of genetic diversity for yield and seedling traits in soybean (Glycine max L. Merrill). *Electronic Journal of Plant Breeding*, **9** (1): 355-360. [Cross Ref]

Narvel, J.M., Fehr, W.R., Chu, W.C., Grant, D. and Shoemaker, R.C. 2000. Simple sequence repeat diversity among soybean plant introductions and elite genotypes. *Crop Science,* **40**: 1452-1458. [Cross Ref]

Saghai-Maroof, K., Soliman, M., Jorgensen, R.A. and Allard, R.W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of National Academy of Sciences, USA.* **81**: 8014-8018. [Cross Ref]

Singh, R.K., Bhatia, V.S., Bhat, K.V., Mohapatra, T., Singh, N.K., Bansal, K.C and Koundal, K.R. 2010. SSR and AFLP based genetic diversity of soybean germplasm differing in photoperiod sensitivity. *Genetics and Molecular Biology,* **33**: 319-324. [Cross Ref]

Tantasawat, P., Trongchuen, J., Prajongjai, T., Jenweerawat, S. and Chaowiset, W. 2011. SSR analysis of soybean (*Glycine max* (L.) Merr.) genetic relationship and variety identification in Thailand. *Australian Journal of Crop Science,* **5**: 283-290.

Wang, L., Guan, R., Zhangxiong, L., Chang, R and Qiu, L. 2006a. Genetic diversity of Chinese cultivated soybean revealed by SSR markers. *Crop Science,* **46**: 1032-1038. [Cross Ref]

Wang, L., Guan, Y., Guan, R., Li, Y., Ma, Y., Dong, Z., Liu, X., Zhang, H., Zhang, Y., Liu, Z., Chang, R., Xu, H., Li, L., Lin, F., Luan, W.,Yan, Z., Ning, X., Zhu, L., Cui, Y., Piao, R., Liu, Y., Chen, P. and Qiu, L. 2006b. Establishment of Chinese soybean (*Glycine max*) core collections with agronomic traits and SSR markers. *Euphytica,* **151**: 215-223. [Cross Ref]

Wang, K.J. and Takahata, Y. 2007. A preliminary comparative evaluation of genetic diversity between Chinese and Japanese wild soybean (*Glycine soja*) germplasm pools using SSR markers. *Genetic Resources and Crop Evolution,* **54**: 157-165. [Cross Ref]

Wang, M., Li, R., Yang, W. and Du, W. 2010. Assessing the genetic diversity of cultivars and wild soybeans using SSR markers. *African Journal of Biotechnology,* **9**: 4857- 4866.