

SSR Marker-Based Genetic Diversity Analysis of Soybean (*Glycine max* L. Merr.) Genotypes for Germplasm Characterization and Parental Selection

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ABSTRACT

Assessment of genetic diversity is essential for the effective utilization of soybean germplasm in breeding programmes. The present study evaluated the genetic diversity among 32 soybean genotypes using 34 simple sequence repeat (SSR) markers. All markers successfully amplified the target loci, generating 82 amplicons, of which 42 were polymorphic, resulting in an overall polymorphism of 51.21%. The markers Satt-149, Satt-235, and Satt-640 exhibited 100% polymorphism and the highest polymorphism information content (PIC), demonstrating superior discriminatory ability among the genotypes. PIC values ranged from 0.18 to 0.74, with a mean of 0.46, indicating moderate to high marker informativeness. Genetic similarity estimated using Jaccard's coefficient ranged from 0.33 to 0.87. The lowest similarity was observed between JS-335 and IVT-22, identifying them as the most genetically divergent genotypes, whereas IVT-25 and IVT-37 showed the highest similarity. UPGMA cluster analysis grouped the 32 genotypes into two major clusters, reflecting their genetic relationships and breeding history. The moderate level of genetic diversity detected among the soybean genotypes demonstrates the effectiveness of SSR markers for germplasm characterization and parental selection. The genetically divergent genotypes identified in this study represent valuable genetic resources for germplasm enhancement, parental selection, and the development of genetically diverse breeding populations, thereby supporting future soybean improvement programmes.

Keywords: *Glycine max* (L.) Merr., SSR markers, Genetic diversity, Polymorphism information content UPGMA Jaccard's similarity coefficient, Seed longevity

1 | INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important grain legumes and the leading oilseed crop, contributing approximately 60% of global oilseed production (FAOSTAT, 2025). It is recognized as an excellent source of high-quality protein and edible oil, with seeds containing approximately 40–42% protein, 18–20% oil, and 11% soluble carbohydrates (Wilson, 2008). Owing to its nutritional, economic, and industrial importance, soybean plays a crucial role in ensuring global food, feed, and nutritional security. The increasing demand for soybean, together with the challenges of climate change and emerging biotic and abiotic stresses, underscores the need to develop genetically diverse and high-yielding cultivars.

Genetic diversity is the cornerstone of crop improvement, providing the variability required for the selection of superior parents and the development of improved cultivars. Accurate assessment of genetic diversity facilitates the identification of genetically divergent germplasm, broadens the breeding gene pool, and enhances the efficiency of breeding programmes (Kujane et al., 2019). Since phenotypic traits are often influenced by environmental factors and genotype × environment interactions, DNA-based molecular markers

have become indispensable for reliable assessment of genetic relationships among soybean genotypes (Mohammadi and Prasanna, 2003; Vinu et al., 2013).

Among the available DNA marker systems, simple sequence repeat (SSR) markers, also known as microsatellites, are widely regarded as one of the most informative and reliable markers for genetic diversity analysis in soybean. SSR markers consist of tandemly repeated short DNA motifs that exhibit high levels of polymorphism due to variation in repeat number among genotypes. Their co-dominant inheritance, multi-allelic nature, high reproducibility, genome-wide distribution, and ease of PCR-based detection make them highly suitable for germplasm characterization and molecular breeding (Maughan et al., 1996; Wang et al., 2008). Furthermore, the high polymorphic information content of SSR markers enables efficient discrimination among closely related soybean genotypes and provides a robust platform for estimating genetic relationships and allelic diversity (Zatybekov et al., 2023).

SSR markers have been extensively employed in soybean for genetic diversity analysis, cultivar identification, seed purity assessment, varietal protection, germplasm conservation, population structure analysis, quantitative trait locus (QTL)

mapping, and marker-assisted breeding (Zatybekov et al., 2023). Their ability to detect multiple alleles at a single locus makes them particularly valuable for identifying genetically diverse parental lines and conserving valuable genetic resources. Moreover, trait-associated SSR markers facilitate the identification of favourable alleles linked to agronomically important characteristics, thereby accelerating soybean improvement programmes (Kujane et al., 2019).

Despite extensive soybean breeding efforts, the genetic base of many cultivated varieties remains relatively narrow due to the repeated use of elite breeding lines. Therefore, systematic assessment of available germplasm using informative SSR markers is essential to identify genetically diverse accessions that can be effectively utilized in breeding programmes. Such information is crucial for broadening the genetic base, improving parental selection, and enhancing the efficiency of marker-assisted breeding.

In the present study, genetic diversity among soybean genotypes was assessed using informative SSR markers associated with important agronomic traits. The study aimed to estimate allelic polymorphism, evaluate genetic

relationships among genotypes, and identify genetically diverse parental lines for soybean improvement. The findings will contribute to the efficient utilization of soybean genetic resources and support the development of high-yielding and resilient cultivars through molecular breeding.

2 | MATERIALS AND METHODS

2.1 | Plant Materials

The experimental material comprised 32 soybean genotypes, including 29 advanced breeding lines (IVTs) and three released cultivars (JS-335, AMC 100-39, and NRC-147), obtained from the Agricultural Research Station, Digraj, Sangli, Maharashtra, India, under Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri. The genotypes represented diverse genetic backgrounds and phenotypic variation. The experiment was conducted during the 2025 cropping season at the Research Farm Unit, College of Agricultural Biotechnology, Loni, Ahilyanagar, Maharashtra. Young leaf samples were collected from 10–15-day-old seedlings for genomic DNA extraction. The soybean genotypes used in the study are listed in Table 1.

Table 1. Soybean genotypes used for SSR-based genetic diversity analysis.

Sr. No.	Soybean genotype	Sr. No.	Soybean genotype
1	IVT-01	17	IVT-25
2	IVT-02	18	IVT-26
3	IVT-06	19	IVT-27
4	IVT-09	20	IVT-28
5	IVT-10	21	IVT-29
6	IVT-11	22	IVT-30
7	IVT-12	23	IVT-31
8	IVT-13	24	IVT-32
9	IVT-14	25	IVT-33
10	IVT-16	26	IVT-34
11	IVT-18	27	IVT-35
12	IVT-20	28	IVT-36
13	IVT-21	29	IVT-37
14	IVT-22	30	JS-335
15	IVT-23	31	AMC 100-39
16	IVT-24	32	NRC-147

2.2 | Genomic DNA Extraction and Quantification

Genomic DNA was extracted from approximately 0.5 g of fresh leaf tissue following the CTAB protocol of Saghai-Maroo et al. (1984) with minor modifications. DNA quality was assessed by electrophoresis on 1.5% agarose gel, while DNA concentration and purity (A260/A280) were determined

using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA). DNA samples with an A260/A280 ratio ranging from 1.71 to 2.00 were diluted to a working concentration of 30 ng μL^{-1} for PCR amplification. The concentration and purity of genomic DNA are presented in Table 2.

Table 2. Genomic DNA concentration and purity of soybean genotypes used for SSR analysis.

Sr. No.	Soybean Genotypes	DNA concentration (ng μL^{-1})	A260/A280	Sr. No.	Soybean Genotypes	DNA concentration (ng μL^{-1})	A260/A280
1	IVT-21	182.3	1.8	17	IVT-34	374.4	1.80
2	IVT-22	493.6	1.8	18	IVT-35	66.8	1.74
3	IVT-23	104.2	1.81	19	IVT-36	81.6	1.78
4	IVT-24	580.1	1.79	20	IVT-37	151.4	1.80
5	IVT-25	118.6	2.0	21	IVT-01	470.5	1.8
6	IVT-26	456.7	1.8	22	IVT-02	401.3	1.8
7	IVT-27	52.7	1.77	23	IVT-11	350.5	1.75
8	IVT-28	275.6	1.9	24	IVT-12	367.8	1.71
9	IVT-30	167.3	1.9	25	IVT-13	244.1	1.74
10	IVT-06	120.5	1.79	26	IVT-14	232.0	1.79
11	IVT-09	146.9	1.81	27	IVT-16	367.5	1.89
12	IVT-10	875.4	1.76	28	IVT-17	300.9	1.73
13	IVT-29	302.7	1.90	29	IVT-18	110.7	1.72
14	IVT-31	97.0	1.96	30	AMC 100-39	180.1	1.8
15	IVT-32	385.6	1.80	31	NRC 147	470.5	1.8
16	IVT-33	227.8	1.83	32	IVT-20	401.3	1.75

2.3 | SSR Marker Analysis and PCR Amplification

Thirty-four SSR markers associated with important agronomic traits were selected for genetic diversity analysis. PCR amplification was carried out in a 15 μL reaction mixture containing 7.5 μL of 2 $\times$ PCR Master Mix, 1.0 μL of SSR primer (16 pmol), 1.0 μL of genomic DNA (30 ng), and 5.5 μL of nuclease-free water. Amplification was performed with

an initial denaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 1 min, 48–50°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. The annealing temperature was adjusted according to the melting temperature (T_m) of each primer. The SSR markers used in the study are listed in Table 3, and the amplification protocol was adapted from Cregan et al. (1999) and Song et al. (2004).

Table 3. SSR markers used for genetic diversity analysis.

Sr. No.	SSR Marker	LG	Forward Primer (5'–3') / Reverse Primer (5'–3')	Ta (°C)
1	Satt123	O	F: TTCAACCATTCAAACATG R: AATTGAAAAGATGTGAAACAT	48–50
2	Satt149	F	F: TTGCACATTCTTTTGGTAAACAGTCATAA R: GTTGGAGGCCATAGTCACATTAATCTTAGA	48–50
3	Satt174	A1	F: TTTCATTCTTTGCCTTCT R: TTCGTAGTCCGTCTTTCAT	48–50
4	Satt181	H	F: TGGCTAGCAGATTGACA R: GGAGCATAGCTGTTAGGA	48–50
5	Satt235	G	F: GCGGGCTTTGCCAAGAAGTTT R: GCGGTGAGGCTGGCTATAAG	48–50
6	Satt377	A2	F: GCGTTTACAGGACCAGAACTTTT R: GCGGGCCAATGGAACCTTAGTCTTTT	48–50
7	Satt467	B2	F: GCGAAGAGCTACATCTAACACAATTCAA R: GCGTTAAGCACGGTCATATTTTCTCATA	48–50
8	Satt511	A1	F: GCGACTTTACTGAAAACCTGGAAA R: GCTTCAAACCAACAAACAACTTA	48–50
9	Satt640	C2	F: AGATACCTACGGAGTGTTTTT R: GGTCCCCGGTGGCTACACAAC	48–50
10	Satt632	A2	F: GGGCTATGAAGGGAATGGAAAGGA R: CCCATATTGAAGATTGAAGTAAT	48–50
11	Satt191	G*	F: CGCGATCATGTCTCTG R: GGGAGTTGGTGTTCCTTGTG	48–50
12	Satt197	B1	F: CACTGCTTTTCCCCTCTCT R: AAGATACCCCAACATTATTTGTAA	48–50
13	Satt225	A1	F: AAAAATGTGTTAGAGCTTGTGTTGTTA R: GCCACACTATTCAGCCACTAC	48–50
14	Satt230	E	F: CCGTCACCGTTAATAAAATAGCAT R: CTCCCCAAATTTAACCTTAAAGA	48–50
15	Satt244	J	F: GCGCCCCATATGTTTAAATTATATGGAG R: GCGATGGGGATATTTCTTTATTATCAG	48–50
16	Satt286	C2	F: GCGGCGTTAATTTATGCCGGAAA R: GCGTTTGGTCTAGAATAGTTCTCA	48–50
17	Satt309	G	F: GCGCCTTCAAATTGGCGTCTT R: GCGCCTTAAATAAAACCCGAAACT	48–50
18	Satt322	C2	F: GGGACCCATCATTAAAGAA R: GTTTGCCGGACACCACA	48–50
19	Satt334	F	F: GCGTTAAGAATGCATTTATGTTTAGTC R: GCGAGTTTTTGGTTGGATTGAGTTG	48–50
20	Satt345	O	F: CCCCTATTTCAAGAGAATAAGGAA R: CCATGCTCTACATCTTCATCATC	48–50
21	Satt354	I	F: GCGAAAATGGACACCAAAAGTAGTTA R: GCGATGCACATCAATTAGAATATACAA	48–50
22	Satt363	C2	F: TAGAGGGATCCAATTAGAATCCAAGAAA R: TCTTCGATCTTCATATTCTTTTGTA	48–50
23	Satt371	C2	F: TGCAAACTAACTGGATTCACTCA R: GAGATCCCGAAATTTAGTGTAACA	48–50
24	Satt409	A2	F: CCTTAGACCATGAATGTCTCGAAGAA R: CTTAAGGACACGTGGAAGATGACTAC	48–50
25	Satt426	B1	F: GCGCCATTATTATTTCTTGAATTGT R: GCGAATGCCTAATGTGATGATAAAAAATA	48–50
26	Satt453	B1	F: GCGGAAAAAACAATAAACAACA R: TAGTGGGGAAGGGAAGTTACC	48–50
27	Satt489	C2	F: GCGTGTGCTTGCTTCTCTAGACTGACT R: GCGTACTACTTACCCTGTTTGTCTAAAA	48–50
28	Satt538	A2	F: GCAGGCTTATCTTAAGACAAGT R: GGGGCGATAAACTAGAACAGGA	48–50
29	Satt565	C1	F: GCGCCCGGAACCTGTAATAACCTAAT R: GCGCTCTCTTATGATGTTTATAATA	48–50
30	Satt591	A1	F: GCGCGACCTTAATGATA R: GCGCCCAAAGCTTAAATTTAATA	48–50
31	Satt601	B2	F: CCTACGCAAGTAACATTGGTTGTCAT R: GCGGCTAAAACGTATTAAATTAAGA	48–50
32	Satt619	A1	F: GGCAGAACTAGTACGCTTCTGATT R: GCGGTTAAGCATAATAGATCAGCCT	48–50
33	Satt665	B1	F: GCGTTGAATTGCTGGTTTAACTCTA R: GCGCATGACTTAATTTTACACCCTTTATT	48–50
34	Satt686	J	F: ACGGAAAATAAATGAACTAAGA R: GCGCTATCAGATAGAGAAGCAGAAGAAT	48–50

Source: SoyBase Soybean Genetics and Genomics Database (USDA-ARS) and the integrated soybean genetic linkage map (Cregan et al., 1999).

2.4 | SSR Allele Scoring and Data Analysis

PCR amplicons were separated by electrophoresis on 1.5% agarose gels prepared in $1\times$ TAE buffer, and fragment sizes were estimated using a 100 bp DNA ladder. Only clear and reproducible bands were scored as present (1) or absent (0) to generate a binary data matrix. Genetic similarity among soybean genotypes was estimated using Jaccard's similarity coefficient (Jaccard, 1908). The polymorphic information content (PIC) of each SSR locus was calculated according to Botstein et al. (1980) as:

$$PIC = 1 - \sum_{i=1}^n p_i^2$$

Where, p_i is the frequency of the i th allele at a given locus.

Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard's similarity matrix generated in NTSYS-pc version 2.1 using the Sequential Agglomerative Hierarchical Nested (SAHN) algorithm (Rohlf, 2000). The resulting dendrogram was used to infer the genetic relationships among the soybean genotypes.

3 | RESULTS AND DISCUSSION

3.1 | SSR Marker Polymorphism

A total of 34 SSR markers were used to assess genetic diversity among the 32 soybean genotypes. All SSR markers successfully amplified the target loci, producing clear and

reproducible banding patterns. The markers generated 82 amplicons, of which 42 were polymorphic, resulting in an overall 51.21% polymorphism (Table 4). The number of amplicons per marker ranged from 2 to 3, while the number of polymorphic amplicons varied from 1 to 3. Among the SSR markers, Satt-149, Satt-235, and Satt-640 exhibited 100% polymorphism, indicating their high discriminatory power for differentiating soybean genotypes (Figure 1). In contrast, Satt-191 and Satt-377 showed the lowest polymorphism (33%), whereas most markers exhibited 50% or 66% polymorphism. The observed variation in polymorphism among the SSR markers reflects differences in allelic diversity at individual loci.

However, polymorphism of 51.21% indicates a moderate level of allelic variation among the evaluated soybean genotypes. Such a level of polymorphism is expected in advanced breeding materials, where repeated selection and the use of related parental lines often reduce overall genetic variability compared with landraces or wild soybean accessions. Nevertheless, the observed polymorphism was sufficient to effectively discriminate among the 32 genotypes. Similar levels of SSR polymorphism have been reported in cultivated soybean germplasm by Maughan et al. (1995), Cregan et al. (1999), Song et al. (2010), and Tantasawat et al. (2011), who attributed the moderate genetic diversity to the relatively narrow genetic base of modern soybean cultivars. Despite this, SSR markers remain effective for germplasm characterization, cultivar identification, and parental selection in soybean breeding programmes.

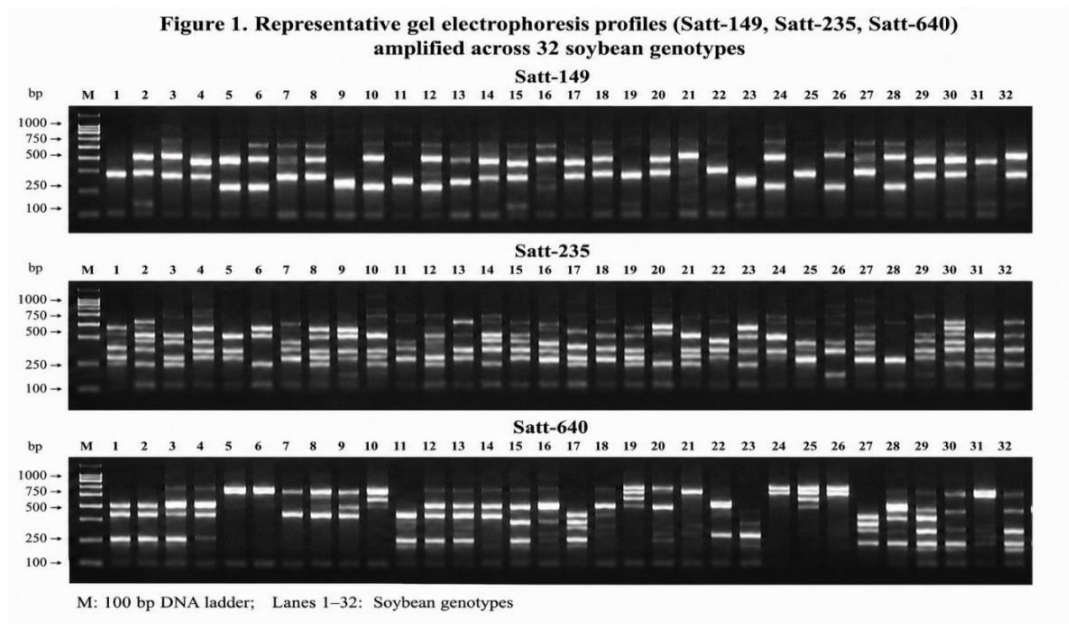


Figure 1. Representative gel electrophoresis profiles (Satt-149, Satt-235, Satt-640) amplified across 32 soybean genotypes.

Table 4. Polymorphism analysis of SSR markers among 32 soybean genotypes.

Sr. No.	SSR Marker	TA	PA	P (%)	Sr. No.	SSR Marker	TA	PA	P (%)
1	Satt-123	2	1	50	18	Satt-322	2	1	50
2	Satt-149	2	2	100	19	Satt-356	3	2	66
3	Satt-168	2	1	50	20	Satt-367	2	1	50
4	Satt-170	2	1	50	21	Satt-377	3	1	33
5	Satt-174	2	1	50	22	Satt-396	2	1	50
6	Satt-181	2	1	50	23	Satt-409	2	1	50
7	Satt-191	3	1	33	24	Satt-429	3	2	66
8	Satt-200	2	1	50	25	Satt-453	3	2	66
9	Satt-215	2	1	50	26	Satt-467	2	1	50
10	Satt-225	2	1	50	27	Satt-511	3	2	66
11	Satt-230	3	2	66	28	Satt-538	3	2	66
12	Satt-235	2	2	100	29	Satt-545	3	2	66
13	Satt-236	2	1	50	30	Satt-591	2	1	50
14	Satt-251	3	2	66	31	Satt-601	2	1	50
15	Satt-277	3	2	66	32	Satt-619	2	1	50
16	Satt-286	3	2	66	33	Satt-640	3	3	100
17	Satt-287	2	1	50	34	Satt-665	3	2	66
Total	34 SSR markers	82	42	51.21					

TA = Total Amplicons; PA = Polymorphic Amplicons; P (%) = Polymorphism (%).

3.2 | Polymorphism Information Content (PIC)

The informativeness of each SSR marker was evaluated using the Polymorphism Information Content (PIC), which measures the discriminatory ability of a marker. The PIC values varied among the SSR loci, reflecting differences in allelic diversity and marker informativeness (Table 4). PIC values ranged from 0.18 to 0.74, with an average of 0.46, indicating moderate to high informativeness of the SSR markers. Markers Satt-149, Satt-235, and Satt-640 exhibited the highest PIC values, confirming their superior discriminatory ability for soybean germplasm characterization.

The observed variation in PIC values may be attributed to differences in the number and frequency of alleles amplified by each SSR locus. Markers possessing multiple alleles with balanced frequencies generally exhibit higher PIC values than markers with fewer alleles or skewed allele frequencies. Overall, the SSR markers employed in the present study were sufficiently informative for genetic diversity analysis and

germplasm characterization. Similar findings regarding SSR marker informativeness in soybean have been reported by Cregan et al. (1999), Song et al. (2010), and Tantasawat et al. (2011).

3.3 | Genetic Similarity Analysis

Genetic similarity among the 32 soybean genotypes was estimated to be using Jaccard's similarity coefficient based on the presence or absence of SSR alleles. The similarity coefficients ranged from 0.33 to 0.87, reflecting moderate to high genetic diversity among the evaluated genotypes (Table 5). The lowest similarity coefficient (0.33) between JS-335 and IVT-22 identified these genotypes as the most genetically divergent and therefore promising parental combinations for hybridization, whereas the highest similarity coefficient (0.87) between IVT-25 and IVT-37 indicated a close genetic relationship, possibly due to a common pedigree or breeding history. Furthermore, the broad range of similarity coefficients confirms the effectiveness of SSR markers in differentiating soybean genotypes, consistent with previous reports by Maughan et al. (1995) and Tantasawat et al. (2011)

Table 5. Summary of genetic similarity among 32 soybean genotypes based on SSR markers.

Parameter	Value
Number of soybean genotypes	32
Number of SSR markers	34
Similarity coefficient	Jaccard's coefficient
Clustering method	UPGMA
Range of genetic similarity	0.33–0.87
Lowest similarity	JS-335 × IVT-22 (0.33)
Highest similarity	IVT-25 × IVT-37 (0.87)
Number of major clusters	2
Most genetically distinct genotype	IVT-22

3.4 | Cluster (UPGMA) Analysis

The genetic relationships among the 32 soybean genotypes were evaluated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on Jaccard's similarity coefficients derived from SSR marker data. The resulting dendrogram classified the genotypes into two major clusters, designated as Cluster I and Cluster II (Figure 2). Cluster I comprised 16 genotypes, including the released cultivars JS-335, AMC 100-39, and NRC-147, whereas the remaining 16 genotypes were assigned to Cluster II.

The clustering pattern reflected the underlying genetic relationships and breeding history of the evaluated materials. Released cultivars were primarily grouped together, indicating a relatively narrow genetic base resulting from the repeated use of elite breeding lines and common parental sources. In contrast, several IVT lines formed distinct subclusters, suggesting the presence of unique allelic combinations and greater genetic divergence. This differentiation indicates that the breeding materials still retain sufficient genetic variability despite selection pressure during cultivar development.

Figure 2. UPGMA dendrogram showing the genetic relationships among 32 soybean genotypes based on Jaccard's similarity coefficients derived from SSR markers.

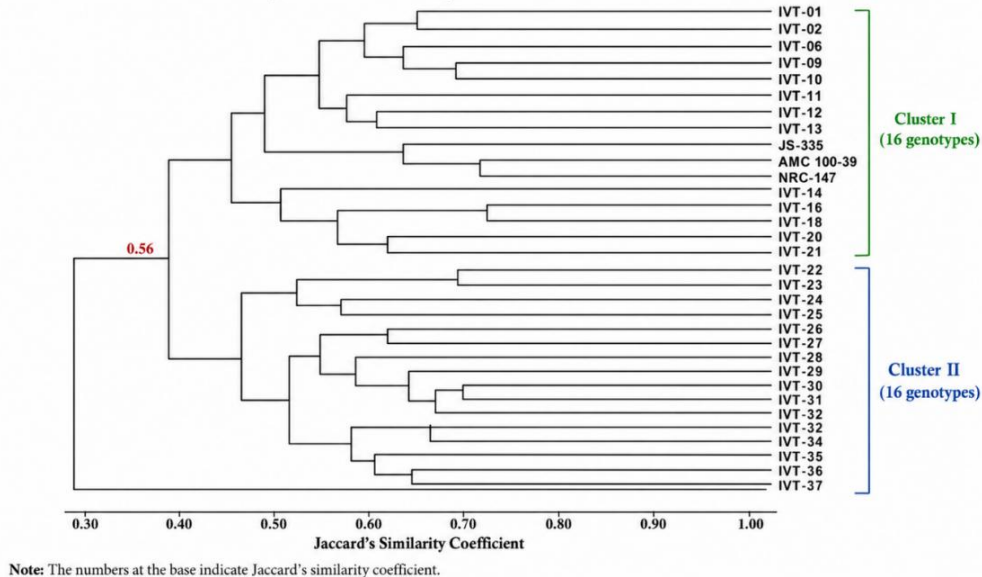


Figure 2. UPGMA dendrogram showing the genetic relationships among 32 soybean genotypes based on Jaccard's similarity coefficients derived from SSR markers.

The clear separation of genotypes into two major clusters demonstrates the effectiveness of SSR markers in resolving genetic relationships among closely related soybean genotypes. The genetically distinct IVT lines identified in this study represent valuable breeding resources for broadening the genetic base and selecting divergent parents for hybridization. Similar clustering patterns have been reported by Diwan and Cregan (1997), Narvel et al. (2000), Song et al. (2010), and Tantasawat et al. (2011), who also observed that SSR-based UPGMA analysis effectively discriminates against soybean germplasm according to genetic background and breeding history.

3.5 | Breeding Implications

The SSR marker analysis revealed considerable genetic diversity among the evaluated soybean genotypes. The low genetic similarity between JS-335 and IVT-22 suggests that these genotypes are promising parental combinations for hybridization to maximize allelic recombination and generate transgressive segregants for economically important traits, including seed longevity and seed yield. Such genetically divergent parents can also be utilized to develop biparental and

recombinant inbred line (RIL) populations for gene mapping and marker validation. Overall, the informative and reproducible SSR markers employed in this study provide a valuable tool for germplasm characterization, parental selection, and marker-assisted breeding aimed at broadening the genetic base and developing improved soybean cultivars.

4 | CONCLUSION

The present study demonstrated that SSR markers are effective tools for assessing genetic diversity among soybean genotypes. Among the 34 SSR markers evaluated, Satt-149, Satt-235, and Satt-640 exhibited high polymorphism and marker informativeness, enabling efficient discrimination of the 32 soybean genotypes. UPGMA clustering and genetic similarity analysis revealed moderate genetic diversity, with JS-335 and IVT-22 identified as the most genetically divergent genotypes and, therefore, promising parental combinations for soybean improvement. The findings provide valuable information for germplasm characterization, parental selection, and the development of mapping populations, thereby supporting marker-assisted breeding and the genetic improvement of soybean, particularly for seed longevity and related agronomic traits.

AUTHOR CONTRIBUTIONS

Amit Adsul conceived and designed the study, performed the experiments, collected and analyzed the data, interpreted the results, prepared the figures and tables, and wrote, reviewed, and approved the final manuscript.

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