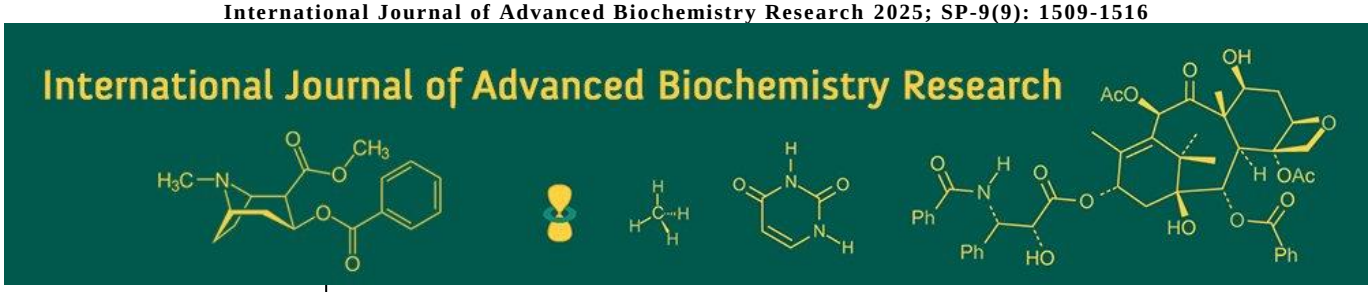

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Genetic differentiation among soybean parental lines of elite vegetable and grain types revealed by molecular markers

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Abstract
Soybean (*Glycine max* (L.) Merrill), a globally important oilseed and protein-rich legume, demands genetic improvement for yield, quality, and stress resilience. Harnessing parental diversity is essential for marker-assisted breeding programs. In this study, eleven soybean genotypes, comprising both grain- and vegetable-type lines, were evaluated for morphological and molecular diversity. Morphological traits such as plant height, flower color, and maturity duration were assessed, revealing substantial variation between grain and vegetable types. Molecular characterization was conducted using 50 simple sequence repeat (SSR) markers, of which 44 amplified successfully, with 18 exhibiting high polymorphism. Polymorphism Information Content (PIC) values ranged from 0.14 to 0.94, indicating moderate to high genetic variability. Cluster analysis clearly differentiated vegetable- and grain-type genotypes into distinct clades, corroborating morphological findings. The study establishes the effectiveness of SSR markers in parental polymorphism surveys. Identified polymorphic markers are directly applicable in hybridity testing, background genome analysis, and marker-assisted backcross breeding to develop nutritionally enhanced and stress-resilient soybean cultivars.

Keywords: Vegetable soybean, MAS, parental polymorphism survey, SSR

Introduction
Soybean (*Glycine max* (L.) Merrill), often referred to as the "Golden Crop" or "Miracle Crop," is one of the most versatile and globally significant legumes of the twentieth century. Owing to its exceptionally high protein content and wide range of applications in food, animal feed, and industrial products, soybean has established itself as the leading oilseed crop worldwide, with a global production of 531 million metric tons in 2024 (USDA, 2024). Nutritionally, soybean seeds contain more than 36% protein, 30% carbohydrates, valuable dietary fiber, vitamins, and minerals, along with approximately 20% oil, making it the most important source of edible oil (IITA). The increasing global population demands enhanced crop productivity, yet the rate of yield improvement is slowing. To address this challenge, modern plant breeding must prioritize traits that ensure yield stability and sustainability, such as durable disease resistance, tolerance to abiotic stresses, and improved nutrient and water use efficiency. Marker-assisted selection (MAS), which employs DNA markers to accelerate breeding, has become a powerful tool in this context (Shinde *et al.*, 2018) [12]. Soybean's high protein content and nutraceutical properties make it highly suitable for human consumption. However, its utilization is constrained by factors such as the off-flavors caused by lipoxygenase isozymes (Anshu *et al.*, 2021) [1] and the presence of anti-nutritional factors (ANFs). Among these, trypsin inhibitors particularly the Kunitz Trypsin Inhibitor (KTI) and Bowman-Birk Inhibitor (BBI) can reduce growth performance in poultry and livestock, necessitating heat treatment to inactivate them. While effective, such processing often lowers the availability of essential amino acids and increases production costs (Patil *et al.*, 2025; Gavande *et al.*, 2024) [11, 2]. Other ANFs, including non-starch polysaccharides (NSPs) and oligosaccharides, are resistant to heat treatment and may cause digestive disturbances, further complicating soybean's food and feed use.

Advances in molecular breeding have provided strategies to address these limitations. Marker-Assisted Backcross Selection (MABS) relies on a preliminary Parental Polymorphism Survey (PPS) to identify informative molecular markers, which enable the mapping of quantitative trait loci (QTLs) associated with desirable traits and facilitate efficient trait introgression (Kumar *et al.*, 2022) [8]. Simple sequence repeats (SSRs), due to their reproducibility, codominant inheritance, and high polymorphism, are particularly effective in PPS and remain widely used despite the growing popularity of single nucleotide polymorphisms (SNPs) (Cregan *et al.*, 1999; Jadhav *et al.*, 2022; Zatybekov *et al.*, 2023) [4, 15, 19]. While SNPs are powerful for genome-wide applications, SSRs continue to provide cost-effective and precise tools for assessing genetic diversity and supporting crop improvement. Harnessing genetic diversity is central to developing soybean cultivars that combine resilience with consumer-preferred traits. Breeding programs must integrate alleles for yield potential, stress tolerance, and nutritional enhancement while retaining key market-oriented characteristics such as seed quality, relative maturity, and processing suitability (Cargil *et al.*, 2021) [18]. Importantly, despite modern tools such as MAS and genome editing, the incorporation of new diversity into elite cultivars remains a slow process, often spanning more than a decade, reflecting the complexity of plant breeding and the need for careful decision-making at each stage. Soybean cultivars are broadly classified into grain-type varieties, which are primarily used for oil extraction and animal feed, and food-type varieties, which are used for traditional fermented (e.g., miso, tempeh, natto) and non-fermented (e.g., tofu, soy flour, soy milk) products (Liu, 1999). Improvement of traits such as protein composition, fatty acid profile (oleic, linoleic, and linolenic acids), sugar content, grain size, hilum color, absence of lipoxygenase enzymes, and cooking time is essential for developing food-type cultivars suitable for direct human consumption. These modifications require confirmation through integrated chemical, physical, and sensory analyses (Carrão-Panizzi, 2000; Meneguce *et al.*, 2005) [16, 17].

Experimental Material

A total of eleven soybean genotypes were selected, comprising five grain types and three vegetable types. The details of the genotypes are as follows:

Table 1: List of genotypes used in investigation

Genotype	Developed/ Released by	Key Description
AMS 100-39 (PDKV Amba)	Dr. PDKV, Akola	Resistant to pod shattering, tolerant to YMV, yield potential ~28-30 q ha^{-1}, Charcoal rot resistance
AMS MB 5-19	Dr. PDKV, Akola	High-yielding, resistant to charcoal rot, stable across environments, tolerant to biotic stresses
NRC 109	ICAR-NSRI Indore	Possesses lipoxygenase-2 null trait, adapted to central Indian agro-ecologies
AMS 1001 (PDKV Yellow Gold)	Dr. PDKV, Akola (2018)	Induced mutant of JS 93-05, 20-36% higher yield than checks, resistant to charcoal rot and YMV, distinct molecular identity (SSR marker Glysatt 180)
JS 93-05	JNKVV, Jabalpur	Widely cultivated, high-yield potential, resistant to root rot
AGS 457	ICAR-RCER Ranchi	Bold seeded, sweet flavored, white flowers, high nutritional value
AGS 465	ICAR-RCER Ranchi	Similar to AGS 457 with slightly extended maturity, good sensory and seed attributes
Swarna Vasundhara	ICAR-RCER Ranchi	Tailored for edamame consumption, high protein (~39% at R8), suitable for fresh use and processing
AMS MB 5-18 (Suvarna Soya)	Dr. PDKV, Akola	Widely used recurrent parent in MAB, source for introgression of null Kunitz trypsin inhibitor (KTI) allele
NRC 101	ICAR-NSRI Indore	One of the first KTI-free soybean genotypes in India, registered with ICAR-NBPGR, donor parent in breeding programs
NRC 127	ICAR-NSRI Indore	Recognized as first KTI-free variety in India, recommended for cultivation in different regions, donor line for nutritional enhancement

Table 2: Genotypes differentiated into grain and vegetable type

Grain type	Vegetable type
AMS-MB-5-19,AMS-MB-5-18,NRC-101,NRC-127, NRC-109, AMS-100-39, JS 93-05, AMS-1001	AGS-457, AGS-465, Swarna Vasundhara

Table 3: List of SSR primers used for Parental Polymorphism Survey

Marker	Forward Primer	Reverse Primer
Sat_126	GGGGGGATGCATATATTGTCTA	AGAAAAAGAGGGAGAATGA
Satt095	TATTTGTTATTGGTGAATTAAGA	ATTCCTGTAGAAATTGATCTG
Satt558	CTCACACCCTTTCATTATCTA	AAATCGCGCATCTAAATTTAC
Satt201	GCGTTGATACTTTCCTAAGACAAT	GGGAGAGAAGGCAATCTAA
Satt152	GCGCTATTCCTATCACAACACA	TAGGGTTGTCACTGTTTTGTTCTTA
Sat_186	GCGACGCGCTAGTCTTATTT	GCGGATGGCTTTTACTTT
Satt258	CGAGGCGATTTGG	CCGAAAAGTGAAACAAGT
Satt211	GAAAAAGCCCACATCCAA	CATGGGCATGCAGTAACA
Sat_003	TGATTTTTGGTGTAGAACTC	CAAATTGGTTAGCTTACTCCA
Satt545	CAATGCCATTCCATATTTGTT	CAATTGCCCTAGTTTTGATAG
Satt591	GCGCGACCTTAATGATA	GCGCCCAAAGCTTAAAATTTAATA
Satt395	CGCGCTAGTTGAATGAATGT	GCGCATTGAGGAATTTTTTAT
Satt457	GTCCGTGTATTTTGTTTTGC	TTATCCATTTTCCCTTTAGTCC
Satt170	GGGAAATCTAAATAAAATGATGGATAT	GGGGTAGTTAAAATTCATCCTTAAAA

Satt142	GGACAACAACAGCGTTTTTAC	TTTGCCACAAAGTTAATTAATGTC
Satt644	TATGCCTCAAACCACAAA	CAGGCCACCATTTTTCTT
Satt661	TGATATGAGCAATGTAGTTCCTCT	TCCATGAAAAAGAAGTTAGAATAGC
Satt315	GCGCGACAACTCTAATGAAAATCT	GCGGAGTTTGATTTTTCAAAAGT
Sat_301	GCGCACAGGACTTAGTGTTATCATTCAATGT	GCGGGGTTCCCATATTCTTGGTATGAAACTA
Satt198	GAGCCAACCAATTAGAGCCAAGTTA	GGCGGTTTGTTTTTTATCTATTCA
Satt557	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA
Satt476	TTTGCTGATTAAAAAAACAAAAACTG	TTGTTAGAATGGGGACTACTTCACTA
Sat_244	GCGTCAACCGGTGAAAAAACCTA	GCGTGGCTGGCAGTAGTCTATATCA
Satt682	GCGTTTAAACTATTTTGTAATTTATTGTGAA	GCGGGGGAAATATTAGAAAAGTGATACATAA
Sat_406	GCGCGTGTGGTGGTTACATTA	GCGTTTGCAGCCATTTCCATTTAC
Satt713	GCGAAACGTATTAATTATGTGTCTTTCTTTA	GCGGTTTGCAGTGTGATATTACAATG
Satt147	CCATCCCTTCCTCCAAATAGAT	CTTCCACACCCTAGTTTAGTGACAA
Satt424	CAACCTGTATTCCACAAAAAATCTCACC	GCGCCCCAATTTGACTATAAATAAAAGT
Sat_227	GCGCAAAATGATTTGGGAAAATAACTTACAA	GCGTTATATACTTTTGGCGAGTTATCC
Sat_379	GCGTTTTGGCTCATCTTTCTTTTA	GCGGCCCTAAGCACAACTGAACCTAT
Sat_265	GCGTGAGTGCCACTTCTCCTG	GCGGCAGCATTAGAACCAAAAAGA
Sat_330	GCGTTAGGATTTAGGATGAGGATAGG	GCGCAAATCAGTTGAGCAATGACTTA
Sat_337	GCGCATGTTTTACAAATTTTGAAGCCTTAG	GCGATCAATCCATTTATGAGGTTAGTTTCTT
Sat_236	GCGGGTGCCTATGGTTCTACGTAGTTAC	GCGTTTGGATTAGATCAATAAAAATCACATT
Sat_276	GCGGAAACCCATCTAGAATATGAAAAACA	GCGTTCTTCTCGAGGTGAGATACAATC
Sat_268	GCGTGCAACATATGACACCATAAAT	GCGTGAGGAGGTTCAAAAATAACAT
Satt507	GCGCTCAGCCTTGTTAAATCACTT	GCGCTACTCTCGTGTCGTTAGTTA
Satt227	GCTCTGCCAAATAGTGTT	CACCCTGGCACATAGA
Sct_034	AATTCTCACTCTCACAACTTC	CCATGGAATAGTTGGGT
Satt212	CCAATCCAAACAAATCCACT	CAGCAATGATGATAATGAATGA
Sat_127	GGGGTTTTGTGTCTAGTCTA	GGGATTAAAAATAACAATACT
Satt181	TGGCTAGCAGATTGACA	GGAGCATAGCTGTTAGGA
Sct_137	TGCTCTTGGGAATCTG	CTCAATAATTCATCATCACTTC
Satt447	CGAAACTACGGTTGATTAT	TCCAAACACTGTCCTTCTT
Sat_141	CGCAATCAAAGACCTGTT	GCCTTGGCTATTTCCTTA
Sat_071	GAGTGCTGATCTATTTGTCA	TTTCTTTTACTGGAATTAACATAT
GMH179	ATACCAGTTGATCTGCATATT	AAAACATCGTCTACTTAAGTC
Sat_044	AAAAAATATTTATAGGTTACATGTG	TTACCACTAAGAATTAGGTCTAA
SSR668	TAACTTTTTAAATAAATTTTAGTAACTC	TCATTATTAGCTAAAACCACATA
Satt195	GTGTAAACGAAAGATGGGATATTCTC	GAAGTGCGACACAAATTTATGAGTA

A set of SSR markers reported by Jadhav *et al.*, 2025 were used for present study.

Methodology

Morphological Characterization

Morphological evaluation of soybean genotypes was carried out to assess variability in key agronomic traits. Traits including plant height, flower color, and days to maturity were recorded at appropriate growth stages.

- **Plant height** was measured from the base of the plant to the tip of the main stem at physiological maturity.
- **Flower color** was documented based on the predominant pigmentation observed during the flowering stage.
- **Days to maturity** were determined as the number of days from sowing until 95% of the pods had reached physiological maturity.

Data were recorded with replication to minimize experimental error and to ensure accuracy in trait assessment.

Molecular Analysis

Genomic DNA Isolation: High-quality genomic DNA was extracted from young leaf tissues using a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980) [10]. Fresh leaf samples were ground into fine powder in liquid nitrogen and homogenized in CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl). The homogenate was incubated at 65 °C for 60 min to promote cell lysis and denaturation of nucleoprotein complexes. Phase separation was achieved through two sequential extractions with chloroform: isoamyl alcohol (24:1, v/v). The aqueous phase was then mixed with CTAB precipitation solution (0.5% CTAB, 0.04 M NaCl) and incubated at room temperature for 1 h, facilitating selective DNA precipitation. The DNA pellet obtained was resuspended in 1.2 M NaCl, subjected to a second chloroform extraction, and subsequently precipitated with isopropanol. The resulting DNA was washed with 70% ethanol, air-dried, and dissolved in nuclease-free water. DNA integrity was verified by electrophoresis on 0.8% agarose gel stained with ethidium bromide, while purity and concentration were assessed using a nanophotometer. Samples with an A260/A280 ratio of ~1.8 were considered of acceptable quality. DNA concentrations were standardized to 30-40 ng/µL for subsequent PCR amplification.

PCR Amplification

PCR reactions were prepared in sterile microcentrifuge tubes maintained on ice to prevent enzyme degradation. Each 10 µL reaction consisted of 3 µL nuclease-free water, 1 µL genomic DNA (30-40 ng/µL), and 6 µL of a master mix containing 5 µL of 2× PCR master mix, 0.5 µL forward primer (10 pmol), and 0.5 µL reverse primer (10 pmol). Reaction mixtures were briefly centrifuged to collect the contents at the tube base and then placed in a programmed thermal cycler for amplification as in fig. 1. After completion of PCR cycles, the amplified products were stored at −20 °C until electrophoresis.

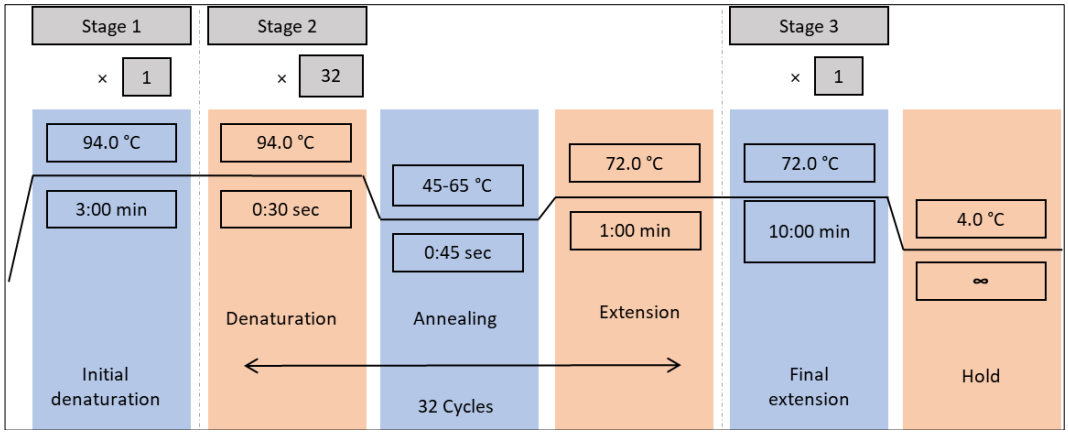

Fig 1: PCR profile used for the parental polymorphism survey

Polyacrylamide Gel Electrophoresis (PAGE)

PCR-amplified fragments were resolved using 8% non-denaturing polyacrylamide gels prepared from a 40% acrylamide:bis-acrylamide stock solution (29:1) in 1× TBE buffer. Polymerization was initiated with 10% ammonium persulfate (APS) and TEMED. The gel solution was carefully poured between assembled glass plates with spacers to avoid bubble formation. After polymerization, the gels were pre-run for 10 min at 120 V to stabilize the matrix. Subsequently, 5 µL of each PCR product was loaded into the wells, and electrophoresis was carried out at 120 V until the tracking dye reached the gel base. Gels were removed, stained with 0.1% ethidium bromide, and visualized under UV transillumination using a gel documentation system. Clear and reproducible banding patterns were scored to assess allelic variation and polymorphism.

Genetic Diversity Analysis

Amplified fragments were scored as present (1) or absent (0) across all genotypes, and only distinct, reproducible bands were considered for analysis. The binary data matrix was used to calculate the Polymorphism Information Content (PIC) for each marker to assess marker informativeness. Pairwise genetic similarity among genotypes was estimated using the Jaccard similarity coefficient. Similarity matrices were generated and subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm to construct dendrograms representing genetic relationships. Principal Coordinate Analysis (PCoA) was also performed to complement cluster analysis and visualize the genetic structure of the population. All statistical analyses were performed using NTSYS-pc version 2.1 (Rohlf, 2000) and DARwin version 6.0 (Perrier & Jacquemoud-Collet, 2006). Polymorphism Information Content (PIC) values were calculated following the formula described by Botstein *et al.* (1980).

Result and Discussion

The morphological characterization of eleven soybean genotypes revealed substantial genetic variation in key agronomic traits, namely plant height, flower color, and maturity period. Such variation is highly relevant for parental polymorphism surveys in breeding programs, as it reflects the potential of these genotypes to serve as diverse parental sources for trait introgression.

Plant Height

Plant height showed considerable variability, ranging from 37.40 cm in AMS-MB-5-18 to 73.23 cm in AMS-100-39. Vegetable-type genotypes such as AGS-457, AGS-465, and Swarna Vasundhara exhibited moderate stature (55-58 cm), which is desirable for ease of pod picking and suitability for mechanized harvesting. In contrast, taller grain-type cultivars such as JS 93-05 and NRC-109 may offer advantages in terms of higher biomass production and potential yield contributions. These findings highlight the differential breeding potential of vegetable-type versus grain-type lines, supporting targeted parental selection for both yield optimization and harvest efficiency (table 3 and fig.2).

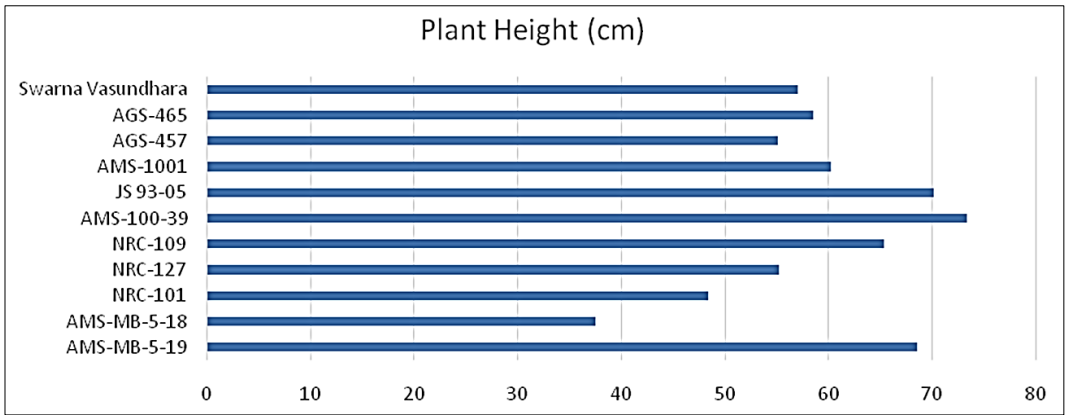

Fig 2: Variation in heights of the soybean genotypes

Flower Color

Flower color exhibited distinct polymorphism, with purple being predominant in AMS-MB-5-19, AMS-MB-5-18, NRC-109, AMS-100-39, JS-93-05, and AMS-1001. White flowers were recorded in NRC-101, NRC-127, AGS-457, and AGS-465, while Swarna Vasundhara displayed a light purple shade. As flower color is controlled by simple Mendelian inheritance, this variation provides a readily scorable morphological marker for varietal identification and polymorphism assessment. Such visible markers are especially useful in field-level differentiation of parental lines and can complement molecular marker-based surveys (table 3 and fig 3).

Maturity Duration

A wide range of variation was also observed in maturity duration. Early-maturing genotypes, including AGS-457 (85 days), AGS-465 (87 days), and Swarna Vasundhara (88 days), are particularly suited for vegetable soybean production, where shorter crop cycles are advantageous. Medium-maturing lines such as NRC-101, NRC-109, JS-93-05, AMS-1001, and AMS-100-39 (90-95 days) align well with the agro-climatic conditions of central India, offering balanced adaptability and yield stability. Late-maturing genotypes, AMS-MB-5-18 (101 days) and NRC-127 (102 days), may be advantageous under extended growing conditions by ensuring prolonged seed filling and stable yields, particularly in regions with longer growing seasons (table 3 and fig 4).

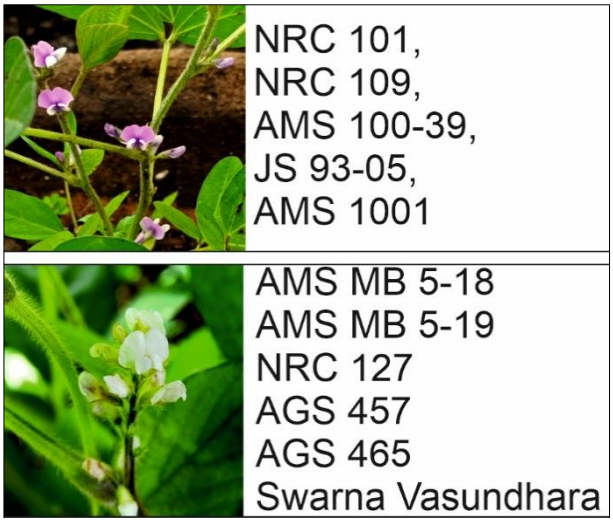

Fig 3: Flower color variation among soybean genotypes

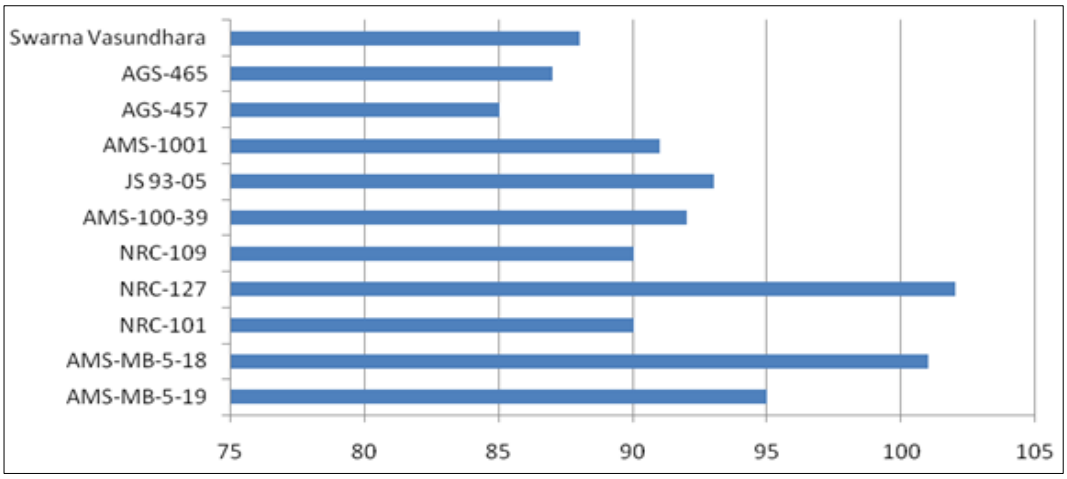

Fig 4: Variation in maturity duration in soybean genotypes

Table 4: Morphological characterization of soybean genotypes

Trait	Plant Height (cm)	Flower Colour	Days to Maturity
AMS-MB-5-19	68.45	White	95
AMS-MB-5-18	37.4	White	101
NRC-101	48.3	Purple	90
NRC-127	55.2	White	102
NRC-109	65.3	Purple	90
AMS-100-39	73.23	Purple	92
JS 93-05	70.1	Purple	93
AMS-1001	60.16	Purple	91
AGS-457	55	White	85
AGS-465	58.5	White	87
Swarna Vasundhara	57	White	88

Molecular Diversity

The electrophoresis profiles generated from the parental polymorphism survey of eight soybean genotypes using 50 SSR markers revealed considerable genetic variability. These markers, distributed across different genomic regions, were highly effective in differentiating the genotypes. Based on Polymorphic Information Content (PIC) values, the markers were classified into three categories: low polymorphism (PIC < 0.4; 8 markers), moderate polymorphism (PIC 0.4-0.7; 18 markers), and high polymorphism (PIC > 0.7; 18 markers). The average PIC value indicated a moderate level of genetic diversity, consistent with earlier reports on soybean germplasm diversity. The gel profiles exhibited distinct and reproducible banding patterns, clearly separating polymorphic loci among the tested genotypes. Strong band separations reflected substantial allelic variation, while uniform banding patterns highlighted genetic similarities. Markers such as Satt 227, Satt 395, Satt 661, and Satt 379 showed high levels of polymorphism, demonstrating their discriminatory potential, whereas markers like Sct_034 and Satt 212 exhibited moderate polymorphism. The variation in band intensity and allele sizes across markers confirmed the reliability of SSRs in detecting genetic polymorphism among soybean parental lines. The highest PIC value (0.94) was recorded for the marker Satt558, indicating its strong discriminatory potential, while the lowest value (0.00) was observed for GMHSP179, suggesting monomorphism at that

locus. Cluster analysis based on Jaccard's similarity coefficient revealed two major clades. Clade A comprised six soybean varieties: AMS 100-39, AMS-MB 5-19, NRC 109, AMS 1001, and JS 93-05, whereas Clade B grouped the vegetable-type aromatic soybean lines AGS 457, AGS 465, and Swarna Vasundhara. The similarity coefficient values ranged from 0.518 to 0.803, demonstrating considerable genetic differentiation among the genotypes. The clear distinction between vegetable-type and grain-type lines in the dendrogram underscores the effectiveness of SSR markers in detecting genetic diversity and clustering genotypes according to their genetic backgrounds.

Table 4: Informativeness of the SSR markers in study

Polymorphic Information Content (PIC)	No. of markers
<0.4	8
0.4-0.7	18
>0.7	18

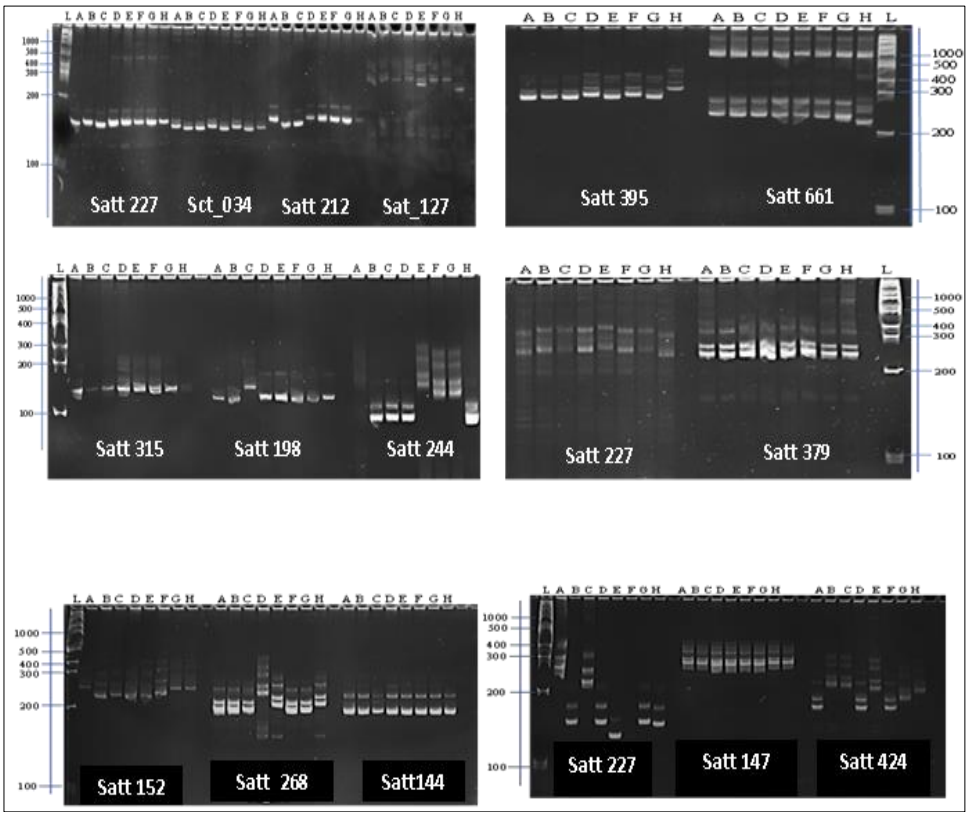

Fig 5: Representative gel picture of polymorphism patter of different SSR markers on soybean genotypes

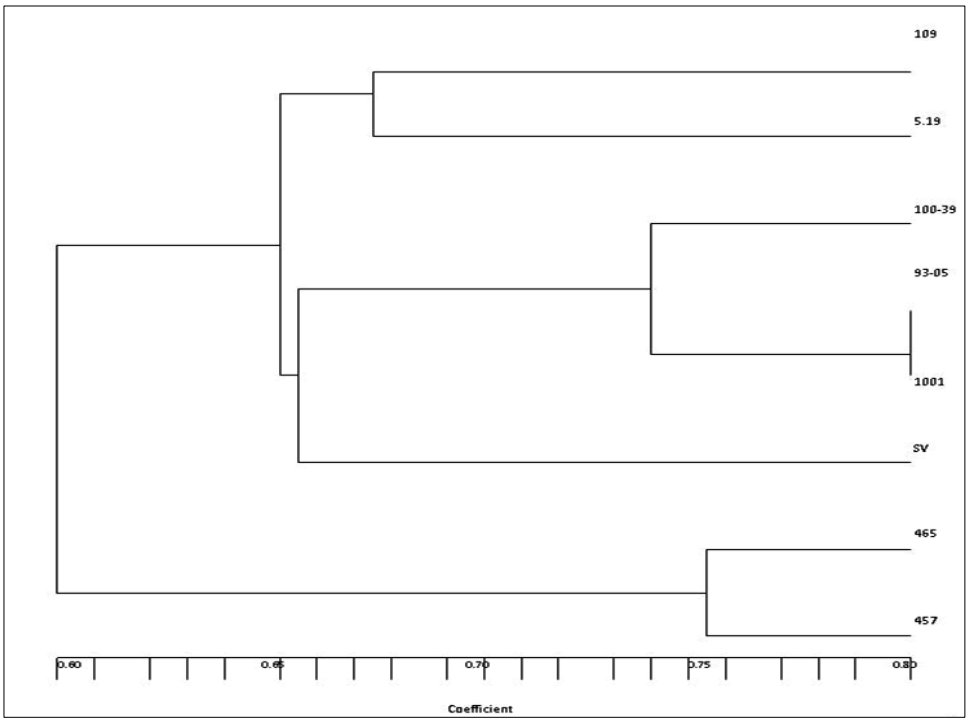

Fig 6: Dendogram of similarity coefficient between eight soybean genotypes

The perusal dendrogram constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm based on Jaccard's similarity coefficient. The x-axis represents the similarity coefficient, ranging from 0.60 to 0.80, indicating the genetic similarity between genotypes. The bifolicious dendogram has two major clad, the major clad A contains six soybean varieties and major clad B contains two aromatic soybean varieties. The major clad comprises 60% similarity between their members. The major clad A is again bifolicious one sub clad Al contains NRC109 and AMS MB 5-19verities with 68% similarity, while second sub clad A2 which is bifolicious and sub divided in to two parts with 66% similarity score. One arm of sub sub clad A2a is differentiated in to two groups with 76% similarity contains 100-39 and another group contains JS93-05 and AMS 1001 with highest 80% similarity. The second arm of major clad A2 contains only suvrnava sundhara which shows similarity of 66% with other 3 members of first arm of A2. The major clad B is bifolicious and contains AGS 465 and AGS 457 soybean which shares about 75.50% similar genetic makeup. This clustering pattern reflects the genetic distinctiveness between grain and vegetable soybean types, aiding in the identification of parental lines for marker-assisted breeding and genetic improvement programs.

Discussion and Conclusion

The present study demonstrated clear morphological and molecular diversity among the evaluated soybean genotypes, reaffirming their potential as valuable parental lines in breeding programs. Morphological traits such as plant height, flower colour, and maturity duration revealed substantial variation, with vegetable-type lines showing moderate plant height and early maturity, favourable for vegetable soybean production and mechanized harvesting, whereas grain-type varieties exhibited taller stature and later maturity, contributing to higher biomass and yield potential. Such variation underscores the genetic richness of the studied germplasm and provides an accessible basis for varietal differentiation and preliminary parental selection. Complementing morphological observations, molecular characterization using 50 SSR markers further revealed significant genetic diversity. Out of the 50 markers tested, 44 produced clear amplification, of which 18 showed high levels of polymorphism. The Polymorphism Information Content (PIC) values ranged widely from 0.14 to 0.99, with markers such as Satt558 proving highly informative. These markers not only validated the genetic distinctiveness observed morphologically but also provided higher resolution in distinguishing allelic variation. Cluster analysis based on SSR profiles separated grain and vegetable types into distinct clades, mirroring their morphological divergence. This concordance between morphological and molecular datasets highlights the reliability of an integrated approach for parental polymorphism surveys. The identification of polymorphic SSR markers holds direct application in marker-assisted backcross breeding (MABB). These markers are particularly useful for hybridity testing, background genome analysis, and estimation of Recurrent Parent Genome Contribution (RPGC). By enabling precise monitoring of recurrent parent genome recovery while introgressing donor traits, breeders can accelerate the development of improved cultivars. This approach is especially relevant for enhancing traits such as resistance to charcoal rot, tolerance to Yellow Mosaic Virus, and nutritional quality improvement (e.g., Kunitz trypsin inhibitor-free soybean lines).

Integrating morphological characterization with molecular polymorphism thus provides a comprehensive and practical framework for breeding. While morphological traits assist in preliminary classification and selection of contrasting parents, SSR markers refine this process by offering precise genetic resolution. Together, they enhance the efficiency of parental selection, support genetic diversity conservation, and ensure targeted genome recovery in MABB pipelines. Overall, this study establishes a robust methodological framework where the combined evaluation of morphology and molecular markers strengthens parental polymorphism surveys and facilitates the development of soybean varieties tailored to diverse agro-climatic conditions and end-use preferences whether for vegetable soybean production with desirable quality traits or for grain-type cultivars optimized for yield and resilience.

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