

MOLECULAR CHARACTERIZATION OF SOYBEAN (*Glycine max*) CULTIVARS USING SSR MARKERS

Vivek D. Pujari, Kuldeep M. Sharma, S.B. Borgaonkar,

Vilasrao Deshmukh College of Agricultural Biotechnology, Latur -413512

(Vasantrao Naik Marathwada Krishi Vidyapeeth-Parbhani-431401

(Parbhani), Maharashtra, India.

MS Received: 02.03.2025; MS Revised:02.04.2026; MS Accepted:03.04.2026)



MS 3473

(RESEARCH PAPER IN AGRICULTURAL BIOTECHNOLOGY,)

**Abstract**

Soybean is a significant leguminous crop, containing 40-44% protein and oil that is free of cholesterol, making up about 20% of its composition. A notable challenge in soybean cultivation is shattering, which is linked to the genetic makeup of various crop species. This study aimed to characterize different soybean cultivars. Twelve cultivars, including both shattering and non-shattering types developed by VNMKV, Parbhani, were examined. To assess the genetic diversity among these cultivars, twelve soybean-specific SSR primers were employed. The diversity identified through these primers ranged from 4.5% to 58%. Findings indicated that the twelve SSR loci displayed a polymorphism rate of 37.5% among the studied genotypes. The selected cultivars could be categorized into shattering and non-shattering groups. Notably, a distinct band of approximately 125 bp was observed exclusively in the non-shattering cultivars using the SSR primer Sat-111; this band was not present in any shattering cultivar. Analysis through a generated dendrogram revealed three clusters. The overall study demonstrated that SSR markers can effectively identify Quantitative Trait Loci (QTL) associated with shattering resistance traits. Molecular and biochemical characterization highlighted that non-shattering cultivars hold potential for enhancing soybean crop improvement via plant breeding initiatives.

**Keywords-** *Glycine max*, shattering and non-shattering, twelve SSR.

**Introduction**

Soybean is often referred to as "nature's versatile" crop due to its rich content of proteins and oils. Given that many diets in India lack sufficient protein both qualitatively and quantitatively, soybean serves as an excellent alternative protein source—often likened to meat for those with limited resources. As the world's leading oilseed crop, soybean accounts for 58% of total oilseed production globally and comprises roughly 40-44% protein, 23% carbohydrates, and 20% cholesterol-free oil (Aslam and Mirza, 1995). Molecular markers are instrumental in revealing genetic diversity at a molecular level (Hill, 1998). DNA markers are numerous, neutral with respect to selection pressure, and can be organized into linkage maps (Thormann et al., 1992). These molecular markers have emerged as powerful tools for breeders seeking new sources of variation while investigating genetic factors responsible for quantitatively inherited traits. Characterization using these markers uncovers both similarities (shared alleles) and differences (distinct alleles) across various cultivars, aiding in tracing gene pools or origins of specific varieties. To foster the development of elite cultivars, it is essential to broaden the genetic base; species whose gene pools have been mapped through markers can facilitate this process by exchanging germplasm. Utilizing markers enables the creation of distinct DNA profiles.

**Table No.1 List of 12 soybean genotypes and their characters**

| Sr. No. | Varieties | Days to 50% flowering | Days for maturity | Distinct characters                           |
|---------|-----------|-----------------------|-------------------|-----------------------------------------------|
| 1       | JS-335    | 40                    | 95                | Round seeds, good germination, Shattering     |
| 2       | MAUS-47   | 40                    | 95                | Good germination, Shattering                  |
| 3       | DS-228    | 39                    | 95-100            | Good germination, Shattering.                 |
| 4       | MAUS-1    | 38                    | 96-98             | Good germination, Shattering, High yielding   |
| 5       | MAUS-2    | 40                    | 95-98             | Good germination, Shattering, high Yielding   |
| 6       | JS-93-05  | 43                    | 90                | Non-shattering, Bold seeded                   |
| 7       | JS-97-52  | 40                    | 101               | Pest tolerant, Non-shattering.                |
| 8       | MAUS-71   | 39                    | 85                | High oil content, Round seeds, Non-shattering |
| 9       | MAUS-81   | 40                    | 104               | High yielding, Early maturity, Non-shattering |
| 10      | MAUS-61   | 40                    | 95                | Non-shattering, Suitable for Intercropping    |
| 11      | MAUS-61-2 | 39                    | 95                | Non-shattering, High oil content              |
| 12      | MAUS-32   | 39                    | 100               | Non-shattering, Good                          |

**Material and Methods**

The molecular characterization of soybean (\**Glycine max*\*) was conducted at the Department of Post Harvest and Food Biotechnology within the College of Agricultural Biotechnology at Latur, affiliated with V.N.M.K.V., Parbhani. SSR analysis is a PCR-based molecular technique widely utilized for characterizing germplasm and assessing genetic diversity across many crops today (Patterson, 1996). The SSR analysis encompasses four primary steps:

- Extraction of total genomic DNA
- Quantification of DNA
- PCR amplification utilizing random primers
- Gel electrophoresis followed by visualization of amplified products

A total of twelve soybean genotypes (\**Glycine max*\*), detailed in Table 1, were sourced from the Soybean Research Station at V.N.M.K.V., Parbhani; seven are classified as shattering while five are designated as non-shattering cultivars. Total genomic DNA was extracted from seedlings that were ten days old and had germinated on moist germination paper within an incubator; eight seedlings from each accession were crushed for DNA isolation purposes.

**Sterilization**

The chemical reagents, tips (20-200  $\mu$ l and 1000  $\mu$ l), eppendorf tubes (1.5 ml and 0.5 ml), PCR tubes (200  $\mu$ l), DNA extraction buffer, mortar and pestle and all other related equipments were autoclaved at 121°C for 15 minutes under 1.06 Kg/cm<sup>2</sup> pressure. The glass wares were also autoclaved after washing and rinsing with double distilled water.

**Isolation of DNA**

The protocol of Cetyl TrimethylAmmonium Bromide (CTAB) DNA extraction method given by Saghai-Marooof (1984) was used with some modifications.

**DNA quantification**

Quality and quantity of DNA was checked by comparing DNA samples with known amount of DNA. The DNA concentration thus estimated and rechecked qualitatively by running on 0.8 % Agarose gel. For preparation of Agarose gel, 0.8 gm of Agarose in 100 ml 1 X TAE buffer was melted by heating till to boiling. After cooling the solution to about 45°C, Ethidium Bromide at 3-5  $\mu$ g/ml was added. To the DNA samples, 6 X gel loading dye were added so that the final concentration was 1 X before

loading. The gel was run at 60 V for 1 hr.

Fifteen soybean specific SSR primers from Sat, Satt, Sct, Sctt series (Imperial Life Sciences (P) Limited, Haryana) were selected (Craig *et al.*, 2000). Twelve primers from different series were screened on the basis of reproductive and scorable amplifications for the analysis of all the twenty genotypes of soybean.

The amplified products were resolved on 2.5 % Agarose gel in 1 X TAE buffer at 100V for 3.5 hr. The gel was stained in Ethidium Bromide. After electrophoresis the gel was carefully taken out of the casting tray and photograph was taken on a gel documentation system (Alphaimager™ 2200).

**Scoring and Data Analysis**

Each amplification product was considered as SSR marker and was scored across all samples. Bands were scored as present (1) or absent (0). Missing and doubtful cases were scored as (9). Molecular weight of the bands was estimated by using 1kb DNA ladder (MBI, Fermentas, U.K.) as standard. All amplifications were repeated at least twice and only reproducible bands were considered for analysis.

**Table 2: Primers selected for SSR analysis of the selected genotypes**

| S. No. | Primers  | Sequence (5'-3')                                                       | G+C Content ( % ) |
|--------|----------|------------------------------------------------------------------------|-------------------|
| 1      | Satt-002 | TGT GGG TAA AAT AGA TAA AAA T<br>ACACCCATTTTATCTATTTT                  | 22.72             |
| 2      | Satt-009 | TGT GGG TAA AAT AGA TAA AAA T<br>ACA CCC ATT TTA TCT ATT TTT A         | 30.43             |
| 3      | Sat-099  | GCG AAA ATG GCA GAG ATA A<br>CGC TTT TAC CGT CTC TAT T                 | 45                |
| 4      | Satt094  | CCA AGT GCC AAT GAA G<br>GGT TCA CGG TTA CTT C                         | 50                |
| 5      | Satt071  | GAG TGC TGA TCT ATT TGT CA<br>CTC ACG ACT AGA TAA ACA GT               | 40                |
| 6      | Sat001   | GCG GAT ACG ACC AAA AAT TGT T<br>CGC CTA TGC TGG TTT TTA ACA A         | 40.90             |
| 7      | Satt-095 | TAT TTG TTA TTG GTG AAT TAA GA<br>ATA AAC AAT AAC CAC TTA ATT CT       | 21.73             |
| 8      | Sat-111  | TTT CTG ATG TAC CCT TTA TGT C<br>AAA GAC TAC ATG GGA AAT ACA G         | 36.36             |
| 9      | Sct-033  | CTT TTA AAT TAT AAT AGC ATG ATC T<br>GAA AAT TTA ATA TTA TCG TAC TAG A | 20                |
| 10     | Sctt-008 | ACG CAG AGA TAG AGA AAG AGA<br>TGC GTC TCT ATC TCT TTC TCT             | 42.85             |
| 11     | Satt032  | GAT CTT TAG CTC CAT GTG T<br>CTA GAA ATC GAG GTA CAC A                 | 42.10             |
| 12     | Satt042  | GAC TTA ATT GCT TGC TAT GA<br>CTG AAT TAA CGA ACG ATA CT               | 35                |

**Results and Discussion**

Among the various tried concentrations of DNA (25, 50, 75 and 100 ng per 10  $\mu$ l reaction mixture), 25 ng was found optimum. Lower quantity of DNA resulted in less intense bands, whereas higher concentrations added background effect.

Among different primer concentrations (10, 15, 20, 25 and 30 pM) tried, 10 pM primer concentration was found to be optimum and it gave complete amplification product. Among different *Taq* DNA polymerase concentrations (0.5 U, 0.6 U and 1 U, 1.25 U) tested, use of 0.6 U *Taq* DNA polymerase enzyme resulted in good amplification. In case of dNTPs, different dNTPs concentrations (100  $\mu$ M, 150  $\mu$ M, and 200  $\mu$ M) tried. The concentration of 200  $\mu$ M resulted in good amplification of genomic DNA.

Different soybean specific SSR primers were used to screen shattering and non- shattering cultivars of soybean. Many loci were similar in

shattering and non- shattering cultivars of soybean using SSR primers. The PCR amplification result showed that 12 SSR primers generated 432 bands.

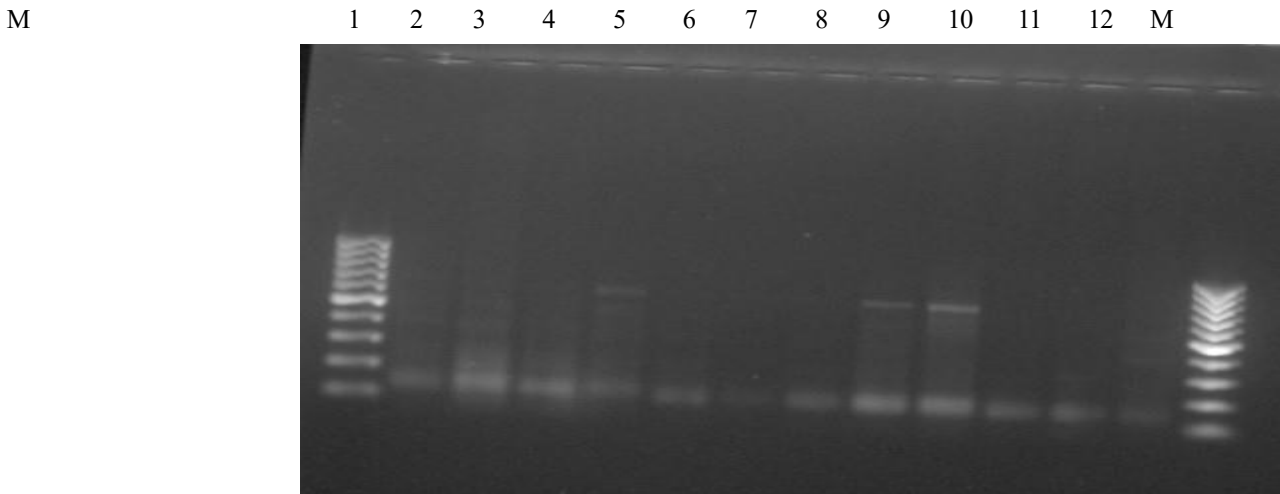
Out of amplified 432 bands, 162 bands were polymorphic and 270 bands were monomorphic among 12 genotypes. The result showed that 12 SSR loci exhibited 37.5% polymorphism among 12 genotypes studied. In case of percentage polymorphism highest percentage of polymorphism was recorded in SSR primer Sat- 009 (58%), Sctt-008 (48%), Satt-094 (45%), and Satt-099 (44.5%). A DNA band of ~ 125 bp was common in cultivars distinguished as non-shattering characteristic of soybean. This specific loci was not amplified in shattering cultivars of soybean using primer Sat 111.

Table 3 showed that total 270 monomorphic bands were observed among 12 genotypes. The average monomorphic bands per SSR loci were 22.5. Also, 162 polymorphic bands were detected among 12 genotypes and

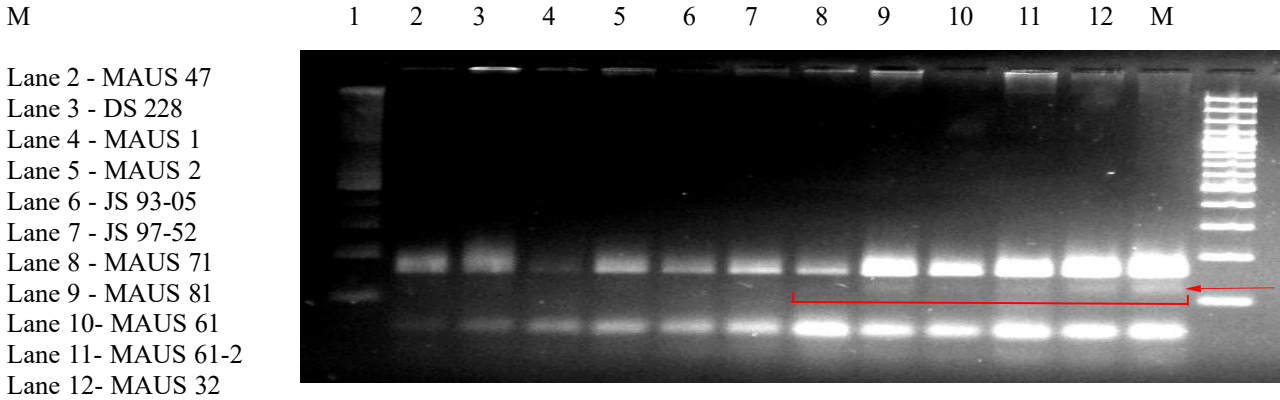
average 13.5 polymorphic bands were detected per locus.

**Table 3: Details of SSR amplicons/bands produced by 12 primers in 12 soybean genotypes.**

| S.No. | Primer   | Total no. of amplicons | No. of monomorphic amplicons | No.of polymorphic amplicons | Percent polymorphism   |
|-------|----------|------------------------|------------------------------|-----------------------------|------------------------|
| 1     | Sat-095  | 24                     | 19                           | 5                           | 20.9                   |
| 2     | satt-002 | 25                     | 15                           | 9                           | 36                     |
| 3     | Sat-071  | 24                     | 14                           | 10                          | 41.7                   |
| 4     | sat-111  | 24                     | 23                           | 1                           | 4.5                    |
| 5     | Sct-033  | 24                     | 19                           | 5                           | 20.9                   |
| 6     | Satt-099 | 36                     | 20                           | 16                          | 44.5                   |
| 7     | Satt-032 | 36                     | 23                           | 13                          | 36.1                   |
| 8     | sat-042  | 48                     | 39                           | 9                           | 18.8                   |
| 9     | Sctt-008 | 48                     | 25                           | 23                          | 48                     |
| 10    | Sat-001  | 48                     | 27                           | 21                          | 43                     |
| 11    | Sat-009  | 48                     | 20                           | 28                          | 58                     |
| 12    | Satt-094 | 48                     | 26                           | 22                          | 45                     |
| Total |          | 432                    | 270<br>(Avg. 62.5 %)         | 162<br>(Avg. 37.5 %)        | 417.4<br>(Avg. 34.78%) |



**Fig.No.1: SSR profile of Soybean genotypes with primer Sat-002**



Lane 2 - MAUS 47  
Lane 3 - DS 228  
Lane 4 - MAUS 1  
Lane 5 - MAUS 2  
Lane 6 - JS 93-05  
Lane 7 - JS 97-52  
Lane 8 - MAUS 71  
Lane 9 - MAUS 81  
Lane 10- MAUS 61  
Lane 11- MAUS 61-2  
Lane 12- MAUS 32

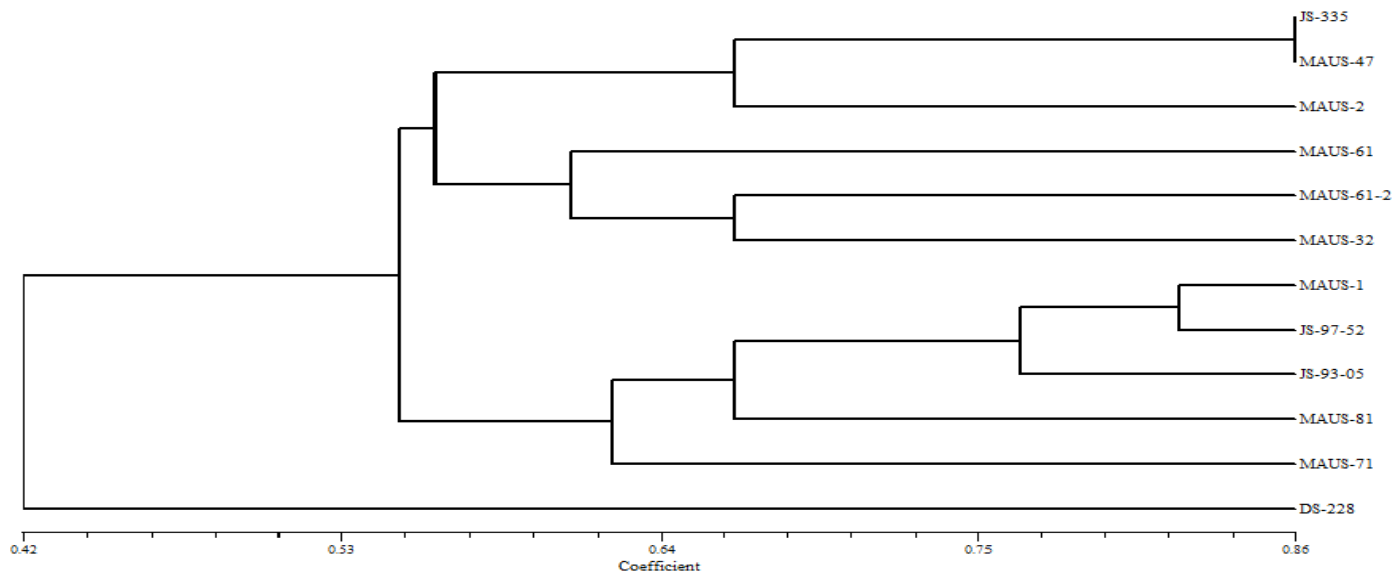
**Fig.No.2: SSR profile of Soybean genotypes with primer SatM- 100 bp ladder Lane 1 - JS335**

**Jaccard's coefficient study**  
Genetic relationship between soybean varieties were determined on the basis of Jaccard's similarity coefficient values. The value of similarity coefficient ranged from 0 to 1. Average similarity coefficient value per variety were calculated which given Table-10 the variety MAUS-1 represented the highest average similarity coefficient value (0.626) and the variety DS-228 represented the lowest average similarity coefficient value (0.424). The average genetic similarity coefficient value was 0.552.

**Cluster analysis**  
A dendrogram was generated by UPGMA cluster analysis based on Jaccard's similarity coefficients. According to cluster analysis, accessions were divided in 3 main clusters viz; cluster A, B and C. Cluster A and B had 54 % similarity. Cluster A contained 6 varieties like, JS-335, MAUS-47, MAUS-2 (all are shattering), MAUS- 61, MAUS-61-2, MAUS-32 (all are non-shattering). In cluster B, varieties MAUS-1 (shattering), JS-97-52, JS-93-05, MAUS-81, and MAUS-71 (non-shattering) were included. In main cluster-C, single variety, DS-228

(shattering) was included. Cluster A and Cluster B were divided into four subclusters. In cluster-A, subcluster-I contained two varieties named as JS-335 (shattering and early) and MAUS-47 (shattering and round seeds). These varieties were 84 % similar. In Subcluster-II, variety MAUS-2 (shattering and high oil content) was included which showed 65% similarity with

varieties included in Subcluster-I. Subcluster-III also had single variety, MAUS-61 (non-shattering and good germination) which had been showing 60 % similarity with varieties included in Subcluster-IV viz; MAUS-61-2 (non-shattering) and MAUS-32 (good germination and non-shattering). Varieties in Subcluster-IV, showed 65 % similarity.



**Fig. 3: Dendrogram generated by UPGMA Analysis based on SSR data showing relationship among 12 soybean genotypes.**

In present study we used NTSYS-PC software and dice similarity coefficient for cluster analysis which divided all 12 varieties into three main clusters; A, B and C. Two main clusters, A and B were divided into four sub clusters on the basis of phenotypic characters like maturity period, flower color, seed shape, oil content, pest and disease resistant once. As per Zengulu *et al.*, (2001) it clearly indicates that geographical origin and phenotypic characters play important role in cluster formation and genetic relationships. The pattern of diversity found by using SSR markers can aid breeders in selecting parental lines to more efficiently exploit the diversity found in gene pool of these selected varieties.

**References:**

1. **Aslam, M. and Mirza, M. S. (1995).** Crop Production Bulletin No.6, Pak.Agric.Res.Council, Islamabad, Pakistan.pg.1.

2. **Craig, P. and Ventor, M. (2000).** List of soybean specific SSR primers.www.soybase.com  
3. **Hill, J. (1998).** Quantitative and ecological aspects of plant breeding. Champn and Hall, ISBN, London.  
4. **Patterson, A. H. C., (1996).** Genome mapping in plants. R.H. Lands Company Austin. USA, pp: 23-26.  
5. **Saghai-Marooof and Saliman, M.A (1984).** Ribosomal spacer length polymorphism in Barley:Mendelian Inheritance, chromosomal allocation and population dynamics. Proc. Natnl. Acad. Science (USA) 81:8014-8018.  
6. **Zengulu, L. and Liuan Q. (2000).**Molecular genetic analysis of US and Chinese ancestral lines. *Crop Sci.*, 41:130-1336.