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# Field Evaluation and Molecular Validation of Soybean Genotypes for Resistance to Yellow Mosaic Disease using SSR Markers

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## ABSTRACT

**Background:** Various biotic factors adversely affect the growth and productivity of soybeans. Viruses are one of them, as they cause significant loss to the yield of soybeans in India. The present study was conducted to identify yellow mosaic virus (YMV) resistant genotypes.

**Methods:** Field screening was performed in disease hotspot conditions to identify YMV-resistant genotypes among 70 soybean genotypes. The field data of a selected set of 40 genotypes was compared with molecular data recorded using gene-specific SSR molecular markers.

**Result:** In a field study involving seventy soybean genotypes, eighteen were highly resistant, twenty-seven were moderately resistant, eighteen were moderately susceptible and six were susceptible to yellow mosaic diseases. Only one genotype, JS 93-05, was highly susceptible to the disease. The genetic relationships among the soybean genotypes were examined using SSR markers and fifteen primers, effectively amplified soybean genomic DNA. The polymorphic information content (PIC) scores were used to assess genetic diversity for specific loci. The PIC values ranged from 0.00 (Sct_199) to 0.84 (Satt 267), with an average of 0.43. The alleles varied from 1 to 3, with an average value of 2.1 and the allele sizes ranged from 60 to 260 base pairs. A UPGMA cluster analysis based on genetic distance data resulted in a dendrogram that grouped the soybean genotypes into two main groups, A (20) and B (20). Group A was further divided into subgroups A1 (16) and A2 (04), while Group B was divided into subgroups B1(07) and B2(13). The highest similarity (0.94) was found between RVS 13-7 and JS 20-116, while the lowest similarity (0.41) was observed between NRC 192 and RVSM 2012-4. The resistant genotypes identified in this study may be used as donors of resistance genes against YMV to develop improved genotypes, which would stand as barriers against the spread of the disease to newer areas and thus boost the production and productivity of soybeans in the country.

**Key words:** Polymorphic information content, Soybean, SSR primers, UPGMA cluster analysis.

## INTRODUCTION

The soybean [*Glycine max* (L.) Merrill], also known as the golden bean, poor man's meat, boneless meat, yellow jewel and the miracle crop of the 20th century, is a highly significant legume crop worldwide (Jain *et al*., 2018, Sivabharathi *et al*., 2023, Barpanda *et al*., 2024). Soybean-based foods are trendy in eastern countries such as China and Japan. Soybean is rich in oil, protein, macronutrients and minerals (Banerjee *et al*., 2023). The central region of the country, comprising Madhya Pradesh (45%), Maharashtra (38%) and Rajasthan (8%), is a significant hub for soybean cultivation (Amrate *et al*., 2024; Mishra *et al*., 2024). In India, soybean cultivation has expanded to approximately 12.07 million hectares, resulting in a total production of about 13.98 million tons and an average productivity of roughly 1158 kg per hectare in the *Kharif* 2022-23 (Annual Report 2023, ICAR- Indian Institute of Soybean Research, Indore). Madhya Pradesh has significantly contributed to India's soybean production, dedicating 5.51 million hectares of land to cultivation and achieving a yield of 5.39 million metric tonnes (Mishra *et al*., 2024).

Soybean yellow mosaic disease is one of the significant soybean diseases in Madhya Pradesh. Yellow Mosaic Disease is caused by *the Mungbean yellow mosaic India virus* (MYMIV) (Amrate *et al*., 2023) transmitted by white flies (*Bemisia tabacci*). The most distinctive symptom of Yellow Mosaic Disease is the presence of contrasting yellow-green patches (mottles) on the leaves. Under severe conditions, infected leaves may turn yellow, leaving the veins green (Amrate *et al*., 2020; Amrate, 2024). Screening soybean

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genotypes for YMV resistance is primarily impaired by the vectors' non-arrival in the field, poor intensity, uneven distribution, environmental conditions, *etc*. Therefore, it is necessary to find some linked molecular markers for effective and efficient genotype screening (Kumar, 2013). Simple sequence repeats (SSRs) are one of the widely used markers for genotypic analysis and molecular mapping, cultivar identification, protecting germplasm, determination of hybrids, analysis of gene pool variation and as diagnostic markers for traits of economic value (Powell *et al*., 1996). In plant due to their species specificity and high reproducibility, simple sequence repeats markers were used in PCR amplification for molecular analysis of soybean. The present investigation was conducted to identify donor parents and SSR marker linked to YMV resistant in soybean.

## MATERIALS AND METHODS

The seventy soybean genotypes were planted on July 1st, 2023, during the *kharif* season, using an augmented block design with a spacing of 45 cm × 7 cm. Each genotype was grown in two rows, with each row being 3 m long, for field screening of the soybean genotype against yellow mosaic disease in the soybean research field (23°12′45N, 79°56′46E) at Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh. Molecular screening using SSR markers was conducted at the Molecular Biology Laboratory, Biotechnology Centre, Jawaharlal Nehru Agriculture University, Jabalpur (M.P).

The experimental area had uniform topography and fertility. The soil was clay loam with a good texture, medium NPK status and good water-holding capacity.

### Experimental material

The study was conducted on seventy genotypes of soybean and collected from All India Coordinated Research Project on Soybean (Table 1).

### Recording of coefficient of infection for YMD

The severity of the symptoms of YMD was evaluated based on a 0-5 rating scale 50 days after sowing (Table 2) and the coefficient of infection was calculated by multiplying the percentage incidence of YMD by the corresponding symptom severity grades. Genotypes were then assigned different reaction categories based on the coefficient of infection, as described by Amrate *et al*. (2020) and (2023).

### Isolation of DNA of soybean genotype

Leaf samples were collected from one month old seedling of soybean genotypes from research field on soybean. Usually fresh and young leaves are used in molecular work and DNA extraction.

### Extraction of genomic DNA and purification

Genomic DNA was isolated using the Sahai-Marof *et al*. (1984) method with suitable modification. The quality of isolated DNA was checked by horizontal submarine gel electrophoresis on 0.8% agarose gel. The purity of DNA was checked by taking the ratio of optical density (OD) using a spectrophotometer at 260 nm to that of 280 nm. The samples with an OD ratio between 1.7 and 1.9 were used in subsequent experiments. If DNA samples show a value beyond this range, they must be repurified.

### PCR amplification

The PCR reaction was carried out in a ten µl reaction volume with various components. The reaction mixture comprised 1.0 µl DNA, 5.7 µl nuclease-free water, 0.7 µl MgCl, , 0.4 µl dNTPs, 1.0 µl primers, 0.2 µl Taq DNA polymerase and 1.0 µl 10× buffer. The reaction mixture underwent a brief spin to ensure thorough mixing. PCR samples were stored at 4°C for short-term use and -20°C for long-term preservation.

The PCR thermal cycling conditions for SSR markers were fine-tuned with various parameters, including initial

**Table 1:** List of soybean genotypes used in the experiment.

| S.N. | Genotype | S.N. | Genotype |
|---|---|---|---|
| 1. | JS 335 (c) | 36. | KDS 10-73 |
| 2. | JS 24-25 | 37. | RVSM 2012-4 |
| 3. | JS 20-29 | 38. | RVS 13-7 |
| 4. | JS 21-13 | 39. | EC 457366 |
| 5. | MACS 1520 | 40. | AMS 243 |
| 6. | EC 280149 | 41. | EC 14117 |
| 7. | DS 1510 | 42. | NRC 192 |
| 8. | JS 20-19 | 43. | NRC -86 |
| 9. | JS 22-11 | 44. | JS 93-05 (c) |
| 10. | AVS-56 | 45. | JS 20-116 |
| 11. | JS 95-60 (c) | 46. | JS 21-70 |
| 12. | JS 25-01 | 47. | JS 20-69 |
| 13. | JS 25-02 | 48. | JS 97-52 |
| 14. | JS 20-98 | 49. | JS 24-32 |
| 15. | JS 21-72 | 50. | NRC 130 |
| 16. | NRC 189 | 51. | NRC 150 |
| 17. | NRC 186 | 52. | NRC 197 |
| 18. | RSC 11-75 | 53. | NRC 201 |
| 19. | Cat- 87 | 54. | JS 21-74 |
| 20. | DS 1318 | 55. | JS 22-09 |
| 21. | JS 20-89 | 56. | PS 1611 |
| 22. | JS 24-31 | 57. | PS 1660 |
| 23. | JS 24-26 | 58. | RSC 11-22 |
| 24. | NRC- 190 | 59. | RSC 11-48 |
| 25. | AMS- 264 | 60. | PS 1689 |
| 26. | AMSMB- 5-19 | 61. | JS 22-14 |
| 27. | AMS-56 | 62. | NRC 154 |
| 28. | EC 350664 | 63. | MACS 1566 |
| 29. | JS 20-56 | 64. | RSC 11-75 |
| 30. | JS 20-78 | 65. | RSC 11-42 |
| 31. | JS 22-17 | 66. | RVSM 16-20 |
| 32. | JS 22-13 | 67. | AMS 2014-1 |
| 33. | JS 22-08 | 68. | JS 20-79 |
| 34. | JSM 230 | 69. | JS 24-24 |
| 35. | JS 20-34 | 70. | KDS 1009 |

denaturation, denaturation, annealing, elongation and final elongation, as outlined in Table 3.

**Agarose gel electrophoresis of SSR-PCR products**

Amplified DNA fragments were loaded in submerged horizontal agarose gel electrophoresis in 2.5 percentage (w/v) agarose gel and visualized by staining with Ethidium bromide. PCR product mixed with loaded dye and loaded into gel wells. The gel was run at a voltage of 80 volts for 1 hour (Fig 1 and 2). The result was recorded in the gel documentation system.

**Data analysis**

NTSYS-PC version 2.02 software was used to observe data analysis. Using the data produced by the cluster analysis, a UPGMA dendrogram was also created (Fig 3 and 4).

**Data scoring**

The polymorphism in SSR was analyzed based on the presence or absence of the SSR bands. The genetic associations among varieties were analyzed by calculating the similarity coefficient (Jaccard, 1908) for pair-wise comparisons based on the proportions of shared bands produced by primers. The polymorphism information content (PIC) of the SSR markers was calculated according to Anderson *et al*. (1993) (Table 4).

## RESULTS AND DISCUSSION

The current study, 70 soybean genotypes were assessed for their resistance to yellow mosaic disease under field conditions. The susceptible varieties (JS 93-05, JS 20-34) were kept in check. The experimental site was one of the hot spot locations for yellow mosaic in soybeans (Amrate *et al*., 2018; Pancheshwar *et al*., 2016; Amrate *et al*., 2020; Amrate *et al*., 2023).

Among the seventy genotypes, eighteen, including JS 24-25, JS 20-29, JS 25-01, DS 1510, NRC 189, JS 24-31, NRC 186, JS 20-89, PS 1660, JS 24-26, JS 24-78, JS 24-32, JS 20-69, JS 22-08, RSC 11-48, JS 20-79, KDS 1009 and JS 22-24, they demonstrated high resistance (Table 5). Additionally, twenty-seven genotypes were moderately resistant and eighteen were susceptible. Six genotypes exhibited susceptibility, including JS 95-60 check, JSM 230, JS 20-34, KDS 10-73, RVS 13-7 and AMS 243, while only one genotype, JS 93-05 (check), was highly susceptible. The present investigation was based on recently evolved soybean genotypes. Similar results were found by Amrate *et al*. (2023), who observed that JS 20-94 showed moderate resistance to Yellow Mosaic Disease. Soybean yellow mosaic disease is one of the significant soybean diseases in Madhya Pradesh. Yellow Mosaic Disease is caused by *the Mungbean yellow mosaic India virus* (MYMIV) (Amrate *et al*., 2023) transmitted by white flies (*Bemisia tabacci*). The most distinctive symptom of Yellow Mosaic Disease is the presence of contrasting yellow-green patches (mottles) on the leaves. Under severe conditions, infected leaves may turn yellow, leaving the veins green (Amrate *et al*., 2020; Amrate, 2024).

The genotypes that showed susceptibility to yellow mosaic were also found susceptible in previous studies (Rani *et al*., 2016; Amrate *et al*., 2023). A similar kind of moderate resistance reaction for JS 20-94 and JS 20-98 was also reported by Amrate *et al*. (2023) and Mishra *et al*. (2022).

During the present study, the genomic DNA of 40 randomly selected soybean genotypes were characterized for genetic diversity using 15 SSR markers to achieve the research goal. The polymorphism information content, or PIC, measures the variation at a specific gene position (Kumar *et al*., 2023). A large number suggests that the plants were most likely significantly different. These PIC

**Table 2:** Criteria for Coefficient of Infection (CI) based designation of disease reaction and category for soybean Yellow Mosaic Disease (YMD) (Amrate *et al*. 2023).

| Appearance of symptoms of YMD on soybean leaves | | Response | Coefficient of | Disease reaction |
|---|---|---|---|---|
| Symptoms | Severity grades | value[a] | Infection (CI)[b] | |
| Healthy plant (symptoms absent) | 0 | 0 | 0 | Absolute resistance (AR) |
| Very mild, yellow mottling in traces (up to 10% leaves) | 1 | 0.1 | >0-2 | Highly resistance (HR) |
| Mid yellow mottling scattered (> 10 to 25% leaves) | 2 | 0.25 | >2-15 | Moderately resistant (MR) |
| Moderate, yellow mottling prominent (> 25 to 50% leaves) | 3 | 0.5 | >15-30 | Moderately susceptible (MS) |
| Moderate to severe yellow mottling prominent (> 50 to 75% leaves) | 4 | 0.75 | >30-60 | Susceptible (S) |
| Severe infection, most of the leaves exhibit complete yellowing (> 75 to 100% leaves) | 5 | 1 | >60-100 | Highly susceptible (HS) |

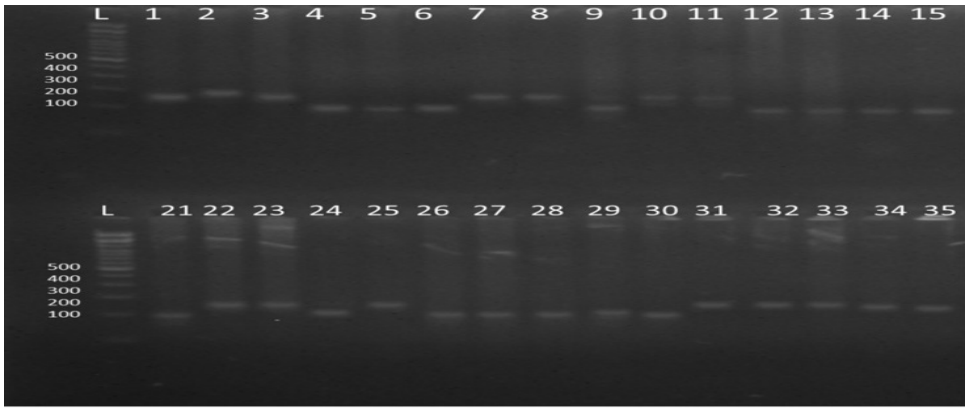
[a]Response value is a corresponding value of symptom severity grades assigned on the basis of symptom pattern and number of leaves showing symptoms.

[b]Coefficient of Infection (CI) is a multiplication of Per cent disease incidence and the corresponding response value of symptoms severity.

**Table 3:** SSR primer used in experiment.

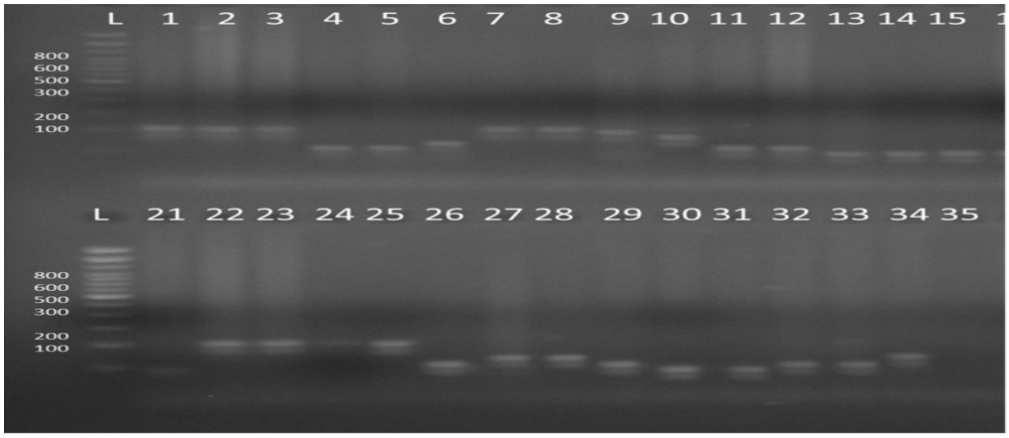
| Primers | DNA sequence | Tm°C |
|---|---|---|
| Satt009 | F- CCAACTTGAAATTACTAGAGAAA | 52 |
| | R- CTTACTAGCGTATTAACCCTT | |
| Satt146 | F- AAGGGATCCCTCAACTGACTG | 52 |
| | R- GTGGTGGTGGTGAAAACTATTAGAA | |
| Sct_199 | F- GCGACAATGGCTATTAGTAACAATCA | 55 |
| | R- GCGATTTTCTATTTTCCTCACAGTG | |
| Satt267 | F- CCGGTCTGACCTATTCTCAT | 52 |
| | R- CACGGCGTATTTTTATTTTG | |
| Sat_281 | F- GGCGGTCATATAAGCCTGATAGGTCGG | 52 |
| | R- GGCGGCAGCCTGTATAGAAACTTCTCAAGC | |
| Satt290 | F- GCGGATTTATAAAACATTAAAAATCA | 55 |
| | R- GCGCGCACGGCTGAAACACTCTCTCA | |
| Satt301 | F- GCGAAACACTCCTAGTTGATTACAAA | 52 |
| | R- GCGATATAATGCACAAAGAAATTAAAGA | |
| Satt352 | F- GCGAATGTATTTTTGTTTCTCCATCAA | 55 |
| | R- TGATAAGCCAAAAAATGGAAGCATAG | |
| Sat_405 | F- GCGTAACCGTGGTAAGTGTGGTGTCGTA | 55 |
| | R-GCGTTTCGTTTTAATTGCTCCTGAAAGT | |
| Satt408 | F- GCGGTCCGTGCTGTTAATTCTATA | 52 |
| | R- GCGTGATTTATTCATGATATATTTTTG | |
| Satt532 | F- GCGCCAATATTATCATGCTTTATGT | 52 |
| | R- GCGTGTAAAAATCTTTGAATCTTGA | |
| Satt541 | F- GCGAATCCATCACACATAAA | 55 |
| | R- GCGGTACTCCCTCCAGAAAAT AACC | |
| Satt554 | F- GCGATATGCTTTGTAAGAAAATTA | 52 |
| | R- GCGCAAGCCCAAATATTACAAATT | |
| Satt560 | F- GCGATCGTGCAAGAAAATA | 52 |
| | R- GCGGTGGACTTCGCCTCAAATAAT | |
| GMSP179 | F- ATACCAGTTGATCTGCATATT | 52 |
| | R- AAAACATCGTCTACTTAAGTC | |

*Note: F- Forward primer, R- Reverse primer.




L = Ladder 1 = JS 20-19, 2 = JS 20-56, 3 = JS 20-98, 4 = JS 20-89, 5 = JS 20-34, 6 = JS 22-08, 7 = JS 22-17, 8 = JS 21-72, 9 = JS 24-26, 10 = JS 24-25, 11 = JS 93-05, 12 = JS 22-13, 13 = JS 24-31, 14 = JS 24-78, 15 = JS 335, 16 = JSM230, 17 = Cat 87, 18 = KDS 10-73, 19 = EC 14117,20 = EC 457366,21 = NRC 86,22 = NRC 186,23 = NRC 189,24 = NRC 190,25 = NRC 192,26 = AMS 56,27 = AMS 243,28 = AMS 264, 29 = AMS MB 5-19,30 = RVS 13-7,31 = RVSM 2012-4,32 = DS 1318,33 = JS 20-79,34 = JS 20-116,35 = JS 95-60,36 = JS 25-01,37 = JS 21-13,38 = JS 20-69,39 = JS 97-52,40 = JS 20-29.

**Fig 1:** SSR profile of soybean genotypes with primer Satt 301.




L = Ladder 1 = JS 20-19, 2 = JS 20-56, 3 = JS 20-98, 4 = JS 20-89, 5 = JS 20-34, 6 = JS 22-08, 7 = JS 22-17, 8 = JS 21-72, 9 = JS 24-26, 10 = JS 24-25, 11 = JS 93-05, 12 = JS 22-13, 13 = JS 24-31, 14 = JS 24-78, 15 = JS 335, 16 = JSM230, 17 = Cat 87, 18 = KDS 10-73, 19 = EC 14117, 20 = EC 457366, 21 = NRC 86, 22 = NRC 186, 23 = NRC 189, 24 = NRC 190, 25 = NRC 192, 26 = AMS 56, 27 = AMS 243, 28 = AMS 264, 29 = AMS MB 5-19, 30 = RVS 13-7, 31 = RVSM 2012-4, 32 = DS 1318, 33 = JS 20-79, 34 = JS 20-116, 35 = JS 95-60, 36 = JS 25-01, 37 = JS 21-13, 38 = JS 20-69, 39 = JS 97-52, 40 = JS 20-29.

**Fig 2:** SSR profile of soybean genotypes with primer GMSP 179.

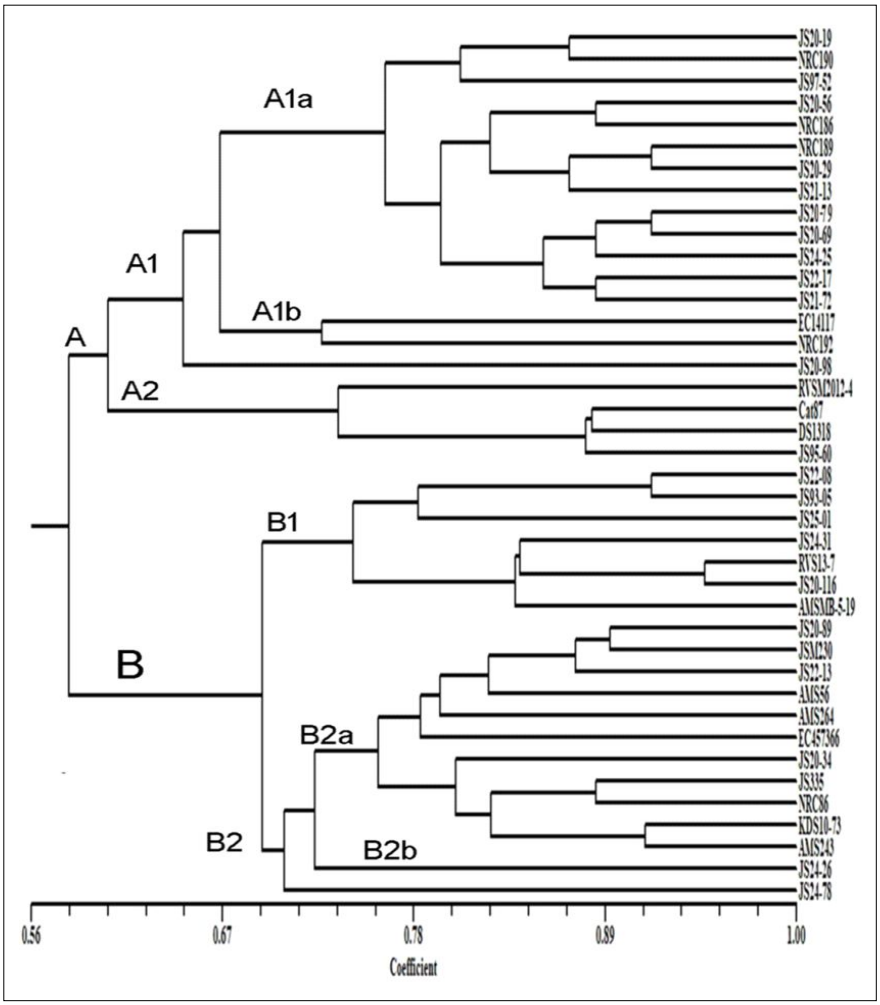



**Fig 3:** Dendrogram generated by UPGMA analysis showing relationship among 40 soybean genotypes based on SSR data.

**Table 4:** PIC (Polymorphic information content) value.

| Primer | No. of alleles | PIC value | Alleles size |
|---|---|---|---|
| Satt 009 | 3 | 0.32 | 70-100 |
| Satt 146 | 1 | 0.00 | 90-100 |
| Sct_199 | 1 | 0.00 | 100-130 |
| Satt 267 | 2 | 0.84 | 100-150 |
| Sat_281 | 2 | 0.46 | 80-100 |
| Satt 290 | 3 | 0.55 | 70-120 |
| Satt 301 | 2 | 0.73 | 80-260 |
| Satt 352 | 2 | 0.32 | 90-140 |
| Sat_405 | 2 | 0.51 | 100-120 |
| Satt 408 | 3 | 0.45 | 90-200 |
| Satt 532 | 1 | 0.00 | 60-90 |
| Satt 541 | 3 | 0.62 | 70-120 |
| Satt 554 | 2 | 0.35 | 100-120 |
| Satt 560 | 2 | 0.48 | 90-110 |
| GMSP 179 | 3 | 0.53 | 70-200 |

values varied from 0 (Sct_199) to 0.84 (Satt 267). Each marker indicates that these plants' genes differ significantly at these specific sites, with an average PIC value of 0.43.

Alleles ranged between 1-3 with an average value of 2.1 and allele size ranged from 60 to 260 base pairs.

Similarly, Kumar *et al*. (2015) also reported two markers (Satt 301 and GMSHP 179) with possible association with yellow mosaic disease in soybeans. Similar results were found by Mishra *et al*. (2022), who observed that JS 335, JS 95-72 and RVS 2001-4 showed highly susceptible expression to yellow mosaic disease. Similarly, Rani *et al*. (2016) identified the genetic basis of 41 soybean genotypes varying in resistance against yellow mosaic virus using 58 simple sequence repeat primers. An average of 2.41 alleles per locus was detected in their study. Kumar *et al*. (2014) found that JS 335 was highly susceptible to yellow mosaic disease at the molecular and phenotypic levels (Table 5 and 6).

**Table 5:** Grouping of genotypes on the basis of per cent incidence of yellow mosaic disease.

| Incidence range | Genotype name | Number of genotypes |
|---|---|---|
| 0-10 | JS 24-25, DS 1510, JS 25-01 ,NRC 186, JS 20-89, JS 24-31, JS 22-08, JS 20-69, PS1660, RSC 11- 48, RSC 11-75, JS 20-79, JS 24-24 | 13 |
| >10-25 | JS 20-29, JS 21-13, JS 20-19, JS 22-11, JS 25-02, JS 20-98, JS 21-72, NRC 189, RSC 11-75, DS 1318, JS 24-26, AMS MB 5-19,JS 20-78, JS 22-17, JS 22-13, JS 20-116, JS 97-52, JS 24-32, NRC 130, NRC 150, NRC 201, PS 1611, RSC 11-22, NRC 154, RSC 11-42, RVSM 16-20, KDS 1009 | 27 |
| >25-50 | MACS 1520, EC 280149, Cat – 87, NRC –190, AMS-264, AMS-56, EC 350664, JS 20-56, RVSM 2012-4, EC 457366, EC 14117, NRC 192, JS 21-70, NRC 197, JS 21- 74, JS 22- 09, PS 1689, JS 22-14, MACS 1566 | 19 |
| >50-75 | JS 335 (check), AVS-56, JS 95-60 (check), JS 20-34, KDS 10-73, RVS 13-7, NRC -86, AMS 2014-1 | 8 |
| >75-100 | JSM 230, AMS 243, JS 93-05 (check) | 3 |

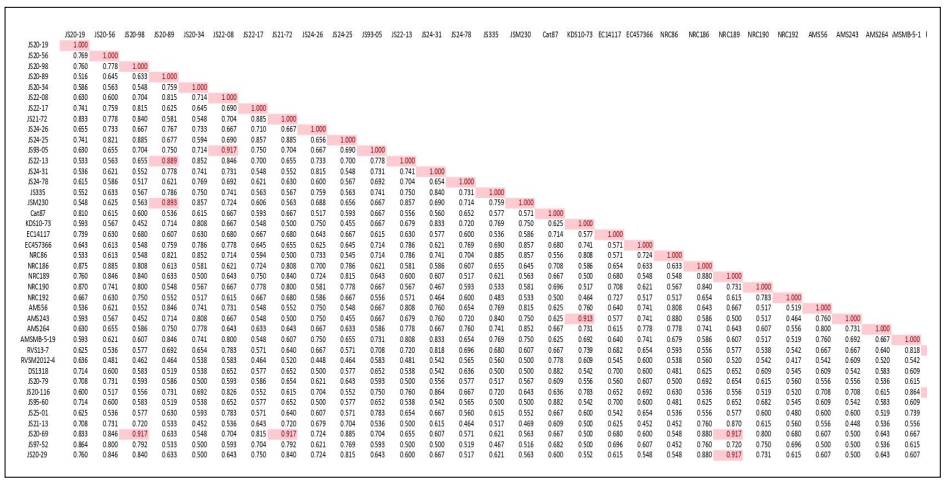


**Fig 4:** Similarity and dissimilarity between the genotypes determined by SSR marker using Jaccard's analysis.

**Table 6:** Genotype wise coefficient of infection (CI), per cent incidence (PI) and reaction for Yellow Mosaic disease in soybean during *kharif* 2023.

| Genotype code | Per cent incidence | Coefficient of infection | Disease reaction (HR to HS) |
|---|---|---|---|
| JS 335 (check) | 53.33 | 26.67 | MS |
| JS 24-25 | 6.67 | 0.67 | HR |
| JS 20-29 | 16.67 | 1.67 | HR |
| JS 21-13 | 20.00 | 5.00 | MR |
| MACS 1520 | 40.00 | 20.00 | MS |
| EC 280149 | 26.67 | 13.33 | MR |
| DS 1510 | 3.33 | 0.33 | HR |
| JS 20-19 | 13.33 | 3.33 | MR |
| JS 22-11 | 20.00 | 5.00 | MR |
| AVS-56 | 53.33 | 26.67 | MS |
| JS 95-60 (check) | 63.33 | 47.50 | S |
| JS 25-01 | 6.67 | 0.67 | HR |
| JS 25-02 | 16.67 | 4.17 | MR |
| JS 20-98 | 13.33 | 3.33 | MR |
| JS 21-72 | 16.67 | 4.17 | MR |
| NRC 189 | 13.33 | 1.33 | HR |
| NRC 186 | 10.00 | 1.00 | HR |
| RSC 11-75 | 23.33 | 5.83 | MR |
| Cat - 87 | 36.67 | 18.33 | MS |
| DS 1318 | 20.00 | 5.00 | MR |
| JS 20-89 | 6.67 | 0.67 | HR |
| JS 24-31 | 6.67 | 0.67 | HR |
| JS 24-26 | 16.67 | 1.67 | HR |
| NRC-190 | 36.67 | 18.33 | MS |
| AMS-264 | 33.33 | 16.67 | MS |
| AMSMB-5-19 | 20.00 | 5.00 | MR |
| AMS-56 | 50.00 | 25.00 | MS |
| EC 350664 | 36.67 | 18.33 | MS |
| JS 20-56 | 33.33 | 16.67 | MS |
| JS 20-78 | 13.33 | 1.33 | HR |
| JS 22-17 | 23.33 | 5.83 | MR |
| JS 22-13 | 13.33 | 3.33 | MR |
| JS 22-08 | 10.00 | 1.00 | HR |
| JSM 230 | 76.67 | 57.50 | S |
| JS 20-34 | 73.33 | 55.00 | S |
| KDS 10-73 | 70.00 | 52.50 | S |
| RVSM 2012-4 | 36.67 | 18.33 | MS |
| RVS 13-7 | 63.33 | 47.50 | S |
| EC 457366 | 46.67 | 23.33 | MS |
| AMS 243 | 76.67 | 57.50 | S |
| EC 14117 | 40.00 | 20.00 | MS |
| NRC 192 | 30.00 | 15.00 | MR |
| NRC -86 | 56.67 | 28.33 | MS |
| JS 93-05 (check) | 83.33 | 62.50 | HS |
| JS 20-116 | 23.33 | 5.83 | MR |
| JS 21-70 | 36.67 | 18.33 | MS |
| JS 20-69 | 6.67 | 0.67 | HR |
| JS 97-52 | 16.67 | 4.17 | MR |
| JS 24-32 | 13.33 | 1.33 | HR |

**Table 6: Continue...**

**Table 6: Continue...**

| | | | |
|---|---|---|---|
| NRC 130 | 16.67 | 4.17 | MR |
| NRC 150 | 23.33 | 5.83 | MR |
| NRC 197 | 40.00 | 20.00 | MS |
| NRC 201 | 16.67 | 4.17 | MR |
| JS 21- 74 | 26.67 | 13.33 | MR |
| JS 22- 09 | 30.00 | 15.00 | MR |
| PS 1611 | 23.33 | 5.83 | MR |
| PS1660 | 6.67 | 0.67 | HR |
| RSC 11-22 | 16.67 | 4.17 | MR |
| RSC 11- 48 | 10.00 | 1.00 | HR |
| PS 1689 | 36.67 | 18.33 | MS |
| JS 22-14 | 26.67 | 13.33 | MR |
| NRC 154 | 13.33 | 3.33 | MR |
| MACS 1566 | 40.00 | 20.00 | MS |
| RSC 11-75 | 10.00 | 2.50 | HR |
| RSC 11-42 | 16.67 | 4.17 | MR |
| RVSM 16-20 | 13.33 | 3.33 | MR |
| AMS 2014-1 | 60.00 | 30.00 | MS |
| JS 20-79 | 6.67 | 0.67 | HR |
| JS 24-24 | 6.67 | 0.67 | HR |
| KDS 1009 | 13.33 | 1.33 | HR |

UPGMA cluster analysis generated a dendrogram using genetic distance data. The genotypes of soybeans were divided into two groups, A and B. Group A was subdivided into A1 and A2. Subgroup A1a contained thirteen genotypes. This group had the most high resistance and moderate resistance. Subgroup A1b contained three genotypes. The A2 subgroup contained four genotypes. Group B is also split into B1 and B2. There are seven genotypes in subgroup B1. B2a and B2b were the divisions of group B. There were eleven genotypes in subgroup B2a and two in subgroup B2b. Group B2 had the most genotypes that showed the most susceptibility to yellow mosaic disease. Similarly, Koutu *et al*. (2019) generated a dendrogram for the clustering of soybean varieties, *i.e.* JS 93-05, JS 20-69, JS 20-29, JS 97-52, JS 95-60, JS 20-93, JS 20-34 and JS 335, which is based on SSR marker analysis. Similar results in soybeans were also reported by Kumar *et al.* (2014); Mishra *et al.* (2022); Sahu *et al*. (2024) and Tiwari *et al.* (2019).

The values of Jaccard's similarity coefficient have been used to establish a genetic relationship between several genotypes of soybeans. The RVS 13-7 and JS 20-116 showed the maximum similarity (0.94), whereas NRC 192 and RVSM 2012-4 showed the lowest similarity (0.41). Seven genotypes had the same similarity (0.91) coefficient among them: JS 22-08, JS 93-05; KDS 10-73, AMS 243; JS 20-98, JS 20-69; JS 21-72, JS 20-69; NRC 189, JS 20-69; NRC 189, JS 20-29; JS 20-69, JS 20-29. Among the seven genotypes, most entries were resistant to yellow mosaic disease in soybeans. Genotypes RVS 13-7 and JS 20-116 showed the maximum similarity because they share the same parentage.

## CONCLUSION

Seventy soybean genotypes were screened in field conditions. Eighteen genotypes were found to be highly resistant, twenty-seven genotypes showed moderate resistance, eighteen soybean genotypes were screened as moderately susceptible, six genotypes were found susceptible and only one genotype showed high susceptibility to yellow mosaic virus. The high Polymorphic Information Content (PIC) value indicates significant variability, demonstrating that the selected markers effectively differentiate between genotypes. Our results showed that closely related genotypes with a common similarity coefficient exhibited similar or dissimilar reactions. To validate the field screening data more accurately, it is recommended to conduct field screening over multiple seasons and locations and to utilize a greater number of SSR primers.

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The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the

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**Informed consent**

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

**Conflict of interest**

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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