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Validation of yellow mosaic disease resistance in soybean (*Glycine max* (L.) Merr.) using SSR markers

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Abstract

The study was carried out for two years (rabi 2023–24 and kharif 2024) to identify promising soybean genotypes resistant to yellow mosaic disease (YMD) and validate SSR markers for YMD resistance. Altogether 10 advanced genotypes were analysed for field resistance to YMD and evaluated for seed yield. Substantial genetic variability was observed for all 9 characters studied. Field screening identified 4 resistant, 2 moderately resistant, 1 moderately susceptible and 3 susceptible genotypes. The resistant lines offer valuable genetic resources for resistance breeding in soybean. The reported SSR marker (Satt 322) for YMD resistance was also validated in these genotypes. A total of 6 genotypes (SL 958 (resistant check), SL 955, DS 3105, DS 3106, PS 1347 and PS 1670) carrying resistant alleles also exhibited YMD resistance (R/MR) under field conditions, while the moderately resistant genotype NRC 128 carried the susceptible allele. The study identified three breeding lines, namely DS 3105, PS 1670 and DS 3106 as possessing superior seed yield alongside YMD resistance. These findings provide valuable insights to accelerate soybean crop improvement using marker assisted breeding.

Keywords: Satt 322; seed yield; SSR markers; yellow mosaic disease

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1. Introduction

Soybean (*Glycine max* (L.) Merr.) is referred to as the “Golden Gift of Nature” to mankind due to its diverse uses as food, animal feed and industrial applications. It is also referred to as the "Protein hope" of the future because its seeds contain approximately 42–45 % protein (1). As a major oilseed crop, soybean contributes over 1/4th of the global protein supply for human food and animal feed (2). The crop plays a major role in meeting plant-based protein requirement and reinforcing its status as a global commodity. The Soybean industry contributes significantly to the economics of many countries, with major producers including the USA, Brazil, Argentina, China and India (3, 4). Beyond its commercial relevance, soybean is recognised for its resilience to challenging climatic conditions and for improving the livelihoods and socio-economic well-being of farmers across India (5).

Although India is the 5th largest producer of soybean, productivity remains considerably lower than that of other major soybean-producing countries such as Brazil, the United States, Argentina and China. Rain-fed cultivation and biotic stresses are the major contributors to low productivity of soybean in India. To enhance productivity in India, it is essential to identify widely adaptable genotypes with resistance to major biotic stresses. In recent years, the increased incidence of the disease in central and southern parts of the country, including Telangana, has severely reduced soybean production to the extent of 30–50 %.

The spread of the disease from the northwestern plains of India to central India in 2015 and subsequently to southern India, emphasises the necessity of developing resistant cultivars to manage this viral infection. High-yielding cultivars must be improved for (yellow mosaic disease (YMD) resistance to mitigate yield losses, thereby contributing to overall food security. Marker assisted selection is the most suitable approach for accelerated development of yellow mosaic disease resistant soybean cultivars.

The virus was first identified in northern India during the early 1970s and has since spread rapidly. Its potential movement into Central India, which is a major region for soybean cultivation, poses a serious threat to the country's soybean industry. Moreover, most of the commonly cultivated soybean varieties in this region are highly vulnerable to YMV infection with a yield loss up to 80 %. The whitefly vector (*Bemisia tabaci*) is the biological agent responsible for transmitting the virus that

causes YMD. Two primary species responsible for the disease are Mungbean yellow mosaic India virus (MYMIV), which is prevalent in northern and central India and Mungbean yellow mosaic virus (MYMV) is prevalent in Southern India (6).

Both MYMIV and MYMV are widely distributed across India and are responsible for outbreaks of YMD in several legume crops such as mungbean, black gram, soybean, cowpea, pigeon pea and horse gram (7). While chemical treatments and cultural practices are employed to manage the virus, these methods are neither efficient nor environmentally sustainable. Hence, the most efficient and eco-friendly strategy to combat YMV is the deployment of genetically resistant varieties. A comprehensive understanding of genetics of disease resistance is crucial for designing effective breeding strategies to develop YMD-resistant cultivars.

Cultivation of high yielding soybean varieties coupled with disease-resistance under unfavourable seasonal conditions will certainly reap economic benefits to the farmers. However, the majority of high-yielding soybean varieties are vulnerable to yellow mosaic disease. To address this key issue, researchers have focused on identifying and introducing resistance genes into susceptible varieties to develop high yielding disease-resistant cultivars.

Punjab, a major hotspot for the disease, has played a significant role in the development of resistant cultivars. Among these, the soybean variety SL 958, released for commercial cultivation, has been identified as a valuable source of resistance to YMD. Given its consistent resistance under field conditions, efforts have been made to map the genomic region(s) governing YMD resistance and identify closely linked markers for their effective deployment in marker assisted selection (MAS). Resistance to YMD in soybean (*Glycine max* L.) is reported to be controlled by either a single dominant gene (8, 9) or by 1–2 recessive genes (10, 11). Quantitative trait loci (QTLs) for YMD resistance have also been mapped to chromosomes 6, 8, 14 and 17 using association mapping or bulked segregant analysis (BSA) (12, 13). Major QTLs for YMD resistance were identified on chromosome 2 and 6 with 19.5 and 27.1 % variation (14). One of the linked SSR marker, Satt 322, was used for QTL introgression; recently YMD resistant soybean advanced breeding lines were developed using this marker (15).

To date, breeding for YMD resistance has been predominantly carried out using conventional approaches. However, with the recent identification of SSR markers linked to QTLs governing MYMIV resistance, MAS has been initiated (14) and improved versions of the cultivar SL 525, which are free of Kunitz trypsin inhibitor (Kti) and resistant to YMD, have been developed (15). Recent studies have clearly shown the utility of SSR markers in resistance breeding for yellow mosaic disease in soybean. During the past few years, the incidence of yellow mosaic disease has shown an increasing trend in Telangana, posing a growing threat to soybean production. Identification of resistant donors along with validated molecular markers for disease resistance is a crucial prerequisite for initiating marker-assisted breeding programmes. Hence, the present study has been carried out to identify potential donors to hasten the development of YMD resistant cultivars in soybean through MAS.

2. Materials and Methods

The study was carried out for two years (rabi, 2023–24 and kharif, 2024) at ARS, Adilabad and the Institute of Biotechnology, Hyderabad, Telangana, to identify YMD resistant soybean genotypes. Meteorological data of ARS, Adilabad for the years 2023–24 and 2024 was given in Supplementary Tables 1 and 2.

The experiment was conducted using 10 genotypes along with two checks (SL 958 and JS 335) in randomised block design with 3 replications in 8 rows of 5 m length with row to row spacing of 45 cm and accommodating 21 plants per meter. Standard crop management practices of PJTAU have been followed. Data was recorded on nine quantitative traits i.e., days to 50 % flowering, days to maturity, plant height (cm), number of branches per plant, number of nodes per plant, internodal length (cm), number of pods per plant, 100 seed weight (g) and grain yield (kg ha^{-1}). The data for days to maturity was recorded on line basis at 90 % pod maturity and each genotype was represented by a single value. Ten plants randomly selected from each replication were used to record data. The genotypes were harvested upon reaching the R8 growth stage. Grain yield was determined for each genotype and expressed as kg ha^{-1}. To estimate seed size, a randomly selected sample of 100 seeds from each genotype was weighed and the results were recorded in grams.

Ten soybean genotypes were evaluated with resistant (SL 958) and susceptible (JS 335) checks for YMD reaction under natural field conditions (Table 1). Pooled analysis of field screening for YMD was performed using R package ‘metan’ (16).

Table 1. List of 10 advanced breeding lines of soybean along with two checks

Sl. No.	Genotype	Sl. No.	Genotype
1	SL 955	7	JS 95-60
2	DS 3105	8	NRC 37
3	DS 3106	9	NRC 157
4	PS 1347	10	NRC 130
5	PS 1670	11	JS 335 (SC)
6	NRC 128	12	SL 958 (RC)

Yellow mosaic disease screening methodology

The infector row technique was followed to ensure uniform disease pressure while screening the genotypes. Each test entry was planted in a single 3 m row across 3 replications; the susceptible check (JS 335) and resistant check (SL 958) were repeated after every five

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test entries. Data were recorded between 50–70 DAS by counting the number of infected plants and the total population and converted to percentage disease index (PDI) as per the formula mentioned. Resistance or susceptibility reaction of the genotype was determined based on the PDI (0–9 scoring) (Table 2) (17).

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total no. of plants examined} \times \text{Maximum disease rating}} \times 100$$

Validation of SSR markers

The genomic DNA was extracted from young leaves of five individual plants for each genotype using the CTAB method as described (18). PCR analysis was carried out in 10 µL reaction using YMD linked SSR marker Satt 322 (FP: GGGACCCATCATTAAGAA and RP: GTTTTGCCGGACACCACA). Around 50 ng of genomic DNA was used for PCR amplification using the following conditions: DNA was denatured at 94 °C for 5 min. followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 51 °C for 40 sec, primer extension at 72 °C for 30 sec, final extension at 72 °C for 7 min. The PCR products were resolved on 3 % metaphore agarose gel stained with ethidium bromide and documented using Gel documentation system from Biorad. Resistant (200 bp) and susceptible (230 bp) alleles were recorded (15).

3. Results and Discussion

Pooled ANOVA showed significant differences for all the traits studied in Table 3. The ANOVA for each season, 2023–24 and 2024 was also performed and given in Supplementary Tables 3 and 4. Superior genotypes for seed yield compared to the best performing check SL 958 were identified. The DS 3105 recorded the highest mean grain yield (2781 kg ha^{-1}), followed by PS 1670 (2648 kg ha^{-1}) and DS 3106 (2308 kg ha^{-1}). The mean performance of soybean lines for yield and yield attributing traits is presented in Table 4.

Screening of soybean advanced breeding lines

Most of the advanced breeding lines displayed a resistant to moderate resistant reaction, with disease scores ranging from 1–3. Out of the ten breeding lines evaluated, 4 lines viz., SL 955, DS 3105, PS 1347 and PS1670 exhibited a resistant reaction with disease score of 1. Additionally, 2 lines viz., NRC 128 and DS 3106 showed a moderately resistant response, with a score of 1.6 to 2.3. The resistant (SL 958) and susceptible (JS 335) checks recorded a score of 1 and 9 respectively, confirming the reliability of the disease screening under natural field conditions. The genotype NRC 37 exhibited moderately susceptible reaction with disease a score of 5, while NRC 130, NRC 157 and JS 95–60 showed susceptible reaction (Fig. 1, Table 5).

Validation of SSR markers for yellow mosaic disease resistance

Recent studies have mapped one major quantitative trait locus (QTL) on chromosome 6 flanked by markers Satt 281 and Satt 322; and another on chromosome two flanked by markers BARCSOYSSR_02_0423 and BARCSOYSSR_02_0425 (14). Their study validated reported marker Satt 322 for YMD resistance in soybean. Hence in our study three plants from each breeding line were randomly selected and analysed using YMD linked SSR marker Satt 322. The molecular data for the resistant and susceptible checks strongly correlated with the phenotypic YMD symptoms. The resistant check displayed allele at 200 bp while susceptible check displayed allele at 230 bp for the linked SSR marker Satt 322 (Fig. 2). All the seven resistant/moderately resistant breeding lines, excluding the moderately resistant NRC 128, displayed the resistant allele (moderately resistant NRC 128 carried the susceptible allele). The genotypes SL 958, SL 955, DS 3105, DS 3106, PS 1347 and PS1670 were homozygous for the resistant allele. All the five YMD susceptible breeding lines (MS/S/HS) carried the susceptible allele (Table 6). Three promising breeding lines with superior seed yield (DS 3105, PS 1670 and DS 3106) carried the resistant allele (Satt 322) and showed resistance (R/MR) under field screening.

Table 2. Disease reaction of soybean genotypes on the basis of 0–9 scale (17)

Phenotypic reaction	Score	Reaction
No symptoms on any plants	0	Immune (I)
Yellow mottle or necrotic mottle in up to 1 % plants	1	Resistant (R)
Yellow mottle or necrotic mottle in up to 1.1–10 % plants	3	Moderately resistant (MR)
Necrotic mottle/mild mottle/mild symptoms on 10.1–25 % plants; no reduction in plant growth; no yield loss	5	Moderately susceptible (MS)
Yellow mottle symptoms not covering the whole leaf lamina on 25.1–50 % plants; reduction in leaf and plant growth	7	Susceptible (S)
Yellow mottle symptoms on more than 50 % plants; severe reduction in leaf and plant growth as well as pod formation and death of plants	9	Highly susceptible (HS)

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Table 3. Analysis of variance for yield and yield attributing traits of soybean

Source of variation	D.F.	Mean sum of squares								
		DFF	DM	PH	NBP	NNP	INL	100-SW	NPP	SY
Replications	2	1.00	0.19	3.77	0.01	0.86	0.05	0.05	38.72	82656.19
Treatments	11	26.73**	138.08**	791.58**	0.44**	16.69**	2.14**	1.46**	172.07**	507522.92**
Error	22	0.76	1.62	15.12	0.04	1.32	0.27	0.09	12.62	23145.44

D.F.: degrees of freedom; ** significance at 1 % probability level; DFF: days to 50 % flowering; DM: days to maturity; PH: plant height in centimeter; NBP: number of branches per plant; NCP: number of nodes per plant; INL: Internodal length in cm; 100-SW: 100 seed weight in gram; NPP: number of pods per plant; SY: seed yield in kg ha^{-1}.

Table 4. The mean performance of soybean lines for yield and yield attributing traits

Sl. No	Genotype	DFF	DM	PH	NBP	NNP	INL	100-SW	NPP	SY
1	SL 958 (RC)	43	109	71.9	1.8	12.7	5.7	11.5	42.3	2024
2	NRC 128	44	111	71.2	2.1	15.0	4.8	11.8	40.4	1987
3	SL 955	45	115	61.8	1.5	11.0	5.7	10.2	49.7	2120
4	DS 3105	38	102	58.2	2.1	10.7	5.5	11.5	58.0	2781
5	DS 3106	41	107	58.4	1.6	11.0	5.4	12.2	45.3	2308
6	PS 1347	37	107	96.0	2.4	15.0	6.4	11.3	42.7	2018
7	PS 1670	40	107	96.8	2.3	17.3	5.6	11.6	54.8	2648
8	JS 95-60	34	96	55.6	1.3	13.3	4.2	10.2	34.1	1449
9	NRC 37	41	107	73.1	1.6	12.0	6.1	10.9	42.9	1947
10	NRC 157	41	104	90.0	1.9	17.7	5.1	11.0	34.0	1562
11	NRC 130	37	90	49.4	1.3	13.3	3.7	10.6	46.4	2055
12	JS 335 (SC)	40	110	58.7	1.5	14.7	4.0	10.0	36.3	1517
	C.D. at 5 %	1.48	2.17	3.18	0.35	1.96	0.89	0.51	6.06	259.28
	C.V. (%)	2.2	1.20	5.55	11.32	8.41	10.05	2.69	8.09	7.44

DFF: days to 50 % flowering; DM: days to maturity; PH: plant height in cm; NBP: number of branches per plant; NCP: number of nodes per plant; INL: Internodal length in cm; 100-SW: 100 seed weight in gram; NPP: number of pods per plant; SY: seed yield in kg ha^{-1}.

Table 5. Disease reaction of genotypes under field condition at ARS, Adilabad

Sl. No.	Entry name	RI	RII	RIII	Score	Disease reaction
1	SL 958 (RC)	1	1	1	1	R
2	NRC 128	3	3	1	2.3	MR
3	SL 955	1	1	1	1	R
4	DS 3105	1	1	1	1	R
5	DS 3106	1	3	1	1.6	MR
6	PS 1347	1	1	1	1	R
7	PS 1670	1	1	1	1	R
8	JS 95-60	7	7	9	7.7	S
9	NRC 37	5	5	5	5	MS
10	NRC 157	7	9	7	7.7	S
11	NRC 130	5	5	5	5	S
12	JS 335 (SC)	9	9	9	9	HS

RC: resistant check; SC: susceptible check; R: resistant; MR: moderate resistant; HS: highly resistant; MS: moderate susceptible; S: susceptible.

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SL 958 (RC) SL 955-R DS 3105-R JS 335-(SC)

Fig. 1. Disease reaction of soybean advanced line under field condition.
RC: resistant check; SC: susceptible check; R: resistant.

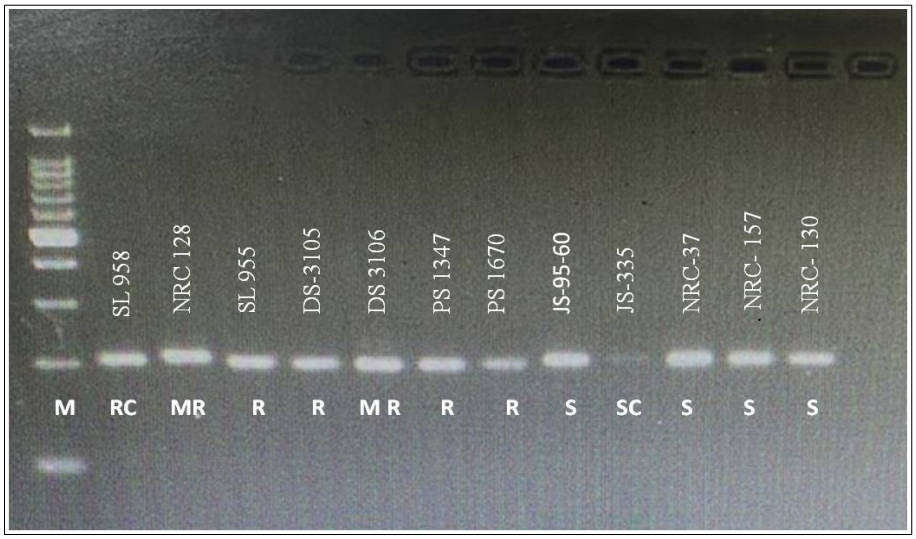


Fig. 2. Validation of SSR markers linked to Yellow mosaic disease resistance in soybean: Satt 322. Disease reaction in filed screening is depicted as R and S.
RC: resistant check; SC: susceptible check; R: resistant; S: susceptible; M: 100bp DNA ladder.

Table 6. Validation of Satt 322 linked to yellow mosaic disease resistance

Sl. No.	Genotype name	Disease reaction	
		Field screening	Marker screening (Satt 322)
1	SL 958 (RC)	R	R (200 bp)
2	NRC 128	MR	S (230 bp)
3	SL 955	R	R (200 bp)
4	DS 3105	R	R (200 bp)
5	DS 3106	MR	R (200 bp)
6	PS 1347	R	R (200 bp)
7	PS 1670	R	R (200 bp)
8	JS 95-60	S	S (230 bp)
9	NRC 37	MS	S (230 bp)

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10	NRC 157	S	S (230 bp)
11	NRC 130	S	S (230 bp)
12	JS 335 (SC)	HS	S (230 bp)

RC: resistant check; SC: susceptible check; R: resistant; HS: highly susceptible; MS: moderately susceptible; S: susceptible.

Soybean is an important grain legume of Indian subcontinent. Being one of the most economical sources of protein (approximately 40 %), soybean has the potential to combat protein malnutrition in developing countries. Yellow mosaic disease is one of the major biotic stresses affecting the crop which can cause up to 80 % yield loss in severe cases. Gradual increase in incidence of yellow mosaic disease has been a major concern for soybean production in the country as the disease spread to central and southern India including Telangana. Being a viral disease, deployment of genetic resistance is the best approach for management. Therefore, it is imperative to introgress YMD resistance in the genetic background of high-yielding varieties. Screening and evaluation of soybean germplasm for yellow mosaic disease resistance is a continuous process to identify potential donors for newly emerging viral strains/vector biotypes (19–21). The significant differences for yield-associated traits confirmed the presence of substantial genetic variability among the tested genotypes and these findings are in agreement with earlier reports (22). The YMV resistant genotypes identified in this study serve as valuable donor parents for resistance breeding in soybean. The susceptible check, JS-335, recorded a highly susceptible reaction, whereas the resistant check, SL-958, consistently confirmed its resistance. The pronounced disease symptoms observed in the susceptible check confirmed adequate disease pressure in the field, validating the effectiveness of the screening process. The existence of variability presents an opportunity for effective utilisation of resistant sources in the soybean breeding programme (23). Similar studies have also been conducted recently and resistant soybean genotypes were identified under field conditions in hotspots (24–28). A total of 34 highly resistant soybean genotypes to YMD under natural disease pressure, highlighting the importance of identifying novel sources of resistance (29). The resistant genotypes offer valuable genetic resources for resistance breeding in soybean. Screening of a set of 92 diverse soybean genotypes identified the genotypes RVS 23-1, RVS 23-8, JS 20-98, PS 1693 and PS 1696 as highly resistant, appearing completely asymptomatic or showing negligible disease symptoms (30). These genotypes are widely used as donors in soybean crop improvement programmes across the country.

The availability of tightly linked DNA markers provides a powerful tool for marker-assisted selection and the efficient development of resistant cultivars. This molecular approach is considerably more rapid than conventional phenotypic screening methods. The integration of marker assisted selection (MAS) with field-based phenotypic screening enhances the precision and effectiveness of the selection process in several field crops. The SSR markers, GMAC7L and Satt 322, linked to the YMD resistance (10) were further validated and Satt 322 was found to be linked with YMD in all the genotypes tested. However, with the recent identification of SSR markers linked to QTLs governing MYMIV resistance, MAS has been initiated (14) and improved versions of SL 525 cultivar for YMD resistance and Kunitz trypsin inhibitor (Kti) free was developed (15). The MYMIV resistance gene was introgressed from *Glycine soja* into the widely cultivated soybean cultivar JS 335 to enhance its resistance to yellow mosaic disease. Four introgression lines, exhibiting MYMIV resistance and superior yield over recurrent parent and checks were previously identified (31). Their study further demonstrated the association of two YMV-linked SSR markers, Satt 063 and BARCSOYSSR_14_1382, with all four introgression lines, thereby validating the successful introgression of the YMV resistance locus.

An attempt has been made in this study to validate previously reported marker (Satt 322) for YMD resistance. A total of six breeding lines homozygous for the resistant allele, with allele sizes of 200 bp were identified. These resistant genotypes SL 958, SL 955, DS 3105, DS 3106, PS 1347 and PS 1670 provides the opportunity to carry out marker assisted selection and hasten the development of resistant cultivars with improved seed yield (10). There are three promising breeding lines, DS 3105, PS 1670 and DS 3106, exhibiting high seed yield coupled with resistance to yellow mosaic disease were identified. Combining high seed yield, YMD resistance and desirable agronomic traits will facilitate the development of stable and farmer-preferred soybean varieties. These genotypes are highly useful for multi-location evaluation to identify stable and well-adapted lines suitable for varietal release. The potential resistant genotypes may be further confirmed using agro inoculation of virulent clones which is a reliable, season-independent technique used to screen soybean germplasm for MYMV/MYMIV bypassing natural whitefly vectors. Genotypes demonstrating superior and consistent performance under particular agro-ecological conditions can be advanced for location-specific varietal development. This approach can facilitate the development of soybean varieties specifically adapted to the production environments of Telangana. The YMD-resistant lines can also be utilised as donors in hybridisation programmes to transfer resistance into elite, high-yielding and adapted soybean genetic background. This would facilitate the development of breeding populations combining disease resistance with superior yield potential.

4. Conclusion

Yellow mosaic disease (YMD) in soybean (*Glycine max* (L.) Merr.) has been gradually spreading from central, western India to southern states in the recent years affecting crop yields. Considering the importance of

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Telangana as a major soybean seed-producing region, identification and development of YMD-resistant soybean varieties adapted to the state is essential for sustainable soybean production and quality seed multiplication. Based on the present study, the genotypes SL 958, SL 955, DS 3105, DS 3106, PS 1347 and PS 1670 carried the resistant allele for the linked marker Satt 322 and exhibited stable resistance under field screening. Earlier reported marker for yellow mosaic disease resistance has been validated in these genotypes. Hence, these genotypes serve as potential donors and marker assisted breeding can be initiated to accelerate the development of yellow mosaic disease resistant varieties of soybean. The genotypes JS 335, JS9-560, NRC 37, NRC 157 and NRC 130 exhibited susceptible reaction in the field and carried the susceptible allele. However, one resistant genotype NRC 128 is an exception as it did not correlate with molecular analysis using linked marker (Satt 322).

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Authors' contributions

MRR carried out field experiment, data recording, data analysis and drafting of manuscript. SNCVLP performed validation with SSR markers, drafting of manuscript. NB conducted field screening of advanced lines for YMD. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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Supplementary Table 1. Meteorological data (rabi, 2023–24), soil type of ARS, Adilabad

Season/ Months	Latitude/ Longitude	Climatic zone	Soil type	Soil texture	Ph level	Temperature (°C)		RH (%)		Evap. (mm/ day)	Actual Rainfall (mm)	Rainy days
						Max.	Min.	I	II			
October, 2023	19.39 °N, 78.32 °E	Tropical semi-arid	Vertisol (predomonantly black)	Deep clayey	7.8-8.4	34.1	19.9	76.2	63.3	0.6	0.0	0
November, 2023						31.5	17.1	80.0	66.6	0.2	26.0	2
December, 2023						29.1	14.1	80.3	59.8	1.6	2.0	0
January, 2024						31.1	15.6	79.0	46.6	2.5	3.1	0
Total	-	-	-	-	-	--	--	--	--	--	31.1	2
Average	-	-	-	-	-	31.5	16.8	78.9	58.9	1.2	--	-

Supplementary Table 2. Meteorological data (kharif, 2024), soil type of ARS, Adilabad

Season/Months	Temperature (°C)		RH (%)		Evaporation (mm/day)	Rainfall (mm)	Rainy days
	Max.	Min.	I	II			
June, 2024	38.4	26.2	74.2	55.3	7.6	137.2	10
July, 2024	31.5	24.3	88.0	81.4	4.3	417.5	19
August, 2024	32.8	44.2	88.4	81.7	3.6	185.7	13
September 2024	32.4	24.3	86.8	78.3	4.3	176.9	9
Total	--	--	--	--	--	917.3	51
Average	33.77	29.75	84.35	74.17	4.95	--	--

Supplementary Table 3. Analysis of variance for yield and yield attributing traits of soybean for the year 2023–24

Source of variation	D.F.	Mean sum of squares								
		DFF	DM	PH	NBP	NNP	INL	100-SW	NPP	SY
Replications	2	4.17	5.02	52.76	0.006	4.04	0.80	0.26	9.00	63838.72
Treatments	11	26.98*	122.98*	769.66	0.37*	16.33*	2.17*	1.55*	158.72*	501182.98*
Error	22	3.98	3.91	22.48	0.043	4.00	1.02	0.15	16.64	127832.84

D.F: degrees of freedom; ** significance at 1 % probability level; DFF: days to 50 % flowering; DM: days to maturity; PH: plant height in cm; NBP: number of branches per plant; NCP: number of nodes per plant; INL: Internodal length in cm; 100-SW: 100 seed weight in g; NPP: number of pods per plant; SY: seed yield in kg ha^{-1}.

Supplementary Table 4. Analysis of variance for yield and yield attributing traits of soybean for the year 2024

Source of variation	D.F.	Mean sum of squares								
		DFF	DM	PH	NBP	NNP	INL	100-SW	NPP	SY
Replications	2	9.38	2.33	40.35	0.004	5.58	0.39	0.10	8.11	14718.82
Treatments	11	37.82*	163.36*	818.18*	0.52*	17.66*	2.23*	1.44*	186.80*	520972.00*
Error	22	3.51	4.15	23.60	0.04	3.86	1.06	0.17	16.72	132298.29

D.F: degrees of freedom; ** significance at 1 % probability level; DFF: days to 50 % flowering; DM: days to maturity; PH: plant height in cm; NBP: number of branches per plant; NCP: number of nodes per plant; INL: internodal length in cm; 100-SW: 100 seed weight in g; NPP: number of pods per plant; SY: seed yield in kg ha^{-1}.

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