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RESEARCH ARTICLE

Resistance profiling of soybean RILs against yellow mosaic virus and charcoal rot under field conditions

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Abstract

Soybean (*Glycine max*) is a vital leguminous crop that faces significant yield losses due to an array of biotic stresses, particularly Yellow Mosaic Virus (YMV) and Charcoal Rot (CR). Present investigation aimed to evaluate the putative resistance of 118 soybean genotypes, includng 115 Recombinant Inbred Lines (RILs) along with three checks *viz.,* JS 97-52, NRC-37 and JS 335, under natural disease pressure at JNKVV, Jabalpur, Madhya Pradesh., India well known a hotspot centre for both YMV and CR diseases for a long time. The genotypes were assessed at different growth stages and disease severity classified based on standard rating scales. Among the tested genotypes, 19 RILs displayed high resistance to YMV, while 63 were resistant including the check (62 RILs; a JS 97-52 check), 6 moderately resistant, 8 moderately susceptible including check NRC 37, 16 susceptible including the check JS-335 (15 RILs; 1 check) and 6 highly susceptible. For CR disease, 21 RILs were identified resistant, 41 moderately resistant including the check, JS 97-52 (40 RILS; a check), 22 moderately susceptible including checks JS 335 and NRC 37 (20 RILS; 2 checks), 8 susceptible and 26 highly susceptible. Markedly, 29 RILs demonstrated resistance against both YMV and CR diseases, allowing them valuable candidates for incorporation as donor parents. The findings highlighted the genetic variability of genotypes to disease resistance and the aptitude for selecting resistant line (s) for soybean improvement. The identified resistant genotype (s) can be utilized in breeding programmes to develop high yielding, disease resistant cultivar (s), contributing to sustainable production and reduced reliance on chemical disease management strategies

Keywords: biotic stresses; Charcoal Rot (CR); disease resistance; Recombinant Inbred Lines (RILs); soybean *(Glycine max)*; Yellow Mosaic Virus (YMV)

Introduction

With higher crude protein content than most commercially grown legumes and pulses, soybeans are an important source of plant derived protein on a global scale (1, 2). Often referred to as a "wonder legume" and "gold from soil" soybeans are prized for their rich nutritional profile and higher protein content. It is a crop that can be used for both animal feed and human nutrition (3-5). It serves as both an oilseed and a legume, making it a crop of dual purpose. Being diploid legume and annual in nature, it is self -pollinated and a member of Fabaceae subfamily (6). Moreover, it is a nutrient rich crop, providing high proteins, essential fats and bio-active compounds like isoflavones and tocopherols. Conventionally, significant in Asian diets, it is effective meat alternative and holds potential for lab grown meat production (7, 8).

Soybean production is unfavourably affected by an array of abiotic and biotic stressors, leading to yield and quality losses. The number of documented soybean diseases has arisen from 50 to over 300, with classified as highly significant. This increase is partly attributed to the intensification of soybean cultivation (9). Although chemical fungicides are India's main tool for controlling different diseases, their widespread usage puts the environment and food safety at risk (10). To overcome this, disease resistant cultivars have been actively created by soybean breeders to improve global productivity and sustainability. Nevertheless, efforts to manage diseases are made more difficult by growing difficulties brought on by climate change (11). It is a *kharif* rainfed, leguminous crop, which encounters severe biotic stresses for instance weeds, insects and diseases (12). The diseases like YMV, Charcoal Rot, RAB and Pod blights *etc*. causes serious devastation in the crop during the different growth stages of the crop (13-15).

Yellow Mosaic Disease (YMD)

YMD poses a significant threat to leguminous crops, resulting in an estimated annual economic loss of approximately US$300 million. In soybean, a crop of considerable economic importance, YMD can lead to yield reductions ranging from 15 % to 75 % (16). The disease is attributed to several begomo viruses, including mung bean yellow mosaic India virus (MYMIV), mung bean yellow mosaic virus (MYMV), horsegram yellow mosaic virus (HgYMV) and Dolichos yellow mosaic virus (17, 18). These pathogens induce characteristic yellow mosaic patterns on soybean leaves and are collectively classified as legume mosaic viruses (LYMVs) (19). It is transmitted by the whitefly (*Bemisia tabaci*), which introduces the virus into the phloem of the host plant. Symptoms initially appear as yellow spots on young leaves, progressing to a mosaic pattern, leading to leaf chlorosis, drying and withering (Fig. 1). Infection reduces photosynthetic efficiency and pod size, ultimately causing substantial yield losses (20). Sustained maximum temperatures above 33 °C for at least five days, coupled with whitefly populations exceeding 12 per plant and a passive monsoon phase lasting over a week, markedly enhance Yellow Mosaic Disease (YMD) incidence in soybean. YMD occurrence exhibits a positive correlation with maximum temperature, sunshine hours and whitefly density, while rainfall and evening relative humidity show negative association. Optimal development and virus transmission efficiency of whitefly vector require temperatures above 26 °C and approximately 60 % relative humidity (21, 22).

Charcoal Rot (CR)

CR caused by *Macrophomina phaseolina*, is a major threat to soybean in India, leading to yield losses of up to 77 % and an estimated 39.2 thousand metric tons annually. While drought conditions exacerbate the disease, significant losses occur even under irrigation, with severity increasing during reproductive stages (23). *Macrophomina phaseolina*, an ascomycete belonging to the family Botryosphaeriaceae, survives in soil as microsclerotia, which germinate between 20-40 °C to infect root tissues. The pathogen affects seedlings, young and mature plants, producing enzymes and toxins that degrade root structures. Following infection, root and stem tissues are rapidly colonized within 2-3 weeks (24). It is characterized by sudden plant wilting and development of a greyish-black discoloration on the lower stems and roots (Fig. 2) (25). Disease incidence is aggravated under high soil and air temperatures (30-35 °C), nutrient deficient soils and conventional tillage during dry years, whereas no till and adequate irrigation mitigate severity by conserving moisture. Thus, moisture stress combined with elevated temperatures creates the most conducive environment for charcoal rot development and progression (26-28). In India, soybean mega varieties like JS 335, JS 93-05 and JS 95-60 have become susceptible to charcoal rot now. The lack of resistant cultivars is a key factor is severe disease outbreaks. This susceptibility may be attributed to the genetic variability of *Macrophomina phaseolina* and its complex pathogenic mechanisms (29).

Breeding programmes sue RIL populations to study the genetic basis of quantitative traits owing to their within-line homogeneity and between -line heterogeneity. This structure allows for repeated field experiments and mapping of phenotypic traits without population structure issues. RILs help to identify contrasting traits between parents and locate related genetic loci (30). To recognise the resistant and susceptible RILs for these 2 economically important diseases of soybean including YMD and CR, all the 118 genotypes were screened under field conditions to search putative resistance with the help of team of plant pathologist. To reduce the yield losses due to diseases and to categorise putative resistant lines/donors for durable resistance, the disease screening has been conducted in the experimental field (Fig. 3).

Materials and Methods

Characteristics of experimental site

Jabalpur, situated at 23.90°N latitude and 79.58°E longitude at an elevation of 411.87 m above mean sea level, experiences a subtropical, semi-arid climate characterized by hot, dry summers and cool, rainy winters. During the experimental crop growth period (July 17 to October 29, 2022), a total of 959.9 mm of rainfall was received, with peak precipitation occurring between August 21-24 (208.1 mm) and August 14-20 (135.7 mm). Temperature arrayed between 29.4 °C (August 14-20 and October 16-22) to 31.9 °C (September 4-10), while relative humidity varied between 93 % (July 24-30) and 40 % (October 23 -29). A total of 41 rainy days were observed, with sunshine hours reaching a maximum of 9.2 hrs (October 16-29) and minimum during July 24-30. Though Jabalpur is hotspot for YMV and CR disease of soybean, the prevailing climatic conditions were favourable for crop growth and development, contributing to a higher disease incidence. The site had medium-black soil with pH 7.5, uniform topography and no standing water. The brief weather record of *Kharif*, 2022 is given in Fig. 4.

Experimental details

The research was conducted during *kharif* 2022 at the AICRP on Soybean Seed Breeding Farm, Department of Genetics and Plant Breeding, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh, India. The experimental material for present investigation comprised a total of 118 genotypes, 115 Recombinant Inbred Lines (RILs) including three checks *viz*., JS 97-52, NRC 37 and JS 335 (Table 1). The RILs 115 series was derived from biparental crossing between the Cat 3293 × NRC 2 and the RILS 107 series was derived from JS 97-52 × NRC 37. The RILs were acquired from NSRI, Indore, Madhya Pradesh, India and were planted employing Augmented Block Design. As the number of genotypes were large and seeds were limited, therefore, augmented block design was applied without replications. Jabalpur being the hotspot for soybean yellow mosaic virus and charcoal rot, the experimental material was subjected to disease screening at field conditions under the guidance of plant pathologist. For yellow mosaic virus the observations were recorded at vegetative stage whereas for charcoal rot it was done at reproductive stage. Throughout the cropping season, every genotype was closely monitored for the onset, progression and severity of any single or complex significant illnesses. According to the following scales, several ratings or grades were given for the severity of yellow mosaic disease and charcoal rot.

Table 1. List of the RILs, along with their pedigree record

S. No.	Name of the RILs	Pedigree	S. No.	Name of the RILs	Pedigree
1	RIL 115-1	Cat 3293 × NRC 2	60	RIL 115-206	Cat 3293 × NRC 2
2	RIL 115-4	Cat 3293 × NRC 2	61	RIL 115-211	Cat 3293 × NRC 2
3	RIL 115-36	Cat 3293 × NRC 2	62	RIL 115-221	Cat 3293 × NRC 2
4	RIL 115-41	Cat 3293 × NRC 2	63	RIL 115-222	Cat 3293 × NRC 2
5	RIL 115-42	Cat 3293 × NRC 2	64	RIL 115-223	Cat 3293 × NRC 2
6	RIL 115-43	Cat 3293 × NRC 2	65	RIL 115-225	Cat 3293 × NRC 2
7	RIL 115-45	Cat 3293 × NRC 2	66	RIL 115-227	Cat 3293 × NRC 2
8	RIL 115-51	Cat 3293 × NRC 2	67	RIL 115-230	Cat 3293 × NRC 2
9	RIL 115-57	Cat 3293 × NRC 2	68	RIL 115-233	Cat 3293 × NRC 2
10	RIL 115-62	Cat 3293 × NRC 2	69	RIL 115-237	Cat 3293 × NRC 2
11	RIL 115-64	Cat 3293 × NRC 2	70	RIL 115-238	Cat 3293 × NRC 2
12	RIL 115-65	Cat 3293 × NRC 2	71	RIL 115-239	Cat 3293 × NRC 2
13	RIL 115-66	Cat 3293 × NRC 2	72	RIL 115-243	Cat 3293 × NRC 2
14	RIL 115-67	Cat 3293 × NRC 2	73	RIL 115-252	Cat 3293 × NRC 2
15	RIL 115-69	Cat 3293 × NRC 2	74	RIL 115-253	Cat 3293 × NRC 2
16	RIL 115-71	Cat 3293 × NRC 2	75	RIL 115-256	Cat 3293 × NRC 2
17	RIL 115-74	Cat 3293 × NRC 2	76	RIL 115-258	Cat 3293 × NRC 2
18	RIL 115-76	Cat 3293 × NRC 2	77	RIL 115-260	Cat 3293 × NRC 2
19	RIL 115-89	Cat 3293 × NRC 2	78	RIL 115-265	Cat 3293 × NRC 2
20	RIL 115-91	Cat 3293 × NRC 2	79	RIL 115-267	Cat 3293 × NRC 2
21	RIL 115-92	Cat 3293 × NRC 2	80	RIL 115-268	Cat 3293 × NRC 2
22	RIL 115-93	Cat 3293 × NRC 2	81	RIL 115-269	Cat 3293 × NRC 2
23	RIL 115-96	Cat 3293 × NRC 2	82	RIL 115-272	Cat 3293 × NRC 2
24	RIL 115-99	Cat 3293 × NRC 2	83	RIL 115-273	Cat 3293 × NRC 2
25	RIL 115-108	Cat 3293 × NRC 2	84	RIL 115-274	Cat 3293 × NRC 2
26	RIL 115-118	Cat 3293 × NRC 2	85	RIL 115-277	Cat 3293 × NRC 2
27	RIL 115-132	Cat 3293 × NRC 2	86	RIL 107-38	JS 97-52 × NRC 37
28	RIL 115-140	Cat 3293 × NRC 2	87	RIL 107-63	JS 97-52 × NRC 37
29	RIL 115-141	Cat 3293 × NRC 2	88	RIL 107-77	JS 97-52 × NRC 37
30	RIL 115-144	Cat 3293 × NRC 2	89	RIL 107-99	JS 97-52 × NRC 37
31	RIL 115-150	Cat 3293 × NRC 2	90	RIL 107-166	JS 97-52 × NRC 37
32	RIL 115-153	Cat 3293 × NRC 2	91	RIL 107-24	JS 97-52 × NRC 37
33	RIL 115-154	Cat 3293 × NRC 2	92	RIL 107-31	JS 97-52 × NRC 37
34	RIL 115-155	Cat 3293 × NRC 2	93	RIL 107-35	JS 97-52 × NRC 37
35	RIL 115-156	Cat 3293 × NRC 2	94	RIL 107-43	JS 97-52 × NRC 37
36	RIL 115-159	Cat 3293 × NRC 2	95	RIL 107-44	JS 97-52 × NRC 37
37	RIL 115-160	Cat 3293 × NRC 2	96	RIL 107-46	JS 97-52 × NRC 37
38	RIL 115-161	Cat 3293 × NRC 2	97	RIL 107-57	JS 97-52 × NRC 37
39	RIL 115-162	Cat 3293 × NRC 2	98	RIL 107-68	JS 97-52 × NRC 37
40	RIL 115-163	Cat 3293 × NRC 2	99	RIL 107-71	JS 97-52 × NRC 37
41	RIL 115-164	Cat 3293 × NRC 2	100	RIL 107-72	JS 97-52 × NRC 37
42	RIL 115-166	Cat 3293 × NRC 2	101	RIL 107-73	JS 97-52 × NRC 37
43	RIL 115-167	Cat 3293 × NRC 2	102	RIL 107-74	JS 97-52 × NRC 37
44	RIL 115-169	Cat 3293 × NRC 2	103	RIL 107-75	JS 97-52 × NRC 37
45	RIL 115-171	Cat 3293 × NRC 2	104	RIL 107-79	JS 97-52 × NRC 37
46	RIL 115-173	Cat 3293 × NRC 2	105	RIL 107-80	JS 97-52 × NRC 37
47	RIL 115-175	Cat 3293 × NRC 2	106	RIL 107-87	JS 97-52 × NRC 37
48	RIL 115-176	Cat 3293 × NRC 2	107	RIL 107-88	JS 97-52 × NRC 37
49	RIL 115-177	Cat 3293 × NRC 2	108	RIL 107-96	JS 97-52 × NRC 37
50	RIL 115-178	Cat 3293 × NRC 2	109	RIL 107-103	JS 97-52 × NRC 37
51	RIL 115-179	Cat 3293 × NRC 2	110	RIL 107-128	JS 97-52 × NRC 37
52	RIL 115-181	Cat 3293 × NRC 2	111	RIL 107-131	JS 97-52 × NRC 37
53	RIL 115-182	Cat 3293 × NRC 2	112	RIL 107-137	JS 97-52 × NRC 37
54	RIL 115-183	Cat 3293 × NRC 2	113	RIL 107-139	JS 97-52 × NRC 37
55	RIL 115-184	Cat 3293 × NRC 2	114	RIL 107-151	JS 97-52 × NRC 37
56	RIL 115-188	Cat 3293 × NRC 2	115	RIL 107-161	JS 97-52 × NRC 37
57	RIL 115-193	Cat 3293 × NRC 2	116	JS 97-52 (C)	PK327 × L129
58	RIL 115-194	Cat 3293 × NRC 2	117	NRC-37 (C)	Gaurav × Punjab 1
59	RIL 115-199	Cat 3293 × NRC 2	118	JS-335 (C)	JS 78-77 × JS 71-05

*(C) – Check

Fig. 1. Symptoms of soybean yellow mosaic disease in field.

Fig. 2. Soybean plant showing symptoms of charcoal rot in field.

Fig. 3. General view of experimental field.

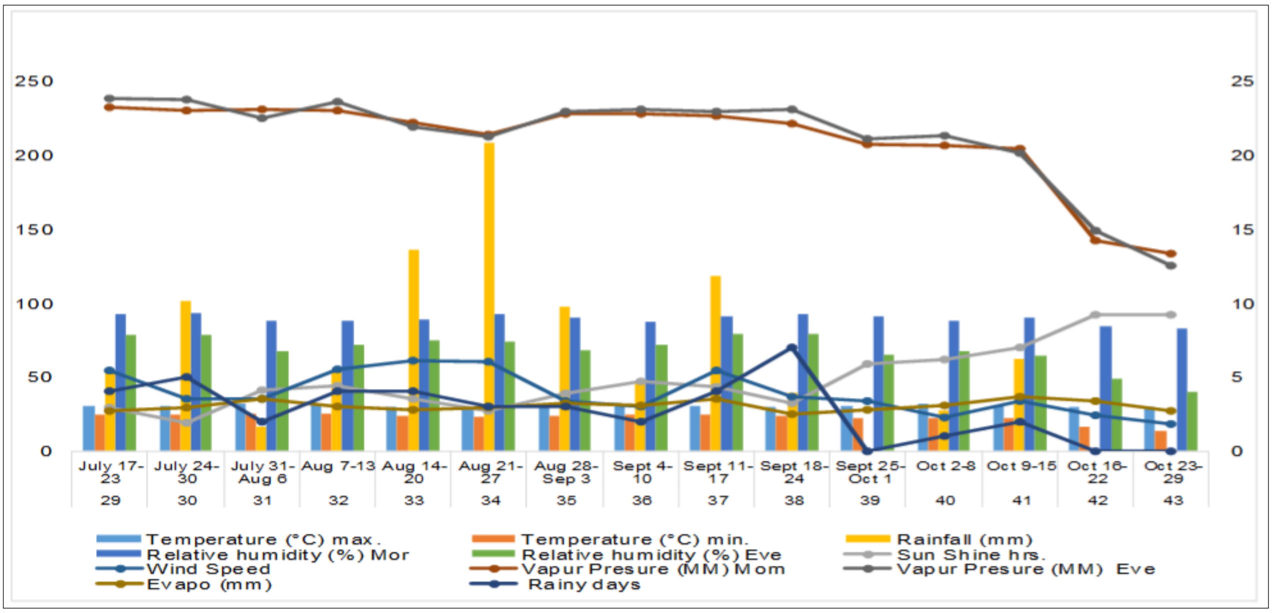

Fig. 4. Meteorological parameters during cropping season, *Kharif*-2022.

Observation recorded and statistical analysis

Based on the percentage of infected leaf area, 0-4 ratings/grades were given to each affected plant (Table 2) (31, 32).

Mortality rates were recorded and genotypes were categorised as described in Table 3 (31, 32).

Moreover, genetic relationships and variability among genotypes for disease resistance were evaluated through qualitative trait analysis. A dendrogram was generated employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to reveal the clustering patterns among genotypes. The analysis was performed employing NTSYSpc software, simplifying the validation of genotype groupings and the assessment of genetic diversity present within the population (33). For the analysis of Shannon-weaver diversity index MS-Excel package was employed.

Results and Discussion

Yellow mosaic virus

Yellow mosaic disease of soybean had initially appeared in the mid-august and severity of disease was comparatively low. Nevertheless, many genotypes displayed prominent symptoms of YMD with high level of severity. Out of 118 genotypes, 19 RILs disclosed highly resistant reaction against YMV. Whereas 63 were found to be resistant including check JS 97-52, 6 moderately resistant and 8 moderately susceptible including check NRC 37. While, 16 RILs exhibited susceptible including check JS 335, rest 6 were found highly susceptible. The coefficient of infection and their reaction is depicted in Table 4, determination of its coefficient of infection in Table 5 and its categorization in Table 6 and Fig. 5 and Fig. 6 respectively. The differential disease responses among the genotypes highlighted the complex nature of resistance mechanisms against YMV. The identification of resistant and highly resistant lines is particularly significant for breeding programmes aimed to develop durable resistance against YMD. The coefficient of infection, as presented in Table 4, further quantifies disease severity across genotypes, while its determination in Table 5 provides insights the extent of infection. Furthermore, the categorization depicted in Table 6 facilitates a structured understanding of disease responses, aiding in the selection of putative potential donor line (s) for resistance breeding.

Charcoal rot

Jabalpur is considered hotspot for charcoal rot of soybean and the circumstances for disease development were favourable and due to this, RILs encountered the severe intensity of charcoal rot infection at the reproductive stage of crop growth and development. Precise screening of CR disease was performed at the experimental field under higher disease pressure. Out of 118 genotypes, only 21 RILs demonstrated absolutely resistant, while 41 were found moderately resistant including check JS 97-52, 22 moderately susceptible along with checks JS 335 and NRC 37, 8 susceptible and 26 were considered as highly susceptible against CR. The disease reaction is depicted in Table 4 and categorization in Table 7 and in Fig. 5 correspondingly. The variation in disease response underscores the complexity of resistance to *M. phaseolina*, which is influenced by both genetic and environmental factors.

The findings from this investigation, provide perilous understandings of the genetic resistance of soybean against CR disease. The disease reaction has been given in Table 4 and its categorization in Table 7 facilitate the identification of putative resistant genotype (s) that may be incorporated into crop improvement programmes intended to breed improved soybean varieties with enhanced tolerance/resistant to charcoal rot. The incidence of highly susceptible line (s) further highlights the necessity for continued screening and the integration of resistance genes into high-yielding cultivars to mitigate yield losses in charcoal rot prone regions.

Dual resistance (Yellow mosaic virus + Charcoal rot)

The categorization for dual resistance for yellow mosaic plus charcoal rot is depicted in Table 8 and Fig. 7 respectively. A total of 29 RILs demonstrated dual resistance against YMV and charcoal rot diseases. The incidence of disease severity against YMV had seen low incidence out of 118 RILs, while resistance for both the diseases *viz.,* YMV (Highly Resistance) + Charcoal Rot (Absolutely resistance) was found in 7 RILs including RIL115-41, RIL115-43, RIL115-64, RIL115-69, RIL115-71, RIL115-230 and RIL107-35, whereas 12 RILs demonstrated resistance for YMV + Absolutely Resistance to charcoal rot *viz*., RIL115-153, RIL115-155, RIL115-159, RIL115-160, RIL115-166, RIL115-176, RIL115-179, RIL115-206, RIL107-38, RIL107-131, RIL107-137 and RIL 107-151. Moderately resistance to YMV + Absolutely resistance to Charcoal rot were evident in 2 RILs *i.e*., RIL107-77 and RIL107-161 and moderately resistance to charcoal rot along with highly resistance to YMV were found in 8 RILs including RIL115-1, RIL115-140, RIL115-141, RIL115-161, RIL107-24, RIL107-31, RIL107-73 and RIL107-79.

Similar studies have also been conducted earlier (17-19, 24, 32, 34- 39). To identify stable and resistant genotypes against

Table 2. Scale for classifying reaction of Yellow mosaic virus

Symptoms	Severity grade	Response value	Coefficient of Infection (CI)	Disease reaction
Symptoms absent	0	0	0-4	Highly resistant
Very mild symptoms up to 25 %leaves	1	0.25	4 to 9	Resistant
Appearance of symptoms in 26- 50 % leaves	2	0.5	10 to 19	Moderately resistant
Appearance of symptoms in 51- 75 % leaves	3	0.75	20 to 39	Moderately susceptible
Severe disease infection in symptoms (>75 % leaves)	4	1	40 to 69	Susceptible
			70 to 100	Highly susceptible

Table 3. Scale for classifying reaction of Charcoal rot disease

Rating	Descriptions	Category
0	No mortality	Absolutely resistant
1	1 % mortality	Highly resistant
3	1.1 to 10 % mortality	Moderately resistant
5	10.1 to 25 % mortality	Moderately susceptible
7	25.1 % to 50 % mortality	Susceptible
9	More than 50 % mortality	Highly susceptible

Table 4. Reaction of RILs of soybean against yellow mosaic virus and charcoal rot of soybean based on the observations recorded on 10 plants

S. No.	Name of RILs	Yellow mosaic virus		Charcoal rot	
		Coefficient of Infection	Reaction	Score	Reaction
1	RIL 115-1	0	HR	3	MR
2	RIL 115-4	0	HR	5	MS
3	RIL 115-36	5	R	5	MS
4	RIL 115-41	2.5	HR	0	AR
5	RIL 115-42	0	HR	5	MS
6	RIL 115-43	0	HR	0	AR
7	RIL 115-45	70	HS	5	MS
8	RIL 115-51	60	S	5	MS
9	RIL 115-57	7.5	R	3	MR
10	RIL 115-62	0	HR	3	MR
11	RIL 115-64	0	HR	0	AR
12	RIL 115-65	90	HS	7	S
13	RIL 115-66	5	R	3	MR
14	RIL 115-67	7.5	R	3	MR
15	RIL 115-69	0	HR	0	AR
16	RIL 115-71	2.5	HR	0	AR
17	RIL 115-74	70	HS	7	S
18	RIL 115-76	60	S	5	MS
19	RIL 115-89	50	S	5	MS
20	RIL 115-91	5	R	3	MR
21	RIL 115-92	7.5	R	3	MR
22	RIL 115-93	60	S	9	HS
23	RIL 115-96	75	HS	9	HS
24	RIL 115-99	40	S	9	HS
25	RIL 115-108	50	S	9	HS
26	RIL 115-118	5	R	3	MR
27	RIL 115-132	5	R	3	MR
28	RIL 115-140	0	HR	3	MR
29	RIL 115-141	0	HR	3	MR
30	RIL 115-144	7.5	R	3	MR
31	RIL 115-150	37.5	MS	9	HS
32	RIL 115-153	5	R	0	AR
33	RIL 115-154	5	R	3	MR
34	RIL 115-155	5	R	0	AR
35	RIL 115-156	7.5	R	3	MR
36	RIL 115-159	7.5	R	0	AR
37	RIL 115-160	7.5	R	0	AR
38	RIL 115-161	0	HR	3	MR
39	RIL 115-162	7.5	R	3	MR
40	RIL 115-163	7.5	R	5	MR
41	RIL 115-164	5	R	3	MR
42	RIL 115-166	0	R	0	AR
43	RIL 115-167	5	R	3	MR
44	RIL 115-169	45	S	9	HS
45	RIL 115-171	5	R	3	MR
46	RIL 115-173	7.5	R	3	MR
47	RIL 115-175	5	R	5	MS
48	RIL 115-176	7.5	R	0	AR
49	RIL 115-177	7.5	R	3	MR
50	RIL 115-178	5	R	5	MS
51	RIL 115-179	5	R	0	AR
52	RIL 115-181	15	MR	5	MS
53	RIL 115-182	52.5	S	9	HS
54	RIL 115-183	37.5	MS	7	S
55	RIL 115-184	60	S	9	HS
56	RIL 115-188	5	R	3	MR
57	RIL 115-193	7.5	R	7	S
58	RIL 115-194	7.5	R	3	MR
59	RIL 115-199	5	R	3	MR
60	RIL 115-206	7.5	R	0	AR
61	RIL 115-211	7.5	R	3	MR
62	RIL 115-221	80	HS	9	HS
63	RIL 115-222	7.5	R	5	MS
64	RIL 115-223	5	R	3	MR
65	RIL 115-225	5	R	3	MR
66	RIL 115-227	60	S	9	HS
67	RIL 115-230	0	HR	0	AR
68	RIL 115-233	60	S	9	HS
69	RIL 115-237	50	S	9	HS
70	RIL 115-238	7.5	R	5	MS

70	RIL 115-238	7.5	R	5	MS
71	RIL 115-239	5	R	5	MS
72	RIL 115-243	30	MS	5	MS
73	RIL 115-252	5	R	3	MR
74	RIL 115-253	5	R	3	MR
75	RIL 115-256	7.5	R	3	MR
76	RIL 115-258	5	R	3	MR
77	RIL 115-260	60	S	9	HS
78	RIL 115-265	5	R	5	MS
79	RIL 115-267	7.5	R	5	MS
80	RIL 115-268	5	R	3	MR
81	RIL 115-269	5	R	5	MS
82	RIL 115-272	7.5	R	5	MS
83	RIL 115-273	52.5	S	9	HS
84	RIL 115-274	80	HS	9	HS
85	RIL 115-277	45	S	7	S
86	RIL 107-38	2.5	R	0	AR
87	RIL 107-63	7.5	R	7	S
88	RIL 107-77	10	MR	0	AR
89	RIL 107-99	15	MR	7	S
90	RIL 107-166	7.5	R	5	MS
91	RIL 107-24	0	HR	3	MR
92	RIL 107-31	2.5	HR	3	MR
93	RIL 107-35	0	HR	0	AR
94	RIL 107-43	5	R	9	HS
95	RIL 107-44	7.5	R	9	HS
96	RIL 107-46	2.5	R	9	HS
97	RIL 107-57	7.5	R	9	HS
98	RIL 107-68	30	MS	9	HS
99	RIL 107-71	2.5	HR	5	MS
100	RIL 107-72	10	MR	7	S
101	RIL 107-73	0	HR	3	MR
102	RIL 107-74	45	MS	9	HS
103	RIL 107-75	5	R	9	HS
104	RIL 107-79	2.5	HR	3	MR
105	RIL 107-80	5	R	9	HS
106	RIL 107-87	22.5	MS	3	MR
107	RIL 107-88	5	R	3	MR
108	RIL 107-96	20	MS	9	HS
109	RIL 107-103	10	MR	9	HS
110	RIL 107-128	7.5	R	3	MR
111	RIL 107-131	5	R	0	AR
112	RIL 107-137	7.5	R	0	AR
113	RIL 107-139	5	R	9	HS
114	RIL 107-151	7.5	R	0	AR
115	RIL 107-161	10	MR	0	AR
116	JS 97-52	5	R	3	MR
117	NRC-37	30	MS	5	MS
118	JS-335	45	S	5	MS

Table 5. Determination of co-efficient of infection for the screening of yellow mosaic disease based on observations recorded on 10 plants

S. No.	Name of RILs	Incidence (%)	Symptoms severity	Response value	Co-efficient of Infection
1	RIL 115-1	0	0	0	0
2	RIL 115-4	0	0	0	0
3	RIL 115-36	20	1	0.25	5
4	RIL 115-41	10	1	0.25	2.5
5	RIL 115-42	0	0	0	0
6	RIL 115-43	0	0	0	0
7	RIL 115-45	70	4	1	70
8	RIL 115-51	80	3	0.75	60
9	RIL 115-57	30	1	0.25	7.5
10	RIL 115-62	0	0	0	0
11	RIL 115-64	0	0	0	0
12	RIL 115-65	90	4	1	90
13	RIL 115-66	20	1	0.25	5
14	RIL 115-67	30	1	0.25	7.5
15	RIL 115-69	0	0	0	0
16	RIL 115-71	10	1	0.25	2.5
17	RIL 115-74	70	4	1	70
18	RIL 115-76	60	4	1	60
19	RIL 115-89	50	4	1	50
20	RIL 115-91	20	1	0.25	5
21	RIL 115-92	30	1	0.25	7.5
22	RIL 115-93	80	3	0.75	60
23	RIL 115-96	100	3	0.75	75
24	RIL 115-99	40	4	1	40
25	RIL 115-108	50	4	1	50

26	RIL 115-118	10	2	0.5	5
27	RIL 115-132	10	2	0.5	5
28	RIL 115-140	0	0	0	0
29	RIL 115-141	0	0	0	0
30	RIL 115-144	30	1	0.25	7.5
31	RIL 115-150	50	3	0.75	37.5
32	RIL 115-153	20	1	0.25	5
33	RIL 115-154	10	2	0.5	5
34	RIL 115-155	10	2	0.5	5
35	RIL 115-156	10	3	0.75	7.5
36	RIL 115-159	30	1	0.25	7.5
37	RIL 115-160	10	3	0.75	7.5
38	RIL 115-161	0	0	0	0
39	RIL 115-162	10	3	0.75	7.5
40	RIL 115-163	10	3	0.75	7.5
41	RIL 115-164	20	1	0.25	5
42	RIL 115-166	0	0	0	0
43	RIL 115-167	20	1	0.25	5
44	RIL 115-169	60	3	0.75	45
45	RIL 115-171	10	2	0.5	5
46	RIL 115-173	10	3	0.75	7.5
47	RIL 115-175	20	1	0.25	5
48	RIL 115-176	30	1	0.25	7.5
49	RIL 115-177	10	3	0.75	7.5
50	RIL 115-178	20	1	0.25	5
51	RIL 115-179	20	1	0.25	5
52	RIL 115-181	30	2	0.5	15
53	RIL 115-182	70	3	0.75	52.5
54	RIL 115-183	50	3	0.75	37.5
55	RIL 115-184	80	3	0.75	60
56	RIL 115-188	20	1	0.25	5
57	RIL 115-193	30	1	0.25	7.5
58	RIL 115-194	30	1	0.25	7.5
59	RIL 115-199	10	2	0.5	5
60	RIL 115-206	10	3	0.75	7.5
61	RIL 115-211	10	3	0.75	7.5
62	RIL 115-221	80	4	1	80
63	RIL 115-222	30	1	0.25	7.5
64	RIL 115-223	20	1	0.25	5
65	RIL 115-225	20	1	0.25	5
66	RIL 115-227	80	3	0.75	60
67	RIL 115-230	0	0	0	0
68	RIL 115-233	60	4	1	60
69	RIL 115-237	50	4	1	50
70	RIL 115-238	30	1	0.25	7.5
71	RIL 115-239	20	1	0.25	5
72	RIL 115-243	40	3	0.75	30
73	RIL 115-252	10	2	0.5	5
74	RIL 115-253	20	1	0.25	5
75	RIL 115-256	30	1	0.25	7.5
76	RIL 115-258	10	2	0.5	5
77	RIL 115-260	80	3	0.75	60
78	RIL 115-265	20	1	0.25	5
79	RIL 115-267	10	3	0.75	7.5
80	RIL 115-268	10	2	0.5	5
81	RIL 115-269	20	1	0.25	5
82	RIL 115-272	30	1	0.25	7.5
83	RIL 115-273	70	3	0.75	52.5
84	RIL 115-274	80	4	1	80
85	RIL 115-277	60	3	0.75	45
86	RIL 107-38	10	1	0.25	2.5
87	RIL 107-63	10	3	0.75	7.5
88	RIL 107-77	20	2	0.5	10
89	RIL 107-99	30	2	0.5	15
90	RIL 107-166	30	1	0.25	7.5
91	RIL 107-24	0	0	0	0
92	RIL 107-31	10	1	0.25	2.5
93	RIL 107-35	0	0	0	0
94	RIL 107-43	20	1	0.25	5
95	RIL 107-44	30	1	0.25	7.5
96	RIL 107-46	10	1	0.25	2.5
97	RIL 107-57	10	3	0.75	7.5
98	RIL 107-68	40	3	0.75	30
99	RIL 107-71	10	1	0.25	2.5
100	RIL 107-72	20	2	0.5	10

101	RIL 107-73	0	0	0	0
102	RIL 107-74	60	3	0.75	45
103	RIL 107-75	10	2	0.5	5
104	RIL 107-79	10	1	0.25	2.5
105	RIL 107-80	10	2	0.5	5
106	RIL 107-87	30	3	0.75	22.5
107	RIL 107-88	20	1	0.25	5
108	RIL 107-96	20	4	1	20
109	RIL 107-103	20	2	0.5	10
110	RIL 107-128	30	1	0.25	7.5
111	RIL 107-131	20	1	0.25	5
112	RIL 107-137	30	1	0.25	7.5
113	RIL 107-139	20	1	0.25	5
114	RIL 107-151	30	1	0.25	7.5
115	RIL 107-161	40	1	0.25	10
116	JS 97-52	10	2	0.5	5
117	NRC-37	80	1	0.25	20
118	JS-335	60	3	0.75	45

Table 6. Categorization of soybean RILs for YMV resistance during *Kharif*, 2022 at Seed Breeding Farm, Jabalpur

Disease reaction	Symptoms	Coefficient of infection (CI)	Genotypes	Total
Highly resistant (HR)	Symptoms absent	0-4	RIL 115-1, RIL 115-4, RIL 115-41, RIL 115-42, RIL 115-43, RIL 115-62, RIL 115-64, RIL 115-69, RIL 115-71, RIL 115-140, RIL 115-141, RIL 115-161, RIL 115-230, RIL 107-24, RIL 107-31, RIL 107-35, RIL 107-71, RIL 107-73, RIL 107-79	19
Resistant (R)	Very mild symptoms up to 25 % leaves	5-9	RIL 115-36, RIL 115-57, RIL 115-66, RIL 115-67, RIL 115-91, RIL 115-92, RIL 115-118, RIL 115-132, RIL 115-144, RIL 115-153, RIL 115-154, RIL 115-155, RIL 115-156, RIL 115-159, RIL 115-160, RIL 115-162, RIL 115-163, RIL 115-164, RIL 115-166, RIL 115-167, RIL 115-171, RIL 115-173, RIL 115-175, RIL 115-176, RIL 115-177, RIL 115-178, RIL 115-179, RIL 115-188, RIL 115-193, RIL 115-194, RIL 115-199, RIL 115-206, RIL 115-211, RIL 115-222, RIL 115-223, RIL 115-225, RIL 115-238, RIL 115-239, RIL 115-252, RIL 115-253, RIL 115-256, RIL 115-258, RIL 115-265, RIL 115-267, RIL 115-268, RIL 115-269, RIL 115-272, RIL 107-38, RIL 107-63, RIL 107-166, RIL 107-43, RIL 107-44, RIL 107-46, RIL 107-57, RIL 107-75, RIL 107-80, RIL 107-88, RIL 107-128, RIL 107-131, RIL 107-137, RIL 107-139, RIL 107-151, JS 97-52	63
Moderately resistant (MR)	Appearance of symptoms in 26-50 % leaves	10-19	RIL 115-181, RIL 107-77, RIL 107-99, RIL 107-72, RIL 107-103, RIL 107-161	6
Moderately susceptible (MS)	Appearance of symptoms in 51-75 % leaves	20-39	RIL 115-150, RIL 115-183, RIL 115-243, RIL 107-68, RIL 107-74, RIL 107-87, RIL 107-9, NRC-37	8
Susceptible (S)	Severe disease symptoms (>75 % leaves)	40-69	RIL 115-51, RIL 115-76, RIL 115-89, RIL 115-93, RIL 115-99, RIL 115-108, RIL 115-169, RIL 115-182, RIL 115-184, RIL 115-227, RIL 115-233, RIL 115-237, RIL 115-260, RIL 115-273, RIL 115-277, JS-335	16
Highly susceptible (HS)		70-100	RIL 115-45, RIL 115-65, RIL 115-74, RIL 115-96, RIL 115-221, RIL 115-274	6
Total				118

Table 7. Categorization of soybean RILs for charcoal rot resistance during *Kharif,* 2022 at Seed Breeding Farm, Jabalpur

S. No.	Categories	Rating	Genotype	Total
1	Absolutely resistant (AR)	0	RIL 115-41, RIL 115-43, RIL 115-64, RIL 115-69, RIL 115-71, RIL 115-153, RIL 115-155, RIL 115-159, RIL 115-160, RIL 115-166, RIL 115-176, RIL 115-179, RIL 115-206, RIL 115-230, RIL 107-38, RIL 107-77, RIL 107-35, RIL 107-131, RIL 107-137, RIL 107-151, RIL 107-161	21
2	Highly resistant (HR)	1	Nil	0
3	Moderately resistant (MR)	3	RIL 115-1, RIL 115-57, RIL 115-62, RIL 115-66, RIL 115-67, RIL 115-91, RIL 115-92, RIL 115-118, RIL 115-132, RIL 115-140, RIL 115-141, RIL 115-144, RIL 115-154, RIL 115-156, RIL 115-161, RIL 115-162, RIL 115-163, RIL 115-164, RIL 115-167, RIL 115-171, RIL 115-173, RIL 115-177, RIL 115-188, RIL 115-194, RIL 115-199, RIL 115-211, RIL 115-223, RIL 115-225, RIL 115-252, RIL 115-253, RIL 115-256, RIL 115-258, RIL 115-268, RIL 107-24, RIL 107-31, RIL 107-73, RIL 107-79, RIL 107-87, RIL 107-88, RIL 107-128, JS 97-52	41
4	Moderately susceptible (MS)	5	RIL 115-4, RIL 115-36, RIL 115-42, RIL 115-45, RIL 115-51, RIL 115-76, RIL 115-89, RIL 115-175, RIL 115-178, RIL 115-181, RIL 115-222, RIL 115-238, RIL 115-239, RIL 115-243, RIL 115-265, RIL 115-267, RIL 115-269, RIL 115-272, RIL 107-166, RIL 107-71, NRC-37, JS-335	22
5	Susceptible (S)	7	RIL 115-65, RIL 115-74, RIL 115-183, RIL 115-193, RIL 115-277, RIL 107-63, RIL 107-99, RIL 107-72	8
6	Highly susceptible (HS)	9	RIL 115-93, RIL 115-96, RIL 115-99, RIL 115-108, RIL 115-150, RIL 115-169, RIL 115-182, RIL 115-184, RIL 115-221, RIL 115-227, RIL 115-233, RIL 115-237, RIL 115-260, RIL 115-273, RIL 115-274, RIL 107-43, RIL 107-44, RIL 107-46, RIL 107-57, RIL 107-68, RIL 107-74, RIL 107-75, RIL 107-80, RIL 107-96, RIL 107-103, RIL 107-139	26
	Total			118

Table 8. Categorization of soybean RILs for dual resistance (YMV + Charcoal rot)

Category	Genotypes	Number
YMV (HR) + CR (AR)	RIL 115-41, RIL 115-43, RIL 115-64, RIL 115-69, RIL 115-71, RIL 115-230, RIL 107-35	7
YMV (R) + CR (AR)	RIL 115-153, RIL 115-155, RIL 115-159, RIL 115-160, RIL 115-166, RIL 115-176, RIL 115-179, RIL 115 -206, RIL 107-38, RIL 107-131, RIL 107-137, RIL 107-151	12
YMV (MR) + CR (AR)	RIL 107-77, RIL 107-161	2
YMV (HR) + CR (MR)	RIL 115-1, RIL 115-140, RIL 115-141, RIL 115-161, RIL 107-24, RIL 107-31, RIL 107-73, RIL 107-79	8
Total		29

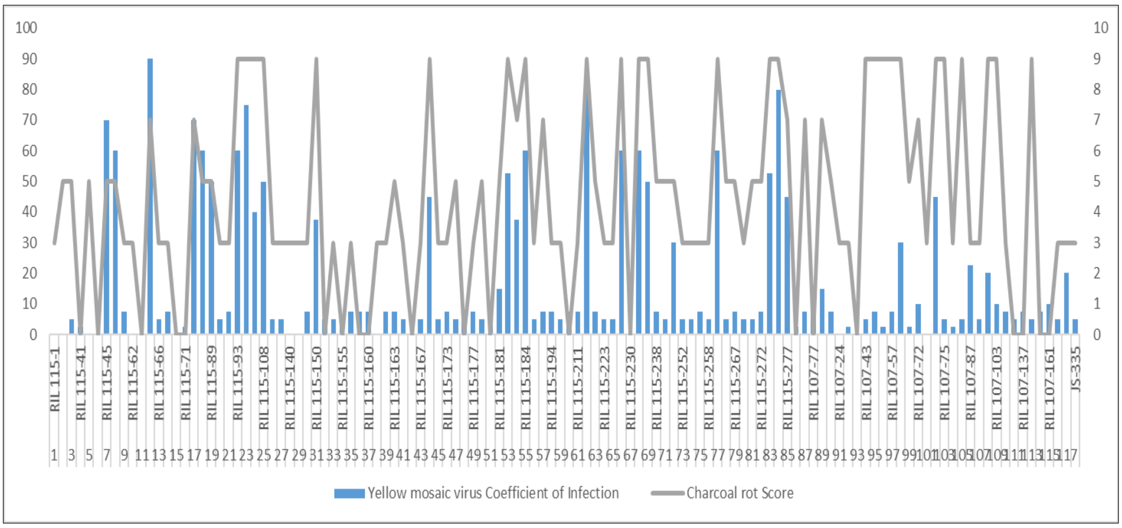

Fig. 5. Graph depicting reaction scores of RILs of soybean against yellow mosaic virus and charcoal rot of soybean.

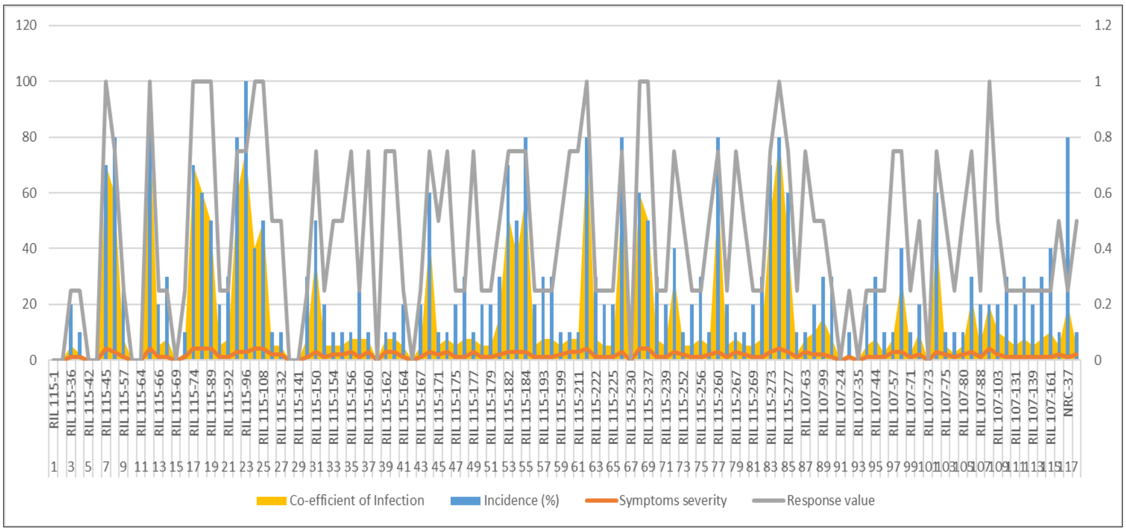

Fig. 6. Graph depicting different parameters for the screening of yellow mosaic disease.

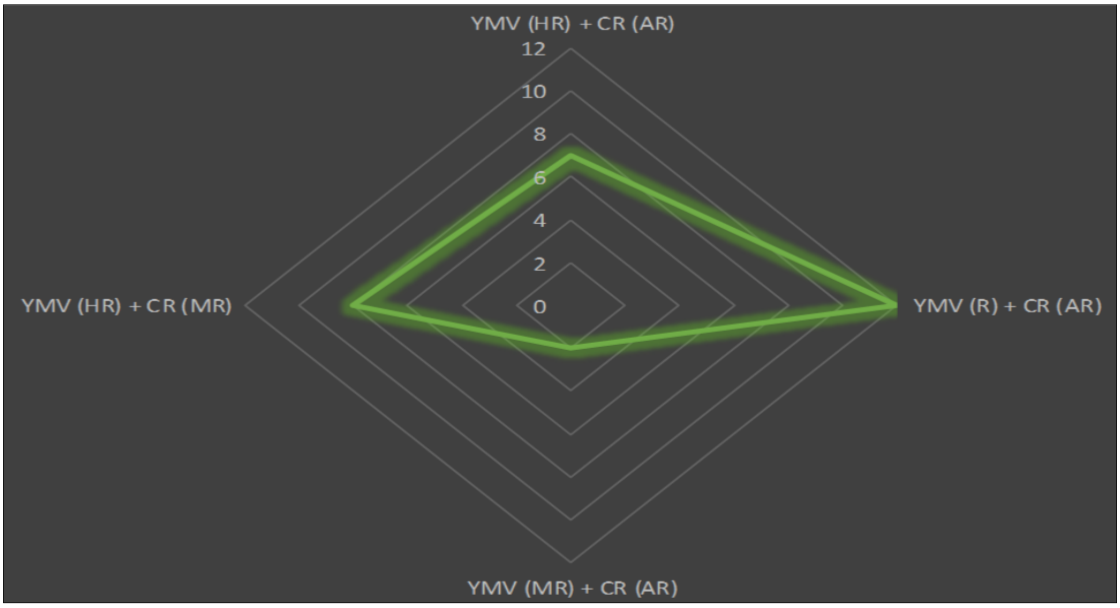

Fig. 7. Soybean RILs depicting dual resistance for YMV + CR.

Yellow Mosaic Disease (YMD) and to characterize the virus causing the disease (17) conducted the study in central India. Over 4 years, 47 soybean genotypes were evaluated under hotspot conditions and DS 3106, SL 955 and JS 21-75 emerged as the most resistant and stable based on WAASBY analysis (17). Similarly, (18) conducted an experiment to identify soybean genotypes with resistance to yellow mosaic disease, charcoal rot and high yield disease pressure. Seventy-eight genotypes were evaluated over three years in sick plots and through artificial inoculation for seedling resistance. Genotypes JS 94-67 and JS 21-05 emerged as promising donors for both YMD and charcoal rot resistance, while JS 22-10 and PS 1613 showed strong seedling resistance to charcoal rot. Notably, JS 21-13 was identified as the most stable and high yielding genotype with resistance to both diseases based on multiple trait selection indices (18). The study focused on identifying soybean germplasm resistant to YMD in Pakistan and uncovering genetic mutations linked to susceptibility. Field screening from 2016-2020 revealed NB-SG soybean as a highly resistant line with stable performance in a YMD hotspot, while several lines including NARC-2021 were highly susceptible. Resistant lines showed significantly higher yields (19). The prevalence and variability of *Macrophomina phaseolina*, the causal agent of charcoal rot were assessed by (24), in key soybean growing districts of Madhya Pradesh during 2018-19. Varietal and cropping pattern influences were evident, with JS 95-60 and JS 93-05 being highly susceptible and cropping systems like Maize-Chickpea showing higher disease incidence (24). A field study was conducted by (32) during *kharif* 2017 evaluated 41 soybean genotypes and 4 checks for resistance to CR and YMV across India. YMV incidence was higher in early growth stages, while CR was severe during the reproductive phase. Among the genotypes, nine were completely resistant to CR and 31 showed high resistance to YMV. Notably six genotypes JS 21-17, AMS 100-39, RVS 2011-1, PS 1611, RSC 11-03 and SKF-SPS-11 exhibited strong resistance to both diseases (32). A total of forty-three soybean genotypes were screened for resistance to soybean Yellow Mosaic Virus (SYMV) under glasshouse conditions using artificial inoculation with viruliferous whiteflies. No genotype displayed high resistance; however, eleven genotypes such as KDS-378 and RKS-118 exhibited moderate resistance. Additionally, six genotypes were moderately susceptible, twenty -one showed susceptibility and five genotypes including JS-335 and JS-20 were identified as highly susceptible (35). In 2020, seven soybean genotypes were evaluated for resistance to charcoal rot in the hotspot region of Amravati, Maharashtra. The genotypes were assessed based on Percent Disease Incidence (PDI), Area under Disease Progress Curve (AUPDC) and grain yield. AMS-1001 exhibited the lowest PDI (2.14) and AUPDC (8.37), along with the highest grain yield, making it a promising candidate for breeding programmes focused on improving both yield and disease resistance in Indian soybean varieties (37).

A dendrogram was constructed based on the phenotypic data for YMD and CR across 118 recombinant inbred lines (RILs) and presented in Fig. 8. The hierarchical clustering analysis classified the genotypes into two primary clusters: a major cluster and a minor cluster. Each of these primary clusters was further subdivided into two subclusters, designated as major and minor subclusters. The minor cluster comprised 41 genotypes, while the major cluster included 77 genotypes. Within the minor cluster, the minor subcluster consisted of 16 RILs, including RIL115-45, RIL115-76, RIL115-89, RIL115-51, RIL115-163, RIL115-243, RIL115-267, RIL115-65, RIL115-74, RIL115-183, RIL115-277, RIL107-63, RIL115-193, RIL107-99, RIL107-72 and RIL115-93. The corresponding major subcluster confined 25 genotypes. Similarly, the major cluster was subdivided into a major subcluster comprising 63 genotypes and a minor subcluster consisting of 14 RILs *viz.*, RIL107-131, RIL107-137, RIL107-151, RIL107-161, RIL115-43, RIL115-64, RIL115-69, RIL115-166, RIL115-230, RIL115-160, RIL107-35, RIL115-206, RIL115-155 and RIL107-77.

The Shannon–Weaver diversity index (H′) was employed to estimate genetic diversity across six categorical responses against the both diseases. The index values arrayed substantially, indicating distinct levels of genetic variation among the genotypes. Specifically, the Shannon index (H′) served to evaluate the frequency distribution of diversity within the population for each disease. Remarkably, the resistant category exhibited the highest diversity for yellow mosaic virus (YMV), with an H′ value of 0.542 (Table 9), while the moderately resistant category displayed the greatest diversity for charcoal rot (CR), with an H′ value of 0.364 (Table 10). These findings suggested that these categories possess the highest genetic variability within the evaluated set. Overall, the observed H′ values remained below 1.5 and, in many cases, below 1.0, reflecting presence of a narrow genetic base among the experimented genotypes. This emphasizes an urgent need for the conservation and broadening of soybean germplasm resources. Identical trends have also been reported in previous studies (40-44), who also employed the Shannon diversity index to assess high variability in qualitative traits of soybean. Based on the Shannon Weaver diversity index, genotypes falling under resistant and moderately resistant categories having higher genetic variability, may aid as potential parental lines in future breeding programmes to breed disease resistance through strategic hybridization and germplasm enhancement.

The identification of soybean genotypes with resistance or tolerance to YMV and CR opens avenues for further research and breeding efforts to develop improved soybean varieties (45).

Table 9. Shannon weaver index of YMV

Category	Highly resistant	Resistant	Moderately resistant	Moderately susceptible	Susceptible	Highly susceptible	Total H value
Shannon weaver H' Index	0.161	0.542	0.051	0.068	0.127	0.051	1.374

Table 10. Shannon weaver index of CR

Category	Absolutely resistant	Moderately resistant	Moderately susceptible	Susceptible	Highly susceptible	Total H value
Shannon weaver H' Index	0.178	0.364	0.17	0.068	0.22	1.492

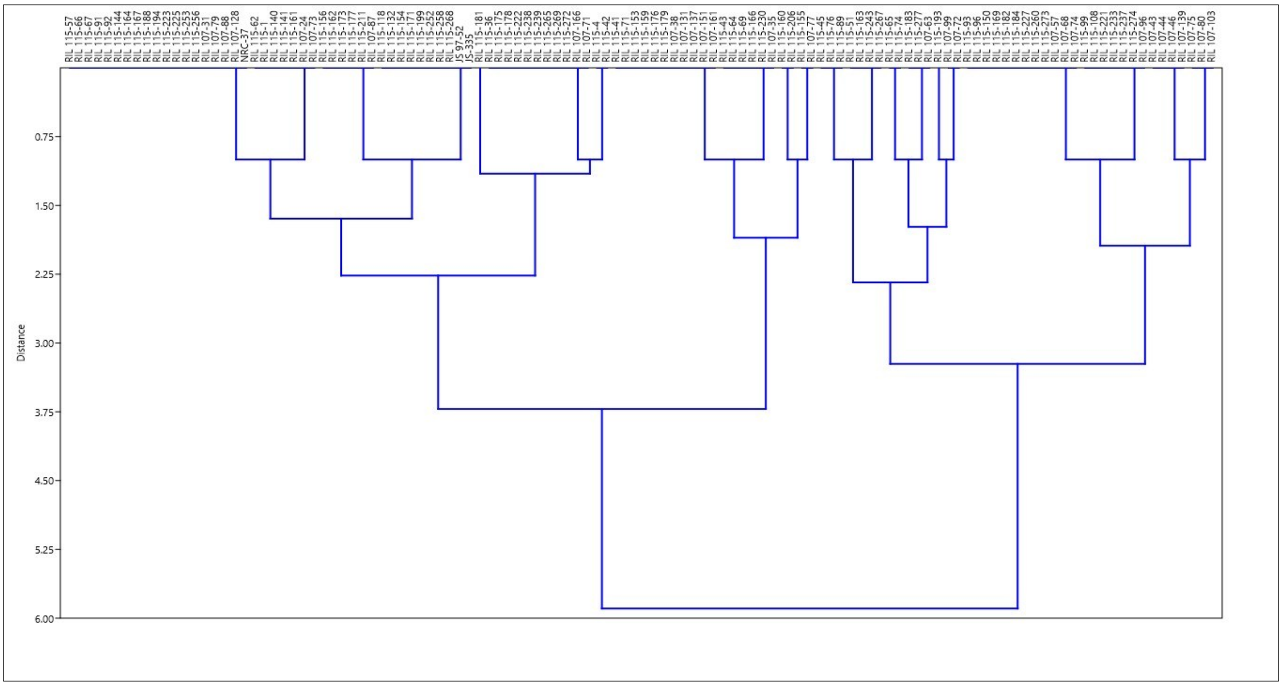

Fig. 8. Dendrogram showing categorization of RILs in different categories for YMV and CR.

These resistant genotype (s) may be used as parental lines in breeding programmes to enhance resistance against YMV, CR or both. Moreover, these provides valuable insights into the genetic diversity present within the germplasm lines (46). Incorporating these resistant traits into elite soybean varieties can help farmers mitigate the losses caused by YMV and CR, ensuring sustainable soybean production (47, 48). Further studies can focus on understanding the genetic basis of resistance and identifying specific molecular markers associated with YMV and CR resistance traits (49, 50).

Conclusion

Developing disease-resistant soybean varieties is decisive for ensuring sustainable production and minimizing yield losses caused by different biotic stresses. The detection of resistant and moderately resistant genotypes delivers a foundation for breeding efforts aimed to enhance resistance to yellow mosaic virus and charcoal rot diseases. The observed genetic variability exist among genotypes highlights the aptitude for selecting superior lines with durable resistance. Incorporating these resistant genotype (s) into breeding programmes may contribute to the development of soybean varieties with improved resilience, reducing the reliance on chemical control measures. Future research should focus on the genetic mechanisms underlying disease resistance in conjunction with the application of molecular breeding techniques to accelerate the development of resistant cultivars, ultimately supporting long-term soybean productivity and stability in disease-prone areas.

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Authors' contributions

RM carried out drafting and writing the manuscript. MKS, MKT, PKA, YS, DKP and AP contributed to the conceptualization of the study and provided essential project supervision. JG, AKP and SS made significant contributions to the formulation of the research framework. All authors critically reviewed and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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