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RESEARCH ARTICLE

Exploring bell pepper (*Capsicum annuum* L. var. *grossum*) germplasm resilient to leaf curl disease

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Abstract

Chili leaf curl is a viral disease that has a negative impact on both the quality and quantity of bell pepper and is transmitted by the vector silverleaf whitefly (*Bemesia tabaci*). Natural screening alone is insufficient for determining the extent of resistance to specific diseases. As a result, natural and artificial screening has been identified as a more precise method for evaluating germplasm, facilitating the identification of resistant genotypes. To determine resistance to chili leaf curl disease, fifty bell pepper genotypes were subjected to both natural and artificial screening at Punjab Agricultural University, Ludhiana, during 2022-23. Two genotypes were found resistant (PAU SM-27, PAU SM-45), seven were moderately resistant (PAU SM-5, PAU SM-16, PAU SM-28, PAU SM-32, PAU SM-43, PAU SM-56 PAU SM-86), eight were susceptible, twenty-five were moderately susceptible, and eight were highly susceptible under natural screening. In contrast, artificial screening identified two moderately resistant (PAU SM-16 and PAU SM-28), twenty-nine susceptible, fourteen moderately susceptible, and five highly susceptible genotypes. Under both natural and artificial screening conditions, the genotypes PAU SM-16 and PAU SM-28 were determined to be moderately resistant. Therefore, these genotypes can be utilized in breeding programs to develop genotypes resistant to chili leaf curl disease.

Keywords: Bell pepper, leaf curl virus, screening, resistance, scaling.

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Introduction

Capsicum annuum L. var. *grossum* is a member of the Solanaceae family with the chromosome number 2n=2x=24. Capsicum is currently cultivated in India on a land area of 24,000 hectares with an annual yield of 3,210,000 metric tons (Anonymous, 2022). With the increasing demand for different types of capsicum on the market, it is anticipated that there will be opportunities to increase the area devoted to the cultivation of this crop. India is the fourth largest capsicum producer in the world (Anonymous, 2019). In India, it is cultivated most frequently in West Bengal, Karnataka, Haryana, Jharkhand, Himachal Pradesh, Madhya Pradesh, Punjab, Maharashtra, Jammu and Kashmir and Uttarakhand (Anonymous, 2022). This crop is extremely susceptible to a variety of diseases, including viral, fungal, bacterial, nematode and phytoplasmal diseases which result in a substantial loss of crop productivity (Muthukumar and Bhaskaran, 2007). Viruses such as chili/pepper leaf curl virus (ChLCV), pepper vein banding virus (PVBV), pepper veinal mottle virus (PVMV), cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) are potential agents that affect the chili pepper crop during the early stages of plant development (Green and Kim, 1991; Ravi *et al.*, 1997; Verma, 2004). Begomoviruses transmitted by whiteflies are responsible for leaf curl disease (Pepper leaf curl disease), which is exceedingly destructive and highly prevalent (Muniyappa and Veeresh, 1984). It causes yield losses of up to 100%. The silverleaf whitefly (*Bemesia tabaci*) is a natural insect vector for leaf curl disease, which is a persistent, circulative and non-propagating carrier (Brown *et al*., 2015).

Typical symptoms of chili leaf curl disease include reduced leaf size, short internodes, thickened leaf margins, upward curling of leaves, discoloration, puckering, stunted growth, and sterile plants. Virus infection during the early phases of plant development results in the premature shedding of flower buds before they reach their maximum size. Thus, leaf curl disease negatively impacted crop growth and resulted in significant economic losses for producers (Kaur *et al*., 2018; Shingote *et al*., 2022). Integrated management of LCV consists of the elimination of infected plants, the implementation of agronomic interventions and the use of pesticides to control vectors with limited success. The use of host plant resistance is a highly effective, cost-effective, environmentally sustainable and long-lasting disease control strategy, particularly for viral-induced diseases (Kumar *et al*., 2022). Due to a lack of specific knowledge regarding the aspect of leaf curl disease in bell pepper, fifty bell pepper genotypes were systematically evaluated for resistance against leaf curl disease under Punjab conditions at the Vegetable research farm, Department of Vegetable Science, Punjab Agricultural University, Ludhiana, during 2022-23. The goal was to identify prospective sources of resistance that could be used in future breeding programs aimed at improving resistance.

Materials and Methods

A set of fifty genotypes of bell pepper (Table 3 and supplementary tables) were examined for resistance to leaf curl disease under both natural and artificial conditions as outlined below at PAU, Ludhiana during 2022-23 (September 2022 to May 2023).

Field screening

To prevent seed-borne diseases, seeds were treated with 0.3% Captan for field screening. The treated seeds were sown in potrays at the end of September 2022 and the plots were monitored regularly. After 25 to 30 days of sowing in the portrays, the germplasm was transplanted at a spacing of 90 x 30 cm^2 using a randomized complete block design (RCBD) in triplicates at PAU, Ludhiana. Observations regarding disease were recorded 30, 60, 90, 120, 150, 180 and 210 days after transplanting. The evaluation of the resistance/susceptibility of the genotypes to ChLCV disease and the calculation of the percent disease index (PDI) were performed using a scale of 0 to 5, as suggested by Sharma *et al*. (2018), with minor modifications (Table 1).

Artificial screening

The same set of 50 germplasm lines (Table 4 and Supplementary Table 2) were subjected to the ChLCV disease under controlled experimental conditions (28/20°C day/night temperature and 70% of relative humidity, RH). The experimental procedure consists of placing the test material into plug trays with a potting mixture (cocopeat: perlite: vermiculite; 3:1:1) in the month of September. The trays were placed in a restricted area among susceptible pepper cultivars (Punjab Surkh). A population of non-viruliferous whiteflies was maintained on cotton plants (*Gossypium hirsutum* L.) cultivated in plastic pots (12 x 8 cm^2) within an insect-proof net house. The source of the leaf curl viral disease was conserved on cultivars that were vulnerable to the virus and maintained within a distinct, confined environment. The non-viruliferous whiteflies raised on cotton were collected in a container and fed on infected plants for 24 hours. During this screening procedure, the virus-infected whiteflies were consistently reintroduced to the plants by shaking the susceptible cultivar placed between the bell pepper genotypes that were vulnerable to infection. The assessment of disease reactions and the calculation of PDI were conducted at particular 15-day intervals till 120 days after sowing. The evaluation was conducted utilizing a scale that had been previously devised by Sharma *et al*. (2018) (Table 2).

Observations recorded under both screening conditions

Number of days to show symptoms

Observations were recorded based on the manifestation of symptoms on each individual genotype plant, and the mean was also recorded. In artificial screening, a susceptible

Table 1: Scale used for natural screening of leaf curl virus disease (modification from Sharma *et al*., 2018)

Symptom	*Symptom severity grade*	*Response value*	*Coefficient of infection*	*Percentage disease index (PDI)*	*Reaction*
Symptomless	0	0.0	0-4.5	<5%	Highly resistant (HR)
25% of curling of leaves	1	0.2	4.6-9.5	5-20%	Resistant (R)
25-50% of curling of leaves	2	0.4	9.6-19.5	20-40%	Moderately resistant (MR)
51-75% curling of leaves	3	0.6	19.6-39.5	40-64%	Moderately susceptible (MS)
>75% curling of leaves	4	0.8	39.6-69.5	64-75%	Susceptible (S)
Stunted growth and severe curling	5	1.0	69.6-100	>75%	Highly susceptible (HS)

Table 2: Phenotypic classes of ChLCD symptoms used for screening of chili reported by Sharma *et al.* (2018)

Symptoms	*Symptom severity grade*	*Response value*	*Coefficient of infection*	*Reaction*
Symptomless	0	0	0	Symptomless
Very mild curling with wavy margins on top 1-2 leaves	1	0.05	0.1-2.5	Highly resistant (HR)
Mild curling, interveinal chlorosis on top 1-2 leaves	2	0.25	2.6-10	Resistant (R)
Curling on top 2-3 leaves, yellowing of interveinal region with leathery texture	3	0.50	10.1-20	Moderately resistant (MR)
Severe curling on more than 50% leaves, yellowing and thickening of veins	4	0.75	20.1-40	Moderately susceptible (MS)
Severe curling of all leaves and stunting	5	1	40.1-70	Susceptible (S)
		1	>70.1	Highly susceptible (HS)

Table 3: Reaction of bell pepper genotypes screened under natural conditions against chili leaf curl virus disease

S. No.	*Name of the genotype*	*(February) 150 DAS*		*(March) 180 DAS*		*(April) 210 DAS*				*Number of days to show symptoms after sowing*	*Disease reaction (April)*
		PDI (%)	*DI (%)*	*PDI (%)*	*DI (%)*	*PDI (%)*	*DI (%)*	*Symptom severity grade*	*Response value*		
1	PAU SM-4	53.33	78.33	70.00	89.99	76.67	100.00	5	1	110.00	HS
2	PAU SM-5	27.50	50.00	28.80	55.55	30.00	60.66	2	0.4	140.00	MR
3	PAU SM-16	10.00	20.00	20.00	40.00	25.00	50.00	2	0.4	156.00	MR
4	PAU SM-27	5.55	5.55	11.11	11.11	14.28	14.28	1	0.2	160.00	R
5	PAU SM-28	23.33	50.00	23.33	50.00	23.33	50.00	2	0.4	148.00	MR
6	PAU SM-32	25.00	33.33	33.33	33.33	40.00	40.00	2	0.4	140.00	MR
7	PAU SM-38	64.44	100.00	74.44	100.00	77.14	100.00	5	1	108.00	HS
8	PAU SM-43	33.00	60.00	33.33	66.67	37.33	78.00	2	0.4	133.00	MR
9	PAU SM-45	14.28	14.28	16.67	19.05	19.04	22.00	1	0.2	154.00	R
10	PAU SM-56	74.07	100.00	75.00	100.00	79.00	100.00	2	0.4	103.00	MR
11	PAU SM-66	88.89	100.00	91.25	100.00	94.44	100.00	5	1	90.00	HS
12	PAU SM-86	20.00	20.00	20.00	25.00	20.80	33.33	2	0.4	150.00	MR
13	PAU SM-89	65.00	100.00	71.43	100.00	82.54	100.00	5	1	92.00	HS
14	PAU SM-92	83.33	100.00	90.00	100.00	100.00	100.00	5	1	86.00	HS
15	PAU SM-93	91.67	100.00	95.00	100.00	100.00	100.00	5	1	83.00	HS
16	PAU SM-98	55.56	91.66	91.67	96.77	100.00	100.00	5	1	89.00	HS
17	PAU SM-100	55.56	100.00	73.33	100.00	86.67	100.00	5	1	91.00	HS
18	PSM-1 (Check)	55.00	74.20	58.33	86.10	63.00	92.00	3	0.6	124.00	MS

Where, PDI =percent disease index, DI =disease incidence, HR= highly resistant, R=resistant, MR=moderately resistant, HS= Highly Resistant, DAS=days after sowing. In this table just represented the HR=highly resistant, R=resistant, MR=moderately resistant, HS=highly susceptible genotypes

cultivar (Punjab Surkh) was placed between the germplasm from the time of sowing and the number of days to exhibit symptoms was computed in terms of the number of days after sowing.

Disease incidence (DI)%

The disease incidence was computed using the formula provided by Banerjee and Kalloo (1987) after the number of affected and total plants from each genotype were recorded.

$$\text{Disease incidence (DI) \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Symptom severity grade and Response value

At a particular time interval, it was recorded for each genotype based on the phenotypic scale depicted in Tables 1 and 2.

Percent Disease Index (PDI)%

PDI was calculated for all experimental material using McKinney's (1923) formula, as shown below:

Percent Disease Index (PDI) =

$$\frac{\text{Number of plants with each disease rating} \times \text{Disease rating scale}}{\text{Total number of plants} \times \text{Highest disease rating scale}} \times 100$$

Where, PDI is <5, 5–20%, 20–40%, 40–64%, >64–75% and >75% were considered as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible, respectively under field screening. However, under artificial screening if the PDI value is 0%, <5%, 6–25%, 26–50%, 51–75% and >75% considered as highly resistant, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible respectively (Tables 1 and 2).

Disease reaction

Each genotype of bell pepper exhibited a distinct disease response based on the PDI, symptom severity grade and response value to ChLCV disease for both natural and artificial screening (Tables 1 and 2).

Results and Discussion

Based on the current and projected landscape of crop and land utilization in India, it is prudent to evaluate and encourage the cultivation of a mixture of enhanced genotypes in close proximity to one another. Specifically, it would be prudent to prioritize the evaluation and promotion of field-resistant and white fly non-preferred genotypes that demonstrate superior general adaptability. Exclusively depending on field screening may be insufficient for evaluating resistance to the ChLCV disease. To facilitate a thorough evaluation of resistance to the ChLCV disease, additional measures such as artificial screening were implemented. The use of artificial screening techniques demonstrates to be a more effective method for introducing inoculum to plants, with promising results in comparison to natural screening methods. The genotypes examined in this study underwent both natural and artificial screening and were recommended as resistant or susceptible cultivars for cultivation in the climate of Punjab based on their response to chili leaf curl disease.

Natural screening for chili leaf curl disease

The natural screening was conducted on the crop sowed in September 2022-23 to determine the susceptibility or resistance of bell pepper plants to the chili leaf curl disease under field conditions. Various environmental conditions can affect field screenings (Harfouche *et al*., 2019). However, traditional breeding and field trials will continue to play an important role in the development of commercial cultivars (Stuthman *et al*., 2007). From 30 days after sowing until the end of the bell pepper crop's life cycle, information regarding the evaluation of disease incidence was recorded. The reported temperatures from October (30 DAS) to January (120 DAS) were notably low, resulting in a small incidence of the disease due to the absence of a thriving whitefly population. Temperatures increased during February (150 DAS), creating favorable conditions for the growth of the whitefly population (Mani, 2022). Consequently, the data presented in Table 3 and Supplementary Table 1 are from

Table 4: Reaction of bell pepper genotypes screened under artificial conditions against chili leaf curl virus disease

S. No.	*Genotypes*	*60 DAS*		*75 DAS*		*90 DAS*		*105 DAS*		*120 DAS*		*150 DAS*					
		PDI (%)	*DI (%)*	*PDI (%)*	*DI (%)*	*PDI (%)*	*DI (%)*	*PDI (%)*	*DI (%)*	*PDI (%)*	*DI (%)*	*PDI (%)*	*DI (%)*	*Symptom severity grade*	*Response value*	*Number of days to show symptoms after sowing*	*Disease reaction (150 DAS)*
1	PAU SM-16	20.00	50.00	20.00	50.00	21.00	52.00	22.67	55.00	22.67	58.00	24.33	60.00	3	0.50	55	MR
2	PAU SM-28	20.89	54.00	20.89	54.00	21.33	54.67	23.35	55.22	24.89	55.56	25.00	58.33	3	0.50	57	MR
3	PAU SM-72	59.00	100.00	62.00	100.00	64.00	100.00	70.00	100.00	80.00	100.00	100.00	100.00	5	1	46	HS
4	PAU SM-78	62.50	100.00	67.86	100.00	70.00	100.00	74.00	100.00	75.00	100.00	81.25	100.00	5	1	48	HS
5	PAU SM-81	50.00	100.00	56.25	100.00	58.33	50.00	61.00	100.00	69.00	100.00	77.00	100.00	5	1	50	HS
6	PAU SM-90	56.00	76.00	64.00	81.00	64.00	81.00	75.00	88.00	89.00	100.00	100.00	100.00	5	1	50	HS
7	PAU SM-96	73.33	100.00	75.23	100.00	75.23	100.00	77.00	100.00	80.00	100.00	82.00	100.00	5	1	53	HS
8	PSM-1 (Check)	45.38	89.23	47.31	92.31	48.46	92.31	48.46	92.31	49.00	100.00	49.00	100.00	5	1	45	S

Where, PDI=percent disease index, DI=disease incidence, HR=highly resistant, R=resistant, MR=moderately resistant, HS=highly susceptible, DAS=days after sowing. In this table just represented the HR=highly resistant, R=resistant, MR=moderately resistant, HS=highly susceptible genotype

150 DAS due to an increase in temperature conditions from 150 DAS to the conclusion of the crop's life cycle, which is conducive to the white fly population and disease incidence. The experimental material became susceptible to infection as a result. The study revealed that various genotypes exhibited a spectrum of infection severity, ranging from mild to severe. Various parameters, including the symptom severity grade, response value, DI, PDI and the number of days between sowing and the onset of symptoms, were examined using the collected data.

Symptom severity grade, Response value and number of days to show symptoms

Throughout all screening intervals, symptom severity grade and response value were registered for each individual plant using the scale presented in Table 1. The number of days for highly susceptible genotypes to exhibit symptoms ranges from 83 DAS (PAU SM-93) to 110 DAS (PAU SM-4), while resistant varieties PAU SM-45 and PAU SM-27 require 154 and 160 DAS, respectively. Table 3 and Supplementary Table 1 show that susceptible genotypes exhibit a shorter incubation period prior to symptom manifestation, whereas resistant genotypes require a longer duration for symptom manifestation.

Disease incidence, Percentage disease index and Disease reaction

Due to the prevailing low temperatures and very high RH, the whitefly population decreased up to 120 DAS, resulting in a lower disease incidence of chili leaf curl disease. The experimental material became infected with chili leaf curl disease 150 days after sowing (DAS) due to an abrupt increase in temperature and the percentage of disease incidence increased until the end of the crop cycle. The disease reaction was evaluated according to Table 1 based on the PDI at 210 DAS. Among these, two genotypes exhibited resistance (PAU SM-27, PAU SM-45) with PDI ranges from (14.28, 19.04%), seven showed moderate resistance (PAU SM-86, PAU SM-5, PAU SM-16, PAU SM-28, PAU SM-43, PAU SM-56, PAU SM-32) having PDI values ranging from (20.80–40%), 25 were moderately susceptible (PAU SM-67, PSM-1, PAU SM-8, PAU SM-12, PAU SM-21, PAU SM-26, PAU SM-30, PAU SM-35, PAU SM-36, PAU SM-41, PAU SM-53, PAU SM-71, PAU SM-72, PAU SM-78, PAU SM-79, PAU SM-81, PAU SM-82, PAU SM-84, PAU SM-91, PAU SM-96, PAU SM-99, PAU SM-101, PAU SM-103, PAU SM-104, PAU SM-20) with PDI values (46.67% - 63.90%), eight were susceptible (PAU SM-95, PAU SM-6, PAU SM-9, PAU SM-61, PAU SM-90, PAU SM-94, PAU SM-102, PAU SM-97) with a range of PDI (66.67% - 75%), and eight were highly susceptible (PAU SM-4, PAU SM-38, PAU SM-66, PAU SM-89, PAU SM-100, PAU SM-92, PAUA SM-93, PAU SM-98) having PDI value ranging from (76.67–100%) as illustrated in Table 3 and supplementary Table 1. The results are similar to those findings of Thakur *et al.* (2019), *i.e.*, analysis of PDI and disease severity values obtained from field screening of thirty genotypes, three genotypes exhibited a high level of resistance (HR): S-343, SL 456 and SL 475, four genotypes were resistant (R) and six genotypes demonstrated moderate resistance (MR). In contrast, Jindal and Dhaliwal (2019) concluded that PSM-1, Royal Wonder and Indra cultivars of bell pepper were susceptible to ChLCV under natural conditions.

Artificial screening for chili leaf curl disease

Relying solely on field screening to evaluate resistance to the ChLCV disease may prove insufficient. Local, seasonal and annual fluctuations have the potential to influence the occurrence and severity of the disease and can influence field assays. In addition, the unequal distribution of disease vectors and the absence of inoculum in certain regions can contribute to the spread of the disease. To facilitate a comprehensive evaluation of resistance to the ChLCV disease, additional measures were implemented. All genotypes were subjected to artificial screening in a poly net house, where favorable conditions for viruliferous whiteflies were created to induce a high inoculum pressure. From 15 to 150 days after sowing and inoculation, the symptom severity grade, response value, DI, PDI and number of days to exhibit symptoms were recorded (Table 4 and supplementary Table 2). Few genotypes exhibited negligible or low disease incidence from 15 to 45 DAS in the present study. Notably, between 60 and 120 DAS, the infection rate was at significant levels; therefore, we analyzed data from 60 to 120 DAS to identify resistance in the germplasm. The study revealed that distinct genotypes exhibited a spectrum of infection severity ranging from moderate to severe. This artificial screening methodology enables a more accurate evaluation of the genotype's resistance to the chili leaf curl disease, thereby overcoming the limitations of field experiments.

Symptom severity grade, Response value and number of days to show symptoms

In accordance with the scale presented in Table 2, the severity level of symptoms and the corresponding response value were recorded for each plant throughout the screening intervals. Under artificial screening conditions, susceptible genotypes exhibited symptoms 30 days after sowing, whereas resistant genotypes PAU SM-16 and PAU SM-28 expressed symptoms 55 and 57 days after sowing, respectively. The susceptible genotypes manifest an earlier onset of disease symptoms, whereas the resistant genotypes manifest a longer duration before the onset of disease symptoms. During artificial screening, symptoms appeared earlier in some genotypes, but there was no progression of disease, and vice versa. These findings are in congruence with Sran *et al.* (2023), *i.e.*, the onset of symptoms in resistant lines varied between 28 and 32 days post-inoculation (dpi), which was substantially later than in the susceptible genotype PAU SM-1 (14 dpi). Artificial screening revealed symptoms of capsicum leaf curl disease earlier than natural screening.

Percentage disease index, Disease incidence and Disease reaction

The resistance levels of fifty genotypes were evaluated using the PDI using data from Table 4 and supplementary Table 2. Two genotypes (PAU SM-16 and PAU SM-28) exhibited moderate resistance, as measured by PDI values of 24.33 and 25%, respectively. In addition, PDI values for five genotypes ranged from 77 to 100%, indicating extreme susceptibility. The susceptible PSM-1 had a 100% of DI and a 49% prevalence of PDI. In a few genotypes screened, the disease incidence percentage was recorded after 30 DAS; however, the disease incidence steadily increased from 45 DAS to 150 DAS. Compared to field screening, artificial screening had the highest percentage of disease incidence recorded (Tables 3, 4 and supplementary tables 1, 2). This study revealed a range of disease incidence among distinct genotypes, including moderately resistant (58.33–60%), susceptible (95.244–100%), moderately susceptible (60–100%) and highly susceptible (100%) genotypes. More or less similar findings have been reported by other researchers (Sharma *et al*., 2018; Nivedha *et al*., 2019; Thakur *et al*., 2019; Singh *et al*., 2021; Sran *et al*., 2023).

PAU SM-16 and PAU SM-28 were identified as moderately resistant genotypes under both natural and artificial conditions, as suggested by our study (Tables 3 and 4). These results were supported by Jindal *et al*. (2018) determined that the cultivar S-343 exhibited significant resistance to chili leaf curl virus disease, as exemplified by a disease incidence of 3.9% on average under natural conditions and 0% under artificial conditions. In contrast, the cultivars PAU SM-20, PAU SM-26, PAU SM-30, PAU SM-71, PAU SM-91 and PAU SM-99 were moderately susceptible under both conditions, according to our research (Supplementary Tables 1 and 2). In contrast, the cultivar FL-201 was highly susceptible to ChLCV disease, with a mean disease incidence of 77.5 and 76.7% under natural and artificial screening conditions, respectively (Jindal *et al*., 2018). During controlled experiments, the consistent and high pressure exerted by whiteflies bearing the virus assisted in identifying accurate levels of resistance. Certain genotypes exhibited a resistance reaction under natural field conditions, but their responses varied when subjected to artificial screening assays that were fabricated. Despite this, it was demonstrated that the genotypes that exhibited resistance to the chili leaf curl disease during artificial screening also exhibited resistance during field screening.

Conclusion

Genotypes of bell pepper, namely PAU SM-16 and PAU SM-28 were discovered to display moderate resistance under both natural and systematic (artificial) screening processes using viruliferous whiteflies as a vector condition. The ChLCV disease is such a significant risk to bell pepper output that the resistant genotypes developed from this study can be utilized as a source of resistance for the chili leaf curl disease in any future breeding programs.

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सारांश

मिर्च की पत्ती का मुड़ना एक वायरल बीमारी है जो बेल मिर्च की गुणवत्ता और मात्रा दोनों पर नकारात्मक प्रभाव डालती है और वेक्टर सिल्वरलीफ व्हाइटफ्लाई (बेमेसिया टैबासी) द्वारा फैलती है। विशिष्ट रोगों के प्रति प्रतिरोध की सीमा निर्धारित करने के लिए अकेले प्राकृतिक जांच अपर्याप्त है। परिणामस्वरूप, प्राकृतिक और कृत्रिम स्क्रीनिंग को जर्मप्लाज्म के मूल्यांकन के लिए एक अधिक सटीक विधि के रूप में पहचाना गया है, जिससे प्रतिरोधी जीनोटाइप की पहचान की सुविधा मिलती है। मिर्च की पत्ती कर्ल रोग के प्रतिरोध को निर्धारित करने के लिए, 2022-23 के दौरान पंजाब कृषि विश्वविद्यालय, लुधियाना में पचास बेल मिर्च के जीनोटाइप को प्राकृतिक और कृत्रिम दोनों तरह से स्क्रीनिंग के अधीन किया गया था। दो जीनोटाइप प्रतिरोधी पाए गए (पीएयू एसएम-27, पीएयू एसएम-45), सात मध्यम प्रतिरोधी थे (पीएयू एसएम-5, पीएयू एसएम-16, पीएयू एसएम-28, पीएयू एसएम-32, पीएयू एसएम-43, पीएयू एसएम- 56 पीएयू एसएम-86), आठ अतिसंवेदनशील थे, पच्चीस मध्यम रूप से संवेदनशील थे, और आठ प्राकृतिक स्क्रीनिंग के तहत अतिसंवेदनशील थे। इसके विपरीत, कृत्रिम जांच ने दो मध्यम प्रतिरोधी (पीएयू एसएम-16 और पीएयू एसएम-28), उनतीस अतिसंवेदनशील, चौदह मध्यम रूप से संवेदनशील और पांच अत्यधिक संवेदनशील जीनोटाइप की पहचान की। प्राकृतिक और कृत्रिम दोनों स्क्रीनिंग स्थितियों के तहत, जीनोटाइप पीएयू एसएम-16 और पीएयू एसएम-28 को मध्यम प्रतिरोधी निर्धारित किया गया था। इसलिए, इन जीनोटाइप का उपयोग प्रजनन कार्यक्रमों में मिर्च पत्ती कर्ल रोग के प्रतिरोधी जीनोटाइप विकसित करने के लिए किया जा सकता है।