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Genetic basis of leaf curl virus resistance in double cross F1 chilli: A monogenic dominant trait

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Abstract

Chilli is a vital crop in Indian agriculture, serving as a major cash crop and export commodity, with substantial contributions to farmers' livelihoods and the national economy. Its significance extends beyond agriculture to nutrition, health and cultural heritage. However, chilli cultivation faces considerable challenges due to susceptibility to pests and diseases, particularly the chilli leaf curl virus (ChLCV), which has emerged as a significant threat in recent years. ChLCV can cause up to 100% crop loss, with widespread damage occurring at all growth stages, jeopardizing the sustainability of chilli cultivation. This underscores the urgent need for effective management strategies, including integrated pest management and the deployment of resistant planting materials. To develop resistant hybrids, understanding the genetic basis of resistance is critical. A study aimed at developing combined resistant lines to chilli leaf curl virus (ChLCV), cucumber mosaic virus (CMV) and chilli veinal mottle virus (ChVMV) employed a double-cross breeding strategy: [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)]. Segregation of double-cross F_1 individuals under natural epiphytotic conditions revealed a 1:1 ratio of resistant to susceptible plants, indicating that resistance to ChLCV is governed by a single dominant gene. The virus associated with this study was identified as the chilli leaf curl virus (Raichur isolate). Chi-square analysis confirmed a good fit to the expected segregation ratio, supporting the hypothesis of monogenic dominant inheritance. The resistant source, IHR 4597, identified in this study, can be effectively utilized for transferring the resistance gene into higher-yielding genotypes. Furthermore, this source offers potential for mapping the resistance gene, facilitating future breeding efforts to enhance ChLCV resistance in chilli.

Key words: Chilli leaf curl virus (ChLCV), monogenic dominant resistance, double-cross hybrid, *Capsicum annuum*, disease resistance breeding, genetic inheritance

Introduction

Chilli (*Capsicum annuum* L.) is a versatile crop valued for its diverse uses as a spice, condiment, medicine, vegetable and ornamental plant. Its pungent fruits, consumed in both green and dried forms are renowned for their unique flavours and high concentrations of vitamins and antioxidants (Ashrafi *et al.,* 2012). The crop originated in Mexico and is spread globally through spice trade routes, reaching India via Portuguese traders in the mid-17th century. By the late 19th century, it had become widely cultivated across the country (Salvador, 2002). Globally, chilli holds a prominent position among spice crops, and in India, it ranks first, accounting total spice export valued at ₹ 1,249,248 lakhs (Spice Board, 2024). The major chilli-growing states in India include Andhra Pradesh, Karnataka, Maharashtra, Odisha, Tamil Nadu, and Madhya Pradesh. The crop exhibits remarkable variability in fruit morphology, pungency, bearing habit, and crop duration, reflecting the diverse agroclimatic conditions and cultivation practices across these regions (Asati and Yadav, 2004).

Chilli exhibits extensive genetic variability due to its long cultivation history and selection practices. Despite being hardy and drought-tolerant, it is vulnerable to various pests and diseases, particularly viral infections, at different growth stages. Over 65 viral diseases affecting chilli have been documented globally, with 11 virus groups reported in India (Devi and Devi, 2020; Rajamanickam and Nakkeeran, 2020; Kumar *et al.,* 2015; Zehra, 2017; Vinaykumar *et al.,* 2023). Over the past decade, begomoviruses (mono- and bipartite) have become a major constraint in chilli production, particularly in Asia. Chilli leaf curl virus (ChLCV) alone can cause up to 100% yield loss, often forcing farmers to abandon fields before harvest (Kavyashri and Nagaraju, 2019). In the Indian subcontinent, ChLCV is linked to over 16 mono- and bipartite begomoviruses transmitted by the whitefly *Bemisia tabaci* cryptic species in a circulative persistent manner (Khan *et al.,* 2006; Kumar *et al.,* 2015; Mishra *et al.,* 2020). Genetic recombination, acquisition of additional DNA components and synergistic interactions among begomoviruses have led to the emergence of novel strains, overcoming host resistance and causing severe symptoms with a broader host range (Lefeuvre *et al.,* 2007).

In India, a wide range of chilli varieties are being cultivated in different states, each adapted to specific agro-climatic zones, enhancing agricultural biodiversity and meeting diverse market demands. The adoption of hybrid varieties over open-pollinated ones offers notable advantages, including increased productivity, resilience and improved crop quality, often with enhanced resistance to pests and diseases. Among hybrids, single-cross hybrids are widely popular and result from a cross between two

inbred lines. These hybrids are highly uniform, exhibit strong hybrid vigour and have a narrower genetic base, although they are more expensive to produce. In contrast, double-cross hybrids, derived from crossing two single-cross hybrids, exhibit greater genetic diversity, broader genetic bases and moderate hybrid vigour. They are less uniform but more cost-effective to produce. With backdrop of above, in the study focused on developing lines resistant to chilli leaf curl virus (ChLCV), cucumber mosaic virus (CMV), and chilli veinal mottle virus (ChVMV) using a double-cross hybrid approach an unique prospective of understanding ChLCV genetics in the double cross hybrid under natural epiphytotic conditions was observed. As, natural resistance tends to outperform artificial screening methods in long-term reliability thus, the obtained results indicate stability and durability of resistant source.

Materials and methods

The present study was conducted during the rainy season (kharif) of 2022 under experimental field conditions at the Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru. The site is situated at 13°58' North latitude, 78°45' East longitude, and an altitude of 890 m above mean sea level. Hesaraghatta experiences a moderately warm climate, with maximum mean temperatures ranging from 30.4°C to 31.2°C (average 29.4°C) and minimum mean temperatures from 17.4°C to 20.8°C (average 18.3°C). The mean relative humidity during the study period was 59.63%.

The nursery for the double-cross F_1 lines of [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)] was established in April 2022. Seeds were sown in plug trays filled with cocopeat and maintained under insect-proof, protected conditions. Transplanting was performed in the last week of May 2022, with plants spaced at 60 × 45 cm. Fertilizers were applied at a rate of 120:60:60 kg N:P_2O_5:K_2O per hectare using urea, single super phosphate, and muriate of potash during the final land preparation. Weekly irrigation was provided, and all recommended cultural practices were adopted accordingly.

Natural screening: Natural screening for disease resistance occurred from May to August, (2022) a period characterized by high natural inoculum pressure due to increased whitefly vector populations under favorable temperature and humidity conditions. Disease reactions for individual plants were recorded 90 days after transplanting (DAT).

Genetics of disease resistance: In the research aimed to develop chilli lines with combined resistance to chilli leaf curl virus (ChLCV), cucumber mosaic virus (CMV), and chilli veinal mottle virus (ChVMV) using a double-cross breeding strategy. The study utilized three parental lines, each contributing resistance to a specific virus: IHR 4597 for ChLCV (Raichur isolate), IHR 2451 for CMV (Guntur isolate) and ChVMV (Bengaluru isolate) (Table 1). The crossing pattern employed was [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)], with IHR 4597 specifically contributing resistance to ChLCV.

Table 1. Genetic source of resistance and their inheritance

Resistant source	Virus	Genetics	Reference
IHR 2451	CMV	Oligogenic recessive	Naresh *et al.*, 2016
IHR 2451	ChVMV	Single recessive	Naresh *et al.*, 2016
IHR 4597	ChLCV	Single dominant	Rajeev *et al.*, 2022

A total of 581 double-cross F_1 plants were raised and transplanted in the experimental field under natural epiphytotic conditions to allow for disease screening under realistic virus pressure. Disease reactions for each plant were assessed 90 days after transplantation (DAT) based on visible symptoms. To confirm infection and validate the phenotypic classification, polymerase chain reaction (PCR) assays were conducted for the presence of virus.

Based on the combined evaluation of symptoms and PCR results, individual plants were categorized as either resistant or susceptible (Table 2).

Table 2. Scale adopted for ChLCV scoring on chilli plants

Score	Disease rating scale	Disease reaction
0	Resistant	No visual symptoms and PCR positive
1	Susceptible	leaf curling, vein thickening, deformed leaves, puckering and yellowing of leaves

Detection of begomovirus: Total DNA was extracted from homogenized samples of symptomatic double-cross F_1 individual lines of [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)], which were screened under natural field conditions, using the CTAB method (Doyle and Doyle, 1987). The extracted DNA was subjected to PCR amplification using begomovirus-specific primers (Table 3) to confirm the presence or absence of the virus. The PCR reaction was carried out in a 25 µL mixture, containing 1.2 µL of DNA template, 2 µL of 25 mM $MgCl_2$, 3 units of Taq DNA polymerase, 2 µL of 2 mM dNTPs, 25 pmol of the primers, and 2.5 µL of 10x PCR buffer. The amplification process was performed in an Eppendorf thermal cycler with the following cycling conditions: an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, primer annealing at 55°C for 60 seconds, primer extension at 72°C for 1 minute 30 seconds and a final extension at 72°C for 20 minutes. The PCR products were visualized on a 4% agarose gel (w/v) stained with ethidium bromide, and the gel was documented using a UVI Tech gel documentation system. The presence or absence of ChLCV-specific PCR bands was determined by comparing the observed band sizes to the expected amplicon size. The electrophoresed PCR products were visualized under UV light and documented using the UVITEC Cambridge gel documentation system.

Table 3. Primer used for detection of ChLCV Begomovirus

Primer Name	Sequence	Expected band size
MKBEG-IF1	5′-TTCCATCCRAACATTCAGGG-3′	1.2 kb
MKBEG-IR1	5′-ATCGTCATTTCTACGCCCG-3'	

Statistical analysis: A Chi-square test for goodness-of fit was conducted with the hypothesis of monogenic control of resistance to ChLCV from IHR 4597. The agreement of the observed values with the expected was tested in the genetic model appropriate for a probability (P) value > 0.05 with degrees of freedom (n-1), where n is the number of classes (Griffiths *et al.*, 2015).

Results

Chilli cultivation faces considerable challenges due to susceptibility to pests and diseases, particularly the chilli leaf curl virus (ChLCV), which has emerged as a significant threat in recent years. Under the natural epiphytotic conditions based on the visual symptoms of chilli leaf curl virus (ChLCV) observed in the 581 double-cross F_1 individual lines of [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)] the plants were categorized into

Fig. 1. Double cross F_1[(MS$_3$B × IHR 2451) × (MS$_3$B × IHR 4597)] plants. R-Resistant; S- susceptible to ChLCV

two groups: resistant and susceptible.

The susceptible plants exhibited several distinct symptoms associated with ChLCV infection. These included the upward curling of leaves, crinkling and puckering of leaf surfaces. There was a reduction in leaf area and the interveinal regions of the leaves showed signs of blistering. Other prominent features of the infected plants included vein banding, where the veins became more pronounced and the shortening of petioles and internodes, resulting in a compact, stunted appearance of the individual plants was noticed. In some cases, the leaves became tightly clustered, a condition known as "bunchy leaves," which is a typical feature of severe virus-induced stunting (Fig. 1). Furthermore, a subset of the susceptible plants exhibited additional symptoms, such as flower bud abscission, poor fruit set and the development of distorted or underdeveloped fruits, leading to a reduced fruit yield (Fig. 2).

Out of the total of 581 plants observed, 298 showed symptoms of susceptibility to ChLCV, while the remaining 283 plants displayed no disease symptoms and grew vigorously, suggesting resistance to the virus. Based on the expected inheritance pattern of a monogenic dominant trait for ChLCV resistance, the plants should segregate in a 1:1 ratio (resistant: susceptible), as per Mendelian genetics. The observed segregation in the study—283 resistant plants and 298 susceptible plants—was consistent with the expected 1:1 ratio, as confirmed by Chi-square analysis (Fig 4), indicating that the resistance to ChLCV in these plants is controlled by a single dominant gene.

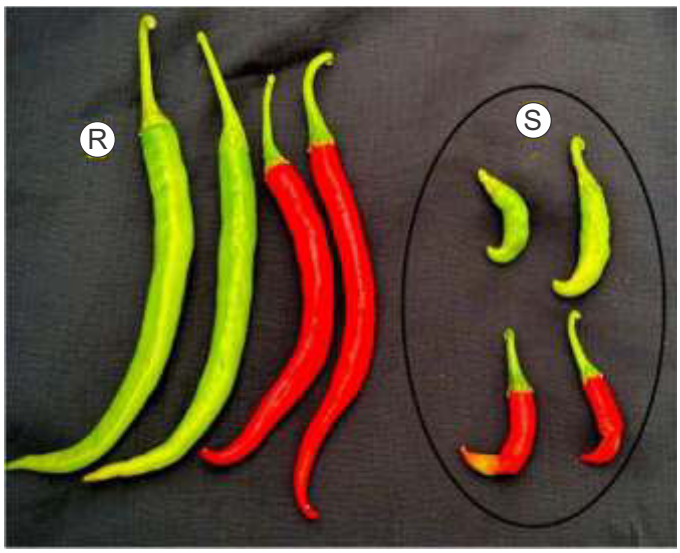

Fig. 2. Double cross F_1[(MS$_3$B × IHR 2451) × (MS$_3$B × IHR 4597)] fruits. R-Resistant; S- susceptible to ChLCV

To further confirm the presence of the virus in the susceptible plants, PCR assays were performed using begomovirus specific primers designed to detect ChLCV. The results showed an amplification product of approximately 1.2 kb in size in the susceptible plants (Fig. 3), indicating presence of ChLCV-specific DNA fragment. This molecular confirmation of infection supported the visual observations of disease symptoms. On sequencing the chilli leaf curl infected samples, it was confirmed

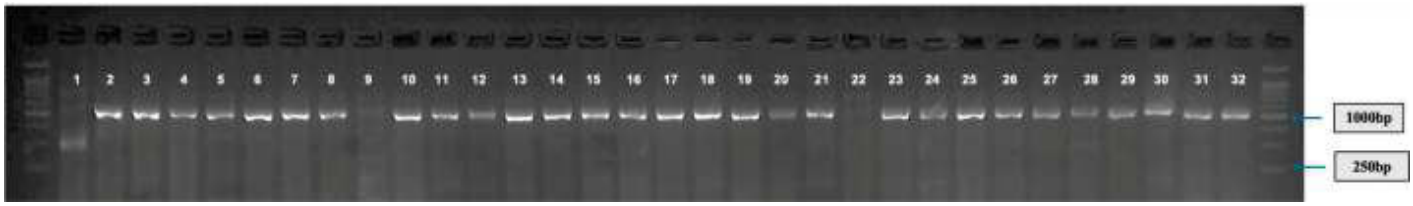

Fig. 3. Confirmation of ChLCV infection susceptibility through PCR assay

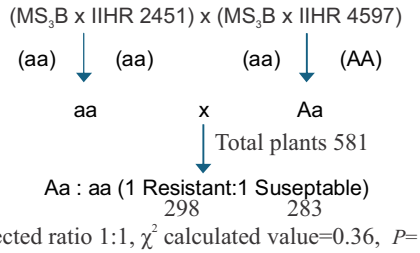

Fig. 4. Segregation of chilli leaf curl virus resistance in Double cross F1 population.

as the Raichur isolate.

Discussion

Chilli is an important vegetable and spice crop, crucial to the food and pharmaceutical sectors, extending its value beyond culinary uses. However, chilli is susceptible to various diseases, particularly viral infections, which pose a major threat and result in significant production losses. While insecticides have been used to manage these pests, resistance and resurgence remain major challenges, making them environmentally unsafe. Host plant resistance provides a more effective and sustainable solution. Genotypes or lines exhibiting leaf curl resistance under natural epiphytotic conditions are particularly valuable in plant breeding, offering greater resilience and adaptability compared to artificially screened individuals.

Sources of resistance to begomoviruses in pepper have been identified in various accessions which includes *C. chinense* accessions BG-3821 from Mexico (Anaya-Lopez *et al.,* 2003) and 'Bhut Jolokia' from India (Adluri *et al.,* 2017). *C. annuum* accessions with reported resistance include DLS-Sel-10, WBC-Sel-5, and several PBC lines (Srivastava *et al.,* 2015; 2017; Kenyon *et al.,* 2014; Kumar *et al.,* 2006; Singh *et al.*, 2016), as well as S-343 (Thakur *et al.,* 2019).

In the present study, a double cross population was developed to incorporate multiple virus resistant lines against CMV, ChVMV, and ChLCV. Each parent in the cross displayed resistance to a specific virus. The crossing strategy involved [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)], with IHR 4597, which exhibited 84% resistance to ChLCV, showing intermediate resistance as reported by Rajeev *et al.* (2022). The double cross F_1 population [(MS_3B × IHR 2451) × (MS_3B × IHR 4597)], when field-planted, displayed symptoms of chilli leaf curl virus (ChLCV) infection. As a result, the individual lines segregated for their resistance to ChLCV with the Chi-square analysis of the segregation, pattern revealed a 1:1 goodness-of-fit ratio with 283 resistant and 298 susceptible plants. This indicates that the resistance to ChLCV follows a monogenic dominant inheritance pattern. Similar studies conducted by Pradeep *et al.* (2020) studied the inheritance of ChLCV resistance by crossing the resistant genotype DLS-Sel-10 with the susceptible Phule Mukta. The F_1 generation showed susceptibility, indicating recessive resistance, governed by a single recessive gene as confirmed by chi-square test. Thakur *et al.* (2020) identified monogenic dominant resistance in the S-343 genotype at Punjab Agricultural University. They used an F_2 mapping population and found two linked SSR markers (CA 516044 and PAU-LC-343-1) on chromosome 6, at genetic distances of 6.8 cM and 8.9 cM, respectively. Garcia-Neria and Rivera Bustamante (2011) reported resistance in BG-3821 to be controlled by two genes with a duplicate recessive epistatic relation, while Rai *et al.* (2014) found a single recessive gene controlling resistance to PepLCV in 'Bhut Jolokia'.

The Chilli Leaf Curl Virus (ChLCV) is a devastating disease affecting chilli plants, transmitted by the vector whitefly. Chilli crops thrive under bright sun (summer season), which unfortunately coincides with the period of high fecundity for whiteflies, leading to the rapid spread of the virus. Therefore, integrated and eco-friendly approaches for controlling the vector, along with the use of resistant varieties by harnessing host plant resistance, are crucial. Understanding the genetics of specific traits is fundamental to any breeding program. In this study, we present our findings on IHR 4597, which shows a monogenic dominant nature of resistance to ChLCV. This segregation of 1:1 ratio for resistant to susceptible plants in a double cross F_1 population of [(MS_3B x IHR 2451) x (MS_3B x IHR 4597)] was observed under natural epiphytotic conditions.

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Compliance: This article does not contain any studies with human or animal subjects performed by any of the authors.

Ethical approval: This article does not contain any studies with human participants performed by any of the authors.

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