Research Article

Assessment of Susceptibility of Seven Sweet Pepper Cultivars to Chili Leaf Curl Virus Infection under Plastic House Conditions

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Abstract | Abstract This study aimed to evaluate the impact of Chili leaf curl virus (ChiLCV) on various growth parameters of seven sweet pepper cultivars grown under plastic house conditions. The evaluation was conducted 85 days after planting and the tested cultivars included King Green, Caiman, Crusader, Carisma, summer, Rio, and California Wonder. Parameters assessed included infection rate and severity, plant height, number of branches, fresh and dry weights of shoot and root systems, fruit number and weight, yield per plant, chlorophyll content, and peroxidase enzyme activity. Results revealed that King Green and Caiman cultivars showed significantly higher tolerance to ChiLCV compared to the others, with no significant difference amongst themselves. These cultivars recorded the lowest disease severity (15.24% and 19.34%, respectively), the highest chlorophyll content (38.32 and 33.58 SPAD), and elevated peroxidase activity (67.21 and 61.54 unit.min^{-1}.g^{-1} fresh weight). In contrast, California Wonder and Rio were the most susceptible cultivars, showing the highest severity rates (58.66% and 52.38%), lowest chlorophyll levels (16.18 and 12.43 SPAD), and reduced peroxidase activity (34.86 and 28.21 unit.min^{-1}.g^{-1} fresh weight). Crusader, Carisma, and summer cultivars displayed intermediate responses. The origin of the virus was confirmed using the polymerase chain reaction (PCR) assay and specialized primers (ChiLCVR/ChiLCVF), and data confirmed specific and expected amplification of the DNA of the virus isolate (ChiLCV) (600 base pairs).

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Keywords | Chili leaf curl virus (ChiLCV), Sweet pepper cultivars, Virus susceptibility, Peroxidase activity, Chlorophyll levels, Various growth parameters

Introduction

Sweet pepper (*Capsicum annuum* L.) is a member of the Solanaceae in which the species is very popular and widely grown throughout the world. Sweet pepper production in Iraq during 2018-2020 were 915 tons from an area of 5860 dunam (Hamza and Ali, 2020). It is a warm season crop and is cultivated in the field as an open crop early spring and protected crop early autumn in Iraq (Muslih and Rasool, 2019).

Viral diseases represent one of the major constraints to pepper production globally today. Approximately 75 viruses are known to infect pepper plants, with

37 species officially recognized by the International Committee on Taxonomy of Viruses (ICTV) (Thomas *et al.*, 2021). Among these, Chili leaf curl virus (ChiLCV) stands out as one of the most destructive pathogens affecting peppers, causing severe yield losses (Shingote *et al.*, 2024). ChiLCV belongs to the genus Begomovirus within the family Geminiviridae. Its genome consists of a circular, single-stranded DNA molecule (ranging from 2600–2700 nitrogenous bases), typically composed of two components: DNA-A and DNA-B (Senanakaye *et al.*, 2012; Shingote *et al.*, 2022). The virus is transmitted by the whitefly vector Bemisia tabaci in a persistent and circulative manner. Symptoms of infection with the virus are represented by vein necrosis, spotting, wrinkling, yellowing of the leaf edges, and their appearance as a boat (Fayyaz *et al.*, 2019; Susmitha *et al.*, 2022; Jones, 2022). ChiLCV was first recorded on C. annum plants in India in 2004 and caused economic losses in pepper crops in many countries, with infection severity reaching 100%. It then spread to European countries, Asia, and Africa (Mishra *et al.*, 2020; Alwan *et al.*, 2025; Talib *et al.*, 2025).

Farmers relied heavily on insecticides to control the whitefly Bemisia tabaci, but complete reliance on pesticides Insect pests have led to numerous environmental and human health risks. Therefore, researchers have resorted to adopting alternative and more environmentally friendly methods to manage plant viruses, including the use of resistant varieties (Prasannathe *et al.*, 2020). Infection with ChiLCV significantly affects photosynthesis and vegetative growth in pepper plants, leading to a decrease in chlorophyll production in the leaves, which affects the effective transport of nutrients within the plant and deteriorates the vegetative performance of infected plants (Kushwah *et al.*, 2019; Ali *et al.*, 2020; Sran *et al.*, 2023).

Also, infection with the virus weakens the ability of plants to absorb light and reduces the plant's ability to produce chlorophyll by affecting the enzymes responsible for chlorophyll production, disrupting the photosynthesis process in general, and accumulating toxic substances inside the plant cells, which prevents the absorption of nutrients such as nitrogen, magnesium, and iron, which are essential elements in the production of chlorophyll (Bhattacharyya *et al.*, 2015; Bhattacharyya and Chakrabotry, 2018). ChiLCV infection leads to a decrease in peroxidase activity along with changes in phenolic components, and an increase in enzyme activity in resistant plants. This enzyme is considered essential for cellular functions, as it enhances the plant host's defenses against pathogens by binding glycoproteins to strengthen the cell wall when exposed to pathogens (Wong *et al.*, 2023). It has a role in the physiological processes of plant life from germination to senescence and may be present on the cell membrane or in the cytoplasm inside the cell (Iqbal *et al.*, 2023). Asif *et al.* (2023) and Singh *et al.* (2024) found that peroxidase is the main enzyme in the biosynthesis of quinine and the deposition of suberin, as well as the synthesis of alpha-toxins. It also works to oxidize phenolic substances when exposed to pathogens, converting phenolic compounds to quinones. It also contributes to the oxidation of phenolic compounds and converting them to Lignin-like substances in plant cell walls at sites of penetration contribute to the symptoms of infection.

Materials and Methods

The experiment was conducted under natural conditions during the spring and summer seasons of 2023–2024 in a plastic house located within the research fields of the Department of Plant Protection, College of Agricultural Engineering Sciences. The total area of the plastic house was 160 m^2. Land preparation involved plowing to a depth of 30–40 cm, followed by leveling and installation of a drip irrigation system. Seven sweet pepper cultivars—Summer, Carisma, Crusader, Ciaman, King Green, Rio, and California Wonder—were used in this study. These cultivars were established as transplants in Styrofoam and sourced from Green Country Nursery in the Al-Yusufiyah region. All are among the most widely cultivated and commercially favored cultivars in various Iraqi provinces (Table 1). Transplanting into the plastic house was carried out on March 17, 2024. The experimental design followed a Randomized Complete Block Design (RCBD) with six replicates per treatment. The plastic house measured 8 meters in width and 20 meters in length. Pepper plants were spaced 40 cm apart within rows, and 1.5 meters between rows. The number of plants in the experimental unit was 8 plants. Crop management practices were carried out periodically until the end of the season, with daily monitoring of plants to record the development of infection symptoms and calculate the percentage and severity of viral infection.

Table 1: *Types, cultivars, producing companies, and origins of the pepper plants used in the study*

Arrangement	Cultivars	Origin	Producing company
1	King green	Holland	Molenzaden
2	California wonder	America	Elite
3	Rio	Holland	Molenzaden
4	Crusader	Switzerland	Syngenta
5	Summer	Holland	Molenzaden
6	Carisma	Spain	Fito
7	Caiman	France	Clause

The Infection rate was calculated using the following formula:

$$\text{Infection rate \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ x } 100$$

(Sharma *et al.*, 2018)

The disease severity was assessed based on the rating scale developed by Sharma *et al.* (2018).

Table 2: *Scale used for natural examination of Chili leaf curl virus (ChiLCV)*

Symptoms	Disease scale	Disease reaction (DR)
Upper leaf spotting and 0–4% transparency (Slight leaf distortion)	1	High resistance (HR)
4–25% Wrinkles, few yellow spots and swelling of leaves	2	Resistant (R)
25–50% Leaf curl, leaf scorch, and vein swelling	3	Moderate susceptibility to infection (MS)
Wrinkling and curling of leaves by 50–75%, stunted growth, and the appearance of blisters on the nodes.	4	Susceptible (S)
75–100% of leaves are wrinkled, small and distorted, boat-like, plant growth is stunted, flowers are small, no fruits or fruits are small, perforated and twisted	5	Highly susceptible (HS)

The studied parameters included plant height and number of branches per plant, measuring plant chlorophyll content, measuring peroxidase activity, and productivity in terms of number of fruits per plant, fruit weight per treatment, fresh and dry weight of the shoot, and fresh and dry weight of the root system 85 days after planting. The experiment was laid out using a Randomized Complete Block Design (RCBD) comprising 7 treatments, each replicated six times. Each experiment unit consist of 8 plants, so the number of experimental plants was 7 x 6 x 8 = 336 plants. Readings were recorded and the results were statistically analyzed using Microsoft Excel at a least significant difference (LSD) of 0.05%.

Genomic DNA extraction from pepper plants

Total DNA was extracted using the Plants Total DNA Mini Kit provided by Geneaid, following the manufacturer's recommended protocol.

Polymerase chain reaction (PCR) analysis

PCR amplification was performed using the Maxime PCR Premix Kit (I-Taq), Cat. No. 25026, manufactured by Bioneer, South Korea. The reaction was carried out using ChiLCV-specific primers:

ChiLCV-F: CATATTCGCCAGACACATTAG
ChiLCV-R: CGTGCCATTTCCTCAAGAC

PCR amplification of ChiLCV DNA was performed under the following thermal cycling conditions:
Initial denaturation at 94°C for 5 minutes
Followed by 35 cycles of: Table 3

Table 3: *shows the steps of thermal cycle for the N-PCR reaction.*

Step	Condition	Time
Denaturation	94	30Sec.
Primer annealing	53	45Sec.
Initial elongation	72	1Min
Final elongation	72	5Min

Agarose gel electrophoresis

A 2% agarose gel was prepared using TBE 1XL buffer, which was dissolved by heating in a microwave at 100 °C for 15 minutes. Afterward, the solution was cooled to approximately 50 °C. Ethidium bromide was added to the gel mixture at a concentration of 10 µg/mL (5 µL per gel volume). The casting tray, equipped with a comb on one end, was set up to form wells within the gel layer. The agarose solution was then poured into the tray and left to solidify at room

temperature for 15 minutes. After solidification, the comb was carefully removed, and the tray was placed into the electrophoresis apparatus containing TBE 1XL buffer.

PCR products (10 µL) were loaded into each well, with 5 µL of a 100 bp DNA ladder loaded into the first well. Electrophoresis was conducted at 135 volts and 80 milliamps for 30 minutes. PCR product visualization was performed using the EZ-Capture MG system from ATTO Corporation, Japan.

Chlorophyll content measurement

Chlorophyll content was measured using a SPAD-502 Plus Chlorophyll Meter. For each experimental unit, three SPAD readings were taken from three separate leaves per plant, and the average of these readings was recorded (Kamarianakis & Panagiotakis, 2023).

Estimation of peroxidase activity in sweet pepper cultivars

Fresh leaves were collected from each of the seven sweet pepper cultivars and transported to the Agricultural Research Directorate in an ice-cooled container. After rinsing with distilled water and blotting dry with filter paper, 1 g of leaf tissue was manually chopped into small pieces. The leaves were then ground in a porcelain mortar with the addition of 2 mL of 0.01 molar sodium phosphate buffer (SPB) at pH 6.5. The sample was centrifuged at 4 °C for 2 minutes at 6000 revolutions per minute. 100 microliters of the supernatant extract were taken and 1.5 ml of 0.05 M Progall solution was added, followed by 100 microliters of 1% hydrogen peroxide. The sample was placed in a spectrophotometer at 420 nm, with 4-6 consecutive readings taken at 15-second intervals. Enzyme activities were calculated from the following formula:

Enzyme units/mL=3× Absorbance÷ ΔTime ×0.001
Where:

- Δ Absorbance: Change in light absorbance.
- Δ Time: Time taken for the change in absorbance

One unit of the enzyme is the activity that results in an increase in light absorbance of 0.01 at 420 nm per min. The amount of peroxidase enzyme was calculated according to Hammerschmidt *et al.* (1982)

Results and Discussion

Detection of ChiLCV in sweet pepper cultivars using polymerase chain reaction (PCR)

Chili leaf curl virus (ChiLCV) in naturally infected sweet pepper leaves showing typical symptoms, was detected after extraction of viral DNA. The PCR was performed with the specific primers (ChiLCVF/ ChiLCVR). The reaction amplified the viral DNA, resulting in the formation of a band of the expected size near 600 base pairs to detect the presence of ChiLCV in the Figure 1. Amplified DNA products using Polymerase Chain Reaction (PCR) from Chili leaf curl virus (ChiLCV) isolates in sweet pepper cultivars, visualized with ethidium bromide stain. DNA fragments appear at a molecular weight of 600 bp. A DNA ladder is shown on the left.

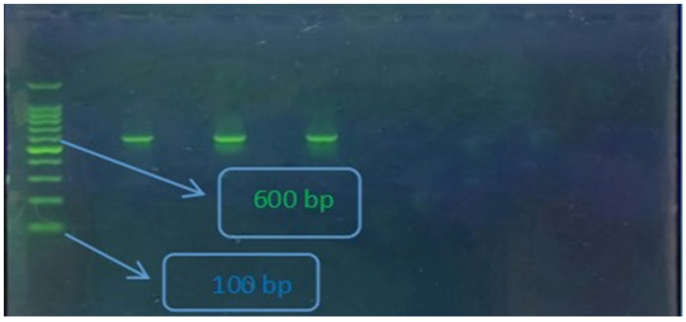

Figure 1: *Amplified DNA products using Polymerase Chain Reaction (PCR) from Chili leaf curl virus (ChiLCV) isolates in sweet pepper cultivars, visualized with ethidium bromide stain. DNA fragments appear at a molecular weight of 600 bp. A DNA ladder is shown on the left.*

Assessment of incidence and severity of chiLCV Infection in sweet pepper cultivars

The results confirmed that all tested sweet pepper cultivars were susceptible to Chili leaf curl virus (ChiLCV), with statistically significant differences among cultivars in terms of infection rate and severity (Figure 2). Notably, the cultivars King Green and Caiman showed significantly lower infection levels, recording the greatest reduction indisease incidence (25.28% and 30.57%) and severity (15.24% and 19.34%), respectively, 85 days after transplanting. It was found that the cultivars California Wonder and Rio of sweet pepper were the most susceptible among the tested cultivars to the virus after 85 days of transplanting, with infection rates of 72.14% and 66.67% and infection severities of 58.66% and 52.38%, respectively (Table 4).

Figure 2: *The visual symptoms of ChiLCV on the susceptible sweet pepper cultivars.*

Table 4: *Effect of ChiLCV on incidence and severity of infection 85 days after transplanting*

Cultivar	Infection severity after 85 days	Impact rating	Infection rate after 85 days
King green	15.24	R	25.28
California wonder	58.66	S	72.14
Crusader	39.60	MS	45.26
Summer	35.67	MS	41.94
Rio	52.38	S	66.67
Carisma	32.11	MS	49.68
Caiman	19.34	R	30.57
LSD (0.5%)	11.965		10.807

Effect of ChiLCV Virus on some vegetative growth indicators

The results showed a clear effect of infection with the ChiLCV virus (Chilli Leaf Curl Virus), 85 days after planting, on plant height and number of branches. Variations were observed in both parameters among all tested varieties.

The two varieties King Green and Ciaman significantly outperformed the other varieties. Moreover, there was a statistically significant difference between these two resistant varieties in terms of plant height after 85 days; their heights were 76.59 cm and 81.49 cm per plant, respectively, with branch numbers reaching 28.16 and 31.33 branches per plant (Figure 3).

In contrast, the varieties California Wonder and Rio recorded the lowest values for both plant height and branch count among the tested cultivars. Their plant heights were 46.23 cm and 50.73 cm per plant (Figure 4), while the average number of branches was 14.82 and 17.74 branches per plant, respectively. These two varieties did not differ significantly from each other, but they differed significantly with moderate differences from other cultivars such as Ciaman, Summer Carisma, and Crusader, and showed highly significant differences compared to the more resistant varieties King Green and Ciaman, as shown in Table 5.

These findings are consistent with Das *et al.* (2021), who reported that infection with the ChiLCV virus caused a significant reduction in plant height and number of branches. This study also aligns with previous studies indicating that virus-affected plants have smaller leaves and shorter branches, giving them a dense and stunted appearance (Senanayake *et al.*, 2012). This is attributed to the impact of the ChiLCV virus on the plant's ability to absorb nutrients and water, which reduces major metabolic processes and weakens overall plant growth, resulting in reduced plant height.

Additionally, the virus affects the plant's ability to produce energy through photosynthesis, reducing the available energy required for branch formation. It also hinders the movement of nutrients within the plant, negatively affecting vegetative growth, decreasing branching, and weakening the plant's ability to form new branches (Zeeshan & Kudada, 2019; Kushwaha, 2019).

This study also confirmed the effect of the ChiLCV virus on the fresh and dry weights of all tested chili pepper varieties infected 85 days after planting. The varieties showed clear differences in fresh and dry weight averages.

The varieties King Green and Ciaman significantly outperformed the rest in terms of fresh shoot weight and dry root weight. For example, the fresh shoot weights were 312.64 g and 324.17 g per plant, respectively, while the fresh root weights were 41.980 g and 46.20 g per plant, respectively. The dry shoot weights were 135.630 g and 142.625 g, and the dry root weights were 22.758 g and 26.338 g per plant, respectively.

In comparison, the varieties California Wonder and Rio gave the lowest statistically significant values for these traits, with no significant difference between them. The fresh shoot weights were 210.69 g and 225.39 g, and the fresh root weights were 24.432 g

Table 5: *Effect of ChiLCV on the height, number of branches, and fresh and dry weight of the shoot and root systems of sweet pepper cultivars*

Cultivar	Plant height after 85 days (cm)	Number of branches after 85 days (branches/plant)	Fresh weight of shoot system (g)	Fresh weight of root system (g)	Dry weight of shoot system (g)	Dry weight of root system (g)
King green	76.59	28.16	312.64	41.980	135.630	22.758
California	46.23	14.82	210.69	24.432	79.335	8.330
Crusader	61.17	21.13	258.26	34.348	115.132	16.073
Summer	65.84	23.16	269.38	36.840	120.213	18.248
Rio	50.73	17.74	225.39	28.192	84.397	11.678
Carisma	59.73	20.08	250.27	34.593	112.212	15.795
Ciaman	81.49	31.33	324.17	46.20	142.625	26.338
LSD(0.5%)	3.828	2.585	22.984	2.277	11.99	2.961

and 28.192 g per plant, respectively. The dry shoot weights were 79.335 g and 84.397 g, and the dry root weights were 8.330 g and 11.678 g per plant, respectively. These values were significantly different from those of the other chili pepper varieties, as presented in Table 5.

These results are consistent with Ghafoor *et al.* (2022), who indicated that the decrease in fresh and dry weights of shoots and roots due to ChiLCV infection may be attributed to a reduction in essential elements such as zinc sulfate, copper sulfate, manganese sulfate, and uric acid, which play important roles in various physiological processes that can alter the plant's response to viral infection.

Figure 3: *shows the difference in plant height between the cultivars king green and california wonder*

Furthermore, Kumar *et al.* (2018); Kushwah *et al.* (2019); and Asif *et al.* (2023) have noted that infection with ChiLCV leads to the accumulation of toxic compounds such as free amino acids and phenolic substances in infected plant tissues. These compounds cause oxidative stress, which reduces the plant's ability to absorb water and nutrients, impairs internal transport processes, and disrupts metabolism. This occurs due to the virus's effect on vascular tissues, limiting the delivery of water and nutrients to different parts of the plant, thus weakening growth and affecting both root and shoot biomass.

Table 6: *Effect of ChiLCV on the yield of sweet pepper cultivars*

Cultivar	Number of fruits (fruit/plant)	Fruit weight (g/fruit)	Yield per plant (g/plant)
King green	11.57	2.100	0.2520
California	2.99	0.7650	0.0833
Crusader	6.28	1.282	0.1570
Summer	8.74	1.678	0.1988
Rio	3.34	0.92	0.0738
Carisma	8.08	1.147	0.1425
Ciaman	14.77	2.250	0.2934
LSD(0.5%)	1.6808	0.4738	0.0577

The results also showed a difference in the rates of the varieties among themselves in the number of fruits formed, fruit weight and plant yield after 85 days of planting. The King green and Caiman varieties of pepper plants achieved a significant superiority over the rest of the tested varieties, as they gave the highest rates of fruit weight, number of fruits and yield per plant with rates of 2.100, 2.250 g/fruit, 11.57, 14.77 fruits/plant and 0.2520, 0.2934 g/plant, respectively. On the other hand, the sensitive varieties California wonder, and Rio gave the lowest rates with rates of 0.7650, 0.92 g/fruit, 2.99, 3.34 fruits/plant and 0.0833, 0.2934 g/plant, respectively, which differed significantly from the rest of the varieties, Table 6.

The results also showed that the King green and Caiman cultivars significantly outperformed the

other tested cultivars in terms of chlorophyll content 85 days after planting, with values of 38.32 and 33.58 SPAD units, respectively. These two cultivars also exhibited the highest peroxidase enzyme levels after 85 days, reaching 67.21 and 61.54 (unit. minute.g. fresh weight), respectively.

Figure 4: *The difference in plant height between the cultivars ciaman and rio.*

Table 7: *Effect of ChiLCV on chlorophyll content and peroxidase enzyme activity in sweet pepper cultivars*

Cultivar	Chlorophyll after 85 days (SPAD)	Peroxidase enzyme after 85 days (unit.min^{-1}.g^{-1} fresh weight)
King green	38.32	67.21
California wonder	16.18	34.86
Crusader	23.67	43.30
Summer	27.53	53.85
Rio	12.43	28.21
Carisma	21.91	49.39
Ciaman	33. 58	61.54
LSD (0.5%)	3.6777	5.295

In contrast, the highly susceptible cultivars, California wonder and Rio, showed the lowest chlorophyll content after 85 days, with values of 16.18 and 12.43 SPAD units, respectively. They also had the lowest peroxidase enzyme levels, at 34.86 and 28.21 (unit. minute.g. fresh weight), respectively (Table 7). These findings are consistent with previous studies indicating that the ChiLCV virus causes a significant decrease in chlorophyll content in susceptible plant varieties compared to resistant ones. This decrease is considered to be due to disruption of the chloroplast membrane system and destruction of the chloroplast envelopes upon infection and which would lead to decline in plant photosynthetic efficiency (Chia *et al.*, 1999; Funayama *et al.*, 1997; Guo *et al.*, 2005; Chaubey & Mishra, 2020). Moreover, elevated concentrations of phenolics, tannins and chlorophyll were detected in the resistant against the susceptible varieties. These may have a preventive role in the defense against pathogens as they may participate in metabolic reactions in relation to plant resistance (Singh *et al.*, 2013; Choubey & Mishra, 2020; Taufik *et al.*, 2024).

Conclusions

This study revealed variability among the sweet pepper cultivars under plastic house for their response against ChiLCV infection. Cultivars King Green and Caiman presented the greatest resistance levels, as manifested by low infection rates and aggressiveness as well as high vegetative growth, chlorophyll content and peroxidase activity. California Wonder and Rio were in contrast to these two, the most sensitive, which exhibited severe disease and great reductions in growth and physiological quality. This information is useful for developing appropriate management strategies, including the cultivation of resistant cultivars for the management of ChiLCV in sweet pepper production systems to complement the growing pool of knowledge on this topic and to assist in the design of environmentally friendly, and efficient strategy.

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Novelty Statement

The most resistant and most susceptible varieties under natural infection conditions have been identified, and these are among the most important varieties cultivated in Iraq.

Author's Contribution

Suadad A. Ibrahim: Conceptualization and overall management, data collection and field research, Wrote abstract, Introduction and methodology
Layla J. Sabr: Wrote results, discussion, conclusion and references.

Generative AI and AI-assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this

manuscript.

Conflict of interest

The authors have declared no conflict of interest

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