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RESEARCH PAPER

Molecular characterization and effectiveness cross-protection of weak strains against super-infection malignant strains *Cucumber mosaic virus* (CMV) on cayenne pepper (*Capsicum annuum* L.) in Lampung, Indonesia

Mahfut[1], Mai Sari[1], Hasriadi Mat Akin[2], Sri Wahyuningsih[1], & Bambang Irawan[1]

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ABSTRACT

Cayenne pepper (*Capsicum annuum* L.) is a horticultural crop of significant economic importance. One of the major threats to its cultivation is the *Cucumber mosaic virus* (CMV), which can cause severe yield losses and crop failures. This study aimed to isolate weak CMV strains from natural populations of cayenne pepper, characterize their molecular properties, and evaluate the effectiveness of cross-protection conferred by weak strains against superinfection by virulent CMV strains. The research was conducted in three stages: virus isolation, molecular characterization, and evaluation of cross-protection effectiveness. Data analysis was performed using the MEGA v.11.0.11 software and analysis of variance (ANOVA). The findings identified the C1 isolate as a highly virulent CMV strain. Molecular analysis of naturally infected samples confirmed the presence of CMV through the amplification of specific DNA bands. Inoculation tests revealed distinct differences between weak and malignant strains, particularly in symptom severity and molecular characteristics, such as genetic distance. Furthermore, the weak strains demonstrated a protective effect, significantly reducing the infection rate of virulent CMV strains. This was evidenced by variations in symptom expression, disease severity, plant resistance, chlorophyll levels, carbohydrate content, and peroxidase enzyme activity. These findings highlight the potential application of weak CMV strains in developing disease management strategies for cayenne pepper cultivation.

Key words: cayenne pepper, cross-protection, *Cucumber mosaic virus* (CMV), molecular characterization, weak strains

INTRODUCTION

Cayenne pepper (*Capsicum annuum* L.) is an important horticultural comodity with high market demand (Marianah, 2020). However, according to the Central Statistics Agency (2022) cayenne pepper production in Lampung, Indonesia, remains insufficient to meet market demand. Several factors contribute to this shortfall, including viral, bacterial, and fungal infections that significanly reduce crop yields (Mahfut, 2020; Mahfut et al., 2020c; Mahfut et al., 2023a; Putra et al., 2024; Mahfut et al., 2020a; Mahfut, 2021; Sukmawati et al., 2021; Mahfut et al., 2020b). Among these, *Cucumber mosaic virus* (CMV) is recognized as the most infectious agent affecting cayenne pepper (Li et al., 2020; Niemann et al., 2021).

Corresponding author:
Mahfut (mahfut.mipa@fmipa.unila.ac.id)

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung. Jl. Prof. Soemantri Brodjonegoro 1, Lampung, Indonesia 35145
[2]Department of Plant Protection, Faculty of Agriculture, Universitas Lampung. Jl. Prof. Soemantri Brodjonegoro 1, Lampung, Indonesia 35145

CMV belongs to the *Geminivirus* family and is transmitted by whiteflies (*Bemisia tabaci*) in a non-persistent manner (Vinodhini et al., 2020). Infected plants exhibit mosaic symptoms on the upper leaves, with young leaves turning from normal or dark green to light yellowish-green stripes. Additionally, infected plants often experience stunted fruit development and reduced fruit formation at the top of the stem (Yu et al., 2000). CMV is widespread in Indonesia and has been reported in cayenne pepper plantations with varying disease incidence rates (Amisnaipa et al., 2024).

Controlling CMV in cayenne pepper presents a greater challenge compared to fungal, bacterial, or nematode pathogens due to the virus's high genetic diversity and broad host range, as well as its transmission by multiple whitefly species (Li et al., 2020; Setiawati et al., 2021). Given these challenges, an effective and efficient control strategy is needed to mitigate CMV infections in cayenne pepper. One promising approach is cross-protection, which involves using a mild virus isolate to protect crops from economic damage caused by a more virulent isolate of the same virus. This mechanism works through several pathways, including interference with viral replication, inhibition of virus particle assembly, restricted translocation within the host plant, and the induction of systemic resistance (Akin, 2022).

The principle of cross-protection relies on introducing a mild strain of CMV to outcompete or antagonize virulent strains, thereby preventing severe symptoms (Raja et al., 2022). However, the success of this approach depends on the interaction between the mild strain, the virulent isolate, and the host plant. In some cases, a mild strain can act as a helper virus to CMV, but in other cases, it may suppress the pathogenic strain, reducing disease severity (Abebe et al., 2021). This technique has been successfully tested in field trials on tomatoes (1988), chili peppers (1989), and cucumbers (1990) (Mahfut et al., 2015; Mahfut et al., 2023b; Mahfut et al., 2023c).

Although CMV infections in cayenne pepper are frequently observed in Lampung, no research has been conducted on using weak strains for cross-protection in controlling this virus. Therefore, molecular analysis of CMV infecting cayenne pepper is essential as a foundation for developing effective control strategies. This study aims to investigate the association between mild CMV strains and their role in suppressing virulent isolates, potentially offering a viable cross-protection strategy for cayenne pepper plantations in Indonesia.

MATERIALS AND METHODS

Research Site. Surveys and sample collection were conducted in South Lampung and Tanggamus Regencies, the two largest cayenne pepper-producing areas in Lampung, Indonesia. A purposive sampling technique was used to select locations with significant cayenne pepper production. Sampling sites included Muara Putih Village (Natar District, South Lampung Regency, 5°17′2.400″ South Latitude and 105°14′9.600″ East Longitude, Sabah Balau Village (Tanjung Bintang District, South Lampung Regency, 5°22′44.4″ South Latitude and 105°19′51.6″ East Longitude, and Srimenganten Village (Pulau Panggung District, Tanggamus Regency, 5°18’29.5” South Latitude and 104°39’34.2” East Longitude).

Cayenne pepper leaves exhibiting symptoms suspected to be caused by CMV—such as mosaic patterns, yellowing, and severe chlorosis—were collected based on the criteria described by Kandito et al. (2020). Leaf samples were placed in envelopes containing silica gel, sealed in plastic bags, and transported to the Laboratory of Agriculture Biotechnology, Faculty of Agriculture and Laboratory of Biomolecular, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung for further analysis.

Molecular Characterization. DNA extraction was performed using the Genomic DNA Mini Kit (Plant) from Geneaid following the manufacturer’s protocol. The extracted DNA was dissolved in TE buffer and stored at -40 ºC. CMV detection was conducted using PCR amplification with universal primers for Begomovirus, following the protocol described by Akin et al. (2013). The primer sequences were: Krusty (Forward): 5’-CCNMRDGGHTGTGARGGNCC-3’ and SPG2 (Reverse): 5’-VDGCRTGVGTRCANGCCAT-3’. The expected PCR product size was approximately 912 bp. The amplification reaction was performed using a Labcycler 48 Gradient Thermocycler (Senso Quest) with the following thermal cycling conditions: initial denaturation at 95 ºC for 3 min, denaturation at 95 ºC for 1 min, annealing at 55 ºC for 30 s, extension at 75 ºC for 1 min 30 s, and final extension at 72 ºC for 10 min. The denaturation to extension stages was carried out for 40 cycles. The amplified DNA fragments were subjected to electrophoresis on a 1% agarose gel at 50 V for 50 min and visualized using the Digi-Doc Imaging System (Major Science). For sequencing analysis, selected PCR products were sent to 1st Base Company, Malaysia, via PT Genetika Science.

Virus Inoculation. A weak CMV strain was inoculated into cayenne pepper plants at 20 days after planting (DAP). Symptom development was monitored, and at 30 DAP, plants that exhibited mild or no symptoms were subsequently inoculated with a virulent CMV strain. Symptom observations continued for three weeks post-inoculation (WPI), following the criteria described by Akin (2005). The severity and progression of disease symptoms were analyzed using the formulas established by Akin (2005) and Hasfiah (2018), in accordance with the methodological framework proposed by Mahfut et al. (2021).

Physiological Analysis. Physiological responses were assessed by measuring chlorophyll and carbohydrate levels as well as peroxidase enzyme activity.

Chlorophyll Analysis: Chlorophyll a, b, and total chlorophyll contents were quantified using a spectrophotometer, following the method described by Mahfut et al. (2021). Calculations were performed based on the formulas provided by Mahfut et al. (2023a).

Carbohydrate Analysis: Carbohydrate content was analyzed using a UV-Vis spectrophotometer at a wavelength of 488 nm, following the protocol outlined by Mahfut et al. (2023c). The analysis involved generating a glucose standard curve, measuring sample absorption, and calculating carbohydrate levels using established formulas (Mahfut et al., 2023a).

Peroxidase Enzyme Activity: Peroxidase activity was analyzed following the method described by Mahfut et al. (2023c). Cayenne pepper, red chili, and tomato leaf samples (1 g each) were ground using a mortar and pestle, and 0.5 mL of 1% H_2O_2 was added. Absorbance was measured at a maximum wavelength of 420 nm using a spectrophotometer.

Data Analysis. Sequencing results were analyzed using BLAST (NCBI) to identify closely related CMV isolates. The percentage identity of the test sample sequences was determined using the Clustal Omega identity matrix from the European Bioinformatics Institute (EMBL). Sequence alignment was performed using ClustalW, and phylogenetic relationships were visualized using the MEGA V.11.0.11 software. Bootstrap analysis (1000 replicates) was conducted to assess branch confidence (Mahfut et al., 2015; Mahfut et al., 2020b; Mahfut et al., 2023b; Mahfut et al., 2023c).

The results of chlorophyll, carbohydrate, and peroxidase enzyme analyses were averaged, and statistical comparisons were conducted using Analysis of Variance (ANOVA) (Mahfut et al., 2021; Mahfut et al., 2023b; Mahfut et al., 2023c).

RESULTS AND DISCUSSION

Virus Source. Surveys conducted revealed that several cayenne pepper plants exhibited symptoms of Cucumber mosaic virus (CMV) infection. Symptom variations were observed across different villages. In Sabah Balau Village, CMV symptoms included yellowing, mosaic patterns, leaf curling, yellow spots, and curled leaf edges. Similarly, in Muara Putih Village, symptoms consisted of yellowing, curling, spotted patterns, and mosaic formations. Srimenganten Village displayed curling, yellowing, mosaic, stunted growth, and curled leaf edges. Symptom variations in CMV-infected cayenne pepper plants from these locations are summarized in Table 1.

DNA Isolation. The isolated DNA underwent a quantitative purity test using UV-Vis Spectrophotometry at wavelengths of 260 and 280. The purity values for samples infected with weak and malignant isolates ranged from 1.4 to 2.2, which is below the standard quality range. According to Li et al. (2020), the ideal purity range for DNA absorption is between 1.8 and 2.0. Values below this threshold indicate possible contamination by impurities such as phenol and other residues carried over during the isolation process.

DNA Amplification using Polymerase Chain Reaction (PCR). DNA amplification process was performed according to Anbiya et al. (2024). Complementary DNA (cDNA) synthesis was conducted using reverse transcriptase at 48 ºC, and the resulting cDNA was amplified to target the CMV coat protein (CP) gene using an annealing temperature of 55 ºC. Electrophoresis of PCR products revealed distinct DNA bands at approximately 657 base pairs (bp) for both weak strain isolates (C1) and malignant strain isolates (C2) from Sabah Balau Village, aligning with findings by Septiana et al. (2024) (Figure 1).

Isolate C2, identified as a virulent strain from Sabah Balau Village, exhibited 98% sequence homology with CMV isolates from Brazil. Choi et al. (2022) reported that CMV isolates typically share more than 90% sequence similarity, classifying them as the same species within the CMV genus. The CMV isolate from Sabah Balau Village appears to have undergone mutations leading to the formation of a new strain. The genetic diversity of CMV isolates in Indonesia suggests that variation is influenced by geographical origin.

Homology Sequence Analysis. BLAST-NCBI analysis indicated the highest sequence homology at 95.45%. Differences in GC base content were observed among isolates, with the malignant strain (C2) having the highest GC content at 44.2%, while the weak strain (C1) had 42%. Alignment analysis revealed mutations such as deletions, insertions, transversions, and transitions. The weak strain (C1) from Tanggamus Regency exhibited 19 deletions, 5 insertions, 100 transversions, and 60 transitions, whereas the malignant strain (C2) from Pringsewu Regency displayed 6 deletions, 9 insertions, 38 transversions, and 32 transitions. The high mutation rate in the weak strain (C1) suggests the possibility of speciation, as CMV infections can drive genetic changes in plants (Mahfut et al., 2024c).

Comparison of Genetic Distances. Genetic distance analysis revealed significant differences between the weak (C1) and malignant (C2) CMV isolates. Nucleotide-based genetic distances were consistently higher than amino acid-based distances, which is a common phenomenon in DNA viruses due to silent mutations (Mahfut et al., 2020c; Mahfut et al., 2023b).

Table 1. Symptoms description of CMV infection in cayenne pepper in South Lampung and Tanggamus Regency

Regency	Districts	Village	Sample Code	Symptoms
Lampung Selatan	Tanjung Bintang	Sabah Balau	TB 1	Leaves turn yellow, and mosaic (Weak)
			TB 2	Leaves turn yellow, and mosaic (Weak)
			TB 3	Yellowing leaves and yellow spots (Malignant)
			TB 4	Yellowing leaves and yellow spots (Malignant)
			TB 5	Yellowing leaves and yellow spots (Malignant)
			TB 6	Mosaic (Malignant)
	Natar	Muara Putih	N 1	Leaves turn yellow, and mosaic (Weak)
			N 2	Leaves turn yellow, and mosaic (Weak)
			N 3	Yellowing leaves and yellow spots (Malignant)
			N 4	Yellowing leaves and yellow spots (Malignant)
			N 5	Mosaic (Malignant)
Tanggamus	Pulau Panggung	Srimenganten	PP 1	Yellowing leaves and yellow spots (Malignant)
			PP 2	Stunted, yellow, and curled (Malignant)
			PP 3	Yellowing leaves and yellow spots (Malignant)
			PP 4	Mosaic and yellowing (Malignant)

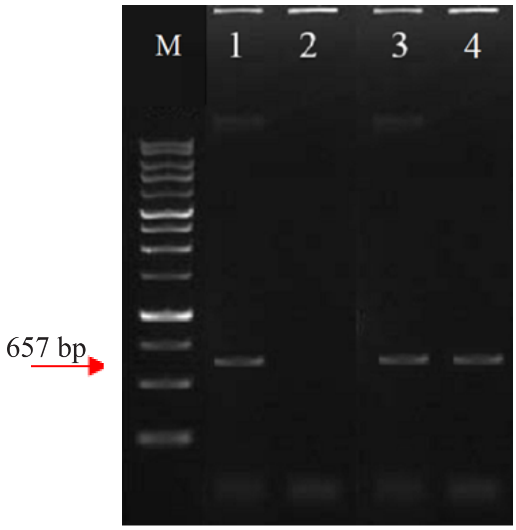

Figure 1. Visualization of PCR amplification results of samples using electrophoresis. M= Marker (1 kb/1000 bp), 1= CMV positive control, 2= CMV negative control, 3= Sample infected with a weak CMV strain isolate (C1), 4= Sample infected with a virulent strain of CMV isolate (C2).

Changes in genome structure are likely caused by polymerization errors. Compared to the nucleotide composition of a weak strain virus (C1) from Dadapan, the CP gene segment of the PepYLCV isolate from Bali exhibited nucleotide differences ranging from 8% to 11%.

Phylogenetic Tree Reconstruction. Phylogenetic analysis revealed that the weak strain isolate (C1) and the malignant strain isolate (C2) from Sabah Balau Village did not cluster together. This separation suggests that geographical factors and high mutation rates contributed to their divergence. The distinct branching pattern in the phylogenetic tree, constructed using 1000× bootstrap analysis (Figure 2), indicates significant evolutionary differences between the two isolates, potentially leading to new CMV strains.

Analysis of Disease Symptoms. Inoculation trials on cayenne pepper, large red chilies, and tomatoes showed varied symptoms of CMV infection, including chlorosis, mosaic patterns, necrosis, leaf malformation,

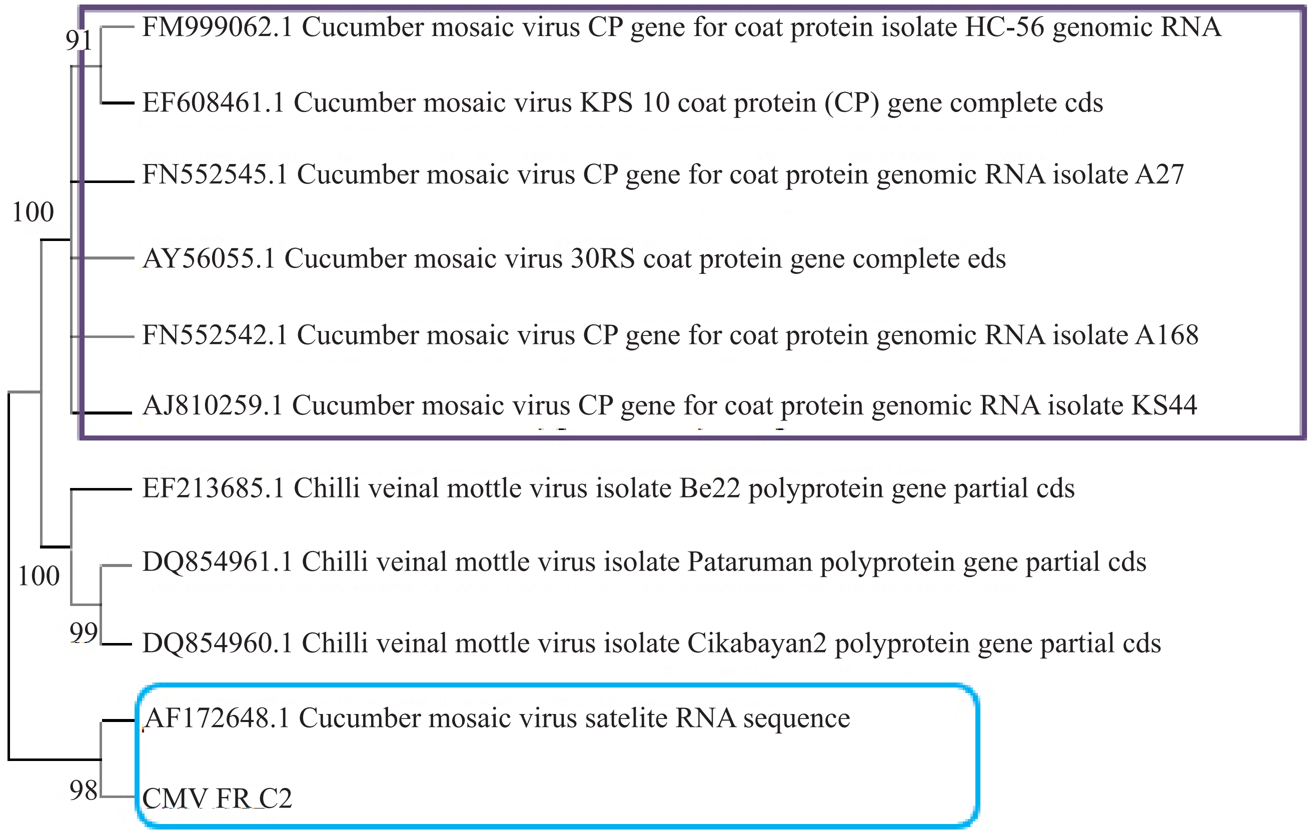

Figure 2. Phylogenetic tree of CMV isolates using 1000 × boostrap.

and asymptomatic responses. The mechanical inoculation process utilized weak, malignant, and mixed CMV strains. Symptom variations are detailed in Table 2.

Symptoms of CMV infection in cayenne pepper, large red chili, and tomato that were inoculated with a weak strain of CMV isolate as reported (Syller & Grupa, 2016) who stated that weak strain isolates from CMV infection could be used as cross-protection agents in protecting plants from malignant strain infections. Dhakar & Geetanjali (2022) also reported that weak strains can protect plants from damage infected by virulent virus isolates with known relatives. Symptoms of CMV infection with inoculation treatment of the virulent strain of CMV isolate are in accordance with the statement (Akin, 2005; Hasfiah, 2018) which stated that chili plants were infected with CMV. Malignant strains of CMV also experience a decrease in the quality and quantity of fruit produced. Apart from being infected with CMV, cayenne pepper, red chili, and tomato plants also experience CMV infection which can damage the plants.

Based on the inoculation with the weak + virulent strain of CMV isolate results, the disease symptoms on cayenne pepper, red chili, and tomato plants infected with CMV had varying symptoms, namely mild mosaic, clear mosaic, deformed leaves, yellowing, and no symptoms of CMV infection. Mosaic symptoms that often appear on the leaves of cayenne pepper, red chili, and tomato during the inoculation process are because mosaic symptoms are the initial symptoms shown by CMV infection in plants, especially cayenne pepper. This is as reported (Nalla et al., 2023) who stated that the mosaic symptoms that first appear on cayenne pepper leaves are influenced by several factors, namely plant age, cultivar, plant genotype and plant growth phase. Other factors that cause variations in CMV symptoms are environmental factors, such as soil fertility and climate on agricultural land (McLaughlin et al., 2022; Septiana et al., 2024).

Disease Progression Analysis. Based on the results of disease development analysis obtained from observing CMV inoculation on the three test plants, it show that there are differences in the level of disease severity and resistance between virulent strains and weak strains. The data that has been obtained is then processed using the method of calculating disease severity and plant resistance which is presented in Table 3 and Table 4.

Based on the results of the analysis of the severity of disease that occurred in test plants infected with CMV in this study, the highest level of disease severity

Table 2. Variation of symptoms of weak and virulent strains of CMV isolates on test plants

Treatment	Plant type	Leaf	Variations in late symptoms of CMV infection	Incubation period (Day-to)
Weak strain	Cayenne 1	V1	MR	21
		V2	TG	30
	Cayenne 2	V1	MR	19
		V2	MJ	27
	Cayenne 3	V1	MR	20
		V2	MR	24
	Red chili	V1	TG	19
		V2	MR	25
	Tomato	V1	MD	24
		V2	TG	30
Malignant strains	Cayenne 1	V1	MJ	18
		V2	MJ	21
	Cayenne 2	V1	MR, MD	25
		V2	MJ	21
	Cayenne 3	V1	MR	20
		V2	MJ	30
	Red chili	V1	MR, MD	17
		V2	MR	22
	Tomato	V1	MD	21
		V2	TG	25
Weak + malignant Strain	Cayenne 1	V1	MR	21
		V2	MJ, MD	30
	Cayenne 2	V1	MJ	19
		V2	MR	26
	Cayenne 3	V1	TG	24
		V2	MR	30
	Red chili	V1	MR	25
		V2	MJ	28
	Tomato	V1	TG	21
		V2	MR	27

MR= Mild mosaic ;MJ = Severe mosaic ;MD = Leaf malformation ;TG = No symptom.

in test plants using virulent strain isolates was 87% at 3 weeks after inoculation. In general, an increase in disease severity will occur if there is a co-infection with one or two viruses compared to if infected with one virus (Chen et al., 2020). This shows that the weak strain isolate is effective in protecting cayenne pepper, red chili, and tomato plants from infection by the virulent strain CMV.

Based on the results, weak strains and weak + malignant strains of CMV showed variations in response to disease resistance compared to malignant strain isolates. The results of virulent strain inoculation in this study showed a very high symptom response to large red chilies, cayenne peppers and tomatoes compared to weak strain isolates. The results of inoculation with the weak malignant strain showed very mild symptoms of CMV infection.

Chlorophyll a Analysis. Based on chlorophyll a analysis using the Tukey test, it shows that the

Table 3. Disease severity in cayenne peppers, large red chilies, and tomatoes inoculated with weak and malignant strains CMV isolate strains

Treatment	Disease severity (%)		
	1 MSI	2 MSI	3 MSI
Control	13.33	20.00	33.33
Weak strain inoculation	33.33	55.00	80.00
Malignant strain inoculation	60.00	80.00	87.00
Weak + malignant strain inoculation	20.00	53.33	55.00

Table 4. Level of resistance of cayenne pepper, large red chili and tomato plants to infection by CMV

Treatment	Plant type	Leaf	Level of resistance	Disease occurrence
Weak strain	Cayenne 1	V1	Very resistant	-
		V2	Resistant	+
	Cayenne 2	V1	Semi resistant	+
		V2	Tolerant	++
	Cayenne 3	V1	Semi resistant	+
		V2	Semi resistant	+
	Red chili	V1	Resistant	+
		V2	Semi resistant	+
	Tomato	V1	Very resistant	-
		V2	Very resistant	-
Malignant strains	Cayenne 1	V1	Very resistant	_
		V2	Tolerant	++
	Cayenne 2	V1	Resistant	+
		V2	Tolerant	++
	Cayenne 3	V1	Very vulnerable	++
		V2	Toleran	++
	Red chili	V1	Resistant	+
		V2	Resistant	+
	Tomato	V1	Very resistant	-
		V2	Very resistant	-
Weak + malignant strain	Cayenne 1	V1	Tolerant	++
		V2	Semi resistant	+
	Cayenne 2	V1	Semi resistant	+
		V2	Very vulnerable	++
	Cayenne 3	V1	Resistant	+
		V2	Very vulnerable	++
	Red chili	V1	Semi resistant	+
		V2	Tolerant	++
	Tomato	V1	Semi resistant	+
		V2	Semi resistant	+

chlorophyll a content in cayenne pepper, red chili, and tomato infected with CMV has different chlorophyll a content. Tomato and cayenne pepper plants had a significant effect on reducing chlorophyll a levels, compared to large red chili plants in the weak strain isolate (C1). Meanwhile, red chili and tomato experienced a decrease in chlorophyll compared to cayenne pepper. The chlorophyll a content will affect photosynthesis which in turn will affect growth (Nuraini et al., 2024; Simamora et al., 2024). In addition, the total leaf area of a plant infected with the virus will decrease. This is due to inhibited growth rates, the process of leaf expansion, an increase in the number of fallen leaves, and chlorophyll production which reduces photosynthesis results (Mahfut et al., 2023c; Aritonang et al., 2024). Results of the Tukey chlorophyll a test in the inoculation treatment of isolates 3 weeks after inoculation was shown in Table 5.

Decrease in chlorophyll a content causes a decrease in the ability to capture light energy. Plants that were inoculated with the virulent strain isolate (C2) had a lower ability to capture light than the weak strain isolate (C1) and the control. An increase in chlorophyll a content in viral infection conditions is related to an increase in chlorophyll protein so it will increase the efficiency of photosynthetic antenna function in Light Harvesting Complex II (LHC II). The malignant strain isolate (C2) had lower chlorophyll levels compared to the weak strain isolate and control isolate, because the malignant strain isolate (C2) maintained leaf structure, compared with a decrease in chlorophyll content.

Chlorophyll b Analysis. Based on chlorophyll b analysis using the Tukey test, it shows that the chlorophyll b content in cayenne pepper, red chili, and tomato infected with CMV has different chlorophyll b content. The weak strain isolate (C1) had a higher chlorophyll content than the malignant strain isolate (C2). An increase in chlorophyll b content in virus-infected conditions is related to an increase in chlorophyll protein so it will increase the efficiency of photosynthetic antenna function in Light Harvesting Complex II (LHC II). In addition, chlorophyll b functions as a photosynthetic antenna that collects light (Mahfut et al., 2020c; Aritonang et al., 2024). This is also proven by the results of research on chlorophyll b content conducted (Mahfut et al., 2021, Mahfut et al., 2023c) on the leaves of orchid plants infected with *Ceratobasidium* and *Trichoderma* showing a decrease in chlorophyll b content. Results of Tukey's chlorophyll b test on inoculation treatment of isolate 3 weeks after inoculation are shown in Table 6.

Total Chlorophyll. Based on the analysis of the total chlorophyll content, it showed that in the treatment of weak strain (C1), virulent strain (C2), and control isolates, the total chlorophyll content in cayenne pepper was 5097 (mg/g tissue), large red chili was 4953 (mg/g tissue), and tomatoes 5415 (mg/g tissue) which were inoculated with the virulent strain (C2) the chlorophyll content was the same as the chlorophyll content inoculated with the weak strain (C1) cayenne pepper 864 (mg/g), large red chili 8802 (mg/g tissue), and tomatoes 5720 (mg/g tissue). However, the virulent strain had a lower total chlorophyll content compared to the weak strain and control isolates. This is also proven by the results of research on total chlorophyll levels conducted (Mahfut et al., 2021, Mahfut et al., 2023c; Mahfut et al., 2024a; Mahfut et al., 2024b) on the leaves of orchid plants infected with

Table 5. Chlorophyll a analysis of isolate inoculation treatment three weeks post-inoculation

Factor B (Species)	Factor A (Treatment)			Marginal mean
	C1(mg/g tissue)	C2 (mg/g tissue)	Ko (mg/g tissue)	
Cayenne	18,254 ± 274.01 b	13,639 ± 193.75 c	28,790 ± 274.01 a	20.22 c
Red chili	26,677 ± 631.66 a	10,737 ± 631.66 b	26,216 ± 631.66 a	21.21 a
Tomato	11,827 ± 264.64 b	10,882 ± 264.64 b	40,968 ± 264.64 a	21.22 a
Marginal mean	18.91 a	11.75 b	31.99 a	

Numbers in the same column followed by different letters indicate significant differences according to the Tukey test at 5% error level.

Table 6. Chlorophyll b analysis of isolate inoculation treatment three weeks post-inoculation

Factor B (Species)	Factor A (Treatment)			Marginal mean
	C1 (mg/g tissue)	C2 (mg/g tissue)	Ko (mg/g tissue)	
Cayenne	15,975 ± 493.49 b	10,287 ± 493.49 c	29,120 ± 697.89 a	18.46 b
Red chili	16.656 ± 956.99 a	10,410 ± 956.99 b	19,532 ± 956.99 a	15.53 a
Tomato	14.605 ± 355.16 b	10,652 ± 355.16 c	25,374 ± 355.16 a	16.87 b
Marginal mean	15.74 b	10.44 b	24.67 a	

Numbers in the same column followed by different letters indicate significant differences according to the Tukey test at 5% error level.

Ceratobasidium and *Trichoderma* showing a decrease in total chlorophyll content. Total chlorophyll Tukey test results in the inoculation treatment of isolates 3 weeks after inoculation on Table 7.

Carbohydrate Analysis. Based on the results of the Tukey test with a 5% level on the carbohydrate content of cayenne peppers, large red chilies and tomatoes with inoculation treatment of the weak strain (C1) and the strong strain (C2) of CMV it shows that the plants have the same effect. The carbohydrate content obtained is stable because it is thought to contain phenol which is formed in cayenne pepper plants, large red chilies and tomatoes. Inoculation of the weak strain (C1) produces a high carbohydrate content because the host plant produces phenol, resulting in the formation of an anti-virus that is useful for resistance in cayenne peppers, large red chilies and tomatoes, so that the CMV virus can be eradicated. Stops being synthesized and cannot damage leaf mesophyll tissue and chloroplasts will not be damaged (Asadudin et al., 2024; Putera et al., 2024). Tukey test results for carbohydrate content in the inoculation treatment of isolates 3 weeks after inoculation are shown in Table 8.

Peroxidase Enzyme Analysis. Based on the treatment of cayenne pepper, red chili, and tomato inoculated with the weak strain isolate (C1), the average peroxidase enzyme activity in cayenne pepper was 8.1771 (g/u/minute), in red chili 7.9420 (g/u/minute), and tomato 5.1870 (g/u/minute). When compared with the control inoculation, the peroxidase enzyme activity will increase by 9.5451 (g/u/minute) for cayenne pepper, 8.59988 (g/u/minute) for red chili, and 8.1391 (g/u/minute) for tomato. This is in accordance with the research of Johnson et al. (2019) that peroxidase enzyme activity will accumulate when plants are induced by viruses. The weak strain virus CMV is one of the plant viruses that has been proven to be effective in cayenne pepper (Pechinger et al., 2019). Tukey test results data on peroxidase enzyme activity in the inoculation treatment of isolates 3 weeks after inoculation was shown in Table 9.

CONCLUSION

The detection results confirmed that a weak strain of Cucumber mosaic virus (CMV) infecting a natural population of cayenne pepper (Capsicum annuum L.) produced a specific band of approximately 657 bp. Molecular characterization of the Coat Protein (CP) gene revealed that the weak strain isolate had the highest number of mutations (100), whereas the malignant strain had the lowest (38). The effectiveness test of the weak CMV strain on test plants demonstrated diverse infection symptoms, including yellowing, leaf malformation, mosaic, and curling at 30 days post-inoculation. These findings provide insight into CMV infection patterns, aiding in preventive measures to mitigate its spread in Lampung. Further research

Table 7. Total chlorophyll of isolate inoculation treatment three weeks post-inoculation

Factor B (Species)	Factor A (Treatment)			Marginal mean
	C1 (mg/g tissue)	C2 (mg/g tissue)	Ko (mg/g tissue)	
Cayenne	8643 ± 379.29 b	5097 ± 379.29 c	16,869 ± 536.40 a	10.20 b
Red chili	8802 ± 899.37 a	4953 ± 899.37 b	8916 ± 899.37 a	7.55 a
Tomato	5720 ± 239.65 b	5415 ± 239.65 b	10,569 ± 239.65 a	7.23 b
Marginal mean	7.72 b	5.15 b	12.13 a	

Numbers in the same column followed by different letters indicate significant differences according to the Tukey test at 5% error level.

Table 8. Carbohydrate content in the inoculation treatment of isolates 3 weeks after inoculation

Factor B (Species)	Factor A (Treatment)			Marginal mean
	C1 (mg/g tissue)	C2 (mg/g tissue)	Ko (mg/g tissue)	
Cayenne	14,929 ± 3291.02 a	14,914 ± 3291.02 a	16,525 ± 3291.02 a	15.45 a
Red chili	18,597 ± 4624.78 a	15,178 ± 4624.78 a	22,274 ± 4624.78 a	18.68 a
Tomato	16,154 ± 3045.74 a	15,176 ± 3045.74 a	17,137 ± 3045.74 a	16.15 a
Marginal mean	16.56 a	15.08 a	18.64 a	

Numbers in the same column followed by different letters indicate significant differences according to the Tukey test at 5% error level.

Table 9. Peroxidase enzyme activity in the inoculation treatment of isolates 3 weeks after inoculation

Factor B (Species)	Factor A (Treatment)			Marginal mean
	C1 (g/u/minute)	C2 (g/u/minute)	Ko (g/u/minute)	
Cayenne	8.18 ± 491.46 b	5.67 ± 491.46 c	9.54 ± 695.03 a	7.79 c
Red chili	7.94 ± 512.11 b	3.87 ± 512.11 c	8.60 ± 512.11 a	6.80 b
Tomato	5.19 ± 464.35 b	4.03 ± 464.35 c	8.14 ± 464.35 a	5.78 b
Marginal mean	7.10 b	4.52 c	8.76 a	

Numbers in the same column followed by different letters indicate significant differences according to the Tukey test at 5% error level

is needed to develop CMV-resistant plant varieties through breeding programs.

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AUTHORS CONTRIBUTIONS

M designed and conducted the experiment, performed data collection, and prepared the manuscript. MS, BI, and SW provided guidance in experiment setup. HMA analyzed and validated the data. AW contributed to writing, editing, reviewing, and finalizing the manuscript. All authors have read and approved the manuscript for publication.

COMPETING INTEREST

The authors declare no conflicts of interest related to this article.

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