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Determinants of symptom variation of *Pepper yellow leaf curl Indonesia virus* in bell pepper and its spread by *Bemisia tabaci*

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Abstract. *Selangga DGW, Listihani L, Temaja IGRM, Wirya GNAS, Sudiarta IP, Yuliadhi KA. 2023. Determinants of symptom variation of* Pepper yellow leaf curl Indonesia virus *in bell pepper and its spread by* Bemisia tabaci. *Biodiversitas 24: 869-877. Pepper yellow leaf curl Indonesia virus* (PYLCIV) is an important virus that infects chili pepper in Indonesia. PYLCIV has been reported in several regions in Indonesia on several host plants, such as chili pepper, tomato, sweet potato, and ornamental plants. PYLCIV infection has never been reported in bell pepper plants, in 2021 we found yellow mosaic symptoms on bell pepper leaves so this research aimed to conduct molecular characterization on the virus causing yellow leaf curl disease in bell pepper and its relationship to distribution and symptoms variations in the field. The research method includes symptom observation, disease incidence, the population of *Bemisia tabaci* in the field, and molecular detection via PCR on PYLCIV obtained from bell pepper leaf and on *B. tabaci* imago. PYLCIV infection in a bell pepper is differentiated into five types of symptoms: mottle, leaf curl and malformation, yellowing, green mosaic, and yellow mosaic. The symptoms type often found in bell pepper plants are yellow mosaic and leaf curl and malformation, with the incidence rates being 19.5% and 21.5%, respectively. The high whitefly population in both symptoms type caused the high incidence rate. Moreover, via PCR, whiteflies on bell pepper with those five symptom types were positive for Begomovirus (*B. tabaci* viruliferous). Bell pepper positive for PYLCIV appeared to have long fruit ripening periods with small sizes, elongated shaped, and abnormal. While healthy bell pepper showed rapid ripening and large and plentiful fruits. The five symptom types of PYLCIV from bell pepper showed the highest nucleotide homology with PYLCIV isolates from Selulung Village, Kintamani Sub-district, Bangli District, Bali Province, Indonesia, obtained from chili pepper with a homology of 99.7-99.8%. The phylogenetic tree showed that the five symptom types PYLCIV in bell pepper formed a group with PYLCIV isolate from Selulung obtained from chili pepper. This research is the first report of PYLCIV infection on bell pepper in Indonesia.

Keywords: Begomovirus, PCR, PYLCIV, symptom variety, viruliferous

INTRODUCTION

Begomovirus is one of the genera in the Geminiviridae family that has the largest number of species and infects most plants compared to the other three genera; Mastrevirus, Curtovirus, dan Topocuvirus (Souza et al. 2022). Begomovirus has caused a lot of damage to various plants, including chili peppers, tomatoes, cucumbers, sweet potatoes, pumpkins, and ornamental plants (Selangga et al. 2018; Listihani et al. 2019a; Fadhila et al. 2020; Shah et al. 2020; Lavanya and Arun 2021; Selangga and Listihani 2021; Selangga et al. 2021; Selangga and Listihani 2022; Listihani et al. 2022b). *Pepper yellow leaf curl virus* (PYLCV) is a group of viruses from the Geminiviridae family, the Begomovirus genus.

PYLCV infection has been reported in chili pepper plants in Central Java, DI Yogyakarta, West Java, and West Sumatra (Fadhila et al. 2020; Sayekti et al. 2021). Since the end of 2002 and early 2003, the incidence of *Pepper yellow leaf curl virus* in old chili plants rapidly increased, reaching 70 to 100% with severe symptoms: yellowing of leaf and stunting of plants. Trisno et al. (2009) reported that the incidence of this disease in West Sumatra reached 60.00-80.83%, while Selangga (2021) reported that yellow leaf curl virus incidence in several chili pepper plantations in Bali province reached 100% with a severity level around 18-87%. Pepper yellow leaf curl disease incidence was also reported in other Indonesian regions, including Lampung 30-100%, North Sumatra 20-80%, South Sumatra 20-60%, and Bengkulu 0-40% (Trisno et al. 2009).

PYLCV infection in chili pepper plants causes yellowing of leaves, while in old chili pepper plants, the leaves at the crown turned bright yellow, and the lower leaves remained green. The plants infected at the early stage remain stunted without bearing any fruit (Agustika et al. 2022). PYLCIV infection causes various symptoms in the Seret cultivar: yellow mosaic, motel, leaf curl, green mosaic, dwarfing, and upward and downward cupping. The symptoms appeared almost in all Bali regions. The yellowing of the leaf, especially on the top (young), is similar to the symptoms of lacking the Fe micronutrient. All symptoms that appear are actually due to the disruption of nutrition flow (photosynthate) from source to sink because the virus in the plant invades the phloem (limited phloem virus) (Folimonova and Tilsner 2018). If the plant

were infected during the generative phase, the fruit produced would be dwarfed and have a hard texture.

The transmission or spread of PYLCIV is accommodated by a vector insect, the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). The higher the population of whitefly, the higher the distribution of PYLCIV. Whiteflies can persistently spread the virus, which means once it feeds on a viral-loaded plant, it will persist in the insect's body throughout its life. Thus, the virus can be transmitted even after the vector is shed (Nigam 2021). The number of whiteflies during transmission will influence the disease incidence rate and viral incubation period (Temaja et al. 2022). The whiteflies' population density in a certain plantation depends on the imago's ability to place eggs and feed. Egg placement and feeding activity are influenced by leaf characteristics and morphology, such as the shape of the leaf, the color, the trichome on the leaf, and chemical compounds produced in the secondary metabolism process (Rosario et al. 2016). In addition, the number, length, and type of trichome on the leaf may influence the population density of whitefly on the plant (Wang et al. 2022).

Bemisia tabaci whitefly is an extremely effective PYLCV vector. PYLCV transmission via *B. tabaci* reaches its maximum (100%) if the acquisition feeding period is over 6 hours (Trisno et al. 2009). An inoculation feeding period of 15 minutes can already cause disease with 20%-40% transmission effectivity (Roy et al. 2021). One adult of *B. tabaci* given an acquisition feeding period of 24 hours and an inoculation feeding period of 48 hours can already transmit the disease for 30%-50% (Trisno et al. 2009). That shows an inoculation source present in a location can potentially cause an epidemic in the field. Active insect vector presence can transmit disease in a short time (Pan et al. 2020).

Research about natural PYLCIV infection in bell pepper has never been reported in the world, including Indonesia. Moreover, PYLCIV spread in bell pepper, and the variation of the symptoms due to the virus has never been reported. Thus, this research aimed to characterize the molecular profile of the virus causing yellow leaf curl disease in bell pepper and the relationship between its distribution and symptom variations in the field. This study is crucial for preventing bell pepper production loss due to PYLCIV infection.

MATERIALS AND METHODS

Location determination and field observation

The research was performed in a greenhouse owned by farmers in Selulung Village, Kintamani Sub-district, Bangli District, Bali Province, Indonesia. The observation was conducted 7 to 63 days after planting (DAP) of pepper variety California wonder with 200 plants/pot with 7 DAP intervals of observation time. The calculation of viral disease incidence based on symptom variations in the field was based on the following equation:

$$DI = \frac{n}{N} x\ 100\%$$

Where:
DI : disease incidence percentage (%)
n : number of infected plants
N : number of observed plants

The pest population was observed by observing the whiteflies found on the surface of the leaf (top, middle, and lower leaf) of every plant. The observation period was the same as the observation of disease incidence. Each plant with variation in symptoms was taken, and viral detection was performed by PCR molecular method using begomovirus universal primers. Each whitefly from infected plants was brought to the plant disease laboratory, Agricultural Faculty, Udayana University, for testing against begomovirus infection. PCR technique was used to detect PYLCIV from the infected plants and whitefly samples which consisted of three phases: extraction, amplification, and DNA visualization.

Total DNA extraction from bell pepper leaf

DNA extraction from leaf samples was performed following the Cetyl-trimethyl-ammonium Bromide (CTAB) method (Doyle and Doyle 1999)**.** First, the leaf (0.1 g) was ground in the pestle, adding liquid nitrogen. The leaf was ground into powder, and then added by 500 µL buffer CTAB (10% cetyl-trimethyl-ammonium bromide, 0.1 M Tris-HCl pH 8, 0.05 M EDTA, 0.5 M NaCl, 1% β-mercapto-ethanol), and the plant sap was moved into 1.5 ml tube. The plant sap was incubated in a water bath at 65 °C for 60 minutes and then turned every 10 minutes to separate lipids and proteins. Next, into the plant sap 500 µL chloroform: isoamyl alcohol (24:1 v/v) was added, vortexed for 5 minutes, and centrifuged at 12,000 rpm for 15 minutes. The supernatant was moved into a new 1.5 ml tube, added by sodium acetate (CH_3COOK) and isopropanol 1/10 and 2/3 of the supernatant volume, respectively, and then turned gently to mix it. The supernatant liquid was incubated overnight or for 4 hours at -20°C. After incubation, the supernatant liquid was centrifuged at 12,000 rpm for 10 minutes to precipitate DNA, and then the fluid was discarded. Next, the pellet was washed by adding 500 µL of ethanol 70%, centrifuged at 8,000 rpm for 5 minutes, and the liquid was discarded. The pellet containing total DNA was then dried at the laminar. Once it was dry, 25 to 50 µL of nuclease-free water or TE buffer (pH 8) was added, and the DNA was ready to be amplified.

Total DNA extraction from whitefly *Bemisia tabaci*

Bemisia tabaci insect stored inside absolute alcohol was extracted by molecular technique to obtain total DNA. *B. tabaci* insects from each location were put inside a 1.5 mL microtube, and 100 µL of extraction buffer CTAB 2% was added. Into the tube, 1 µL of proteinase K was added then the solid content was crushed finely using a micropestle. The suspension was incubated at 65°C for 3 minutes. A mixture of Chloroform: Isoamyl alcohol (CI) (24:1) as much as 100 µL was added into the suspension and then vortexed for 3 minutes. The suspension was centrifuged for 15 minutes at 10,000 rpm to produce supernatant. As much

as 60 µL of the supernatant obtained was moved into a new 1.5 mL tube. The supernatant added 44 µL of isopropanol and 6 µL sodium acetate 3 M (pH 5.2). The mixture was incubated in the refrigerator at -20°C for 3 hours or (overnight). Afterward, the tube was centrifuged at 10,000 rpm for 10 minutes. The supernatant formed was discarded, and a pellet containing total DNA was left. The pellet was washed with 100 µL of 80% ethanol and was recentrifuged at 8,000 rpm for 5 minutes. The supernatant formed was discarded again. The total DNA pellet was suspended with 20 µL of Tris-EDTA (TE).

Begomovirus DNA amplification

The extracted DNA (from the plant and the *B. tabaci*) was amplified by PCR, using a pair of Begomovirus universal primers (SPG 1 dan SPG 2) to amplify the TrAP and Rep region with desired DNA fragment being ≈ 912 bp. The nucleotide sequence of primer SPG1 was 5'-CCCCKGTGCGWRAATCCAT-3', and for SPG2 was 5'-ATCCVAAYWTYCAGGGAGCT-3' (Li et al. 2004). The amplification was performed by using a thermal cycler (GeneAmp PCR System 9700) with amplification reaction (total volume 25 µL) consisting of 12.5 µL Dream Taq Green Master Mix (Thermo Scientific, US), 9.5 µL ultra-pure water, primer SPG 1 and SPG 2 as much as 1.0 µL each, and template DNA as much as 1.0 µL. The amplification reaction was initiated by heating at 94°C for 5 minutes. Then, the amplification stage was conducted in 35 cycles with the following stages: denaturation at 94°C for 1 minute, annealing at 50 °C for 1 minute, and extension at 72°C for 1 minute. Finally, the cycle ended at 72°C for 7 minutes and was managed at 4°C until the tubes were taken.

DNA visualisation and analysis

The Begomovirus DNA from amplification was analyzed by electrophoresis using 1% agarose gel in TBE (Tris-Boric acid- EDTA) 0.5X. The agarose gel was heated in the microwave for two to three minutes until it dissolved, and stain FluoroVue™ Nucleic Acid Gel Stain (Smobio, Taiwan) was poured into the mold. It was then left for hardening. After hardening, the agarose gel was moved to an electrophoresis tank containing TBE 0.5X buffer. Electrophoresis was performed under 50 V for 50 minutes. DNA from PCR positive of Begomovirus was sent to FirstBase (Malaysia) to have its nucleotide sequenced. The nucleotide homology was analyzed by BioEdit software. The genetic relationship was analyzed by MEGA V6.0 software with 1,000 times bootstrap repetitions. The result obtained was compared with data from Genbank.

RESULTS AND DISCUSSION

Different symptoms were found in bell pepper plants of the same variety (Table 1; Figure 1). Generally, the infection symptoms of *Pepper yellow leaf curl Indonesia virus* (PYLCIV) are differentiated into five symptoms: mottle, leaf curl and malformation, yellowing, green mosaic, and yellow mosaic. These changes are due to viral infection that causes the leaves to suffer chlorosis and disturbances in photosynthesis. The disappearance of chlorophyll causes chlorosis in plants due to a viral attack. The yellow disease on chili pepper can reduce the photosynthesis rate due to the decreasing number of chlorophylls per leaf. Thus, causing a suboptimal amount of photosynthate (Carretero et al. 2011; Hamblin et al. 2014; Xue et al. 2014). The PYLCIV infection symptoms in bell pepper first appeared when the plant reached 28 DAP. The symptoms often found on bell pepper plants were yellow mosaic and leaf curl and malformation, with the incidence rate being 19.5% dan 21.5%, respectively (Table 1). Several factors influence the high disease incidence rate, which is genotype, plant condition, time of infection, the acquisition feeding period, inoculation feeding period, and the number of insect vector (Listihani et al. 2018; Listihani et al. 2019b; Listihani et al. 2020; Sutrawati et al. 2021; Damayanti et al. 2022; Listihani et al. 2022a; Pandawani et al. 2022; Selangga et al. 2022). As per PCR results, all the plants with the symptoms were positive for Begomovirus. The symptom variation of PYLCIV in chili pepper plants has been reported by Selangga and Listihani (2021), Selangga et al. (2021), and Temaja et al. (2022).

Symptom variations due to virus infection can be caused by several factors: virus strain, plant genotype, the age of the plant when infected, environmental conditions, and vector activity (Lukman et al. 2019; Roy et al. 2021; Kwak et al. 2022; Lestari et al. 2022; Temaja et al. 2022). In this research, the symptom variations observed on bell pepper plants were caused by differences in age upon PYLCIV infection. In addition, the disease symptoms in chili peppers appeared to be more severe (Selangga et al. 2021) compared to the bell pepper in this research. Different defense responses between the two plants proved that every strain has different resistance against viruses. According to War et al. (2012), Sauban et al. (2016), Shittu et al. (2019), and Kaur et al. (2022), generally, there are two types of defense mechanisms in plants: structural defense and biochemical defense. Structural defense is the structural properties functioning as a physical barrier that hinders pathogens from gaining access and spreading inside the plant. The biochemical defense is biochemical reactions occurring inside plant cells and tissues, which produce compounds toxic to pathogens or create a condition that inhibits pathogen growth inside the plant. The defense mechanism of bell pepper is assumed to be related to leaf morphological structure and the ability of the plant to produce inhibitory compounds. Other than that, the cause of symptom variety is the whitefly activity. The whiteflies inside the greenhouses probably originated from plants around the greenhouse, consisting of chili peppers, tomatoes, strawberries, napa cabbages, bok choy, carrots, and cabbages.

Besides the various symptoms, PYLCIV infection caused changes in bell pepper quality and size (Figure 2). Bell pepper positives of PYLVIC via PCR appeared to take longer to ripen, and the fruits produced were small with abnormal, elongated shapes. On the other hand, healthy bell pepper (PYLCIV negative via PCR) showed rapid fruit

ripening and big and normal fruits in large quantities. That proves viral infection lowered the quantity and quality of fruits. In previous research, it has been reported that Begomovirus infection has caused a 56.3% production loss in yellow pumpkin production and lowered harvest quality (Selangga and Listihani 2022).

When the bell pepper plant reached 14 days after planting, *B. tabaci* whitefly had started to be found on the plants of each symptom (Table 2). The whitefly population started to increase when the plant reached 35 DAP, and its population peaked at 63 DAP. Out of the five symptoms, the highest whitefly population was found in bell pepper plants with the leaf curl and malformation and the yellow mosaic symptom. The high whitefly population in the two symptom types also caused a high incidence rate (Tables 1 and 2). Whiteflies found on bell peppers exhibiting the five symptoms have been confirmed via PCR using Begomovirus universal primer to be viruliferous. The population and activity of whitefly in the field greatly influence the spread and incidence of PYLCIV in the field. This research result is similar to a previous study, which found that the whitefly population peaked when the plants were at 63 DAP (Temaja et al. 2022). When the bell pepper plant reached 63 DAP, the highest whitefly population was four insects per leaf. In contrast, in the research by Temaja et al. (2022), in chili pepper plants aged 63 DAP, the highest whitefly population was 4.8 insects per leaf.

The number of insect vectors is one of the determining factors for incidence rate and virus incubation period. The higher the number of insects, the higher the incidence rate and the shorter the incubation period, and vice versa (Listihani et al. 2022a).

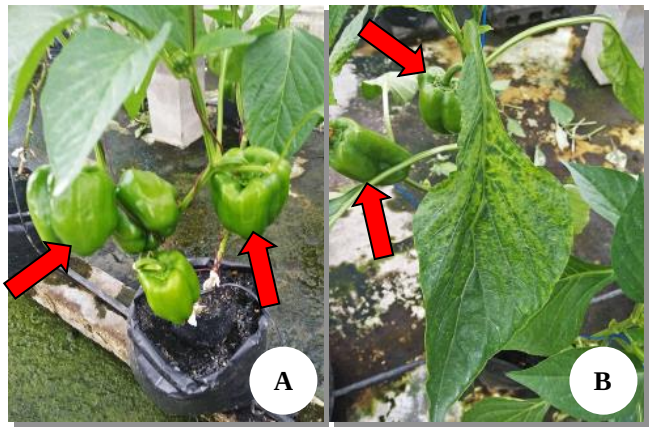

Figure 2. Fruit size and shape on bell pepper plants at the same plant age: A. Healthy plants; B. Diseased plants infected with PYLCIV

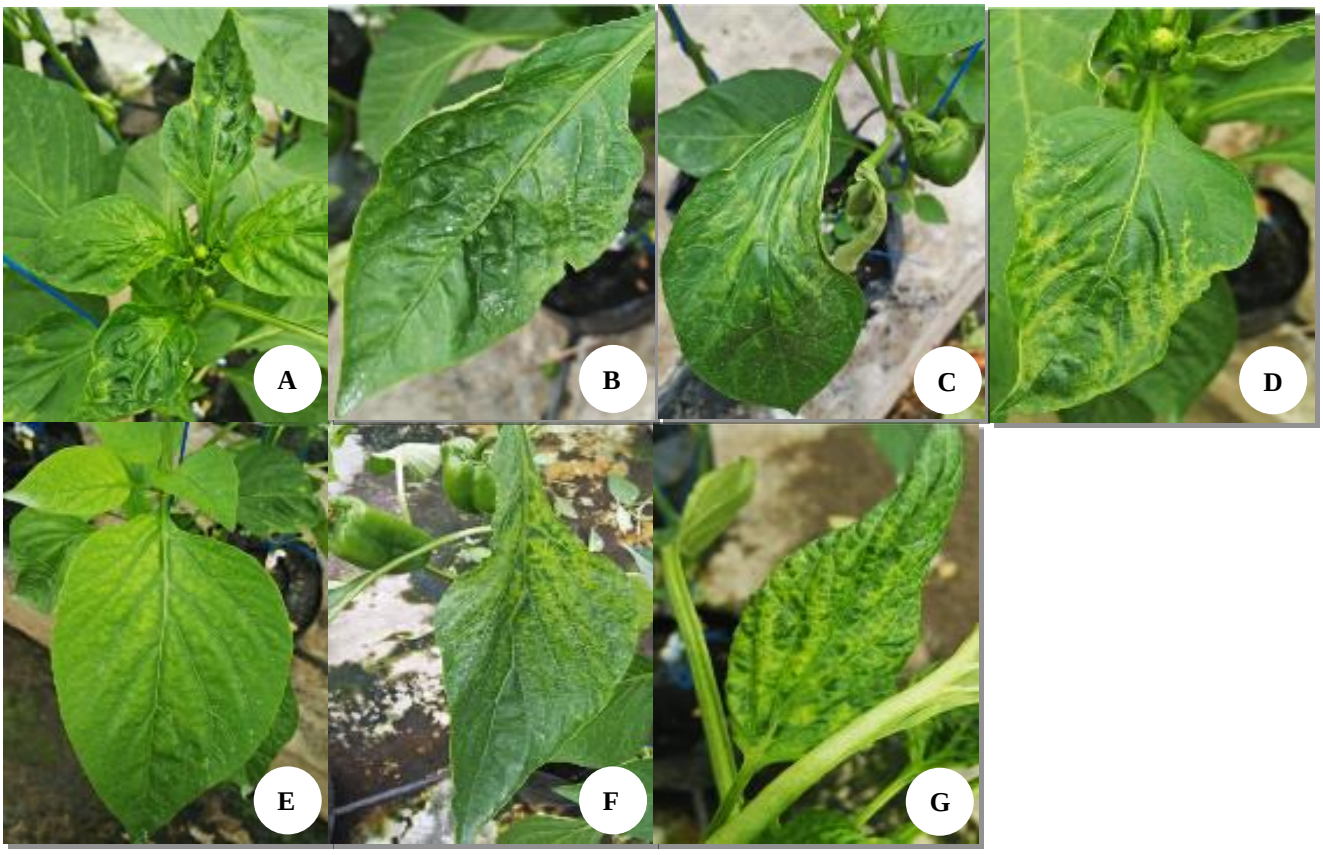

Figure 1. Symptoms on bell pepper plants infected by PYLCIV: A. Mottle; B-D. Leaf curl and malformation; E. Yellowing; F. Green mosaic; G. Yellow mosaic

The insect population is influenced by leaf characteristics and morphology, such as leaf shape, leaf color, leach trichome, and chemical compounds produced by the secondary metabolism process (Rodríguez-López et al. 2012; Lucatti et al. 2014; Hasanuzzaman et al. 2016; Triwidodo and Listihani 2020). Triwidodo and Listihani et al. (2020) further explained that the number, length, and type of trichomes on leaves might influence the insect population density or plants. Trichome is a morphological feature most influential on insect egg placement on plants. The number of eggs placed on leaves with dense and tight trichomes tends to be considerably fewer than those on few and sparse trichomes (Deng et al. 2013; Triwidodo and Listihani 2020). The previous research also reported that Begomovirus incidence in the field is influenced by whitefly feeding activity (Luan et al. 2014; Czosnek et al. 2017; Roy et al. 2021). Feeding activity by an initial instar will support its growth into the last instar, and the feeding activity during the last instar will support it in turning into pupae. Whitefly feeds by sucking plant fluids. Whitefly sucks fluid from plants by placing and penetrating their stylet inside. Plant fluid sucked turns into nutrition for the whitefly to continue its life after hatching. When stabbing its stylet, the whitefly also releases the virus it carries in its body. Therefore, the activity and population of insect vectors are crucial in determining their ability to transmit viruses.

Five plant samples for each symptom were used to detect Begomovirus via PCR using Begomovirus universal primer. Virus detection was performed to ensure that the symptoms appearing on the bell pepper plants were due to Begomovirus infection. DNA bands around 912 bp in length were managed to be amplified on all bell pepper samples (Table 1; Figure 3). This result showed that the five symptoms appearing on the bell pepper plants are Begomovirus viral infection symptoms.

The DNA products from amplification were further used in nucleotide sequencing, where the result was used for sequence analysis. Based on nucleotide homology analysis, it was known that the bell pepper samples with the five symptoms have sequence similarities with PYLCIV isolates in the Genbank database, around 93.6%-99.8% (Table 3). The five symptoms of PYLCIV from bell pepper plants showed the closest homology with PYLCIV isolates from Selulung, Kintamani Sub-district, Bangli Regency, Bali, Indonesia, obtained from chili pepper plants with similarity reaching 99.7%-99.8%. The phylogenetic tree showed that the five PYLCIV symptoms in bell pepper plants form a group with PYLCIV isolates from Selulung, Kintamani, obtained from chili pepper (Figure 4). Thus, it is confirmed that the samples obtained from the field are associated with PYLCIV infection.

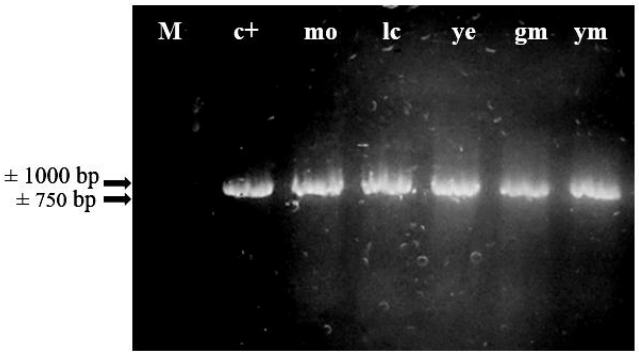

Figure 3. Results of PYLCIV DNA amplification on bell pepper from Bedugul. (M) DNA marker (1 kb ladder); (c+) PYLCIV-blanga 1 (LC381268) as positive control; (mo) mosaic symptom; (lc) leaf curl and malformation; (ye) yellowing; (gm) green mosaic; (ym) yellow mosaic, respectively

Table 1. Incidence of yellow leaf curl disease at different ages of bell pepper plants

Fields symptoms	Disease incidence (%) at plant age (DAP)								Begomovirus detection by PCR
	14	21	28	35	42	49	56	63	
Mottle	0	0	0	2.5	3	7.5	9.5	11.5	+
Leaf curl and malformation	0	0	0.5	3.5	6.5	11.5	16	21.5	+
Yellowing	0	0	0	2	3	8.5	9	13.5	+
Green mosaic	0	0	0	1.5	3.5	8.5	11.5	17	+
Yellow mosaic	0	0	1	4	5.5	10	13.5	19.5	+

Note: DAP (day after planting).

Table 2. Vector population of *Bemisia tabaci* on yellow leaf curl disease at different ages of paprika plants

Fields symptoms	Vector population of *Bemisia tabaci* (individual/leaf) at plant age (DAP)								*B. tabaci* viruliverous Begomovirus by PCR
	14	21	28	35	42	49	56	63	
Mottle	0	2	1	3	2	2	1	2	+
Leaf curl and malformation	1	2	2	3	3	4	3	4	+
Yellowing	0	1	1	3	2	1	1	2	+
Green mosaic	1	1	1	2	3	2	3	3	+
Yellow mosaic	1	2	1	3	3	2	4	4	+

Note: DAP (day after planting).

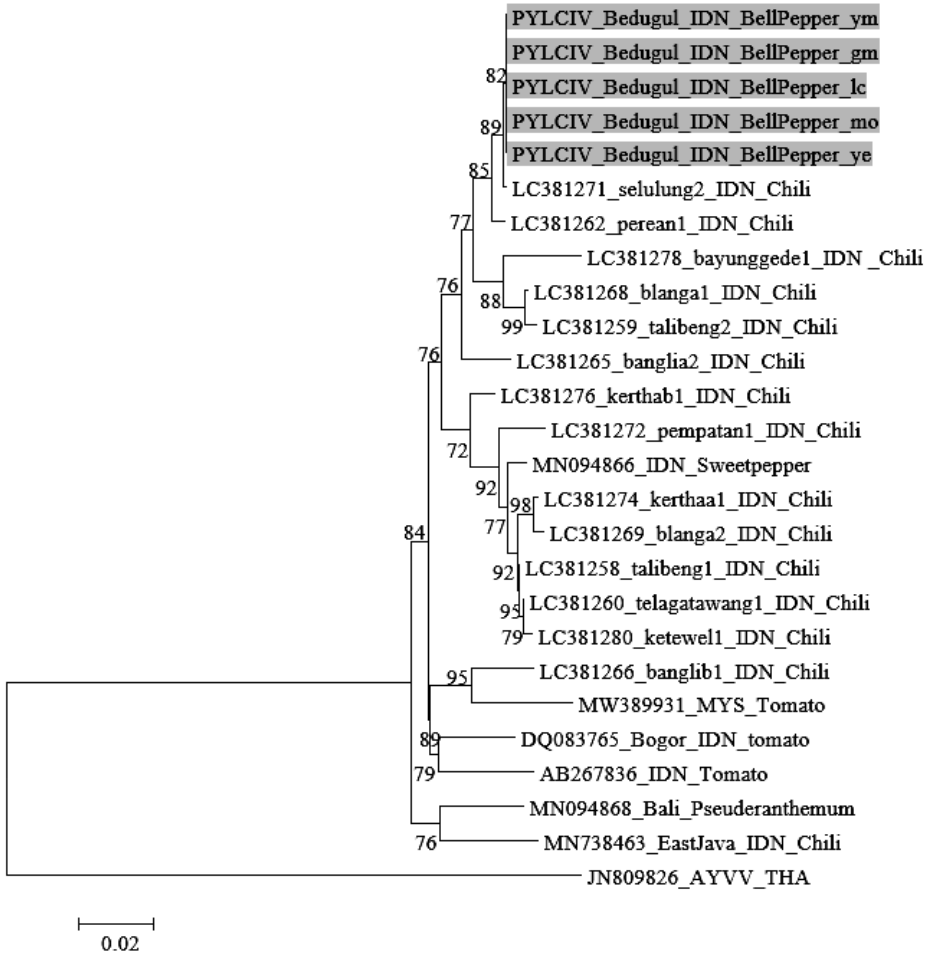

Figure 4. Phylogenetic analysis of PYLCIV infecting bell pepper in Bedugul based on an alignment of partial nucleotide sequences of the DNA-A using Mega 6.06 (Algorithm Neighbor Joining with 1,000 bootstrap replicates). *Ageratum yellow vein virus* (AYVV) from Thailand (THA) is included as an outgroup. Indonesia (IDN), Malaysia (MYS), mottle symptom (mo), leaf curl and malformation (lc), yellowing (ye), green mosaic (gm), and yellow mosaic (ym)

Kintamani is the center of the vegetable nursery in Bali, including chili pepper and bell pepper plants. Farmers in Bali usually buy bell pepper seedlings from the region. That causes the high nucleotide homology between PYLCIV isolates obtained from bell pepper in Bedugul and PYLCIV from the chili pepper plant in Kintamani. The spread of PYLCIV in vegetables in Bali is due to the presence of inoculum sources during vegetable nursing in the Kintamani Sub-district.

PYLCIV infection has spread widely in several chili pepper plantation centers in Central Java, West Java, Yogyakarta, Lampung, South Sumatra, Bengkulu, Jambi, and West Sumatra (Trisno et al. 2009; Sayekti et al. 2021). The incidence rate and distribution varied. In 2021 in Bali, the disease incidence for yellow curl leaf disease in chili pepper reached 100% in all areas with dominant symptoms: yellow mosaic (Selangga et al. 2021). The high infection rate is related to the whitefly population as its vector. Trisno et al. (2009) and Temaja et al. (2022) stated that the percentage of plants infected by viruses would increase along with the increase of viruliferous whitefly.

Generally, plants have defense genes required to inhibit pathogen infection. Plant defense genes are differentiated into dominant and recessive genes. Dominant defense genes may activate through plant-defense gene signal activation upon virus infection. The dominant defense gene mechanism is related to hypersensitive response (HR) through cell death programming, which prevents the virus from infecting other cells (De Ronde et al. 2014; Balint-Kurti 2019; Baebler et al. 2020). The recessive defense gene is related to translation initiation called host factors for infection, replication, translation, and transfer between cells by the virus, such as elF4G (De Ronde et al. 2014; Baebler et al. 2020). Several mutant strains with defense genes against Potyvirus (PVY) infection have been produced. The protein expressed by this recessive gene directly influences virus replication within plant cells (Quenouille et al. 2013; Baebler et al. 2020).

Table 3. Homology nucleotide of PYLCIV Bedugul isolate on bell pepper plants based on different symptoms against isolates from other countries available on GenBank

Isolate source	Isolate	Host	Homology of nucleotide (%)					Accession number
			mo	lc	ye	gm	ym	
IDN	Bedugul_mo	Bell pepper		99.9	99.9	99.9	99.9	-
IDN	Bedugul_lc	Bell pepper	99.9		99.9	99.9	99.9	-
IDN	Bedugul_ye	Bell pepper	99.9	99.9		99.9	99.9	-
IDN	Bedugul_gm	Bell pepper	99.9	99.9	99.9		99.9	-
IDN	Bedugul_ym	Bell pepper	99.9	99.9	99.9	99.9		-
IDN	Selulung_2	Chili pepper	99.8	99.7	99.8	99.8	99.7	LC381271
IDN	Perean_1	Chili pepper	99.2	99.2	99.3	99.1	99.3	LC381262
IDN	Blanga_1	Chili pepper	98.0	98.2	98.0	98.2	98.0	LC381268
IDN	Talibeng_2	Chili pepper	97.8	97.8	97.7	97.7	97.7	LC381259
IDN	Kertha_b1	Chili pepper	97.1	97.0	97.1	97.0	97.0	LC381276
IDN	Bangle_a2	Chili pepper	97.3	97.4	97.3	97.4	97.3	LC381265
IDN	Bayung gede_1	Chili pepper	96.1	96.2	96.1	96.1	96.3	LC381278
IDN	Talibeng_1	Chili pepper	95.9	95.8	95.9	95.8	95.8	LC381258
IDN	Indonesia	Sweet pepper	95.8	95.8	95.7	95.7	95.8	MN094866
IDN	Telaga tawang_1	Chili pepper	95.7	95.6	95.6	95.6	95.5	LC381260
IDN	Ketewel_1	Chili pepper	95.6	95.4	95.5	95.4	95.4	LC381280
IDN	Bangli_b1	Chili pepper	95.5	95.5	95.5	95.4	95.5	LC381266
IDN	Bogor	Tomato	95.5	95.5	95.4	95.5	95.4	DQ083765
IDN	Kertha_a1	Chili pepper	95.2	95.2	95.3	95.3	95.2	LC381274
IDN	Pempatan_1	Chili pepper	95.2	95.3	95.2	95.2	95.2	LC381272
IDN	Blanga_2	Chili pepper	95.0	95.0	95.0	95.1	95.1	LC381269
IDN	Indonesia	Tomato	95.1	95.2	95.2	95.1	95.1	AB267836
IDN	Bali	Yellow-vein eranthemum	94.2	94.1	94.1	94.2	94.2	MN094868
MYS	Malaysia_pep	Tomato	93.9	93.9	93.8	93.9	93.8	MW389931
IDN	East Java	Chili pepper	93.8	93.7	93.6	93.6	93.7	MN738463
AYVV-THA*	Kamphaengsaen	Katuk	76.5	76.6	76.5	76.5	76.4	JN809826

Note: mo: mottle symptom, lc: leaf curl and malformation, ye: yellowing, gm: green mosaic, and ym: yellow mosaic. *AYVV, *Ageratum yellow vein virus* from Thailand (THA), is included as an outgroup.

Chili pepper plants attacked by pests will have slow growth, fail to bud, and die. That is due to the pests attacking the growth point of the plant, causing young leaves to turn up small (vegetative phase) and for the plant to not bud (generative phase). In addition, virus infection in chili pepper plants also causes slow growth and failure to bud if the infection occurs during the vulnerable phase. Several chili pepper plants with virus inoculated can form budding at the growth point, but the budding will fail to grow and fall. Ganefianti et al. (2017) and Ojinaga et al. (2022) stated that the vulnerable phase of plants against virus infection occurs during the nursery phase when the chili pepper has two or six-leaved cotyledons. Therefore, two leaves chili pepper plant is the most vulnerable phase against virus infection, which can lower the growth and production of chili pepper.

The whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) is an exceedingly effective PYLCIV vector with a wide host range. *B. tabaci* attacks over 600 species of plants (Macfadyen et al. 2018; Romba et al. 2018; Costa et al. 2019). The PYLCIV effectivity transmission via insect vector depends on the acquisition and inoculation feeding period and the number of insects involved. Differences in viral strain also cause transmission effectivity differences. The transmission will be more effective if the insect vector and the virus strain originate from the same location (Trisno et al. 2009). The spread of PYLCIV by whitefly is persistent so that the virus can stay in the insect's body for a long time. Moreno-Delafuente et al. (2013) and Temaja et al. (2022) stated that an adult viruliferous whitefly can transmit viruses until death. One viruliferous whitefly can cause 40% of infections (Moreno-Delafuente et al. 2013). The presence of whitefly as an effective vector with a wide range of hosts allows a rapid and difficult-to-control spread of yellow curl leaf disease in chili pepper.

In conclusion, *Pepper yellow leaf curl Indonesia virus* has infected bell pepper plants in Bedugul, Bali, with a disease incidence rate of 11.5-21.5 on plants aged 63 DAP. The dominant symptoms in bell pepper plants were leaf curl, malformation, and yellow mosaic. PYLCIV isolates from bell pepper plants showed the highest nucleotide homology, with isolates from Selulung, Kintamani obtained from chili pepper plants. The spread of PYLCIV in the field was caused by viruliferous whitefly.

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