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Effect of Screen House on Disease Severity and Coat Protein Diversity of *Begomovirus*-infected *Capsicum frutescens* L. 'Cempluk' from Indonesia

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ABSTRACT

Chili can be infected by *Begomovirus* through whiteflies (*Bemisia tabaci*) serving as a vector insect. *Begomovirus* infection causes dwarf plants and yellow curly leaves. The molecular detection of *Begomovirus* coat protein gene may serve as a preliminary identification of *Begomovirus*. This study was conducted to observe the differences in the symptom severity of *Begomovirus* infection in chilies (*Capsicum frutescens* L. 'Cempluk') planted inside and outside a screen house. This study also observed whether or not using a screen house in chili farming affects the diversity of the coat protein of *Begomovirus*. Symptom observation and sampling were conducted in Madurejo, Prambanan, Sleman. Molecular detection was performed by amplifying the coat protein (CP) gene using the universal primer Krusty and Homer. Results showed 7 plant samples with DNA bands ± 550 bp and confirmed that the plants were positively infected with *Begomovirus*. The amplified bands were purified and sequenced. The nucleotide sequences were analyzed using BLASTn, followed by phylogenetic analysis using MEGA. Planting chili in the screen house resulted in low disease severity and good crop conditions. The coat protein sequence showed different strains of *Begomovirus* infected the chili plants inside and outside the screen house. *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) was found inside the screen house while PepYLCIV [Ageratum] was dominant outside the screen house. Both strains are closely related to other *Pepper yellow leaf curl virus* (PepYLCV) from various regions in Indonesia. Optical

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manipulation using an ultraviolet screen or screen house was effective in reducing *Begomovirus* infection and improving plant performance.

Keywords: *Begomovirus*, coat protein, disease severity, *Pepper yellow leaf curl Indonesia virus*, plant defense mechanism, plant hormones

INTRODUCTION

Yellow leaf curling caused by *Begomovirus* in chili has long been a problem in Indonesia and has caused crop failure in various regions. Yellow leaf curling in chili was first reported in 1999 in West Java as being caused by geminiviruses, mainly from the genus *Begomovirus* (Rusli et al., 1999). Since 2000, this disease has spread in Yogyakarta and Central Java and has reduced chili production (Sulandari et al., 2006).

Begomovirus-infected chilies are characterized by the presence of specific yellow mosaics on the leaves, curling of leaves from the edges, and stunted plant growth (Sulandari et al., 2006). *Begomovirus* infections are usually transmitted by whitefly (*Bemisia tabaci*), which is a viruliferous insect that feeds on plant phloem by injecting enzymes and secreting sap. It reduces plant strength or, in the case of severe attacks, kills the host (Horowitz et al., 2011; Kumar et al., 2017). The whitefly population increases in high humidity and causes crop failure. *Begomovirus* infection in chili plants may reduce harvest by 20%–100% (Setiawati et al., 2005).

Begomovirus is the largest genus in the *Geminiviridae* family. It infects a wide range of hosts, such as cultivated plants, weeds around plants, monocotyledonous plants, and dicotyledonous plants in tropical or temperate climates. Some of its strains that were reported to have infected chili and tomato plants were the *Tomato leaf curl Java virus* (Kon et al., 2006), *Pepper yellow leaf curl Indonesia virus*, *Tomato leaf curl Philippine virus* (Sakamto et al., 2005), *Ageratum yellow vein virus* (Tsai et al., 2009), and *Tomato yellow leaf curl Kanchaburi virus* (Kenyon et al., 2014). Its genome consists of ssDNA components DNA-A and DNA-B, and each genome size measures 2.5–2.8 kb (Snehi et al., 2011). *Begomovirus* is divided into two groups according to complete nucleotide sequences: bipartite and monopartite. The bipartite group has two types of ssDNA components, namely, DNA-A and DNA-B; the monopartite group has one circular homologous genomic DNA with DNA-A. Open reading frames (ORFs) in DNA-A and DNA-B in *Begomovirus* genomes produce several types of proteins with different functions. One of the most important ORFs is AV1/CP. This gene is responsible for expressing the protein coat that forms viral capsids. Furthermore, the CP gene regulates the transmission of the virus to whiteflies and host plants. The CP gene is the most conserved genome region among all genes/ORFs (Snehi et al., 2011; Wartig et al., 1997).

Begomoviruses tend to rejoin, thus causing new *Begomovirus* strains and new plant diseases in various host plants (Chakraborty et al., 2003; Varma & Malathi, 2003). Therefore, the detection of *Begomovirus* particles is needed to determine infected plants and find the source of infection. The assessment of viral diseases can be conducted by morphological observation and molecular detection. Symptom monitoring is a simple, fast, and inexpensive method for assessing the presence of *Begomovirus* infection. However, the results often vary due to subjective interpretations and effects of environmental conditions, hence the need to conduct reliable assessments, such as molecular detection (González-Pérez et al., 2011). The application of polymerase chain reaction (PCR) techniques to detect viral infections in plants is a sensitive, reliable, reproducible, and effective method, especially for large samples (Lopez et al., 2008). Previous studies reported the use of molecular methods to detect *Begomovirus* infections in chilies and eggplants (Maruthi et al., 2007), okra (Venkataravanappa et al., 2018), and tomatoes (Kusumaningrum et al., 2015). The present study was conducted to determine the effect of using screen houses in chili (*Capsicum frutescens* L. 'Cempluk') cultivation on the severity of *Begomovirus* infection and the diversity of *Begomovirus* coat proteins that cause yellow leaf curling in chilies using molecular methods.

METHODS

Morphological Observation and Sample Collection

Observation and sampling were performed in Madurejo, Sleman, Yogyakarta. Symptom observations were carried out in two study areas, namely, outside and inside a screen house in February–October 2018. A total of 469 and 41 chili plants (*Capsicum frutescens* L. 'Cempluk') were planted outside and inside the screen house. Virus infection in chili was classified into six scale groups on the basis of the plant height and scale of virus infection established by Srivastava et al. (2017) with a few modifications. The scale was as follows: 0: healthy plants, scale 1: yellow spot leaves, scale 2: yellow spots and moderately curved leaves, scale 3: yellow spots and curved leaves, scale 4: yellow and curly leaves, scale 5: fully yellow and curly leaves, stunted plants. Disease incidence (DI) was calculated using the following formula (Srivastava et al., 2017):

$$DI = \frac{Number\ of\ infected\ plants}{Total\ plants} \times 100\%$$

The scoring results were then converted into the disease severity (DS) index using the following formula to reveal DS (S. Islam et al.., 2010):

$$DS = \frac{\sum(ni \times Vi)}{Z \times N} \times 100\%$$

where, n_i = sum of plant in each score, V_i = score of symptoms, Z = value of highest

symptom, and N = total number of observed plants.

DNA Isolation and Amplification of Coat Protein Gene

DNA was extracted from chili leaf samples by using the commercial plant DNA extraction kit Illustra Phytopure™ and by following the processes described by Daryono and Natsuaki (2002) with slight modification. Furthermore, DNA was checked quantitatively using NanoDrop™ spectrophotometry with λ 260/280 nm. Universal primers for *Begomovirus* coat protein, namely, Krusty and Homer (Krusty [Forward]: 5′CCNMRDGGHTGTGARGGNCC′3; Homer [Reverse]: 5′SVDGCRTGVGTRCANGCCAT′3), were utilized to amplify the partial coat protein of *Begomovirus* by using the PCR thermal cycler (Revill et al., 2003). Infected plants show a DNA band at about ~550 bp in electrophoresis visualization. A PCR mix (25 µL) containing 12.5 µL PCR kit Bioline 2x MyTaq™ HS RedMix (Bioline, United Kingdom), 1.5 µL for each primer (10 pmol), 1.5 µL DNA samples (100 pmol), 0.5 µL $MgCl_2$, and 7.5 µL distilled water was used. The PCR reaction was started with initial denaturation at 95°C for 5 min; continued with 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and then followed by a final extension at 72°C for 5 min. The PCR results were analyzed using 2% of agarose gel, which was stained with FloroSafe DNA stain (First BASE, Singapore).

DNA Sequence Analysis

Positive PCR samples were sent to First BASE, Singapore for sequencing. Then, nucleotide sequences were assembled using GeneStudio™ software, followed by BLASTn analysis for comparing sequences with other coat protein gene sequences of *Begomovirus* in GenBank. A phylogenetic tree was constructed using MEGA 7.0 with 2,000 bootstraps and then edited manually.

RESULTS

Observed Symptoms on Leaves

The symptom observation of *Begomovirus* infection was focused on leaf morphology. However, some of the plants observed were found to be dead due to wilting rather than the *Begomovirus* infection. Seven and nine dead plants were found inside and outside the screen house, respectively. The differences in the responses of the plants inside and outside the screen house to the *Begomovirus* infection are detailed in Table 1. The results of the study showed that the incidence and severity of the disease was

Table 1
Response of chili to natural Begomovirus *infection*

	Symptomatic plants/ total plants	Disease incidence (%)	Disease severity (DS)
Chili inside screen house	25/41	60.9	42
Chili at field	455/464	97	53.8

greater in the chili plants outside the screen house than in those planted inside the screen house. The use of a screen house thereby reduced the *Begomovirus* infection because it protected the plants from whiteflies while minimizing the effects of environmental stress on plant development.

The severity of the morphological symptoms on the leaves of the chili plants was grouped into six categories according to the observed symptoms (Figure 1). This scale determination was based on the study of Srivastava et al. (2017). The most common morphological symptoms in the chilies planted outside the screen house were yellow and curved leaves (scale 3). Those observed in the chilies inside the screen house indicated relatively health plants despite the presence of yellow spots on the leaves (scale 1). The real difference was that no plant inside the screen house showed yellow and curly leaves (scale 4). The most severe symptoms observed in both locations were completely yellow and curly leaves and stunted growth.

Effect of *Begomovirus* Infection on Plant Height

One symptom of *Begomovirus* infection is stunted plant growth. Hence, plant height measurements were carried out on the plants in both study areas. The chilies planted outside the screen house were measured to be about 126–135 cm tall, whereas those planted in the screen house were taller with a height of 142–149 cm. Rank analysis in SPSS® software also showed a significant difference in the average heights of the chili plants outside and inside the screen house (Table 2). This condition supports the previous explanation that planting chilies in a screen house provides good plant conditions.

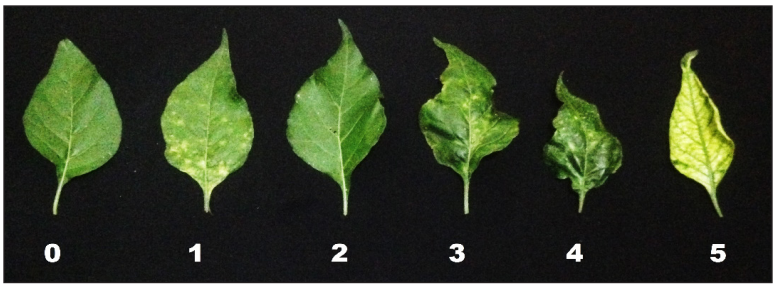

Figure 1. Morphological comparison of leaves based on the scale of virus infection: 0: healthy plants, 1: yellow spot leaves, 2: yellow spots and moderately curved leaves, 3: yellow spots and curved leaves, 4: yellow and curly leaves, 5: yellow and curly leaves, stunted plants, respectively

Table 2
Differences in average plant heights of chilies planted inside and outside the screen house

	Location	N	Mean rank	Sum of rank
Plant height	Screen house	34	303	10302
	Field	469	248.3	116454
	Total	503		

Molecular Diagnosis of *Begomovirus* Infection

Total DNA was extracted from 16 plant samples in the six infection scale groups; that is, eight samples were obtained from each of the two locations. A set of primers (Krusty and Homer) was used to amplify the CP gene region using the PCR technique. However, only 7 samples from the 16 samples were amplified; these samples showed a target DNA band at ±550 bp and were positively infected by *Begomovirus* (Figure 2). A total of five samples from outside the screen house produced the targeted bands, whereas only two samples from inside the screen house did. *Begomovirus* DNA was only found in the samples in the 3–5 scale; none was found in those in the 0–2 scale. This result indicated that the severity of the symptoms was correlated with the concentration of virus particles. The low severity of symptoms indicated low viral DNA concentrations and vice versa.

Diversity of Coat Protein Sequences

Each of the two samples from each location was sequenced to analyze the CP gene sequence. Then, the obtained sequences were analyzed using BLASTn to compare them with other nucleotide sequences in GenBank as the initial identification of *Begomovirus* strains. Table 3 shows that the two locations have the potential to be infected by the *Pepper yellow leaf curl Indonesia virus* with a CP percentage of similarity of >97%. According to the CP sequences, the samples from the screen house showed a close genetic relationship

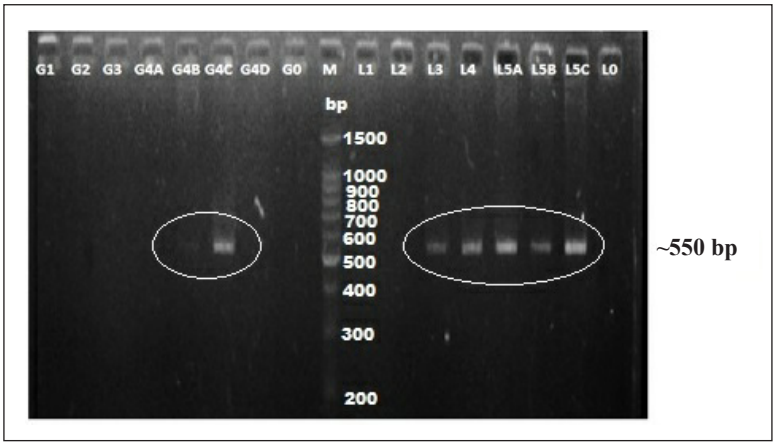

Figure 2. Electrophoresis visualization of CP gene amplification
Note. G: inside screen house samples; L: outside screen house samples; 0–6: infection scale

Table 3
Sequence analysis of Begomovirus *CP gene*

Sample	% Identity	Potential *Begomovirus* species	GenBank accession
Inside screen house	99.52	PepYLCIV	AB267834
Outside screen house	97.12	PepYLCIV [Ageratum]	AB267838

with the *Pepper yellow leaf curl Indonesia virus*, whereas the samples from outside the screen house showed CP sequences with 97.12% similarity to the *Pepper yellow leaf curl Indonesia virus* [Ageratum]. However, this analysis cannot be used for final identification as it may only serve as an indication of the prevalence of *Begomovirus* strains that cause curly leaves in chili 'Cempluk' planted inside and outside a screen house.

The nucleotide sequence of the CP gene from each location was compared with 22 nucleotide sequences from different virus strains to evaluate their genetic relationship. The genome samples used in the comparison came from several plants, such as *Capsicum frutescens*/chili, *Capsicum annum*, *Solanum lycopersicum*/tomato, and *Ageratum* (weeds). The analysis was performed by constructing a phylogenetic tree in MEGA 7.0. The phylogenetic tree showed that the two samples from the two locations are closely related to PepYLCIV isolated from various regions in Indonesia, such as West Sumatra, Banda Aceh, and Bogor (Figure 3). Begomoviruses detected in the plants from the inside and outside of the screen house were grouped into different clades. The samples isolated from inside the screen

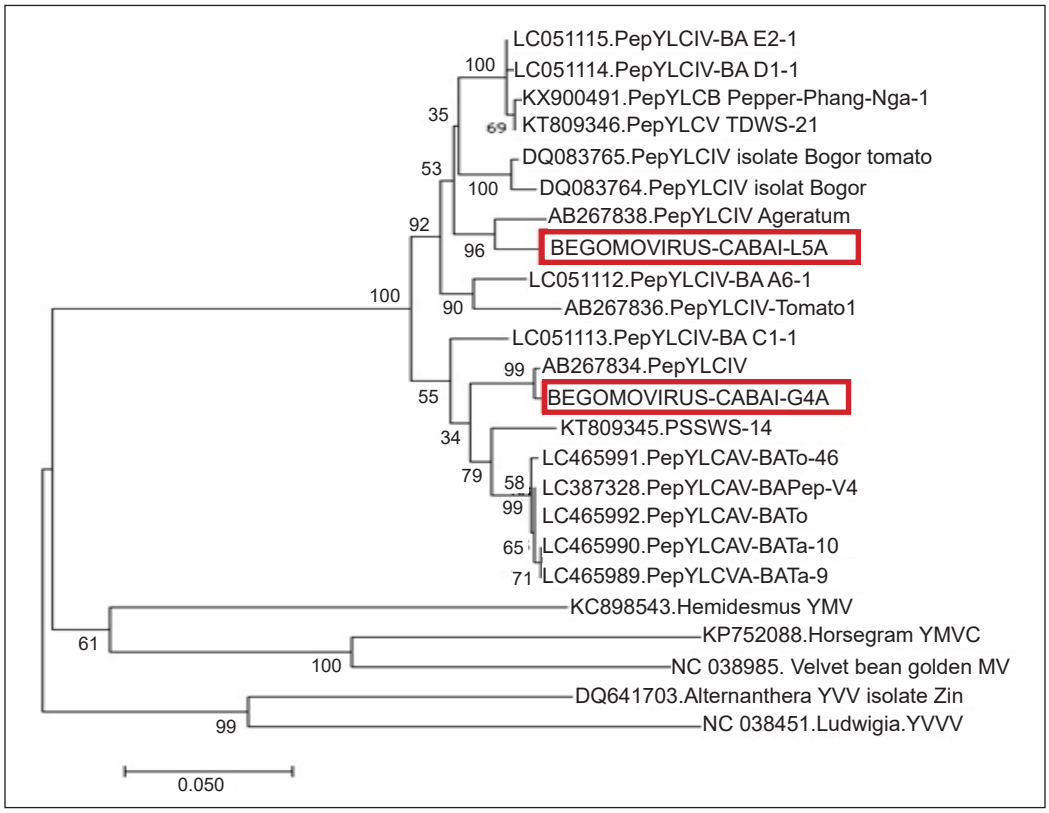

Figure 3. Phylogenetic tree of the partial CP gene of *Begomovirus* constructed using the neighbor joining algorithm with 2,000 bootstraps

house showed a close genetic relationship with PepYLCIV isolated from Bogor, West Java. The samples from outside the screen house showed a close genetic relationship with PepYLCIV [Ageratum] (Figure 3). These results indirectly indicated that the source of the *Begomovirus* infection outside the screen house came from nearby plantation crops or infected weeds.

DISCUSSION

Begomovirus infection in chili is a serious problem in Indonesian agriculture. Chili is one of the horticultural commodities with high economic value. Chili plants show several obvious symptoms when infected with *Begomovirus*; these symptoms include yellow spots on the leaf surface, vein clearing, thickening at the leaf bones, and cupping (Sulandari et al., 2006). Morphological observation is a simple way to identify the early symptoms of *Begomovirus* infection (González-Pérez et al., 2011). The results of symptom observations are usually converted into DI and DS to obtain a reliable estimation for plant response determination (Bock et al., 2015). The present study showed that DS inside the screen house was lower than that outside the screen house (Table 1). This result might be due to the fact that the condition of the plants in the screen house was more controlled than that outside the screen house. Inside the screen house, the plants were not exposed to abiotic stress, such as high temperatures, rain, and extreme temperature changes. This condition can affect plant resistance to *Begomovirus* infection. Simultaneous abiotic and biotic stresses can exert antagonistic, synergistic, or additive effects on plants, and these effects may lead to increased or decreased susceptibility to stresses. The great damage in the field with uncontrolled conditions can be attributed to secondary stress from a combination of two stressors exerting a negative or additive effect on plants (Asselbergh et al., 2008). Suleman et al. (2001) reported that drought stress in common beans infected with *Macrophomina phaseolina* leads to other symptoms. The same result was found when exogenous ABA was applied to detached tomato leaves; such application increases the susceptibility of wild type plants to *Botrytis cinerea* (Audenaert et al., 2002).

The results of the current study also showed that planting in the screen house led to a reduction in the population of whiteflies. Such outcome was deduced as the cause of the reduction in *Begomovirus* infection because this virus is only transmitted by whiteflies. This result was also reflected in the low scale of yellow leaf curling symptoms found in the plants in the screen house. Meanwhile, the plants outside the screen house showed diverse symptoms. This result may be due to the uncontrolled growth of whitefly infestation because *Begomovirus* can be carried by whitefly infestation from nearby weeds or crops that have been infected previously. Some wild plants (*Hyptis brevipes*, *Physalis floridana*, and *Crotalaria juncea*) and weeds (*Ageratum conyzoides*) are often found

around chili fields, and they are susceptible to *Begomovirus* infections (Sulandari et al., 2006).

As *Begomovirus* cannot be restrained directly, the most effective strategies are controlling the population of *Bemisia tabaci*. Whitefly, from the family Aleyrodidae and the Homoptera order, is a complex cryptic species of small insects with a piercing and sucking mouth type. These insects are distributed globally in tropical, subtropical, and low-climate regions (Wang et al., 2018). When infecting plants, whiteflies cause the shrinkage of plant nutrients as they spread the virus. Through their stylet, whiteflies suck the phloem liquid, thereby decreasing and even draining the nutrients in the leaves completely and causing the leaves to turn yellow. The reduction of leaf nutrition also causes a slope in plant photosynthesis activity while weakening the plants (Horowitz et al., 2011). Whitefly infestations affect plants in three stages of growth: nursery, flowering, and fruiting. Whiteflies in the nymph and imago stages suck the juice of plants through their mouth resting in a protected position in the rostrum. Whiteflies infect intercellular plant tissues and introduce fluid into the phloem, thereby inhibiting photosynthesis and affecting fruit conditions (Mohamed, 2012). Chemical control is not highly effective in preventing infestations because virus acquisition, as well as the development of virus resistance, requires a short time. Previous studies revealed the effects of insecticides on whiteflies after virus acquisition (Antignus, 2010). However, after a certain period of time, the effectiveness of insecticides decreases due to adaptations that cause resistance to insecticides; moreover, the relatively high toxicity of these insecticides for non-target organisms (including arthropods and humans) makes them ineffective in the efforts to control whitefly infestations (Mascarin et al., 2013; Wang et al., 2018).

Whiteflies only rely on their vision for navigation and orientation because their olfactory reaction is poor. They are sensitive to ultraviolet (UV) and the visible range of the electromagnetic spectrum. Numerous studies also reported that a disruption in UV vision might cause a disturbance in dispersal or orientation (Antignus, 2010). The screen of a screen house eliminates UV spectrum between 280 and 380 nm, thereby significantly diminishing the infestation of insects (including thrips, aphids, and whiteflies) on crop plants. The use of a screen has also been reported to reduce *Tomato yellow leaf curl virus* and *Cucumber yellowing stunting disorder virus* infections in tomato and cucumber (Kumar & Phoeling, 2006). Thus, using a screen house could hinder the entrance of whiteflies, which disrupt plant development. Although whiteflies were still found inside the screen house in the current study, they were fewer than those found outside the screen house.

Dramatic morphological and physiological changes in virus-infected plants result in the reduction of crop yield. Many previous reports revealed that virus infections can disrupt metabolic and

physiological processes, such as respiration, transpiration, and photosynthesis (Tajul et al., 2011). The results of the current study are in agreement with those of Khalil et al. (2014), who reported that *Begomovirus* infections in tomato cause the reduction of root length, number of plant leaves, shoot height, and the fresh and dry weight of shoots and roots of plants. The decrease in morphological quality and quantity might be related to the reduction of photosynthesis level due to the disruption of photosynthesis pigments. *Begomovirus* infections have been reported to cause mineral deficiency that increases the degradation/damage of the chloroplast thylakoid membrane (Khalil et al., 2014). Radwan et al. (2007) also revealed that *Zucchini yellow mosaic virus*-infected plants present decreased chlorophyll pigments. The disruption of chlorophyll pigments may be related to the production of plant defense hormones, such as salicylic acid (SA) and jasmonic acid (JA). JA and SA elicit the expressions of specific hormone-responsive genes that restrict invading pathogens (Spoel et al., 2007). Meanwhile, chloroplasts also play a role in innate immunity by restricting viral spread and systemic infections; they also serve as the site for defense hormone production. Chloroplasts undergo structural and functional damage as they become the main target of virus infections (Bhattacharyya & Chakraborty, 2018).

In the current work, the molecular detection of *Begomovirus* was conducted using coat protein genes. The coat protein gene sequence can be used to detect the presence of *Begomovirus* infection quickly and accurately with molecular characterization. CP gene sequences have conserved areas near the 5′ and 3′ ends and varied regions at the 5′ end along 200 nucleotides (Sinha et al., 2013). Thus, mutations in CP are associated with the emergence of *Begomovirus* variations (Subiastuti et al., 2019). Moreover, this sequencing has been used for the early identification of begomoviruses associated with cultivation plants in Mexico. However, the sequencing of the full DNA-A of *Begomovirus* needs to be carried out to achieve precise and accurate identification results (Hernandez-Zepeda et al., 2007). Previous studies also used this method for the quick identification of *Begomovirus* in melon (Subiastuti et al., 2019), tobacco (Widarta et al., 2017), tomato (Kusumaningrum et al., 2015), bitter melon (Tiwari et al., 2010), and mung bean (M. N. Islam et al., 2012).

Sample L5A was planted outside the screen house. Wild plants and weeds, as well as overlapping planting systems, were found near this study location. This condition facilitates the great prevalence of mixed infections (Subiastuti et al., 2019). The result herein also indicates that the sources of *Begomovirus* infection are nearby plantation crops or weeds that have been infected. *Ageratum* is one of the most common weeds found in fields near plantation crops, and they might be the source of the *Begomovirus* infection in this study. This phenomenon is in agreement with the results obtained by Shibuya et al. (2007) and Sakata et al. (2008), who found

that the Indonesia yellow vein disease in *Ageratum conyzoides* plants is caused by PepYLCIV. As reported by Mubin et al. (2009), weeds are reservoirs of *Begomovirus* that may be responsible for viral infections in crop plants. Weeds also facilitate virus recombination as they frequently harbor multiple viruses; this process results in new viruses/new strains. Various studies also reported *Begomovirus* infection in weeds. Graham et al. (2007) found that weeds of the genus *Sida* persistently harbored several begomoviruses originating from pseudo-recombination or molecular recombination; this finding led to the identification of *Sida micrantha mosaic virus*. Moreover, *Deinbollia mosaic virus*, a weed-infecting *Begomovirus*, has been reported to infect Solanaceae and Euphorbiaceae (Kyallo et al., 2017).

The L5 samples in the current work showed possible differences in PepYLCIV strains that infected the chili plants outside the screen house. This difference might be due to the condition of the plants in the screen house being more homogeneous than that outside the screen house. Further research is needed to obtain a full sequencing of the viral genome to complete the identification of the differences in the *Begomovirus* strains in the plants inside and outside the screen house and to determine potential polymorphism in both strains associated with DI or DS. The presence or absence of betasatellite and differences in DNA-A sequences might influence the severity of the disease in plants and host determination (Mansoor et al., 2003; Zubair et al., 2017).

CONCLUSION

Optical manipulation using a UV screen or a screen house was effective in reducing either *Begomovirus* infection or whitefly population. Understanding the source of infection will help to prevent the spread of infection. Molecular detection offers a fast method to detect the source of *Begomovirus* infection accurately while detecting its diversity. These strategies are expected to be a sustainable agriculture effort as they may reduce the use of insecticides and serve as alternatives to the development of resistant cultivars for controlling *Begomovirus* infections.

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