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THE PRIMARY INOCULUM SOURCES IN THE EPIDEMIOLOGY OF *PEPPER YELLOW LEAF CURL INDONESIA VIRUS* ON CHILI PLANTS

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A B S T R A C T

Pepper yellow leaf curl Indonesia virus (*PepYLCIV*), a Begomovirus in Geminiviridae, is transmitted in a persistent manner by *Beminisa tabaci* Genn. (Hemiptera: Aleyrodidae). The virus has caused tremendous damages to chili plants in Indonesia. The disease had been reported non seed-transmissible and exclusively transmitted by whitefly, however, our field observations and previous reports showed that high disease incidence was present in the absence of vector. Therefore, the present study was conducted to determine the roles and the importance of seed, vector in the seedbed, and vector in the field as a source of primary inoculum for disease development. To determine whether the disease is seed-transmissible, seeds from *PepYLCIV*-infected plants were sown in cage and sprayed with insecticide weekly to prevent *B. tabaci* infestation. Vector population and *PepYLCIV* incidence were monitored weekly. To determine whether the vector acted as inoculum source in the seedbed, certified seeds of chili cv. Pilar were sown in caged and uncaged seedbeds. To determine whether vector acted as inoculum source in the field, caged seedlings were planted in open field (uncaged). Our results indicated that *PepYLCIV* is seed transmissible with an incidence of 2.5%. The highest disease infection was found on uncaged plants both in the seedbed and in the field. Therefore, the use of healthy seeds and protecting seedbed from vector infestation is very important in preventing infection on the seedlings. Besides that, any control measures in a frame of integrated pest management should be employed to suppress the vector population in the field.

Keywords: Primary inoculum source, *PepYLCIV*, chili, epidemiology, seed-borne disease.

INTRODUCTION

In Indonesia, chilies (*Capsicum annum* L. *and C. frutescens* L.) are important horticultural crops. They have high contents of nutritions, vitamins, and minerals (Harpenas and Dermawan, 2010). Besides this, chilies have higher economic values compared to other main crops in the country, such as rice and corn. Average national chili production is about 2.7 million tons annually with an average productivity of 3.5 tons/ha (BPS, 2021). *Pepper yellow leaf curl Indonesia virus* (*PepYLCIV*), a Begomovirus of Geminiviridae, and its vector *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) have become major limiting factors of chili production in the country.

The vector can cause direct damage to plant by sucking the plant sap, reducing yield up to 80% (Inayati and Marwoto, 2015), while the virus infection can cause total yield loss (Munandar and Suwandi 2021, Santoso *et al.,* 2016; Setiawati *et al.,* 2007). *PepYLCIV*-infected plants show typical symptoms of leaf yellowing, vein clearing, vein thickening, curling, cupping; smaller leaf and fruits; and stunted plants (Dombrovsky *et al.,* 2010; Sukada *et al.,* 2014; Sulandari, 2006).

Pepper yellow leaf curl Indonesia virus is transmitted by *B. tabaci* in a persistent, non-propagative manner (Adilah and Hidayat, 2014; Putri *et al.,* 2018) and it is not seed-transmissible (Rusli *et al.,* 2000; Sulandari *et al.,* 2001; Sulandari, 2006). However, recently, Fadhila *et al.* (2020)

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indicated that *PepYLCIV* was detected in seeds obtained from *PepYLCIV*-infected plants. Furthermore, the seedlings generated from the seeds were also confirmed infected by the virus. Vivaldi (2017) reported that high incidence of the viral disease was found although the vector population was low in the field. Thus, seeds might play an important role in the spread of the virus. This is new information that could change our current understanding of the disease epidemiology, especially the inoculum source of the pathogen in the field.

Therefore, there are three possible primary inoculum sources of *PepYLCIV* in the field. Firstly, seeds obtained from plants naturally infected by the virus in the previous season could act as inoculum source. Viral diseases vertically transmitted from one plant generation to the next through seeds have a potential to spread quickly in a long distance and cause epidemic in a large area. Besides that, a seed transmissible virus infects young seedlings immediately after emergence causing high disease incidence and severity later in the season that could reduce yield substantially. Secondly, viruliferous whitefly could act as primary inoculum source of inoculum by introducing virus into the seedbed. Infected seedlings act as secondary inoculum source for the development of infection on the other seedlings and other plants after transplanting. Lastly, viruliferous whitefly introduces virus into a new chili plantation. From the early infected plants, the virus could be transmitted to the other plants as long as the vector is present in the field.

Pepper yellow leaf curl Indonesia virus is a damaging disease of chili, thus, it is necessary to develop an integrated management approach based upon the epidemiological study of the disease. Inoculum source is very important in epidemiology of a plant diseases because it affects how and when a pathogen infects a plant. Plants infected early in the season have higher infection in comparison to plants infected late in the season (Kandito *et al.,* 2021; Koeda *et al.,* 2018). These in turn affect the disease intensity in plant and the management strategy appropriate for its control. The current study was conducted to elucidate the roles and the importance of seed, vector in the seedbed, and vector in the field as a primary source of inoculum for *PepYLCIV* disease.

MATERIALS AND METHODS

Experimental site: Field experiments were conducted at the Agricultural Experiment Station, Faculty of Agriculture, Hasanuddin University (5°07'42" S, 119°28'47" E), Makassar, Indonesia, from July 2020 to March 2021. The station is an ideal site for studies on the epidemiology of *PepYLCIV* because the virus and its vector, *B. tabaci* have been continuously present and causing severe damages to chili plants for the previous five years.

Seeds as primary *PepYLCIV* inoculum source: This field experiment was carried out to determine if *PepYLCIV* can spread through seeds. Seeds of seven chili cultivars: Pilar, Lado, Laris, Bara, Bhaskara, Kastilo, and Kopei, obtained from plants naturally infected by *PepYLCIV* in the field, were used in this experiment. One hundred seeds of each cultivar were sown in a seedbed confined within a whitefly-proof cage (6 x 1.5 x 0.8 m (L x W x H) covered with fine cloth (80 mesh) (Gunaeni, 2015). The seedbed was raised about 20 cm using a hoe and the top soil was mixed with a chicken manure compost, equivalent to two tons per ha. The seedlings were sprayed with imidacloprid (Confidor 200SL) once per week to prevent whitefly infestation and *PepYLCIV* infection on the seedlings. The seedbed was manually weeded and watered as needed and the seedlings were ready for transplanting six weeks after emergence. Seedlings were observed weekly to determine the number of *B. tabaci* eggs, nymphs, and adults present on each seedling. Disease incidence on the seedlings was also determined on weekly basis by calculating the percentage of infected seedlings for each cultivar.

Thirty seedlings for each cultivar were individually transplanted into plastic pots, containing growth medium of soil and chicken manure mixture (1:1). The pots were placed in the field and confined in whitefly-proof cages, covered with fine cloth (80 mesh) as described before. The plant maintenance followed the standard practice for watering and fertilizing. The plants were sprayed with imidacloprid (Confidor 200SL) every seven days to protect the plants from whitefly infestation and virus infection. Weekly observations were conducted to determine the vector population, disease incidence, and disease severity on each cultivar.

Vector populations were assessed by observing three leaves per plant (Hendrival *et al.,* 2011; Nasruddin *et al.,* 2020). The leaves were turned slowly and then counted all adults present. The same leaves were brought to our laboratory for egg and nymphs counting under a dissecting microscope (100-200x). Disease incidence was determined by calculating the percentage of plants infected by the chili leaf curl disease. Disease severity

was calculated using scoring system based on the symptom severity, ranging from 0 - 5: 0 = no symptoms, 1 = leaf yellowing; 2 = leaf yellowing and curling; 3 = leaf yellowing; curling, cupping up or down; 4 = leaf yellowing, curling, cupping up and down; and 5 = leaf yellowing, curling, cupping up and down, and plant stunted (Gaswanto *et al.,* 2016). Scores were then input into the following formula:

$$DS = \frac{\sum ni \ x \ vi}{N \ x \ Z} x \ 100\%$$

DS = diseases severity, ni = number of plants showing a particular score, N = number of plants observed, and Z = the highest score of the scoring system (5).

Whitefly adults as a source of primary inoculum in the seedbed: This field experiment was conducted to determine if *B. tabaci* can act as primary inoculum source for seedling infection in the nursery. Certified seeds of Chili cv. Pilar were used in this study. Treatments consisted of caged seedbed and uncaged seedbed. For the caged seedbed, the seedbed was confined in a cage as described before (Gunaeni, 2015). Treatments were arranged in randomized complete block design with three replications of one seedbed each. Each seedbed contained 150 seeds that were scattered evenly on the seedbed. Caged seedlings were treated with insecticide imidacloprid (Confidor 200SL) weekly to establish an insect-free nursery. While, the uncaged seedlings were left unsprayed to develop whitefly infestation. The seedlings were fertilized with NPK 16-16-16 (1 g/liter of water), starting two weeks after sowing and every 10 days thereafter, until the seedlings were ready for transplanting (6 weeks after sowing).

Vector population was assessed by observing 10 seedlings per seedbed. Leaves were turned slowly and then counted all adults present. The same leaves were brought to the Laboratory of Insect in Relation to Plant Disease, Hasanuddin University, for eggs and nymphs counting under a dissecting microscope (100-200x). Disease incidence was determined by calculating the percentage of plants infected by the chili leaf curl disease. Disease severity was measured by using scoring system based on the symptom severity as described by Gaswanto *et al*. (2016).

Whitefly adults as primary inoculum source in the field: This field experiment was conducted to determine if *B. tabaci* can act as primary inoculum source for plant infection in the field. Caged seedlings were planted in uncaged field. Caged seedlings used in this experiment came from the experiment described in the previous section. Each treatment had four replications of a plot each (3 rows wide and 2 m long), containing 15 plants. One day before transplanting, soil was evenly mixed with chicken manure fertilizer equivalent to two tons per ha before it was covered with silver plastic mulch (Hafizah and Mukarramah, 2017).

Five weeks old seedlings were transplanted in the field with planting space of 60 cm between rows and 60 cm between plants within a row. Cage treatment was installed one day before transplanting. Cage size used was 3 x 2 x 1.5 m (L x W x H) with a frame made of 0.75 in plastic pipe, covered with fine cloth (80 mesh).

Plants were maintained following the local recommended practices. First fertilization was applied one day before transplanting using NPK (16:16:16) at the rate of 50 kg per ha (Moekasan *et al.,* 2015). Subsequent fertilizer applications started 21 days after transplanting and every seven days thereafter for a total of 16 applications. Total fertilizer used was equivalent to 200 kg per ha for the whole season. Plants were irrigated as needed using overhead sprinklers and weeds were manually controlled using a hoe.

Number of whitefly eggs, nymphs, and adults; *PepYLCIV* incidence and severity were *assessed every seven days for a total of 16 observations. For whitefly population assessment,* from each replication plot, three plants were randomly selected. From each plant sample, three leaves were randomly selected, one from each canopy stratum, lower, middle, and upper (Hendrival *et al.,* 2011). Adults were counted in the field, while eggs and nymphs were counted under a dissecting microscope as explained before.

Disease incidence was determined by calculating the percentage of plants infected by the chili leaf curl disease in each replication plot. Disease severity was assessed using scoring system based on the symptom severity as described before (Gaswanto *et al.,* 2016).

Relationship between occurrence time of primary infection and disease severity at the end of the season: Twenty plants infected during the vegetative stage of the plant and 20 plants infected during the generative stage of the plant were randomly selected and marked for diseases severity rating at the end of the season. Disease severity was assessed using scoring system based on the symptom severity as described by Gaswanto *et al.,* 2016).

Confirmation *PepYLCIV* infection: To confirm that plants expressing yellowing and curling symptoms were

infected by *PepYLCIV*, a molecular detection using a PCR procedure was performed. Leaf showing typical symptoms of *PepYLCIV* curling,yellowing, and blisters were picked up to detect the presence of *PepYLCIV*. Total plant DNA extraction was performed using CTAB procedures. Viral DNA was amplified with a polymerase chain reaction procedure (PCR) using primers to specifically detect 385 bp of DNA-B (forward: 5′-TGT CCT CAT CGT AGT CAC ACA-3; reverse: 5′-GAA GAT AGT CTG TAC CGT CAT GTA C-3) (Koeda *et al.,* 2018). A thermal cycler (Gene Amp, PCR System 9700 PE Applied Bio–system) was used to amplify the virus DNA under the following conditions: preheating at 95^{0}C (1 min), then 35 cycles of denaturation at 95^{0}C (15s), annealing at 58^{0}C (15s), and extension at 72^{0}C (30s), followed by a final extension at 72^{0}C (5 min).

DATA ANALYSIS

The numbers of whitefly eggs, nymphs, and adults as well as the incidence and severity of *PepYLCIV* were subjected to an analysis of variance (ANOVA). If significant differences among the treatments were detected, the means were separated using the Duncan's multiple range test (P = 0.05) (SPSS version 27, 2020).

RESULTS

Seeds as primary *PepYLCIV* inoculum source: In this experiment, seeds of each tested cultivar obtained from *PepYLCIV*-infected plants were planted in a seedbed confined within a whitefly-proof cage. The seedlings remained caged and sprayed weekly with insecticide until they were transplanted to the field at the age of six weeks after sowing. During nursery time, no eggs, nymphs, and adults of *B. tabaci* were found on the seedlings. However, *PepYLCIV* infection was found on Pilar with an incidence of 7.8%. No seedlings of the other tested cultivars expressed the virus symptoms (Data not shown).

At the age of six weeks, the seedlings were transplanted in the field and they were confined in insect-proof cages and weekly sprayed with insecticide throughout the rest of the season. No eggs, nymphs, and adults of *B. tabaci* were found in the cages during the study. However, one week after transplanting, all cultivars showed *PepYLCIV* symptoms with varying incidence and severity, from 3.3 - 23.3% and 0.7 - 6.7%, respectively (Table 1). The disease incidence and severity steadily increased as the season progressed. Twelve weeks after transplanting, disease incidence on different cultivars ranged from 76.7 to 100% . The highest incidence and severity were found in Pilar and Laris (100%), which significantly higher than the incidence in Bara. Similarly, the disease severity also steadily increased as the season progressed. The highest severity was also present on Laris and Pilar, though no significant differences were detected among cultivars.

Table 1. Average incidence and severity of *PepYLCIV* infection on seven chili cultivars planted from seeds obtained from infected plants in the field

Cultivar	Week after transplanting						
	1	3	5	7	9	10	12
	Disease Incidence (%)						
Bara	6.7 b	20.0 ab	36.7 b	40.0b	63.3 bc	63.3 b	76.7 b
Bhaskara	6.7 b	6.7 b	16.7 b	40.1b	73.3 abc	73.3 ab	80.0 ab
Kastilo	6.7 b	10.0 b	26.7 b	53.3b	86.7 abc	86.7 ab	86.7 ab
Kopei	3.3 b	16.7 ab	36.7 b	43.3b	93.3 ab	93.3 ab	96.7 ab
Lado	16.7 b	23.3 ab	30.0 b	43.3b	60.0 c	60.0 ab	83.3 ab
Laris	6.7 b	16.7 ab	33.3 b	63.3b	90.0 abc	90.0 a	100.0 a
Pilar	23.3 a	36.7 a	63.3 a	90.0a	96.7 a	100.0 a	100.0 a
	Disease severity (%)						
Bara	2.7 a	5.3 a	10.0 ab	10.0 b	16.0 b	17.3 b	33.3 a
Bhaskara	2.7 a	2.7 a	4.7 b	10.0 b	22.0 ab	25.3 bc	27.3 a
Kastilo	2.7 a	4.0 a	9.3 ab	14.7 b	24.0 ab	26.0 bc	28.0 a
Kopei	0.7 a	4.0 a	8.0 ab	10.7 b	28.0 ab	31.3 ab	36.0 a
Lado	4.0 a	6.7 a	9.3 ab	10.7 b	18.0 a	22.0 bc	34.7 a
Laris	2.7 a	4.7 a	10.0 ab	18.7 ab	28.7 ab	30.0 ab	37.3 a
Pilar	6.7 a	9.3 a	16.0 a	26.7 a	32.0 a	37.3 a	41.3 a

Numbers followed by the same letter within the same column are not significantly different (Duncan's multiple range test, P = 0.05).

As the *PepYLCIV* disease incidence increased, the disease severity also increased (Figure 2). The severity of *PepYLCIV* was significantly, positively correlated with the the disease incidence ($y = 0.3534x - 1.7306$, $R^2 = 0.9377$, $P < 0.01$) (Figure 1).

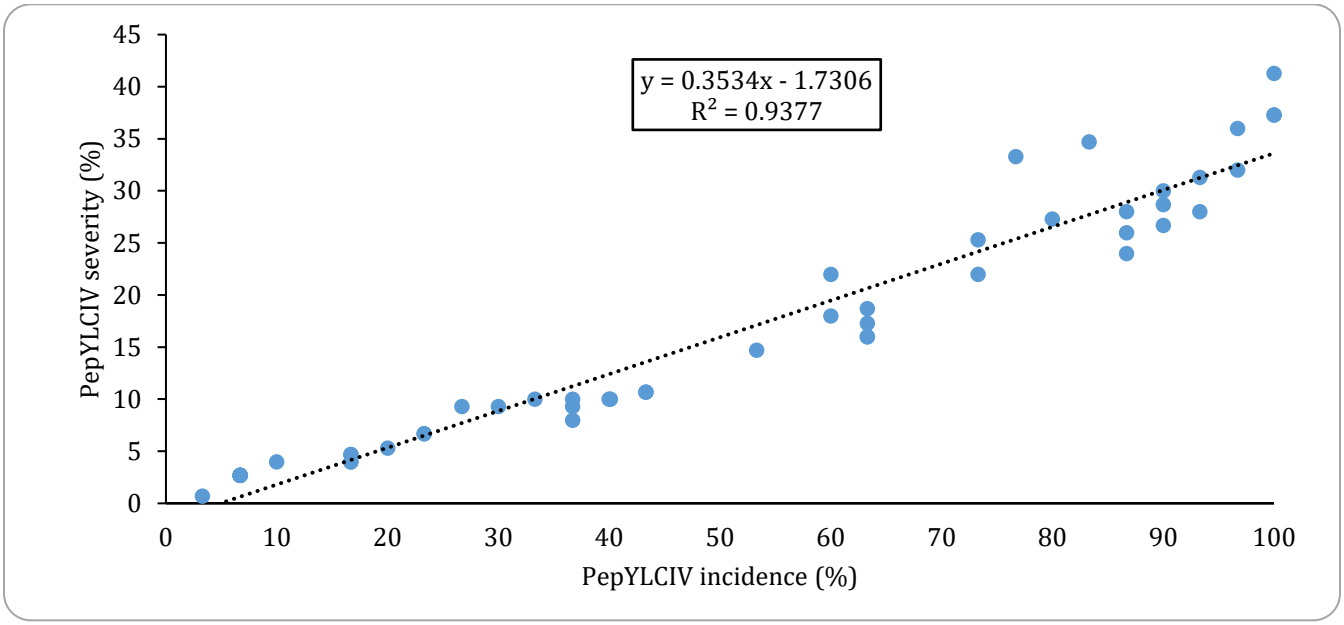

Figure 1. Relationship between *PepYLCIV* incidence and severity during the season

Whitefly as primary inoculum source in the seedbed: Whitefly eggs, nymphs, and adults were not found in caged seedbed during the seedling stage of the plant. However, eggs were found on the uncaged seedlings starting two weeks after seeds were sown, and one week later nymphs and adults were present on the uncaged seedlings (Table 2).

Table 1. Average numbers of eggs, nymphs, and adults of *B. tabaci* as well as incidence of *PepYLCIV* in the nursery.

Treatment	No. eggs, nymphs, and adults per plant / disease incidence (%)				
	Week after transplanting				
	1	2	3	4	5
			Egg		
Caged seedbed	0.0	0.0	0.0	0.0	0.0
Uncaged seedbed	0.0	0.1	4.9	0.5	2.5
			Nymph		
Caged seedbed	0.0	0.0	0.0	0.0	0.0
Uncaged seedbed	0.0	0.0	1.0	1.6	0.5
			Adult		
Caged seedbed	0.0	0.0	0.0	0.0	0.0
Uncaged seedbed	0.0	0.0	0.3	0.6	0.4
			Disease incidence		
Caged seedbed	0.0	0.00	0.0	0.0	0.0
Uncaged seedbed	0.0	0.00	0.0	0.4	1.8

None of the caged seedlings expressed *PepYLCIV* virus symptoms during the nursery period, however, the uncaged seedlings showed symptoms with an incidence of 0.4% in the fourth week and then 1.8% in the fifth week after the seeds were sown (Tabel 2).

Whitefly as primary inoculum source in the field: To determine the role of *B. tabaci* as inoculum source in the field, certified seeds of chili cv. Pilar were planted in caged seedbed until the seedlings were transplanted in the field at the age of six weeks. In the field, the plants were left uncaged for the rest of the season. Vector population, *PepYLCIV* incidence and severity during the season are shown in Figure 3. *Bemisia tabaci* was present in the field from week 1 to week 10 after transplanting. Eggs were found till week 9 and both nymphs and adults were found until week 10. The peaks of egg number and nymph number occurred in week 4, while the peak of adult number occurred in week 2 after transplanting. From week 11 till the end of the experiment (week 16), no vectors were present in the field.

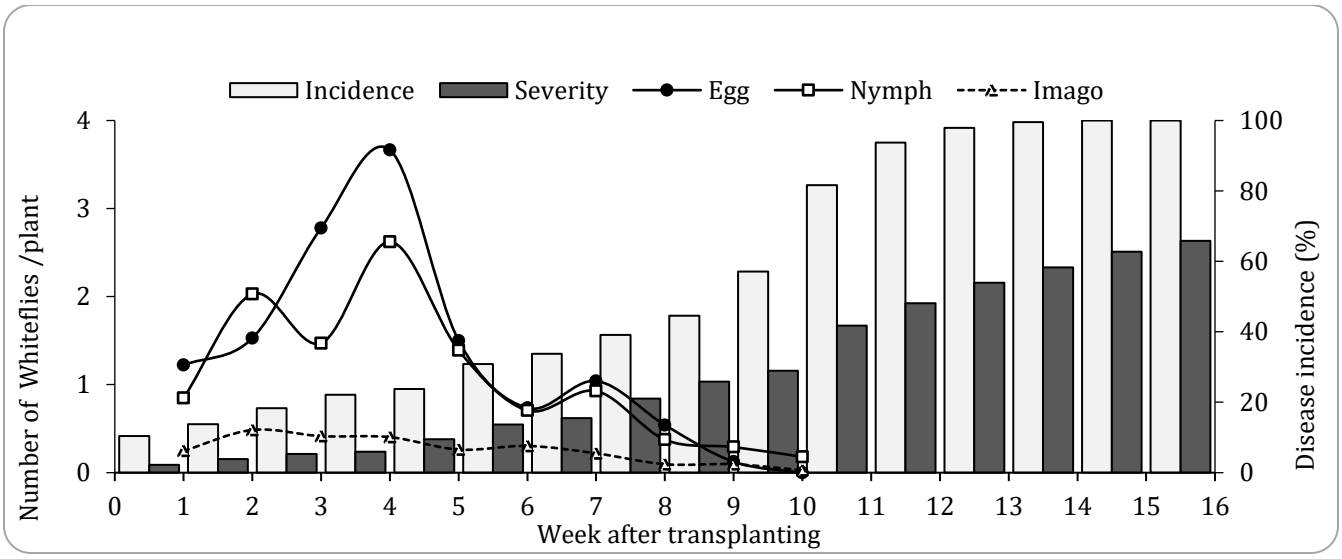

Figure 2. Populations of eggs, nymphs and imago as well as the incidence and severity of *PepYLCIV* disease in the field during the 16 weeks observation

Disease presence in the field was detected one week after transplanting, with incidence and severity were 10.4 and 2.2%, respectively. Disease incidence and severity continuously increased as the season progressed, although the vector was not found from week 11 to 16. In the end of the season, the peaks of disease incidence and disease severity of 100% and 65.8% were reached in week 16, respectively.

Relationship between occurrence time of primary infection and disease severity at the end of the season: Plants infected early in the season (vegetative stage of the plant) had significantly higher severity rate (78.2%) than the disease severity on plants infected later in the season (generative stage of plant) (45.5%) (Figure 4).

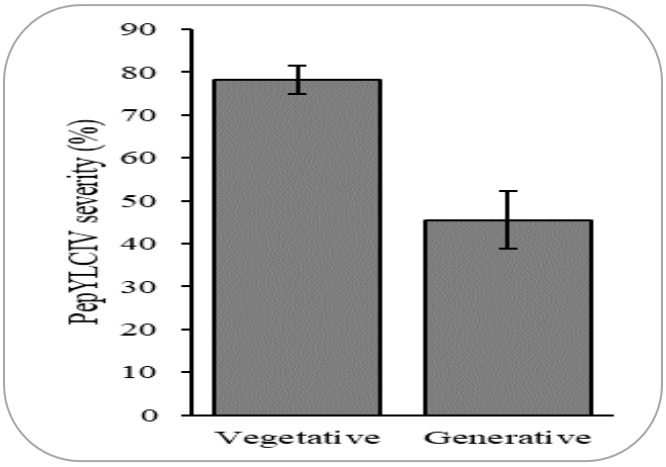

Figure 3. Disease severity in the end of the season on the plants infected in vegetative and generative growth stages.

Confirmation *PepYLCIV* infection: The result of the PCR analysis showed clear band of 385 bp, confirming that the symptomatic leaf samples tested (Pilar) contained DNA-B of *PepYLCIV*. While no band developed on samples from healthy plants (K-). This confirmed that the symptomatic plants were infected by the *PepYLCIV* (Figure 5).

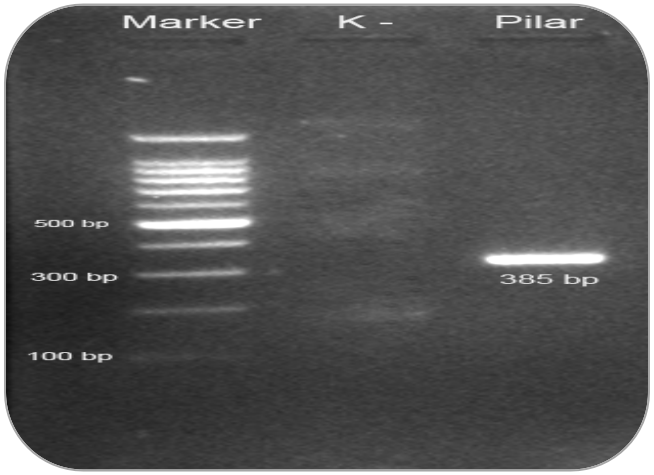

Figure 4. Agarose gel electrophoresis stained with ethidium bromide for detection of *PepYLCIV* in symptomatic leaf samples. K- = healthy sample, Pilar = symptomatic sample.

DISCUSSION

To determine whether *PepYLCIV* is seed transmissible, seeds of seven chili cultivars obtained from *PepYLCIV*-infected plants in the field were sown in caged seedbeds and then transplanted into insect-proof cages in the field. To assure that the seedlings and plants were insect-free, they were sprayed weekly with insecticide.

Although no vector was found on the plants during the experiment, infected plants were present both in the seedbed and in the field (Table 1). The result suggested that *PepYLCIV* can be vertically transmitted from one plant generation to next through seeds. This is in agreement with Fadhila *et al*. (2020) that *PepYLCIV* is detectable in seeds and can be transmitted from the seeds to new seedlings. Several other begomoviruses have been reported seed transmissible, including Tomato yellow leafcurl virus (TYLCV) (Kil *et al.,* 2017) and Squash mosaic virus (SqMV) (Lestari and Nurhayati, 2014). Transmission through seeds is very effective in helping the virus to spread quickly in space and time. This is probably one of the reasons why the disease has spread quickly in many parts of the country, for example, the disease was reported for the first time in West Java in 1999 (Rusli *et al.,* 2000) and 4 years later it has been found all over Java Island (Sulandari *et al.,* 2006). Infected seedlings grown from infected seeds could act as inoculum source for the secondary infections both in seedbed and the field. This finding has an implication on chili seed production system. Thus, *PepYLCIV*-free seeds should be included as one of the requirements for seed certification. Our results also showed that none of the cultivars tested was resistant to the virus when plants are grown from infected seeds. In all cultivars, there was a strong positive correlation between *PepYLCIV* disease and disease severity (Figure 1).

Seedlings from certified seeds of chili cv. Pilar grown in caged seedbeds were not infested by *B. tabaci* and neither were any of the seedlings infected by *PepYLCIV* during the nursery period. In contrast, the seedlings grown in uncaged seedbeds were infested by the whitefly and infected by the virus (Table 2). This indicated that *B. tabaci* could act as inoculum source in the nursery by bringing in the virus from outside the nursery even with low population (0.4 adults/plant). Thus, protecting the nursery from the vector infestation is very important to prevent the disease onset at early stage of the plant growth. This in line with Lapidot *et al*. (2014) reporting that plant can be protected from whitefly infestation and the virus it transmits by using physical barriers, such as cages and screen houses.

Plants originated from healthy seeds sown in caged seedbeds and then transplanted in uncaged field were infested by *B. tabaci* and infected by *PepYLCIV* (Figure 2). Vector insects were found from week 1 to 10 after transplanting but from week 11 till the end of the season, no vectors were found in the field probably due to the high rainfall rate during that period. Whitefly population is negatively correlated with the rainfall rate (Nasruddin *at al*., 2021; Sudiono and Purnomo, 2009). Virus spread most likely happened early in the season when vector population was present in the field and the virus continuously replicated in the plants so more and more plants were expressing symptoms as the season progressed. Chandra *et al*. (2016) and Alif *et al.* (2018) reported that plants infected with low concentration of virus do not express the typical symptoms of the disease until later in the season. Therefore, the results confirmed that the vector could act as inoculum source for infection in the field.

Time of the primary infection affected the disease severity at the end of the season. Plants infected in the vegetative stage had higher disease severity than did the plants infected later in the generative growth stage at the end of the season (Figure 3). The difference is due to the longer time available for the virus to replicate when primary infection took place early in the season (Widyastuti and Hidayat, 2005).

Our results confirmed that symptomatic plants observed in the current experiments were caused by *PepYLCIV* (Figure 4). The results indicated that the disease could spread through seeds, whitefly in seedbed, and whitefly in the field. Though, in general, it has been believed that *PepYLCIV* is not seed transmissible (Rusli *et al.,* 2000; Sulandari *et al.,* 2001; Sulandari, 2006). However, our results are in agreement with the study results by Fadhilla *et al*. (2020) that confirmed the presence of *PepYLCIV* in chili seeds and seedlings. The seed-borne nature of the virus has implications on seed certification system in which the virus was not currently included in the certification requirements. Besides that, most farmers used uncertified seeds and even used their own seeds from previous season. This practice could exacerbate the spread and the incidence of the disease. Primary infection of *PepYLCIV* could also happen in seedbeds and the field due to viruliferous whitefly infestation.

CONCLUSION

Although previous studies reported that *PepYLCIV* does not spread through seeds, our results indicated otherwise, the virus is transmissible through seeds obtained from *PepYLCIV*-infected plants in the previous season. Infected seeds became primary inoculum source in the seedbed. Vector, *B. tabaci*, could become primary

inoculum source in the seedbed and the field after transplanting. Regardless the primary source of the inoculum, if the vector population was present in the field, by the end of the season the disease incidence reached 100%. Thus, our results suggested that *PepYLCIV* should be managed by using an integrated approach using healthy-seeds, caged seedbed, and if necessary, using safer insecticides to suppress the vector population in the field.

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Sudarsono Sudarsono	:	Conduct research and analyzed the data
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Andi Nasruddin	:	Supervised the study, wrote the final draft of the manuscript, and acted as the corresponding author