BIODIVERSITAS
Volume 23, Number 10, October 2022
Pages: 5360-5366

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231046

Relationship between viruliferous *Bemisia tabaci* population and disease incidence of *Pepper yellow leaf curl Indonesia virus* in chili pepper

I GEDE RAI MAYA TEMAJA[1,♥], DEWA GEDE WIRYANGGA SELANGGA[1], TRISNA AGUNG PHABIOLA[1], KHAMDAN KHALIMI[1], LISTIHANI LISTIHANI[2]

[1]Faculty of Agriculture, Universitas Udayana. Jl. Raya Kampus Unud Jimbaran, Kuta Sel., Badung 80361, Bali, Indonesia.
Tel./Fax.: +62-361-701954, ♥email: raitemaja@unud.ac.id
[2]Faculty of Agriculture and Business, Universitas Mahasaraswati Denpasar. Jl. Kamboja No.11A, Dangin Puri Kangin, Kec. Denpasar Utara, Denpasar 80233, Bali, Indonesia

Manuscript received: 22 August 2022. Revision accepted: 17 October 2022.

Abstract. *Temaja IGRM, Selangga DGW, Phabiola TA, Khalimi K, Listihani L. 2022. Relationship between viruliferous* Bemisia tabaci *population and disease incidence of* Pepper yellow leaf curl Indonesia virus *in chili pepper. Biodiversitas 23: 5360-5366. Bemisia tabaci* Genn is an important pest in horticulture crops. Until today, *B. tabaci* has always been found in chili pepper and causes damage. In addition to a direct injury to the chili pepper, it is also the vector of Begomovirus. There is no research till date regarding the relationship between *B. tabaci* with Begomovirus which caused damage to chili pepper crops. Thus, the aim of this research was to analyze the interaction between *B. tabaci* population with the disease incidence of Begomovirus on chili peppers crops, as well as the percentage of viruliferous *B. tabaci*. This research noted *B. tabaci* population dynamics, disease incidence of pepper yellow leaf curl disease, and the percentage of viruliferous *B. tabaci* via PCR by using Begomovirus universal primer and DNA analysis confirmation. The highest imago population occurred at 6 WAP with the average density 27.21 imago/yellow sticky card, while the highest nymph population occurred at 8 WAP with the average density of 4.26 nymph/leaf. The high population of *B. tabaci* nymph and imago caused the incidence of Begomovirus disease to reach 83.07% at 12 WAP. Result showed that high population of *B. tabaci* accelerated the speed of virus in the field. The confirmation result of Begomovirus in *B. tabaci* body showed that the virus was *Pepper yellow leaf curl Indonesia virus* (PYLCIV). The highest homology obtained was between PYLCIV isolates from Sekaan, Bangli with isolates from Bayung Gede, Bangli which ranged from 99.6 to 99.8%. Further phylogenetic analysis showed that PYLCIV isolates from Sekaan, Bali formed a group with the isolate from Bayung Gede, Bangli, which was from a different group with other isolates. This proved that *B. tabaci* carries and transmits PYLCIV to chili peppers in Bali.

Keywords: Begomovirus, chili pepper, PCR, PYLCIV, viruliferous whitefly

INTRODUCTION

Bemisia tabaci Genn is considered a polyphagous insect and is widely spread across subtropical and tropical regions (Das et al. 2022). *B. tabaci* is known to attack more than 600 species of plants and causes the formation of chlorotic spots on leaves and clogging of stomata closure due to honeydew (Legg et al. 2014; Macfadyen et al. 2018). *B. tabaci* turned into an important pest in plants in Florida, such as vegetables, fruits, nuts, and medicinal plants (Simmons et al. 2019; Li et al. 2021).

Bemisia tabaci host species come from the families Asteraceae, Malvaceae, Solanaceae, Cruciferaceae, Lamiaceae, Euphorbiaceae, Cucurbitaceae, Fabaceae, Bignoniaceae, Lythraceae and Zygophyllaceae (Mascarin et al. 2013; Sulistyo and Inayati 2016; Lima et al. 2017; Macfadyen et al. 2018; Costa et al. 2019; Li et al. 2021). The host species are mostly associated with the Fabaceae family, while Bignoniaceae, Lythraceae and Zygophyllaceae only have one species each (Song et al. 2014; Li et al. 2021). The *B. tabaci* host found around red chili pepper crops included cultivated plants and weeds. Weed act as a Geminivirus alternative host, as shelters for parasitoid imago, as well as alternative hosts or food sources such as nectar and pollen for predator and parasitoid (Harth et al. 2016).

Bemisia tabaci is an insect vector for plant virus from genus Geminivirus (Geminiviridae), Crinivirus (Closteroviridae) and Carlavirus or Ipomovirus (Potyviridae) (Mauck et al. 2012). Geminivirus is a group of viruses that get transmitted by *B. tabaci* the most (Mauck et al. 2012). For the last three decades, Begomovirus infection has caused production loss in Solanaceae crops, especially tomatoes (*Solanum lycopersicum* L.), chili pepper (*Capsicum* spp.), and eggplants (*Solanum melongena* L.), in many tropical and subtropical regions in the world (Kenyon et al. 2014). The yield loss caused by Begomovirus on individual pumpkin plants was 56.3%, and the disease resulted in reduced quality of harvested fruits (Selangga and Listihani 2022). Begomovirus has been reported in Indonesia in tomato, eggplant, cucumber, pumpkin and sweet potato (Listihani et al. 2019; Listihani et al. 2022; Selangga and Listihani 2022). In Indonesia, Begomovirus infection in chili pepper was first reported in Java and several years later Selangga et al. (2019), Selangga and Listihani (2021), Selangga et al. (2022) reported that chili pepper crops in several regions in (Bali Province) had been infected by Begomovirus. The

main type of Begomovirus in chili pepper is *Pepper yellow leaf curl virus* (PYLCV). This virus can cause huge production loss (Ashwathappa et al. 2020). Disease incidence Geminivirus causing high yield loss, especially in chili pepper (Zaidi et al. 2017). Yield loss due to Geminivirus may reach 100% (Kenyon et al. 2014). The severity of Geminivirus attack is related to the population of *B. tabaci*, as Saeed and Samad (2017) reported.

Natural transmission of Begomovirus occurs through insect vector *B. tabaci* in persistent circulative nature (Czosnek et al. 2017). The study of Begomovirus transmission showed that the most efficient transmission occurs with acquisition feeding period for 24 hours and inoculation feeding period of 24 hours (Pan et al. 2018; Selangga et al. 2019). According to Shalev et al. (2016) one adult *B. tabaci* vector after sucking inoculum source for 24 hours and going through inoculation period for 48 hours, can transmit the disease with an incidence rate of 30-50%. Luan et al. (2014) reported the number of whitefly during the transmission influences disease incidence and virus incubation period; a whitefly can cause 50% transmission with incubation period of 13-29 days after inoculation (DAI), while five whiteflies can cause 70% transmission with incubation period of 12-29 DAI. This shows that the density of whitefly population within a plantation can potentially be a source of virus transmission.

There have been no reports on the association of *B. tabaci* population with the incidence rate of *Pepper yellow leaf curl Indonesia virus* (PYLCIV) disease, as well as the percentage of viruliferous whitefly in chili pepper. This research can strengthen the understanding of the relationship between Begomovirus disease incidence and viruliferous *B. tabaci* population in chili pepper plantations. This knowledge can be used as a reference in pest and important disease control in chili pepper. Thus, the aim of this research was to analyze the interaction relationship between *B. tabaci* and Begomovirus in the field.

MATERIALS AND METHODS

Bemisia tabaci population dynamics

The research was performed in chili pepper plantation owned by the residents in Sekaan Village, Kintamani Sub-district, Bangli District, Bali, Indonesia. The disease incidence was calculated based on the number of plants with symptoms/ total number of observed plants × 100, observation was done when the plants were aged 1 to 15 weeks after planting (WAP). The observation of population dynamics was conducted to study the development of *B. tabaci* nymph and imago populations in chili pepper plantations. The techniques used to observe *B. tabaci* population dynamic were by sampling nymph and imago stadia samples. The yellow leaf curl disease incidence and *B. tabaci* nymph and imago population observation was performed every week, from when the plants age reached 1 to 15 weeks after planting (WAP). The *B. tabaci* nymph and imago sampling were as follows:

***Bemisia tabaci* nymph sampling:** *B. tabaci* nymph population was calculated by taking sample plants from all chili pepper population at the research plot. Plant sampling pattern was performed systematically. The sample plants were determined by following planting row with 8 plants gap. The total number of all sample plants in one plot was 40 which spread evenly on five beds, so from each bed 8 plant samples were taken out of the total 60 plants per bed. The leaf sampling method was performed randomly, and the plants' upper, middle, and lower leaves were sampled from each sample plant. From each sample plant, six leaves were obtained (two from upper region, two from middle region, and two from lower region). The leaves were stored in a plastic bag for examination of *B. tabaci* nymph by using stereo microscope.

***Bemisia tabaci* imago sampling:** The yellow sticky card method was often used to observe *B. tabaci* imago population. *B. tabaci* imago sampling was performed by using yellow sticky cards in the size of (21.5 cm x 15 cm). *B. tabaci* imago captured on yellow sticky cards was calculated under a stereo microscope.

The percentage of viruliferous *Bemisia tabaci* containing Begomovirus by PCR

Bemisia tabaci samples were obtained from 3 chili pepper plantations area in Sekaan Village, Kintamani Sub-district, Bangli District, Bali, Indonesia. *B. tabaci* sample types obtained from chili pepper were nymph and imago. The nymph and imago were used for total DNA extraction. After arriving at the laboratory, nymph and imago were stored at -20°C. Viruliferous *B. tabaci* percentage observation by PCR was performed every week, from 1 week after planting (WAP) until 15 WAP.

Detection of Begomovirus viruliferous *Bemisia tabaci* by PCR

One *B. tabaci* insect was stored in absolute alcohol and then extracted by molecular technique to obtain total DNA. *B. tabaci* from each location was placed in a 1mL microtube and added 100 µL of extraction buffer CTAB 2%. Furthermore, 1 µL proteinase K was added and then mixture was ground finely by 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 then centrifuged for 15 minutes at 10,000 rpm. 60 µL supernatant was transferred to a new 1.5 mL microtube, followed by the addition of 44 µL of isopropanol and 6 µL of sodium acetate 3 M (pH 5.2), and the mixture was incubated at -20°C for 3 hours or overnight. Afterward, the tube was centrifuged at 10,000 rpm for 10 minutes. The obtained pellet was washed with 100 µL of ethanol 80% and then recentrifuged at 8,000 rpm for 5 minutes. The supernatant was then discarded again, and total DNA pellet was resuspended by 20 µL Tris-EDTA solution.

Target virus DNA amplification was performed by using ready to go PCR read (Amersham Pharmacia Biotech. Inc.). The amplification reaction comprised of 2 µL template DNA, 1 µL each of primer SPG1 (5'-

CCCCKGTGCGWRA ATCCAT-3') / SPG2 (5'-ATCCVAAYWTYCAGGGAGCT-3') which was 912 pb in length (Li et al. 2004) with 1 µM concentration, and then added distilled water until the total volume reached 25 µL. Amplification was performed using a PCR machine (Gene Amp. PCR System 9700 PE Applied Biosystem) for 35 cycles, with the stages being initial preheating at 94ºC for 5 minutes, followed by DNA separation at 94ºC for 1 minute, primer attachment on template DNA at 50ºC for 1 minute, DNA synthesis at 72ºC for 1 minute, last cycle at 72ºC for 1 minute, and then cooled to 4.0ºC

Amplified DNA was visualized by electrophoresis on agarose gel 1% with 50 volt for 50 minutes. Agarose gel dye used was FlouroVue TM (Smobio, Taiwan). The electrophoresis result was visualized by ultraviolet transilluminator and documented.

Confirmation of Begomovirus from *Bemisia tabaci* by DNA sequencing

DNA confirmation of Begomovirus from *B. tabaci* was followed by sequencing analysis. DNA from PCR results that were positive for Begomovirus was sent to FirstBase (Malaysia) to have the nucleotide base sorted. Nucleotide and amino acid homology were analyzed by BioEdit software. The relationship was analyzed by MEGA V6.0 software with 1000 bootstraps as repetition. The analysis result was compared with data obtained from GenBank.

RESULTS AND DISCUSSION

Bemisia tabaci imago was found in chili pepper crop plantation from 1 WAP with average density of 18.05 imago/yellow sticky card (Figure 1A). When the plants reached 5 WAP, imago population was still relatively high, and the highest imago population was found at 6 WAP with the average density being 27.21 imago/yellow sticky cards. The presence of *B. tabaci* in chili pepper comes from vegetables around chili pepper plantation that serve as the host of *B. tabaci*. The *B. tabaci* imago population showed a sharp decline after 6 WAP and even reached the lowest population on 13 WAP with 5.18 imago/yellow sticky card. The *B. tabaci* imago population elevated once again after 13 WAP up to 15 WAP (Table 1).

Bemisia tabaci was always found in the field as its host species was extant around chili pepper plantations. *B. tabaci* host which existed around chili pepper plantations were *Solanum lycopersicum*, *C. esculenta*, *Solanum melongena*, *Ageratum* spp., and *M. esculenta*. In line with Choi and Park (2015. An increase in one *B. tabaci* host species will elevate *B. tabaci* imago population by as much as 1.53 imago/yellow sticky cards (Choi and Park 2015).

The *B. tabaci* nymph population result showed that nymph population was found on chili pepper with the average density of 0.21 nymph/leaf from 1 WAP onwards (Figure 1B). The nymph population increased on 6 WAP, and highest nymph population occurred during 8 WAP with the average density of 4.26 nymph/leaf. Nymph population experienced sharp decline after 10 WAP up to13 WAP. The decline in the nymph population was caused by insecticide application performed by the farmers, high rainfall conditions, and the presence of parasitoids in the field (El-Sherbeni et al. 2019). The population of parasitoid *Eretmocerus* sp. elevated along with the increase of *B. tabaci* population, causing *B. tabaci* nymph to be infested and eventually population declined (Pedro et al. 2021).

Table 1. Presentation of viruliferous *Bemisia tabaci* of PYLCIV by PCR on chili pepper plantations in Sekaan Village, Bangli, Bali, Indonesia

Plant ages (WAP)	Total samples	Presentation of *Bemisia tabaci* viruliferous PYLCIV (%)		
		Sekaan 1	Sekaan 2	Sekaan 3
1	20	0 (0/20)	0 (0/20)	0 (0/20)
2	22	0 (0/22)	4.54 (1/22)	0 (0/22)
3	21	0 (0/21)	0 (0/21)	0 (0/21)
4	21	4.76 (1/21)	9.52 (2/21)	0 (0/21)
5	23	4.35 (1/23)	8.69 (2/23)	4.35 (1/23)
6	20	10 (2/20)	10 (2/20)	5 (1/20)
7	22	9.09 (2/22)	13.63 (3/22)	9.09 (2/22)
8	20	15 (3/20)	15 (3/20)	15 (3/20)
9	22	22.72 (5/22)	18.18 (4/22)	13.63 (3/22)
10	21	23.80 (5/21)	28.57 (6/21)	28.57 (6/21)
11	23	30.43 (7/23)	34.78 (8/23)	26.08 (6/23)
12	23	39.13 (9/23)	39.13 (9/23)	34.78 (8/23)
13	21	38.09 (8/21)	38.09 (8/21)	33.33 (7/21)
14	22	36.36 (8/22)	36.36 (8/22)	31.18 (7/22)
15	20	35 (7/20)	30 (6/20)	25 (5//20)

Note: WAP: week after planting

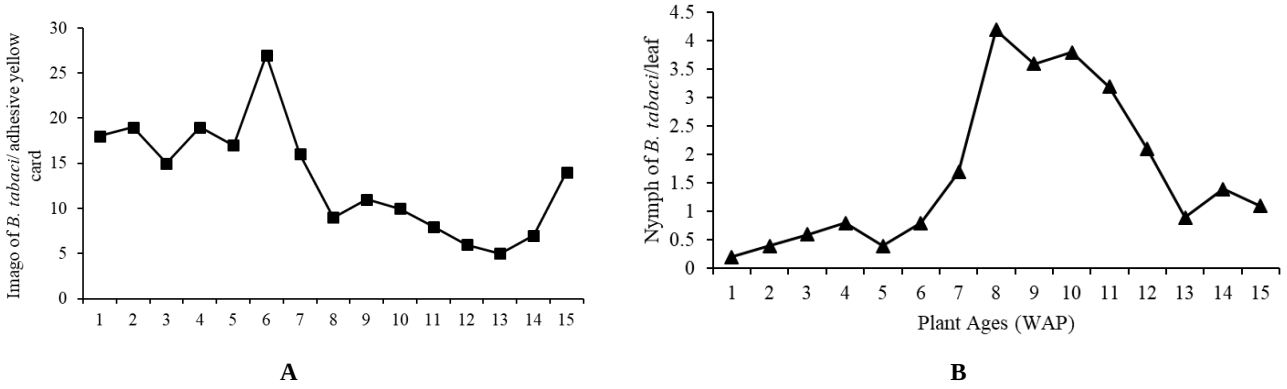

Figure 1. The population development of imago (A) and nymphs (B) of *Bemisia tabaci* on a chili pepper plantation in Sekaan Village, Bangli, Bali, Indonesia

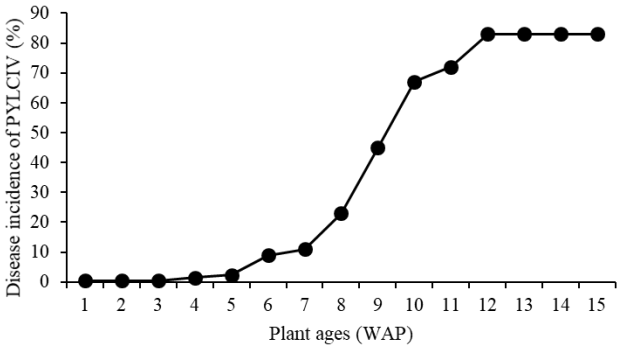

Figure 2. The development of the disease incidence of PYLCIV disease in chili pepper plantations in Sekaan Village, Bangli, Bali, Indonesia

The present study found an increase in the nymph population of *B. tabaci* after insecticide application. Patra and Hath (2022) reported that insecticide application did not give satisfactory results in controlling *B. tabaci*. Gogi et al. (2021) reported that the increase in *B. tabaci* population in area where insecticide was applied was due to the decrease of natural competitors caused by wide spectrum insecticide application. Excessive insecticide application gives negative impacts on the environment, including the elimination of natural competitors.

The incidence of disease caused by PYLCIV in chili pepper on 1-3 WAP showed (0.4%) low incidence rate and showed elevation on 4 WAP (Figure 2). The highest disease incidence was 83.07% when the plants were aged 12 WAP. PYLCIV disease incidence in the field did not show elevation after the plants reached 12 WAP. The farmers in Sekaan Village, Bangli, Bali, Indonesia, implemented monoculture and intercropping planting patterns. The incidence and severity of the disease are found to be more severe in plantations with monoculture planting patterns compared to the intercropping pattern (Selangga et al. 2021). According to Selangga and Listihani (2021), intercropping patterns with chili pepper among other crops from different families within a plantation and the use of resistant plant cultivars can lower disease infection levels and show high production yield in the field. However, until now, no such chili cultivar has been found which is resistant to Begomovirus (Selangga et al. 2022). PYLCIV was found in the field as many of its hosts, such as Solanaceae, Cucurbitaceae, and weed, were present on the field. PYLCIV infection has been reported on different plants and weeds, including tomatoes, eggplants, cucumbers, *Ageratum* spp. and Ludwigia (Annisaa et al. 2021). The PYLCIV host plants and weed around chili plantation is the inoculum source for PYLCIV in the field.

The elevation of *B. tabaci* imago and nymph after 5 WAP caused an increase in PYLCIV disease incidence in the field, reaching up to 83.07% (Figures 3A and 3B). The number of insect vectors is one factor influencing the high incidence of viral disease (Listihani et al. 2022). The high number of insect vectors increases disease incidence and shortens virus incubation and vice versa (Pan et al. 2018). Insect vectors' activity and feeding behavior strongly determine the ability to transmit virus. The percentage of viruliferous *B. tabaci* with PYLCIV was very low in proportion when the plants were aged 1-3 WAP, where it ranged around 0-4.54%. The highest percentage of PYLCIV viruliferous *B. tabaci* at 12 WAP was 39.13%. The high *B. tabaci* population in the field was followed by the high incidence of PYLCIV disease, which also led to a high percentage of *B. tabaci* carrying Begomovirus. This showed that whitefly vector is the main cause of Begomovirus transmission in the field.

The presence of *B. tabaci* influences the chance of PYLCIV disease transmission. Moreover, the spread of disease depends on the presence of virus particle in *B. tabaci* body. The damage caused by virus sometimes cause major loss compared to the direct damage by *B. tabaci* attack. Viruses use plant proteins to replicate and cause plants to lack protein for metabolic processes, so that plants experience leaf discoloration, malformations, and stunting (Listihani et al. 2019; Listihani et al. 2020; Sutrawati et al. 2021; Damayanti et al. 2022; Pandawani et al. 2022; Selangga et al. 2022).

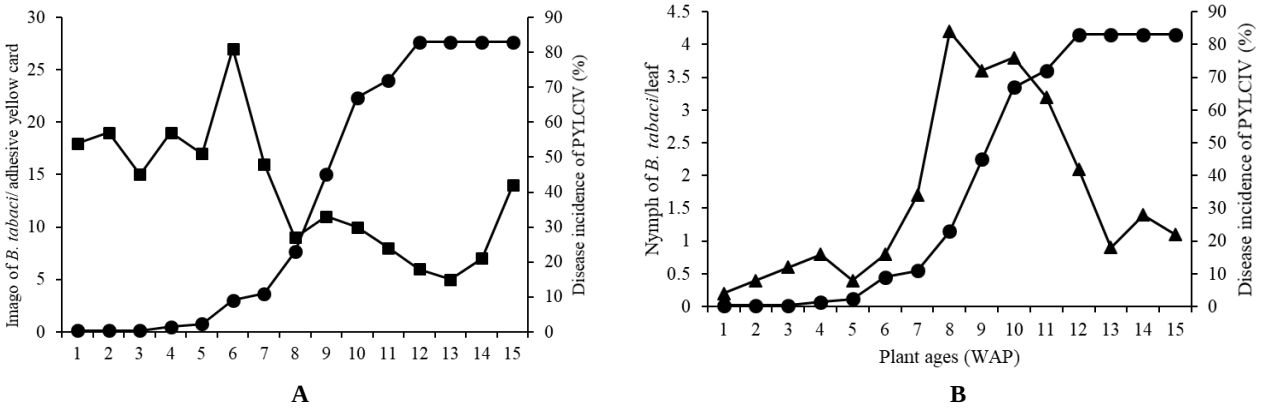

Figure 3. Relationship between imago population of *Bemisia tabaci* with disease incidence of PYLCIV (A) and population of nymphs of *B. tabaci* with disease incidence of PYLCIV (B) on chili pepper plantations in Sekaan Village, Bangli, Bali, Indonesia

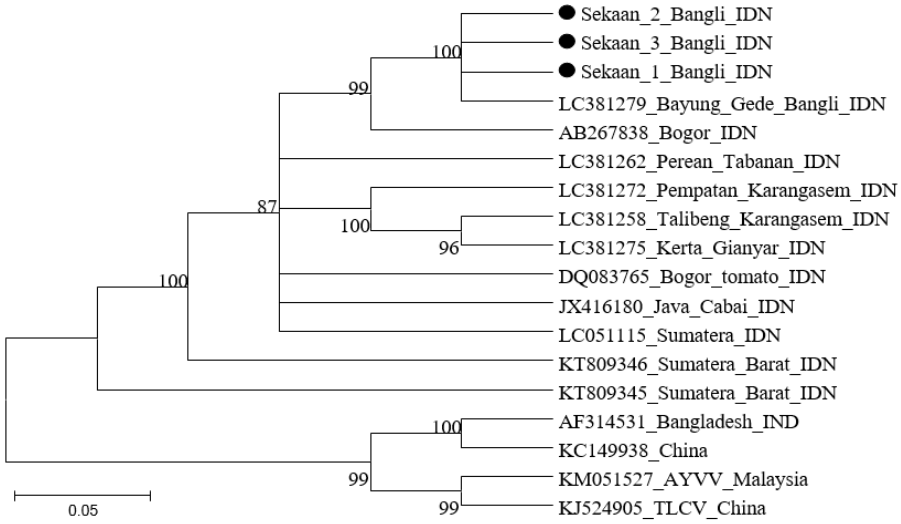

Figure 4. Phylogenetic analysis of Begomovirus isolates infecting chili pepper in Sekaan Village, Bangli, Bali, Indonesia based on partial nucleotide sequence alignment of DNA-A using Mega 6.06 (Neighbor-Joining Algorithm with 1,000 bootstraps replicates). *Ageratum yellow vein virus* (AYVV) from Malaysia and *Tobacco leaf curl virus* (TLCV) from China as outgroups. IDN (Indonesia), IND (India), isolates marked with black dots are Sekaan, Bangli, Bali isolates

Table 2. Homology of nucleotide PYLCIV from viruliferous *Bemisia tabaci* in chili pepper cultivation in Sekaan Village, Bangli, Bali, Indonesia (IDN)

Isolate sources	Hosts	Accession number	Homology (%) PYLCIV		
			Sekaan 1	Sekaan 2	Sekaan 3
Bayung_Gede_Bangli_IDN	Chili	LC381279	99.8	99.6	99.7
Bogor_IDN	*Ageratum* spp.	AB267838	98.2	98.3	98.4
Perean_Tengah_Tabanan_IDN	Chili	LC381262	96.3	96.2	96.4
Bogor	Tomato	DQ083765	96.7	96.6	96.8
Java-IDN	Chili	DQ083765	95.4	95.2	95.3
Talibeng_Karangasem _IDN	Chili	JX416180	94.2	94.3	94.4
Pempatan_Karangasem_IDN	Chili	LC381272	94.3	94,4	94.5
Kerta_Gianyar_IDN	Chili	LC381275	94.2	94.3	94,3
Sumatera_IDN	Chili	LC051115	94.4	94.5	94.2
Sumatera_Barat_IDN	Chili	KT809346	93.4	93.3	93,2
Sumatera_Barat_IDN	Chili	KT809345	90.4	90.3	90,5
Bangladesh	Chili	AF314531	77.1	77.3	77.4
China	Chili	LC051113	76.3	76.2	76,4
AYVV_Malaysia	*Ageratum* spp.	KM051527	66.2	66.4	66.3
TLCV _China	*Ageratum* spp.	KJ524905	61.2	61.4	61.3

Costa et al. (2019) reported that soybean crops infected with Begomovirus resulted in10 -70% yield loss. Homology analysis of the sequences between three Begomovirus isolates obtained from Sekaan Village, Bangli, Bali, which came from insect bodies with several *Pepper yellow leaf curl Indonesia virus* (PYLCIV) isolates from Bali, Java, and Sumatera showed homology ranged from 90.3% to 99.8% (Table 2). The highest homology was between PYLCIV isolate from Sekaan, Bangli with the isolate from Bayung Gede, Bangli ranging from 99.6% to 99.8%. The *Begomovirus* isolate from Sekaan, Bali, showed low homology if compared with PYLCV sequences from other countries. which was 76.2% to 76.4% and 77.1% to 77.4% with PYLCV from China and India, respectively. Further phylogenetic analysis showed that the isolate from Sekaan, Bali, formed one group with the isolate from Bayung Gede, Bangli (Figure 4). The PYLCV isolates from China and India were in one group (Figure 4).

Nucleotide sequence analysis of DNA fragments from amplification showed sequence variation between Begomovirus isolates from Bali in AC1 gene (*replication-associated protein*) and part of AC2 (*transcriptional activator protein*) region. The effect of these nucleotide base differences needs to be analyzed further with the codon impact that influences the amino acid alignment. Begomovirus isolate from *B. tabaci* body was proven to be PYLCIV from Bangli, Bali. According to Rubio et al. (2013), viral DNA has broad genetic variations and rapid evolution. Rubio et al. (2020) also explained that the genetic diversity between virus isolates is caused by virus evolution which points to environmental suitability. The genetic variations in virus genome can be caused by mutation or recombination. The high rate of mutation in viral DNA is a consequence of the lack of proofreading mechanism in viral DNA polymerase. Furthermore, recombination between the DNA genome of certain virus species causes the birth of diversity in virus biological characteristics (Rubio et al. 2013). Viruses have great potential for high genetic variability due to rapid replication and generation of large populations. Viruses with RNA genomes, which comprise the majority of plant viruses, and viroids have the highest mutation rate of any replicant group, because RNA polymerase has no proofreading activity (Rubio et al. 2020).

This research showed that there is high probability in *B. tabaci* population carrying Begomovirus and transmitting it to chili pepper. The viruliferous *B. tabaci* percentage information is strongly related to the increase of Begomovirus disease incidence in the field. The higher the *B. tabaci* population, the faster the spread of PYLCIV in the field. This research recommends that controlling the vector *B. tabaci* in the field is very effective in suppressing the spread and infection of PYLCIV in the field. In addition, good sanitation practiced by cleaning weed before and during chili planting can prevent the provision of alternative hosts from *B. tabaci* and PYLCIV. The present research provides information to farmers not to use vulnerable plant cultivar continuously that can lead to PYLCIV epidemic in the field. Thus, new control strategy based on preventive and curative system can be developed for regional management of the insect and viruses.

In conclusion, the high population of *B. tabaci* in chili plantations caused the high incidence of PYLCIV disease. One whitefly insect can carry PYLCIV in its body. The percentage of PYLCIV viruliferous *B. tabaci* in chili plantations reached 39.13%. The homology of PYLCIV from *B. tabaci* in chili plantation in Sekaan Village, Bangli, was highest with PYLCIV from chili leaves in Bayung Gede Village, Bangli, Bali, Indonesia.

ACKNOWLEDGMENTS

This research was funded by Udayana University with the contract number B/78.700/UN 14.4.A/PT.01.03/2022. This work was supported by I Putu Sudiarta and Ni Putu Pandawani who provided feedback for this paper.

REFERENCES

Annisaa NW, Hidayat P, Giyanto, Hidayat SH, Lee S. 2021. Multiple infections of Begomovirus on its host plants. IOP Conf Ser: Earth Environ Sci 694: 1-8. DOI: 10.1088/1755-1315/694/1/012047.

Ashwathappa KV, Venkataravanappa V, Lakshminarayana RCN, Krishna RM. 2020. Association of *Tomato leaf curl New Delhi virus* with mosaic and leaf curl disease of chrysanthemum and its whitefly crypticspecies. Indian Phytopathol 73 (3): 533-542. DOI: 10.1007/s42360-020-00214-1.

Choi WS, Park JJ. 2015. Development of sequential sampling plan for *Bemisia tabaci* in paprika greenhouses. Korean J Appl Entomol 54 (3): 159-167. DOI: 10.5656/KSAE.2015.06.0.019.

Costa TL, Sarmento RA, de Araujo TA, Pereira PS, Silva RS, Lopes MC, Picanco MC. 2019. Economic injury levels and sequential sampling plans for *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype B on open-field melon crops. Crop Prot 125: 104887. DOI: 10.1016/j.cropro.2019.104887.

Czosnek H, Hariton-Shalev A, Sobol I, Gorovits R, Ghanim M. 2017. The incredible journey of Begomoviruses in their whitefly vector. Viruses 9 (10): 273. DOI: 10.3390/v9100273.

Damayanti TA, Nurjannah T, Listihani L, Hidayat SH, Wiyono S. 2022. Characterization of a variant isolate of *Zucchini yellow mosaic virus* infecting green kabocha (*Cucurbita maxima* L.) in Bogor, Indonesia. Arch Phytopathol Plant Prot 55 (1): 121-128. DOI: 10.1080/03235408.2021.2003604.

Das S, Rahman M, Dash PK, Mitra M, Kamal MM. 2022. Transmission attributes of Asian I silverleaf whitefly (*Bemisia tabaci*) modulating the spread of chili leaf curl virus disease in chili (*Capsicum* spp.). Arch Phytopathol Plant Prot 55 (6): 699-719. DOI: 10.1080/03235408.2022.2040698.

El-Sherbeni AEHED, Khaleid MS, AbdAllah SAEA, Ali OSM. 2019. Effect of some insecticides alone and in combination with salicylic acid against aphid, *Aphis gossypii*, and whitefly *Bemisia tabaci* on the cotton field. Bull Natl Res Cent 43 (1): 1-7. DOI: 10.1186/s42269-019-0103-0.

Gogi MD, Syed AH, Atta B, Sufyan M, Arif MJ, Arshad M, Nawaz A, Khan MA, Mukhtar A, Liburd OE. 2021. Efficacy of biorational insecticides against *Bemisia tabaci* (Genn.) and their selectivity for its parasitoid *Encarsia formosa* Gahan on *Bt* cotton. Sci Rep 11 (1): 1-12. DOI: 10.1038/s41598-021-81585-x.

Harth JE, Winsor JA, Weakland DR, Nowak KJ, Ferrari MJ, Stephenson AG. 2016. Effects of virus infection on pollen production and pollen performance: Implication for the spread of resistance alleles. Am J Bot 103 (3): 577-583. DOI: 10.3732/ajb.1500165.

Kenyon L, Tsai WS, Shih SL, Lee LM. 2014. Emergence and diversity of Begomoviruses infecting solanaceous crops in East and Southeast Asia. Virus Res 186: 104-113. DOI: 10.1016/j.virusres.2013.12.026.

Legg JP, Shirima R, Tajebe LS, Guastella D, Boniface S, Jeremiah S, Nsami E, Chikoti P, Rapisarda C. 2014. Biology and management of

Bemisia whitefly vectors of cassava virus pandemics in Africa. Pest Manag Sci 70 (10): 1446-1453. DOI: 10.1002/ps.3793.

Li R, Salih S, Hurtt S. 2004. Detection of Geminiviruses in sweetpotato by polymerase chain reaction. Plant Dis 88 (12): 1347-1351. DOI: 10.1094/PDIS.2004.88.12.1347.

Li Y, Mbata GN, Punnuri S, Simmons AM, Shapiro-Ilan DI. 2021. *Bemisia tabaci* on vegetables in the Southern United States: incidence, impact, and management. Insects 12 (3): 198. DOI: 10.3390/insects12030198

Lima CH, Sarmento RA, Pereira PS, Galdino TV, Santos, FA, Silva J, Picanco MC. 2017. Feasible sampling plan for *Bemisia tabaci* control decision-making in watermelon fields. Pest Manag Sci 73: 2345-2352. DOI: 10.1002/ps.4621.

Listihani L, Ariati PEP, Yuniti IGAD, Selangga DGW. 2022. The brown planthopper (*Nilaparvata lugens*) attack and its genetic diversity on rice in Bali, Indonesia. Biodiversitas 23 (9): 4696-4704. DOI: 10.13057/biodiv/d230936.

Listihani L, Damayanti TA, Hidayat SH, Wiyono S. 2019. Molecular characterization of Begomovirus on cucumber in Java. Jurnal Perlindungan Tanaman Indonesia 23 (2): 197-204. DOI: 10.22146/jpti.41402. [Indonesian]

Listihani L, Damayanti TA, Hidayat SH, Wiyono S. 2020. First report of *Cucurbit aphid-borne yellows virus* on cucumber in Java, Indonesia. J Gen Plant Pathol 86 (3): 219-223. DOI: 10.1007/s10327-019-00905-2.

Listihani L, Yuniti IGAD, Lestari PFK, Ariati PEP. 2022. First report of *Sweet potato leaf curl virus* (SPLCV) on *Ipomoea batatas* in Bali, Indonesia. Indian Phytopathol 75 (2): 595-598. DOI: 10.1007/s42360-022-00489-6.

Listihani L, Hidayat SH, Wiyono S, Damayanti TA. 2019. Characteristic of *Tobacco mosaic virus* isolated from cucumber and tobacco collected from East Java, Indonesia. Biodiversitas 20: 2937-2942. DOI: 10.13057/biodiv/d201023.

Luan JB, Wang XW, Colvin J, Liu SS. 2014. Plant-mediated whitefly-begomovirus interactions: research progress and future prospects. Bull Entomol Res 104: 267-276. DOI: 10.1017/S000748531400011X.

Macfadyen S, Paull C, Boykin LM, De Barro P, Maruthi MN, Otim M, Kelyebi A, Vassao DG, Sseruwangi P, Tay WT, Delatte H, Seguni Z, Colvin J, Omongo CA. 2018. Cassava whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in East African farming landscapes: a review of the factors determining abundance. Bull Entomol Res 108 (5): 565-582. DOI: 10.1017/S0007485318000032.

Mascarin GM, Kobori NN, Quintela ED, Delalibera I. 2013. The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. Biol Control 66 (3): 209-218. DOI: 10.1016/j.biocontrol.2013.05.001.

Mauck K, Bosque-Perez NA, Eigenbrode SD, De Moraes CM, Mescher MC. 2012. Transmission mechanism shape pathogen effects on host-vector interaction: Evidence from plant viruses. Funct Ecol 26:1162-1175. DOI: 10.1111/j.1365-2435.2012.02026.x

Pan LL, Chen QF, Guo T, Wang XR, Li P. 2018. Differential efficiency of a begomovirus to cross the midgut of different species of whiteflies results in variation of virus transmission by the vectors. Sci China Life Sci 61: 1254-1265. DOI: 10.1007/s11427-017-9283-4.

Pandawani NP, Listihani L, Widnyana IK, Ariati PEP, Selangga DGW. 2022. High impact of *Clerodendrum paniculatum* leaf extract to suppress Zucchini yellow mosaic virus infection in zucchini plants. Biodiversitas 23 (6): 2914-2919. DOI: 10.13057/biodiv/d230618.

Patra B, Hath TK. 2022. Insecticide resistance in whiteflies *Bemisia tabaci* (Gennadius): current global status. Insecticides. DOI: 10.5772/intechopen.101954

Pedro LP, López-Gallego E, Pérez-Marcos M, Ramírez-Soria MJ, Sanchez JA. 2021. Native natural enemies in Mediterranean melon fields can provide levels of pest control similar to conventional pest management with broad-spectrum pesticides. Biol Control 164: 104778. DOI: 10.1016/j.biocontrol.2021.104778.

Rubio L, Galipienso L, Ferriol I. 2020. Detection of plant viruses and disease management: relevance of genetic diversity and evolution. Front Plant Sci 11: 1092. DOI: 10.3389/fpls.2020.01092.

Rubio L, Guerri J, Moreno P. 2013. Genetic variability and evolutionary dynamics of viruses of the family Closteroviridae. Front Microbiol 4: 151. DOI: 10.3389/fmicb.2013.00151.

Saeed ST, Samad A. 2017. Emerging threats of begomoviruses to the cultivation of medicinal and aromatic crops and their management strategies. Virus Dis 28 (1): 1-17. DOI: 10.1007/s13337-016-0358-0.

Selangga DGW, Hidayat SH, Susila AD, Wiyono S. 2019. The effect of silica (SiO2) to the severity of yellow leaf curl disease on chili pepper. Jurnal Perlindungan Tanaman Indonesia 23 (1): 54-60. DOI: 10.22146/jpti.38951. [Indonesian]

Selangga DGW, Listihani L. 2022. Squash leaf curl virus: species of begomovirus as the cause of butternut squash yield losses in Indonesia. Hayati J Biosci 29 (6): 806-813. DOI: 10.4308/hjb.29.6.806-813.

Selangga DGW, Listihani. 2021. Molecular identification of *Pepper yellow leaf curl Indonesia virus* on chili pepper in Nusa Penida Island. J Trop Plant Pests Dis 21: 97-102. DOI: 10.23960/jhptt.22197-102.

Selangga DGW, Temaja IGRM, Wirya GNAS, Sudiarta IP, Listihani L. 2022. First report of *Papaya ringspot virus*-watermelon strain on melon (*Cucumis melo* L.) in Bali, Indonesia. Indian Phytopathol 75 (3): 911-914. DOI: 10.1007/s42360-022-00519-3.

Selangga DGW, Wiyono S, Susila AD, Hidayat SH. 2022. Distribution and identification of *Pepper yellow leaf curl Indonesia virus* infecting chili pepper in Bali Island. J Fitopatol Indones 17 (6): 217-224. DOI: 10.14692/jfi.17.6.217-224. [Indonesian]

Shalev AH, Sobol I, Ghanim M, Liu SS, Czosnek H. 2016. The whitefly Bemisia tabaci knottin-1 gene is implicated in regulating the quantity of *Tomato yellow leaf curl virus* ingested and transmitted by the insect. Viruses 8 (7): 205. DOI: 10.3390/ v8070205.

Simmons AM, Jarret RL, Cantrell CL, Levi A. 2019. *Citrullus ecirrhosus*: Wild source of resistance against *Bemisia tabaci* (Hemiptera: Aleyrodidae) for cultivated watermelon. J Econ Entomol 112 (5): 2425-2432. DOI: 10.1093/jee/toz069.

Song JH, Lee KJ, Yang YT, Lee SC. 2014. Sampling plan for *Bemisia tabaci* adults by using yellow-color sticky traps in tomato greenhouses. Korean J Appl Entomol 53: 375-380. DOI: 10.5656/KSAE.2014.10.0.044.

Sulistyo A, Inayati A. 2016. Mechanisms of antixenosis, antibiosis, and tolerance of fourteen soybean genotypes in response to whiteflies (*Bemisia tabaci*). Biodiversitas 17: 447-453. DOI: 10.13057/biodiv/d170207.

Sutrawati M, Ganefianti DW, Sipriyadi S, Wibowo RH, Agustin Z, Listihani, Selangga DGW. 2021. Disease incidence and molecular diversity of tungro virus on rice (*Oryza sativa*) in Bengkulu, Indonesia. Intl J Agric Technol 17 (5): 1973-1984.

Zaidi SS, Martin DP, Imran A, Muhammad F, Shahid M. 2017. *Tomato leaf curl New Delhi virus*: a wide spread bipartite begomovirus in the territory of monopartite begomoviruses. Mol Plant Pathol 18: 901-911. DOI: 10.1111/mpp.12481.