Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

OLECULAR DETECTION AND IDENTIFICATION OF *BEGOMOVIRUSES* INFECTING EGGPLANT IN LAMPUNG PROVINCE, INDONESIA

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A B S T R A C T

Begomovirus infecting eggplants were detected by the polymerase chain reaction (PCR) in total nucleic acid preparations of eggplant leaf samples. This study aimed to detect and identify the Begomoviruses infecting eggplants of Yumi F-1 variety from the leaf samples collected from Pajar Agung Village, Pringsewu Regency, Lampung Province, Indonesia. The identified TYLCKaV was inoculated to eggplants, tomatoes, and chili peppers to evaluate its phathogenicity according to disease simptoms. The Results confirmed that Begomoviruses are responsible for eggplant leaves. Based on phylogenetic tree of AC2 and ORF AC1 sequence, the Begomovirus showed high homology with *Tomato yellow leaf curl* Kanchanaburi *virus* (TYLCKaV) infecting *Capsicum annum* and *C. melongena*. The result showed that the inoculated plants expressed symptom variations, such as yellowing, leaf edges rolling up, apical shoots to permeate, vein banding, little leaf, and dwarf.

Keywords: *Begomovirus*, eggplant, *Tomato yellow leaf curl* Kanchanaburi *virus*, whitefly.

INTRODUCTION

Plant virus diseases that have emerged in the last 3 decades have limited the production of important crops in tropical and subtropical regions around the world. Begomovirus belonging to the family Geminiviridae is one of the largest plant virus genera with over 400 reported (Olive & Castillo, 2020). The Geminiviridae family consists of 9 genera (Begomovirus, *Mastrevirus, Curtovirus, Topocuvirus, Turncurtovirus, Becurtovirus, Eragrovirus, Capulavirus* and *Grablovirus*), distinguished by host range, insect vector and genomic organization (Varsani *et al.* 2014, 2017; Roumagnac *et al.* 2015). The majority of the family *Geminiviridae* belong to the genus *Begomovirus*, which is transmitted by the whitefly (*Bemisia tabaci*) (Varma & Malathi 2003). *Begomovirus* have monopartite or bipartite circular single-stranded DNA (ssDNA) genomes, encapsidated in geminate particles (Fauquet & Stanley, 2005). Begomovirus have either monopartite or bipartite according to the presence of one or two (DNA-A and DNA-B) genome components, each of about 2.7 kb in size (Stanley, 1985). Both the monopartite and bipartite *Begomovirus*es have the DNA-A component which encodes all essential proteins for virus functions, while the bipartite *Begomovirus*es still contain the DNA- B component (Fundong, 2013). DNA A has six open reading frames (ORFs): AV1, AV2, AC1, AC2, AC3, and AC4 protein (Stanley, 1985; Marwal *et al.,* 2013). AV1 is coat protein (CP) gene, the main component that plays a role in encapsidation of the viral genome (ssDNA), virus particle formation, viral movement, and vector transmission (Snehi *et al.* 2017). AC2 encodes transcription activator protein (TrAP), activates expression from the viral coat protein (CP) gene (Hartitz *et al.* 1999). AC1 is replication initiator protein (ReP), a multitasking protein that replicates viral genome (Kushwaha *et al.* 2017). While DNA B has two ORFs, BV1 and BC1 (Fundong, 2013). BV1 encodes nuclear shuttle protein (nsp) and BC1 encodes movement protein virus in the plant (mp) (Fundong, 2013). The 50 ~ 200 nucleotide CP gene is highly variable and useful as an informative

Submitted: August 08, 2022
Revised: October17, 2022
Accepted for Publication: December 01, 2021
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region for predicting taxonomic relationships within the genus Begomovirus (Fundong, 2013). Full-length CP gene sequences can be used for rapid detection followed by prediction of *Begomovirus* species identification (Brown *et al.* 2001).

Begomoviruses are known to infect a variety of plants and wild plants in tropical and subtropical regions (Navas-Castillo *et al.,* 2011). In Indonesia, severe yield losses have been reported due to *Begomovirus* especially in *Solanaceae* (tomato, tobacco, and eggplant)(Subiastuti *et al.,* 2019). Five species of *Begomovirus*es have been reported, especially in tomato and pepper, such as *Tomato leaf curl Java virus*, *Tomato leaf curl Philippine virus*, *Pepper yellow leaf curl Indonesia virus*, *Ageratum yellow vein virus,* (Tsai *et al.* 2009), *Tomato leaf curl New Delhi virus* (ToLCNDV), and *Tomato yellow leaf curl* Kanchanaburi *virus* (Kon *et al.* 2006; Sukamto *et al.* 2009); Pratap *et al.* 2011; Kenyon *et al.* 2014). In the field, the incidence of disease is that most of the plants are infected by a mixture of viruses, such as chilies infected with *Pepper yellow leaf curl* Indonesian *virus* (PepYLCIV), *Tomato yellow leaf curl* Kanchanaburi *virus* (TYLCKaV) and *Ageratum yellow vein virus* (AYVV). (Subiastuti *et al.,* 2019). In addition, *Pepper yellow leaf curl* Aceh *virus* (PepYLCAV) have been reported infected chili, tomato, and tobacco (Kesumawati *et al.* 2019).

Regarding begomoviruses infecting eggplat, very few studies have been carried out in eggplant. Kesumawati *et al* (2020) reported *Tomato yellow leaf curl* Kanchanaburi *virus* (TYLCKaV) infecting eggplant. One of the most important steps in controlling plant diseases is identification of pathogens (Webster et al., 2004). Therefore, this study is focused on molecular detection and molecular variation based on Begomoviruses genom that infect eggplant. This research aims to: (1) detect *Begomovirus* infecting eggplant with universal primers Krusty/Homer according to AV1 gene (coat protein-CP); (2) identification the genetic variation of *Begomovirus* infecting eggplant with degenerate primers SPG1/SPG2 according to AC2 gene (transcription activator protein-TrAP) and AC1 gene (replication initiator protein-ReP); (3) to investigate the pathogenicity of *Begomovirus* infecting eggplant on various *Solanaceae* plants. The result will be useful in developing a strategy to control *Begomovirus* infection in Indonesia, spesifically in Lampung Province, Indonesia.

MATERIALS AND METHODS

Research site : The study was conducted in January to December 2021. It was done in Pringsewu Regency, Lampung Province and the Biotechnology Laboratory, Department of Plant Protection, Agriculture Faculty, Lampung University (Indonesia).

Field disease and collected samples: Observations of disease incidence of Bomovirus disease on eggplant plantation was carried out Pajar Agung Village. The disease intensity of *Begomovirus* infection on eggplant leaves using formula: IP= [Σ(ni x zi)/(N x Z) x 100%], with i = 0-4 disease symptom scores, ni = sum of plant symptoms with the score value, zi = value of symptoms score, N= sum of plant, and Z = hight score of disease symptoms (Ganefianti *et al.* 2015). Disease intensity was evaluated according to the following scale by Lapidot *et al.* (2001) with modification (Table 1).

Table 1. Disaes intensity of *Begomovirus* infection on eggplant

Score (Category)	Description
0 (Healthty)	no visible symptoms
1 (Mild)	very slight yellowing of leaflet margins on apical leaf
2 (Moderate)	some yellowing and minor curling of leaflet ends
3 (Severe)	a wide range of leaf yellowing, curling, and cupping, with some reduction in size, yet plants continue to develop
4 (Failure)	very severe plant stunting and yellowing, pronounced leaf cupping and curling; then plants stop growth

Leaf samples of eggplant which typical mosaic symptoms accompanied by yellowing and severe chlorosis suspected of being infected with *Begomovirus* were collected at Pajar Agung Village, Pringsewu Regency, Lampung Province, Indonesia. The leaves samples were classified base on disease symptoms, no visible symptom, mild, moderate, and severe according to a score catagories (Table 1).

Figure 1. Typical disease symptoms on eggplant leaves, mosaics accompanied by symptoms, severe chlorosis, and stunting suspected infected by *Begomovirus*

Inoculation of Begomovirus using Whitefly vector: Whitefly (*Bemisia tabaci*) used to inoculate Begomovirus from eggplant to evaluate the pathogenicity on various *Solanaceae* plants (eggplant, chili, and tomato). Whitefly colonies were reared on eggplants and grown in glass-covered cages. Adult whiteflies were acquisition access period (AAP) for 48 haurs on *Begomovirus*-infected eggplant and inoculation access period (IAP) for 72 hours. Inoculated plant were grown for 7 days to evaluated disease incubation period.

PCR Amplifcation of Begomovirus DNA fragments: The total nucleic acids were extracted from eggplant leaves (with symptoms) according to procedure of Genomic DNA Mini Kit (Plant_Geneaid). To detect begomovirus are associated with the mosaic disease of eggplats, two sets of Begomovirus universal and degenerate primers were used in PCR (Tabel 1). The first set of primers (Krusty and Homer) designed to amplifythe core of coat protein of Begomovirus and produces 550 bp DNA fragment. The second set of primers (SPG1 and SPG2) had been designed to amplify

the conserved regions in the open reading frames (ORFs) AC2 and ORF AC1 of the Begomovirus and produces 912 bp DNA pragment. The PCR amplification were analyzed using 1% agarose gel electrophoresis using 50V power for 55 minutes, stained with ethidium bromide in 1x Tris Borate EDTA (TBE) buffer, and viewed under ultraviolet light.

Table 2. Oligonucleotide primers used for the amplification of Begomovirus DNA fragments

Primer	Sequence amplified	Primer sequence	DNA Fragment size (bp)	anneling temperature/
(Krusty (Forward) Homer (Reverse)	Core CP	5'-CCNMRDGGHTGTGAR GGNCC'-3 5-SVD GCRTGV GTR CAN GCCAT-3	550	55
(SPG1 (Forward SPG2 (Reverse))	AC1 and AC2	5′-CCCCKG TGCGWRAAT CCAT-3′ 5′-ATCCVA AYWTYCAGGGAGCTA A-3′)	912	55

Note: K = G or T; M = A or C; R = A or G; S = C or G; W = A or T; Y = C or T

PCR compositions in one tube PCR were 10 µl (for sample "B, C, D, E, F, G, H") containing 5 µl MyTaqTM HS Red Mix 2x, 1 µl for each primer, 1 µl DNA samples, and 2 µL Water for Injection (WI), then 25 µl (for sample "A" process to sequencing) containing 12.5 µl MyTaqTM HS Red Mix 2x, 1 µl for each primer, 1 µl DNA samples, and 12.5 µL Water for Injection (WI). PCR reaction began with an initial denaturation at 95ºC for 3 minutes continued with 40 cycles of denaturation at 95ºC for 1 minute, annealing at 55ºC for 30 seconds, and extention 75ºC for 90 seconds then followed by a final extension at 72ºC for 10 minutes (Kandito *et al.* 2020). Then the PCR results were analyzed using 1% agarose gel electrophoresis using 50 V power for 55 minutes, stained with ethidium bromide in 1x Tris Borate EDTA (TBE) buffer, and viewed under ultraviolet light.

The DNA frames of AC2 and ORF AC1 were sequenced and used to study genetic biodiversity among Begomovirus. Analysis sequence of the DNA fragment was carried out with the MEGA v.11 program and the Basic Local Assessment Search Tool (BLAST) program on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov) to compare the target virus sequences with the registered-nucleotide sequences of viruses from other countries in Genbank. Nucleotide and amino acid homology levels were obtained by the ClustalW multiple alignments. The phylogenetic analysis uses the unweighted pair group method with arithmetic mean (UPGMA) with 1000 bootstrap replicates

RESULTS

Field observation and sample collections: The field observation eggplant leaves suspected infected by Begomovirus was in Pajar Agung Village, Pringsewu Regency, Lampung Province, Indonesia showed that Yumi F-1. The results revealed that based on disease symptoms on eggplant leaves varied from leaf curl, yellowing, and mosaic with disease incidence was 100% and the diseases severity was 33.36%. The symptoms category on the polupation of egglants were 73.5% eggplants on mild category, 2.3 % eggplants on moderate category, 22.3% eggplants on severe category, and 2% eggplants on failure.

Molecular detection: PCR Amplification using the two universal Krusty/Homer and degerate SPG1/SPG2 primers were amplified the target sequences on *Begomovirus*es genome. The universal Krusty/Homer primers amplified 550 kb DNA correspond to the a part of the coat protein gene and SPG1/SPG2 primers amplified 867 kb DNA of AC2 and ORF AC1 open reading fames. Both universal primers Krusty/Homer and SPG1/SPG2 confirmed that samples collected from field were infected by Begomovirus (Figure 4).

Genetic variation of Begomovirus: PCR amplified sequence of the 867 bp DNA fragment were designed to amplify conserve region of AC2 and ORF AC1 open reading fames was used to study genetic variation among Begomovirus. Based on phylogenetic analysis showed that Begomovirus infected eggplants identified as *Tomato yellow leaf curl* Kanchanaburi *virus* (TYLCKaV) and have high homology to TYLCKaV isolated from Capsicum annum (Figure 1).

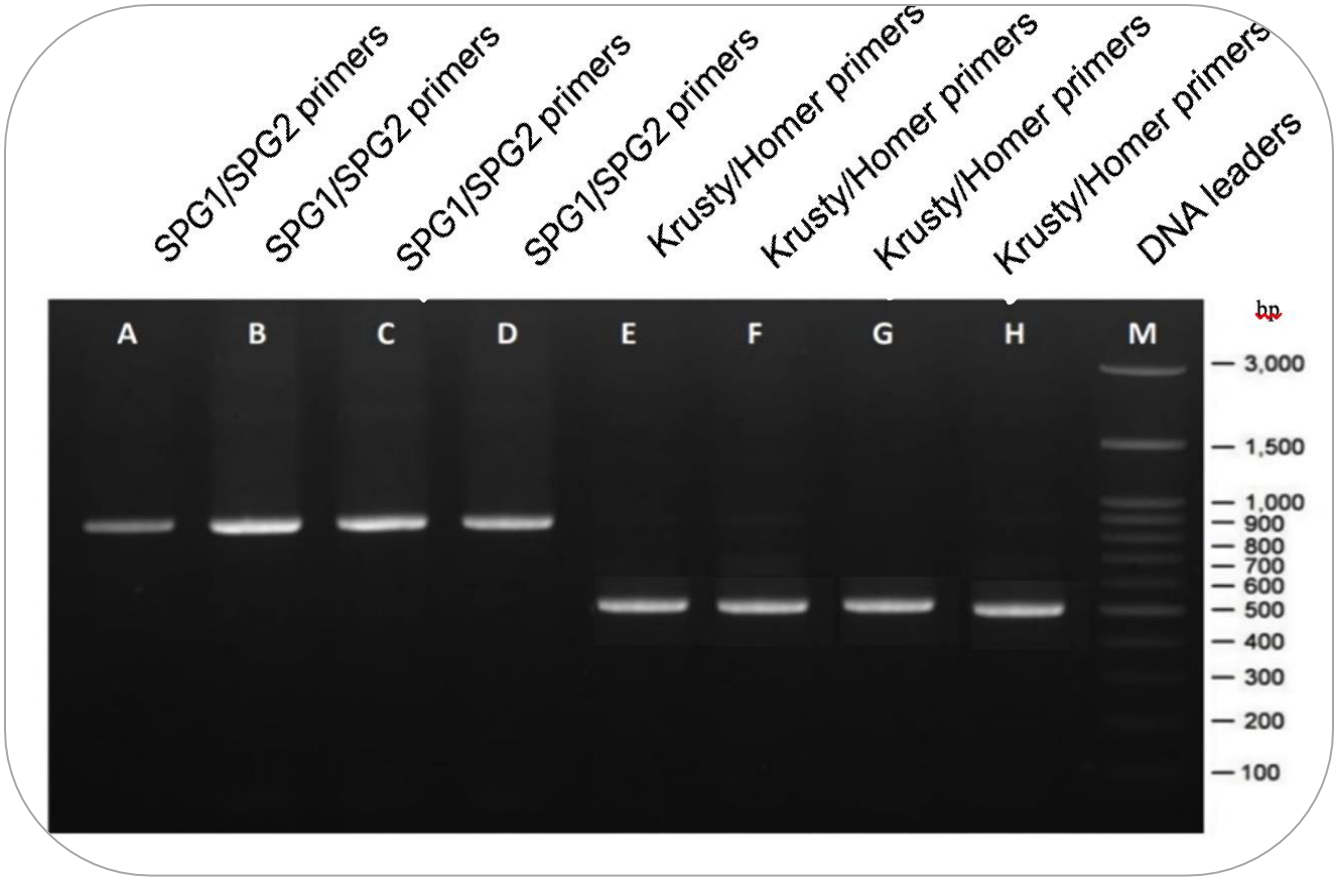

Figure 2. PCR amplification from leaf extract eggplant of Begomovirus unsing universal primers SPG1/SPG2 produced ~867 bp DNA fragment and Krusty/Homer produced ~550 bp DNA fragment.

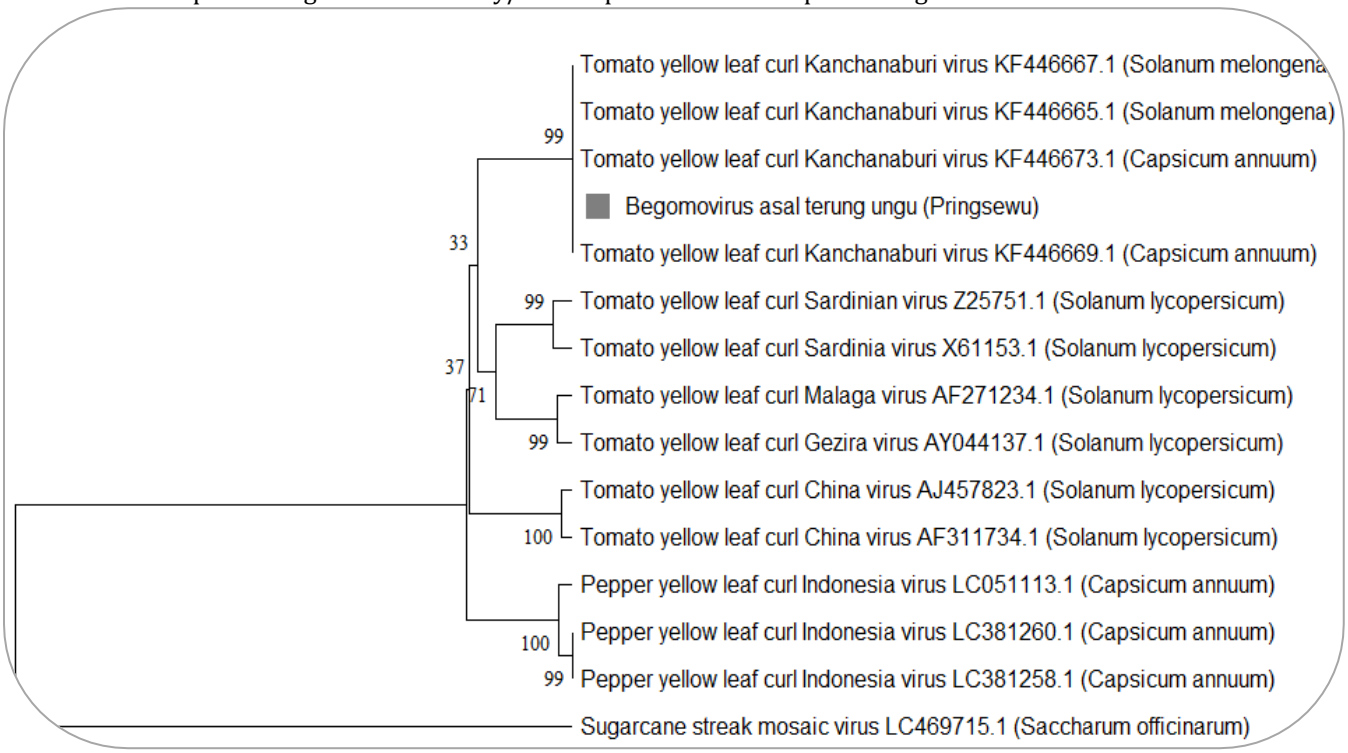

Figure 3. Phylogenetic analysis result using the UPGMA with 1000 bootstrap replicates

Pathogenicity of the Begomovirus on solanacea plants: Identified *Tomato yellow leaf curl* Kanchanaburi *virus* (TYLCKaV) from eggplants were evaluated its pathogenicity on other solanacea plants (tomatoes, chili peppers, and eggplants). Viruliferous whiteflies (*Bemisia tabaci*) were used to inoculate the TYLCKaV to test plants, tomatoes, chili peppers, and eggplants. The disease symptoms on inoculated plant were characterized based on visible symptoms. The result showed that infected plants expressed symproms, leaf curl or malformation, mosaic, yellowing, and dwarf on all infected plants and vein clearing on eggplants (Figure x).

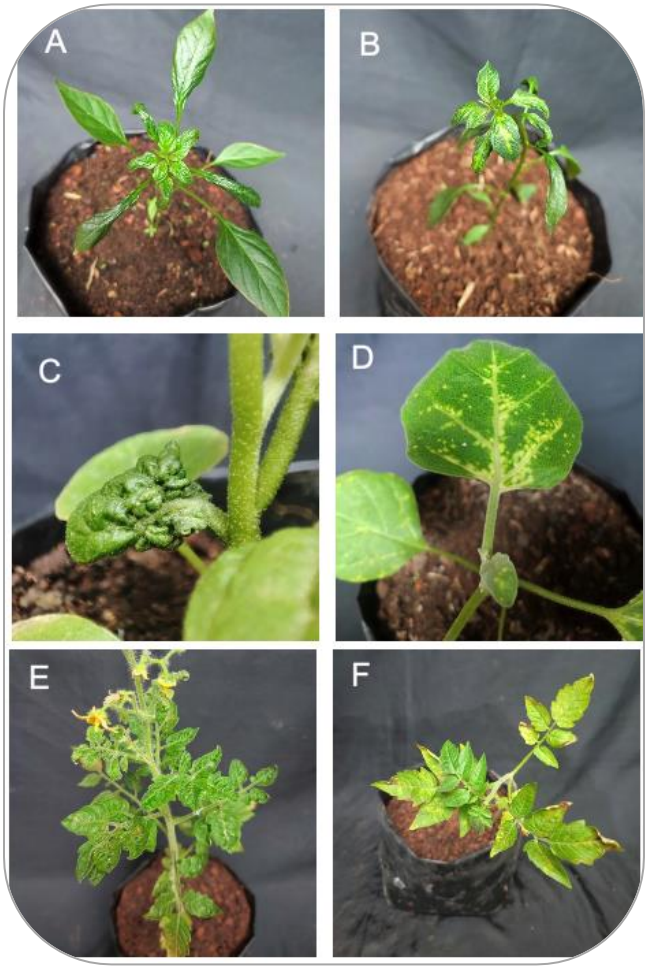

Figure 4. Typical symptoms of the TYLCKaV from eggplant on solanaceae plants, eggplants (A,B); chili peppers (C,D), and tomato (E,F)

DISCUSSION

The total DNA was isolated from eggplant plants expressing typical Begomovirus symptoms. These confirmed that most of the genomic DNA could be used for PCR amplification using universal primers. This research were proved that Boths universal primers Krusty/Homer and degenerate primers SPG1/SPG2 can be used to detect Begomovirus in eggplant leaves collected from Pringsewu using PCR techniques.

The use of these two types of primers, served to test the capability of the two pairs of primers in amplifying *Begomovirus* on eggplant. The results suggest these two sets of primers successfully amplify the *Begomovirus*, but there are differences in the quality of the DNA ribbon. Plant samples that use degenerate primers SPG1/SPG2 produce more vivid DNA ribbons than universal primers Krusty/Homer. Therefore, DNA amplification using degenerate primers SPG1/SPG2 is more effective and efficient than universal primers Krusty/Homer for identification of *Begomovirus*.

The difference in the quality of this DNA ribbon is because degenerate primers SPG1/SPG2 can amplify a viral genome of the same family, thus enabling it to amplify different sequen from different proteins (Iserte *et al.* 2013), while the universal primers Krusty/Homer can amplify the viral genome of the same genus only (Daidoji *et al.* 2021). Therefore, degenerate primers SPG1/SPG2 have a broader genome range compared to the universal primers Krusty/Homer.

Begomovirus infection in Indonesia was first reported in West Java in 1999 and spread into Central Java in 2003 (Subiastuti, 2019). The first infection was PePYLCIV in Pepper (Rusli *et al.* 1999). Meanwhile, in 1998 tomato-infecting *Begomovirus* was found in Lembang and named as *Tomato yellow leaf curl* Indonesia *virus* (TYLCIDV) (Tsai *et al.* 2006) and it was the first report about *Begomovirus* infecting a member of *Solanaceae* in Indonesia. Then in 2006, *Tomato leaf curl* Java *virus* (ToLCJaV) was reported infecting tomatoes in Bogor (Kon *et al.* 2006). Thenceforth, *Begomovirus* infecting *Solanaceae* developed into *Tomato yellow leaf curl* Kanchanaburi *virus* (TYLCKaV). After several proper identifications, TYLCKaV isolate from Indonesia was first found in eggplant and it is very similar to that initially identified in Thailand (Kenyon *et al.* 2014).

TYLCKaV is a member of the *Begomovirus* genus in the Old World and most of them are reported to attack eggplants, tobaccos, tomatoes, and chillies (Kintasari *et al.* 2013; Kusumaningrum *et al.* 2015). The symptoms of TYLCKaV in eggplants induce chlorotic and mosaic symptoms. While the infection in tomato plants is known to cause apical shoots to permeate and dwarf (Tang *et al.* 2013). Then the symptoms in chili plants such as thickening on the leaf bone, leaf edges rolling up,

narrowing, and bright yellow leaves (Windarningsih 2019).

TYLCV is a monopartite *Begomovirus* transmitted by the whitefly, *Bemisia tabaci*. TYLCV epidemics tend to be associated with high populations of the whitefly vector. TYLCV was believed to be inoculated only by whitefly-mediated transmission (Stanley *et al.* 2001).

CONCLUSION

The molecular detection with universal primers Krusty/Homer can be used for detect *Begomovirus* according to AV1 gene (coat protein-CP). While the genetic variation of *Begomovirus* infecting eggplant with degenerate primers SPG1/SPG2 according to AC2 gene (transcription activator protein-TrAP) and AC1 gene (replication initiator protein-ReP) was identify as Tomato Yellow Leaf Curl Kanchanaburi Virus (TYLCKaV).

ACKNOWLEDGEMENTS

The authors would like to thank the farmers in South Lampung, Pesawaran and Central Lampung for giving permission to collect data and samples.

FUNDING

This research was funded by Dipa Faculty of Agriculture in 2022

ETHICAL APPROVAL

This article does not contain any studies with human participants performed by any of the authors.

DISCLOSURE STATEMENT

The authors declared that they have no conflict of interest.

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HMA, NN, and SH;	:	Conceptualization
EF and SH;	:	Molecular test
EF;	:	Data analysis:
SH and EF;	:	Writing—original draft preparation
all authors;	:	Writing—review and editing
SH	:	Funding acquisition