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BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF *BEGOMOVIRUS* MILD STRAINS AND THEIR ELUCIDATION FOR CROSS PROTECTION AGAINST EGGPLANT *BEGOMOVIRUS* (*SOLANUM MELONGENA* L.)

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A B S T R A C T

One alternative form of controlling disease caused by *Begomovirus* infection which infects eggplant plants (*Solanum melongena* L) is by utilizing the principle of cross protection. This study aims to obtain mild strains of *Begomovirus* from natural populations in *Solanacea* plants, identify molecular characteristics *of Begomoviruses* that infect eggplant based on AV1, AC1, and AC2 gene sequences, determine the pathogenicity of mild strains of *Begomovirus* in several *Solanacea* plants, and determine the effectiveness of mild strains *of Begomovirus* against super infection of the severe strain *Begomovirus*. Identification of *Begomovirus* used *the universal primer* Krusty/Homer which amplified the AV1 gene sequence at ±550 bp and *the degenerate primer* SPG1/SPG2 which amplified the AC1 and AC2 gene sequences at ±912 bp. The results obtained from this study showed that eggplant from a natural population from Pesawaran was a mild strain *of Begomovirus*. Phylogenetic analysis and genetic variation *of Begomovirus* showed that *Begomovirus* from South Lampung was identified as *tomato leaf curl kanchanaburi virus* (*TYLCKaV*). Inoculation of mild strains and severe strains *of Begomovirus* on eggplant and red pepper plants showed symptoms of yellow spots on leaves and leaf curl. The mild strain *of Begomovirus* from Pesawaran was able to suppress superinfection of the severe *Begomovirus strain*.

Keywords: Cross protection, *Begomovirus* , Eggplant, Pesawaran, *Solanacea*.

INTRODUCTION

Eggplant (*Solanum melongena* L) is the fifth largest horticultural crop in the world after tomatoes, potatoes, pepper and tobacco. Global eggplant production continues to increase and in 2014 production reached 50.19 million and constituted one third of total global tomato production (Hirakawa *et al*, 2014). China's eggplant production reached 28.4 million tons or 57% of the world's total eggplant production, followed by India with a production of 13.4 million tons (27% of world production), Egypt 1.2 million tons, Turkey 0.82 million tonnes, and Iran 0.75 million tonnes (FAO, 2014).

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One important virus that has been reported to infect eggplant is *Begomovirus* (Schippers, 2000). *Begomovirus* is one of the largest genera in the *Geminiviridae* family with a relatively wide distribution, i.e. almost entirely in agricultural cultivation. In Asia, two *Begomovirus* species have been reported that infect eggplant, namely *Tomato yellow leaf curl kanchanaburi virus* in Thailand (Green *et al*., 2003) and *tomato leaf curl new dehli virus* in India (Pratap *et al*., 2011). Abubakar *et al.* (2022) reported decreased crop productivity due to *Begomovirus* infection ranging from 20% -100% and based on a survey conducted in the Nagpur area, India during the 2009 to 2010 period it was found that the incidence of disease due to *Begomovirus* infection in eggplant plants was 60-65% (Pratap *et al*., 2011).

Virus infections in plants are generally difficult to detect and efforts to control them are also quite difficult. Virus-affected plants cannot be controlled by any chemical treatment (Sneki *et al*., 2015). Along with technological developments, the

challenge arises to find methods of controlling plant viruses that are effective and without side effects. Cross protection is a method of controlling plant viruses with a fairly high success rate. In various countries cross protection has been widely applied and proven effective, some evidence is that cross protection has been successful in controlling *Citrus tristeza virus, Papaya ring-spot virus, Zucchini yellow mosaic virus, Tomato mosaic virus* (Lecoq, 1998). In addition, cross-protection was able to suppress *African cassava mosaic virus* (ACMV) infection so that it could maintain cassava production in Uganda (Owor *et al*., 2004). Thus, this study purpose to obtain mild strains of *Begomovirus* from natural populations in plants of the *Solanaceae family* that have potential as cross-protection agents, pathogenicity of mild strains *of Begomovirus* in several plants of *the Solanaceae family* , identify the molecular character *of Begomovirus* that infects eggplant (*S. melongena* L.) based on the AV1, AC1 and AC2 gene sequences, as well as knowing the effectiveness of mild *Begomovirus strains* against superinfection of severe *Begomovirus strains.*

MATERIALS AND METHODS

The study was conducted at the Biotechnology Laboratory, Faculty of Agriculture, University of Lampung from September 2022 to August 2023.

Eggplant plant exploration in the field: Exploration was carried out in three regions in Lampung province, namely Pesawaran Regency, South Lampung Regency and Pringsewu Regency. This activity was carried out with the purpose of obtaining eggplant plant samples that were used as a source of mild strain inoculums. The criteria for plants that can be used as a source of mild strain inoculums are eggplant plants with severe symptoms with an attack intensity of >50% in one field. To calculate the occurrence and severity of the disease using the formula proposed by Nelson *et al.* (1999) which is modified as follows. Disease Occurrence = ((n/N) x 100%) where n: number of diseased plants, N: number of plants observed. While the formula for disease severity is as follows KP : [Σ(ni x zi)/(NXZ) x 100%] , Disease severity (%), ni : Number of diseased plants, zi : Value of symptomatic plants with score (0-4), N : the total number of plants observed, and Z : the score of the highest plant. Score 0: healthy plants, no symptoms 0%, score 1: mild symptoms > 2% -25%, score 2: moderate symptoms > 26% -50%, score 3: severe symptoms > 51% -75%, and score 4: very severe symptoms > 76% -100%.

Preparation of test plants: The test plants used were eggplant (*Solanum melongena* L.) variety SS 110 and chili pepper (*Capsicum annum* L.) variety ENNO 1433, each of which consisted of 8 plants. The test plants were planted in polybags filled with planting media in the form of soil and rice husks with a ratio of 2:1.

Molecular detection of *Begomovirus* in purple eggplant and inoculated plants using PCR technique: Molecular detection was carried out in several stages, namely DNA extraction, DNA amplification, and visualization of the amplification results. The DNA extraction steps were carried out according to the Genomic DNA Kit (*Plant)* protocol from Geneaid which consisted of plant tissue separation, lysis, DNA binding, washing, and DNA elution. After the extraction stage is complete, then proceed with the DNA amplification stage using two types of primer, namely the *universal primer* Krusty (*forward*) (5'CCNMRDGGHTGTGARGGNCC'3) And Homer (*reverse*) (5'SVDGCRTGVGTRCANGCCAT'3) DNA targets 550 bp and *degenerate primers* (5'CCCCKGTGCGWRATTCCAT'3) and SPG1 (*forward*) SPG2 (*reverse*) (5'ATCCVAAYWTYCAGGGAG GAGCTAA'3) DNA targets 912 bp. The DNA amplification stage was carried out for 40 cycles consisting of *pre-denaturation* at 95 °C for 3 minutes, *denaturation* at 95 °C for 1 minute, *annealing* at 55 °C for 30 seconds, elongation *at* 75 °C for 1 minute 30 seconds, and *final extension* temperature 72 °C for 10 minutes (Kandito *et al.,* 2020). The results of DNA amplification were analyzed using 1% agarose. during ± 50 minutes with a voltage of 50 volts. The agarose gel was stained with ethidium bromide in 1x Tris Borate (TBE) buffer and viewed with a UV trans illuminator.

***Begomovirus* Genetic Variations Based on AV1, AC1 and AC2 Gene Sequences:** Two amplified eggplant samples were sequenced, and the sequenced data were then confirmed to GenBank using the *Basic Local Alignment Search Tool* (BLAST) program at *the National Center for Biotechnology Information* (NCBI). The test sample sequences were aligned *using* the *Clustal W Multiple Alignment* MEGA v11. Then the test sample was visualized in the form of a dendrogram using the *Molecular Evolutionary Genetic Analysis Software* (MEGA) v11 program with the *Neighbor-Joining Tree* using bootstrap 1000 replicates.

Pathogenicity Test of Mild Strains *of Begomovirus* in *Solanacea Plants:* Inoculation of *Begomovirus* to plants consists of two stages, namely inoculation of Inoculation of *Begomovirus* to plants consists of two stages, namely inoculation of mild strains *of Begomovirus* and inoculation of severe strains *of Begomovirus.* Inoculation was carried out mechanically with the sap method. The liquid sap composition consists of 1 g of eggplant leaves with mild symptoms, 1 g of eggplant leaves with severe symptoms, 5 ml of phosphate buffer, 0.1 g of carborudum. The stages of

inoculation of the mild strain *of Begomovirus* are pounded leaves of eggplant with mild symptoms which have been added with phosphate buffer until smooth. After that, filter the sap liquid with sterile gauze. Then 0.1 g of carborundum was added to the sap. After that it was applied to the plants with a cotton bud on 2 leaves until evenly distributed, then left for 2 minutes and cleaned of carborundum residues attached to the leaves with distilled water. After that the plants were incubated for 1 week. The same steps were carried out for severe strain inoculation. However, at this stage the leaves of the eggplant plant used are the ones symptoms. After the inoculation stage was completed, the plants were incubated for 4 weeks in a closed room to see the response of the plants after inoculation.

***Begomovirus* host range assay:** Papaya plants were used as test plants. Five papaya plants were inoculated with *Begomovirus* with the help of vector (*Bemisia tabaci*) then incubated in a lid for 4 weeks and observed the symptoms that appeared.

Measurement of the purity of the amplified DNA sample: A total of eight samples of amplified DNA consisted of eggplant with severe *Begomovirus* field strain, eggplant with mild strain in *Begomovirus* field, eggplant inoculated with mild *Begomovirus strain*, eggplant inoculated with severe *Begomovirus strain,* eggplant inoculated with mild and severe *Begomovirus strains* , chili pepper inoculated with strains *Begomovirus* mild, chili pepper inoculation of severe *Begomovirus strains*, and chili pepper inoculation of mild and severe *Begomovirus strains.* The purity of the eight samples was measured using a uv-vis spectrophotometer at a wavelength of 260 nm and 280 nm.

RESULTS

Eggplant exploration in the field: exploration was carried out in 3 areas including Pesawaran, Pringsewu, and South Lampung. In the Pesawaran area, plants were obtained as the inoculum source for the mild strain *of Begomovirus* and in the South Lampung region, the source of the inoculum for the severe *Begomovirus strain was obtained* (Figure 1). The disease incidence rate in the region reached 100% with a severity of 54.2% for each Pesawaran, 48.5% for Pringsewu, and 68.14% for South Lampung.

Figure 1. Pattern of *Begomovirus* infection spread in the field (a), eggplant mild strain (b), and eggplant severe strain (c).

Molecular detection of *Begomovirus*: DNA amplification using two types of primers Krusty/Homer amplified at 550 bp and SPG1/SPG2 could amplify at 912 bp (Figure 2).

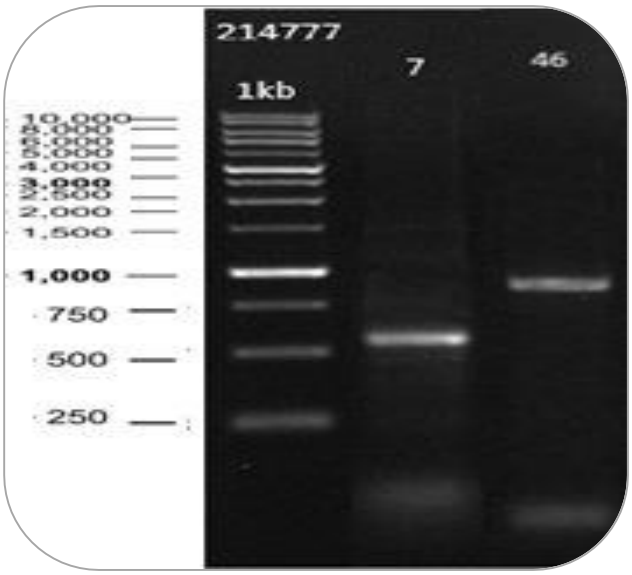

Figure 2. Visualization of *Begomovirus* DNA measuring ± 510 bp and ± 912 bp (based on AV1 and AC2 genes, AC1). Marker (1kb), eggplant samples from South Lampung (7), and eggplant samples from Pesawaran (46).

***Begomovirus* genetic variation:** based on phylogenetic analysis Eggplant samples from South Lampung with Krusty/Homer primers were identified as *Tomato yellow leaf curl Kanchanaburi virus* (*TYLCVKaV*) and are related to *Tomato yellow leaf curl Kanchanaburi virus isolate Cambodia* with 72% homology (Figure 3).

Pathogenicity test of mild strains of *Begomovirus* on Solanaceae plants: Inoculation of mild strains of *Begomovirus* on Solanaceae plants (eggplant and chili pepper) showed a response in the form of yellow spot symptoms on some test eggplant plants and on all test chili plants symptoms of curling on young leaves, but redness disease in each test plant was low (Figure 4). The average incubation period for eggplant plants ranges from 21 days after incubation (DAI) and for chili plants the incubation period ranges from 7-21 days after incubation (DAI).

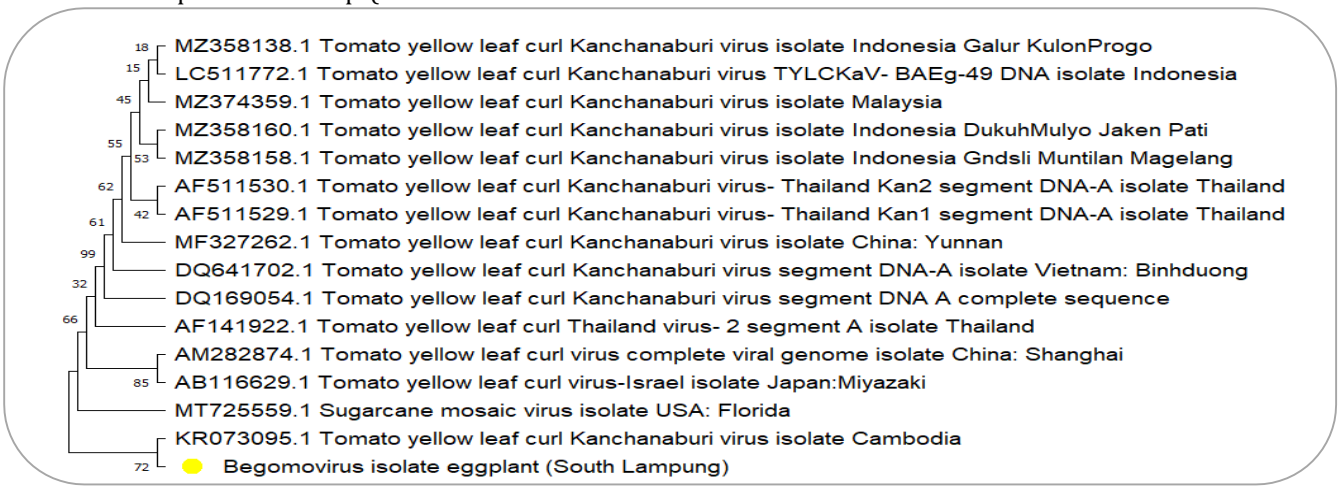

Figure 3. The eggplant phylogeny tree from South Lampung using the *Neighbor-Joining Tree* method

A B

Figure 4. Variation of *Begomovirus* symptoms in the test plants. Yellow spots on purple eggplant (a) and curly leaves on chilies pepper (b).

***Begomovirus* host range test:** the results of *Begomovirus* inoculation on papaya plants showed a symptom response in the form of yellow spots on leaves and leaf curl (Figure 5).

A B

Figure 5. Variation of symptoms of *Begomovirus* infection in papaya plants. Yellowing leaves (a) and curly leaves (b).

Measuring the purity of the amplified DNA samples: the purity of the DNA samples included two field eggplant samples, 3 inoculated eggplant samples, and 3 inoculated chili samples showed absorbance values ranging from 0.99 to 1.00 (Table 1).

Table 1. Absorbance values of the amplified DNA samples

Sample Name	λ260	λ280	absorbance value
The eggplant is a severe strain of field *Begomovirus*	0.146	0.147	1.006
Eggplant is a mild strain of field *Begomovirus*	0.178	0.177	1.005
Eggplant inoculation of mild and severe strains of *Begomovirus*	0.212	0.213	0.995
Eggplant inoculated with a mild strain of *Begomovirus*	0.181	0.181	1,000
Eggplant inoculated with the severe *Begomovirus* strain	0.175	0.175	1,000
Chili pepper inoculation of mild and severe strains of *Begomovirus*	0.168	0.168	1,000
Chili pepper inoculation of a mild strain of *Begomovirus*	0.172	0.173	0.994
Chili pepper inoculation of the severe *Begomovirus* strain	0.176	0.177	0.994

DISCUSSION

Begomovirus is a genus in the *Geminiviridae* family which has many hosts, one of which is a plant in the *Solanaceae* family. Kintasari *et al.* (2013) reported that in 2013 a *Begomovirus* had been found infecting Solanacea plants in West Java, Central Java and the Special Region of Yogyakarta with symptoms in the form of mosaics and yellowing. Eggplant *Begomovirus* isolateSouth Lampung was identified as *Tomato yellow leaf curl Kanchanaburi virus* (*TYLCVKaV*) with a variety of symptoms in the form of leaf mosaic and yellowing leaves. Kenyon *et al.* (2013) reported that the first Indonesian *TYLCVKaV* isolate that infected eggplant plants had similarities to *TYLCVKaV* originating from Thailand. The host range test for *TYLCVKaV* was carried out on papaya plants showing positive results, namely symptoms of yellow spots on the leaves and curly leaves. Even though according to research by Dickey *et al.* (2012) papaya is not a host plant for *TYLCVKaV*, papaya can host *Begomovirus*.

Phylogenetic analysis of the eggplant *Begomovirus* isolate

from South Lampung was identified as *TYLCVKaV* and related to *Tomato yellow leaf curl Kanchanaburi virus isolate Cambodia* with 72% homology. Hillis and Bull (1993) stated that phylogenetic analysis purpose to see the kinship between organisms based on their evolutionary relationship. The greater the bootstrap value used, the higher the confidence of the reconstructed tree topology. Bootstrap analysis with values of 70 % or higher indicates a reliable categorization.

Eggplant and chili plants which were inoculated with mild strains of *Begomovirus* mechanically showed symptoms of yellow spots and leaf curl. The incubation period for eggplant plants ranges from 21 day after incubation (DAI) and chili plants ranges from 7-21 day after incubation (DAI). Rimbaud *et al.* (2015) on disease control based on visual symptoms, the incubation period is important because it relates to the time of appearance of symptoms. The faster the incubation period, the faster control can be carried out, so that the incubation period plays a very important role in the spread of the virus. Although in general the success rate of inoculation by mechanical means is low. *Begomovirus* infects plant phloem tissue and if mechanical transmission is carried out, the virus can only be introduced to plant epidermal tissue. The success of mechanical transmission depends on the concentration of the virus in the solution (sap), the source of the inoculum, the resistance of the virus in the sap, the method of preparation, the host plant and the environment (Akin, 2006). Thus the inoculated mild strain of *Begomovirus* could suppress the superinfection of the severe *Begomovirus* strain in the test plants. The application of plant virus cross protection is quite widely applied in various countries as one of the measures to protect plants and prevent loss of production. One of the successful implementation of cross protection was stated by Walkey *et al*. (1992) reported success with cross protected C. pepo which resulted in an increase in marketable fruit ranging from 0 to 43% in one study and 56 to 63% in another trial. They attributed these variable yield benefits to the amount of time between mild strain and severe strain inoculation, noting that 14 days were required for effective protection. In addition, the application of cross-protection was carried out to protect cassava plants from infection with a malignant strain of the *African cassava mosaic virus (ACMV)* virus in Uganda (Owor *et al.*, 2014). Several cases of plant virus infection globally were successfully controlled by applying cross protection, the *Zucchini yellow mosaic virus* which affected global cucurbit production, the *papaya ringspot virus* which attacked the papaya plant in Hawaii and affected papaya production in many countries, and the *cocoa swollen shoot virus* which threatens cocoa production in Africa (Ziebell and Carr, 2010).

A purity test of the inoculated samples was also carried out and the average absorbance was found to be in the range of 0.99 -1.00, which means that the purity of the virus in the samples was not good. Sambrook *et al.* (1989) suggests a good purity value ranging from 1.8 to 2.0. This range is generally required as a requirement in molecular analysis.

CONCLUSION

Based on the results above, a weak strain of *Begomovirus* from a natural population of eggplant plants from Pesawaran was able to suppress *Begomovirus* superinfection with a response in the form of yellow spot symptoms on the test plants. *Begomovirus* isolate from South Lampung was identified as *tomato yellow leaf curl kanchanaburi virus* and is related to isolate from Cambodia.

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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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Hasriadi M. Akin	:	Designed research methodology, laying a strong foundation.
Selvi Helina	:	Responsible for accurate data collection and in-depth analysis.
Nuryasin	:	Ensured statistical integrity with a profound understanding of data analysis.
Saipul Abbas	:	Conducted thorough article reviews and plagiarism checks.
Muhammad Nurdin	:	Provided critical insights into interpreting research results.
Reni Safitri	:	Wrote articles, presenting findings with comprehensive synthesis.