

# Begomovirus detection in the whitefly Bemisia spp. on eggplant Solanum melongena L. leaves

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### ABSTRACT

*Begomovirus* infects various species of the Solanaceae family and reduces crop productivity. It is important to note that *Bemisia tabaci* is a vector that contributes to increasing infection. Therefore, this study aims to identify and modify DNA extraction methods, as well as detect the presence of *Begomovirus* in whitefly and eggplants (*Solanum melongena* L.) plantations. *S. melongena* leaves showing infection symptoms were collected from eggplant cultivation in Rejodani, Sleman, D.I. Yogyakarta, Indonesia. The identification of whitefly collected from the leaves was performed microscopically, DNA extraction was carried out using lysis and cetyltrimethylammonium bromide (CTAB) buffer, while the presence of *Begomovirus* was detected using polymerase chain reaction. Based on the results, the whitefly is a part of *Bemisia* genus, the DNA quantification showed that *Bemisia* spp. has low purity, while *S. melongena* is slightly purer. However, *Begomovirus* presence was detected in both sample since the DNA band appeared in ±580 bp. It was concluded that DNA from *Begomovirus* in the whitefly *Bemisia* spp. and *S. melongena* can be extracted using lysis and CTAB buffer, respectively. It was also shown that *Begomovirus* infects *S. melongena* similarly to its vector, *Bemisia* spp., in Rejodani eggplant plantations.

# **1. INTRODUCTION**

*Begomovirus* infection has attacked various crops in Indonesia, specifically the Solanaceae and Cucurbitaceae families [1,2]. It is also known as ssDNA virus and a member of the Geminivirus family with great diversity. According to the International Committee on Taxonomy of Viruses, the total species of *Begomovirus* is about 424. The infection in Cucurbitaceae and Solanaceae families often shows several symptoms such as stunting, mosaics, curls [2], yellow mosaics in eggplant's leaves [1], green mosaics, and yellow mosaics in melon's leaves [3]. In Solanaceae plants, particularly *Capsicum annum L., Begomovirus* tends to reduce productivity. A positive correlation was also reported between the presence of *Begomovirus* and plant growth inhibition such as stem diameter, the number of fruits, and mass of fruit [4].

Since *Begomovirus* is transmitted by whiteflies, higher infection cases are likely to occur. One of the popular species of whitefly is *Bemisia tabaci*, with a unique reproduction named arrhenotoky

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parthenogenesis, which enhances the yield. The higher the population, the greater the tendency of the virus to spread faster [5,6]. The type of *Begomovirus* inside the vector is circularly persistent, settling in a long period without any replication. Therefore, when *B. tabaci* carrying *Begomovirus* sucks the phloem sap, infection simultaneously spreads out and infects the plant [7].

Detection of *Begomovirus* in plant leaves through the polymerase chain reaction (PCR) method has been carried out on eggplant, melon, chili, watermelon, papaya, and pepper plants [2,8]. DNA extraction is an initial step in *Begomovirus* detection using PCR. A common simple extraction method to obtain DNA from insects is lysis buffer [9], while plant leaves DNA extraction can be performed using cetyltrimethylammonium bromide (CTAB) [10]. It is important to note that there are only a few reports on the use of lysis and CTAB buffers to detect *Begomovirus*. Therefore, this study aims to detect *Begomovirus* in *B. tabaci* and its host with a brief extraction method to predict *Begomovirus* incidence.

### 2. MATERIALS AND METHODS

### 2.1. Sample Collection and Identification

The symptomatic eggplant leaves and whitefly were collected in pairs at an eggplant plantation in Rejodani, Sleman, Yogyakarta, Indonesia, in October 2021. A total of 15–25 whitefly (code: s) were collected from the underside of yellow and mosaic leaves into a microtube

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containing 70% alcohol. In addition, whitefly was sampled using a respirator for the observations with light microscopy and stereo zoom. The whole body color and wings type were identified according to the manual identification book titled *Borror and Delong's Introduction to The Study of Insects* [11]. Eggplant leaves were collected and stored in a zip lock with chill condition inside an icebox. Several codes were used as sample identifiers including yellow and yellow mosaic leaves with code of k and km, respectively.

# 2.2. Whitefly DNA Extraction

Whitefly DNA extraction was performed using lysis buffer [9], with several modifications such as dithiothreitol replacement with  $\beta$ -mercaptoethanol [12]. The samples were washed using a sterile ddH<sub>2</sub>O, then it was crushed simultaneously with the 100 µL lysis buffer solution containing 0.01 M Tris-HCl pH 8.0, 0.01 M NaCl, 0.005 M CaCl2, 0.002 M EDTA pH 8.0, 2% sodium dodecyl sulfate (SDS),  $\beta$ -mercaptoethanol, and 0.25 mg/mL of the Proteinase K. Furthermore, the crushed samples were incubated overnight at 55°C and centrifuged to produce supernatant and pellet. The supernatant was stored at -20°C as the DNA sample.

### 2.3. Solanum melongena DNA Extraction

CTAB buffer containing 2% CTAB, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 0.02 M EDTA pH 8.0, and 1% PVP [10] was heated at 60°C using a water bath. Afterward, 0.4 gr of S. melongena leaves were crushed in 2 mL of chilled CTAB buffer, and 10 µL RNase was added to the supernatant, while the microtube was inverted to obtain a completely dissolve sample. Since it was homogenous, the sample was incubated at 37°C for 10 min, then 2  $\mu$ L  $\beta$ -mercaptoethanol was added and incubated at 65°C for 30 min in a water bath with microtubes inverted every 10 min. The sample was cooled at room temperature for 3 min, while 2 mL of 1:1 of phenol: Chloroform was added to the sample, and the microtube was inverted reverse for 3 min. The homogenous sample was centrifuged at 12000 rpm, 10 min, at 25°C, then chloroform was added in a ratio of 1:1 to the supernatant and centrifuged at 12000 rpm, 10 min, at 25°C. Furthermore, 3 M Na-acetate pH 5.2 up to 1/10 of the supernatant volume and cold isopropanol 1:1 were added to the supernatant. The samples were inverted and incubated for 1 h at -20°C and then centrifuged at 12000 rpm, 10 min, at 25°C to obtain the pellet. The DNA pellet was washed with 500 µL of 70% alcohol, then centrifuged at 12000 rpm, 10 min, at 25°C. The 70% alcohol was removed from the microtube and DNA pellet was air dry, while 30 µL of TE buffer was added and stored at -20°C.

### 2.4. Begomovirus Coat Protein (CP) Gene Amplification

A total of 2.5 µL DNA samples were dropped onto the Nanodrop surface and measured at 260, 280, and 230 nm for quantity and quality. The amplification of the *Begomovirus*' CP gene was carried out by

PCR. About 5  $\mu$ L of 5x PCR master mix (Smobio), 1  $\mu$ L of each 10 mM forward primer 5'CCNMRDGGHTGTGARGGNCC'3 and reverse primer 5'SVDGCRTGVGTRCANGCCAT'3 [13], 1  $\mu$ L MgCl<sub>2</sub>, 9  $\mu$ L ddH<sub>2</sub>O, and 8  $\mu$ L of template DNA were added into the PCR tube. PCR reaction was performed with an initial denaturation at 95°C for 5 min, and 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 45 s. The last cycle of post elongation was performed at 72°C for 5 min. The amplicon was analyzed using 1% agarose gel electrophoresis and visualized using UV light.

# 3. RESULTS AND DISCUSSION

#### 3.1. Symptoms of Begomovirus Infection

The symptoms of *Begomovirus* infection in eggplants are stunting, mosaic, yellow, and curling in the leaves [1,2]. The eggplant at the Rejodani area is an interlude plant to the chili garden with most of the plants receiving direct sunlight without any canopy. About 13 out of the 25 eggplant trees show symptoms of infection in the form of yellow and yellow mosaics leaves. Based on the observations, most of the plants have similar symptoms, yellow, and yellow mosaic [Figure 1a and b].

Whitefly was widely found on the underside of the eggplants' leaves [Figure 1c and d], which were exposed to direct sunlight since it is stimulated by the green-yellow light spectrum reflected by the leaves [12]. Based on the results, Whitefly *Bemisia* spp. exists on a variety of plants, including chili as the main crop on this plantation. *B. tabaci* has a higher survival ability when living on eggplant plants than other hosts such as cucumber and pepper [14,15], also, it tends to lay eggs on dense trichomes leaves to attach to the body [6].

### 3.2. Whitefly Identification

Based on the results, the overall body length of the whitefly *Bemisia* spp. is  $\pm 0.6$  mm, and it consists of the head/caput, thorax, and abdomen [Figure 2a and b]. The caput comprises antennae, eyes, and mouth, which each has seven segments, provides few ommatidia, and is a piercing-sucking type that cannot be observed using light microscope. On the thorax area, there are 3 pairs of legs with long femur and tibia, as well as two pairs of wings with a relatively equal wingspan and little venation. Based on microscopic observations, the prothoracic and mesothorax wings are the same size. Both wings are membranous, opaque white in color, and covered in white dust. Therefore, these insects can be classified into the Order *Hemiptera*, *Aleyrodidae* Family [14].

According to Martin and Mound [16], there are  $\pm 41$  species in the genus *Bemisia*, which can be identified based on the morphological characteristics of the pupa since the adult phase has an undistinguishable morphology. *B. tabaci* species are known as vectors of various plant viruses, including *Begomovirus* [17]. Whitefly *Bemisia* spp. in Rejodani eggplant plantation might belong to the

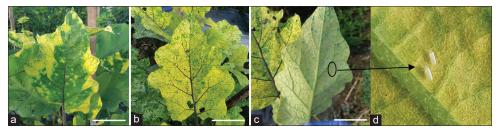


Figure 1: *Begomovirus* symptoms in *S. melongena* leaves and whitefly on the underside of leaves.(a) Yellow mosaic symptom; (b) Yellow symptom; (c and d) Whitefly; Bar=5 cm.

*B. tabaci* species when it carries *Begomovirus*. Although there is no other supporting evidence, *Trialeurodes ricini*, and *T. vaporariorum* have been reported to carry *Begomovirus* [18-20]. The whole body and wings of *Bemisia* spp. is more oblong and slenderer than the whitefly genus *Trialeuroides*. Comparing the morphology, the species observed in Rejodani plantation were similar to *Bemisia* spp., specifically in its phenotypic body and wings.

# 3.3 *Bemisia* spp. and *S. melongena* DNA Extraction Using Lysis and CTAB Buffer

In general, DNA extraction consists of lysis, contaminant removal, precipitation, and washing. In this study, DNA extraction was conducted for *Bemisia* spp. using lysis buffer containing 0.01 M Tris-HCl pH 8.0, 0.01 M NaCl, 0.005 M CaCl<sub>2</sub>, 0.002 M EDTA pH 8.0, 2% SDS,  $\beta$ -mercaptoethanol, and 0.25 mg/mL of the Proteinase-K. Cell lysis was performed using micro pestle, while lysis buffer containing CaCl<sub>2</sub> and SDS played a role in weakening cell membranes as well as dissolving labile fragments [21,22] Furthermore, Tris and EDTA pH 8.0 maintained pH stability and bind ions in charge of maintaining the integrity of cell membranes [23,24]. The removal of contaminants such as the protein was carried out with Proteinase-K and  $\beta$ -mercaptoethanol. The protease enzyme was used to break peptide bonds while the sulfur bonds in proteins were separated using  $\beta$ -mercaptoethanol [25-27]. The DNA precipitation process was performed by Na<sup>+</sup> from NaCl [24].

S. melongena DNA extraction was conducted with CTAB buffer. The detail CTAB procedure referred to Nugroho *et al.* [10] along with other modifications in sample weight, buffer volume per reaction, cold isopropanol, time, and temperature incubation of precipitation [10]. Cell lysis was performed by CTAB as an amphipathic cationic detergent [28]. Tris and EDTA were used to stabilize pH and assist the process of membrane lysis, as well as DNase inactivation [21,22]. The removal of RNA, protein, carbohydrates, tannins, polyphenols, lipids contamination from DNA was assisted by RNase,  $\beta$ -mercaptoethanol, Proteinase-K, PVP, phenol, and chloroform [24]. After removing the contaminant, Na-acetate and isopropanol precipitated the DNA for an hour at -20°C to obtain DNA pellet [28]. The washing step was carried out with 70% ethanol to reduce excess salt [23].

Based on these results, most of the DNA pellets were colored brownish indicating that PVP was not optimal enough in binding the polyphenols located in the vacuole leading to the oxidation of polyphenols which are then turned into brown [24,29]. Although the pellets were brownish, a higher density was obtained indicating that the extraction method with CTAB buffer, modification of 0.4 gr sample weight, 2 mL buffer in each reaction, precipitation with Na-acetate, and cold isopropanol for 1 h in a  $-20^{\circ}$ C is suitable to produce a large amount of DNA.

Table 1 shows the quantity and purity of DNA, although *Bemisia* spp. samples, namely, S1 and S2, had poor purity, all samples had a high DNA concentration. According to a previous study, A260/A280 ratio near 1.8 indicates pure DNA [30]. Since the whitefly extraction method is quite brief with no separation of contaminant and washing step, poor quality of *B. tabaci* DNA was obtained. The overall *Bemisia* spp. in the *S. melongena* sample has slight purer DNA even though the quantity was low. This condition occurred due to the more complex extraction steps in *S. melongena* extraction.

# 3.4. Begomovirus Detection

The amplification of *Begomovirus* CP gene is shown in Figure 3, and both *Bemisia* spp. and *S. melongena* samples were positive *Begomovirus* 

Table 1: The quantity and purity of DNA.

| S. no. | Sample | DNA concentration (ng/µL) | DNA purity A <sub>260</sub> /A <sub>280</sub> |
|--------|--------|---------------------------|---|
| 1      | S1     | 1632                      | 1.764   |
| 2      | S2     | 1736                      | 1.772   |
| 3      | S3     | 1484                      | 1.828   |
| 4      | S4     | 1536                      | 1.864   |
| 5      | K1     | 719.7                     | 2.018   |
| 6      | K2     | 1749                      | 1.885   |
| 7      | K3     | 816.4                     | 1.985   |
| 8      | KM1    | 1855                      | 1.971   |

S. melongena: Solanum melongena, S: Bemisia spp., K: S. melongena with yellowing leaves, KM: S. melongena with yellow mosaic leaves.

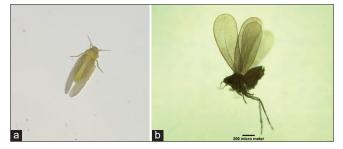


Figure 2: *Bemisia* spp. observed using stereo zoom (a) and light microscope  $(4 \times 10)$  (b) Bar= 200  $\mu$ m.

with an existing band in  $\pm 580$  bp. According to Table 1, Bemisia spp. samples (S1, S2) collected from yellow leaves are not pure enough, but proper amplification occurred and produced a specific DNA band as demonstrated in Figure 3. The band of S4 sample appeared weak, fade but specific, in comparison, all S. melongena samples both yellow and mosaic leaves namely K1, K2, K3, and KM1 produced clear and fair band in ±580 bp. Begomovirus infection was observed from yellow and mosaic leaves, but the presence in Bemisia spp. cannot be observed morphologically. However, Bemisia spp. Samples, namely, S1, S2, S3, and S4 were collected in pairs with S. melongena leaves, indicating the probability of Begomovirus presence in Bemisia spp. is relatively high. When B. tabaci sucks infected leaves, the phloem sap along with Begomovirus will be sucked through the stylets. The virions pass through the esophagus, midgut (middle intestine) to hemolymph, and then they are translocated to the primary salivary glands (PSGs) of *B. tabaci* [31].

The presence of *Begomovirus* on *B. tabaci* can be detected after four to seven hours of phloem suction. From the primary salivary glands, the infection spreads through the saliva when it makes contact with phloem [31]. As a vector, all *Bemisia* spp. detected positive are able to spread the virus by sucking the phloem sap. This virus remains stable inside the body due to the interaction of CP to GroEL from bacteria endosymbiont and heat-shocked protein 16 (HSP 16) from *Bemisia* spp. [31,32]. According to Rana *et al.* [32], these two proteins have a role in virus transmission. The presence of symbiotic bacteria that secretes GroEL protein might cause the virus to remain stable due to the interaction with the viral coat. Virions protected by GroEL can be released into the PSG [33], while the presence of heat-shocked protein 16 (HSP 16) or BtHSP16 *Bemisia* spp. serves as a chaperone in stabilizing other proteins. This interaction can prevent virus aggregation during transmission [31].

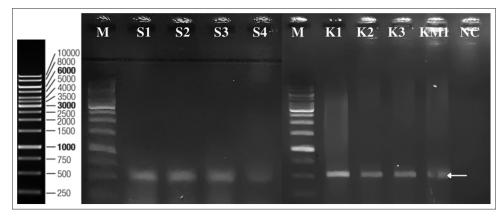


Figure 3: Amplification of coat protein gene in *Bemisia* spp. and S. melongena leaves in 1% agarose. M: 1kb marker, S: *Bemisia* spp., K: S. melongena with yellowing leaves, KM: S. melongena with yellow mosaic leaves, NC: Control.

# 4. CONCLUSION

Based on the results, *Begomovirus* DNA in the whitefly *Bemisia* spp. can be extracted using lysis buffer, while in *S. melongena*, it can be extracted using CTAB buffer. *Bemisia* spp. on the lower leaves side of *S. melongena* was found as a potential vector to spread the *Begomovirus* infection in plantations.

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### 6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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# 8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

### 9. ETHICAL APPROVALS

Ethics approval is not required for this type of study in the country where the study was conducted.

### **10. DATA AVAILABILITY**

Data sharing not applicable - no new data generated.

# **11. PUBLISHER'S NOTE**

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