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Begomovirus Infection Patterns in Melons of Indonesian Lowlands: Growth Trends Among Ecosystems and Genotypes

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ABSTRACT

Melon plants are challenged with diseases caused by begomovirus. To understand the epidemiology, ecology, and evolution of this genus, information about its temporal and spatial patterns of infection in specific ecosystems is needed. This paper aimed to report begomovirus infections in melon plants cultivated in greenhouse ecosystems under local climactic conditions in the Indonesian lowlands. To achieve this goal, we determined the begomovirus infection rates, plant physiological characteristics, and whitefly number. The data obtained were then analyzed using analysis of variance (ANOVA) and Pearson's correlation. We documented the symptoms of begomovirus infection in melon plants cultivated in Indonesian lowlands. We also documented begomovirus infections in melon plants, which increased from the first exposure to after the reproductive phase. In different artificial ecosystems, we explained differential patterns of begomovirus infections in melon plants. Finally, we captured the variation in infection conditions found among melon host genotypes.

1. Introduction

Melon (*Cucumis melo* L.) is a horticultural product with a high market value. Total world melon production in 2017 amounted to 31,948,349 tons, representing a 2.02% increase over the previous year (FAO 2019). Global melon production has increased over the past few years. This agricultural sector is predicted to stimulate the world economy with the amount of money in circulation covering it. In Indonesia, melon consumption per capita in 2011 reached 0.42 kg and grew by 166.67% over the previous year (Agriculture Information System and Data Center 2012). However, as is the case with many other agricultural crops, melons are also faced with problems caused by viruses. One such virus is begomovirus.

Begomovirus, a member genus of the family Geminiviridae, is one of the most widely studied viruses of the last decade. This is due to the widespread impact of this virus throughout the world, especially in artificial ecosystems. The genome of this virus can be arranged as a monopartite (one molecule) or bipartite (two molecules) single-stranded circular DNA, termed DNA-A and DNA-B, and transmitted persistently and circulatively by *Bemisia tabaci* (Genn.) (Jones 2003; Fauquet *et al.* 2008). Because of this global distribution of its vectors, begomovirus has become a major threat to vegetable crops in tropical and semitropical regions (Lapidot and Friedmann 2002; Mansoor *et al.* 2003; Rojas *et al.* 2005; Seal *et al.* 2006; Varma and Malathi 2003). Especially for melon, several begomovirus species that has been reported to infect this plant are *Tomato yellow leaf curl Kanchanaburi virus* (Kikkawa *et al.* 2023), *Tomato leaf curl Palampur virus* (Shahid 2023), *Tomato leaf curl New Delhi virus* (Chang *et al.* 2023), and *Cotton leaf curl Gezira virus* (Gambley *et al.* 2020).

Exposure that occurs between begomovirus, the host, and the vector supposedly differs between artificial ecosystems, spatially, and temporally. To understand the epidemiology, ecology, and evolution of this genus, spatial and temporal information about its infection in specific ecosystems is needed. Studies related to the dynamics of space and time from begomovirus infections have been conducted by several researchers. Sufrin-Ringwald and Lapidot (2011), for example, compared multiple begomovirus infections in melon plants between different seasons. Rodrigues *et al.* (2019)

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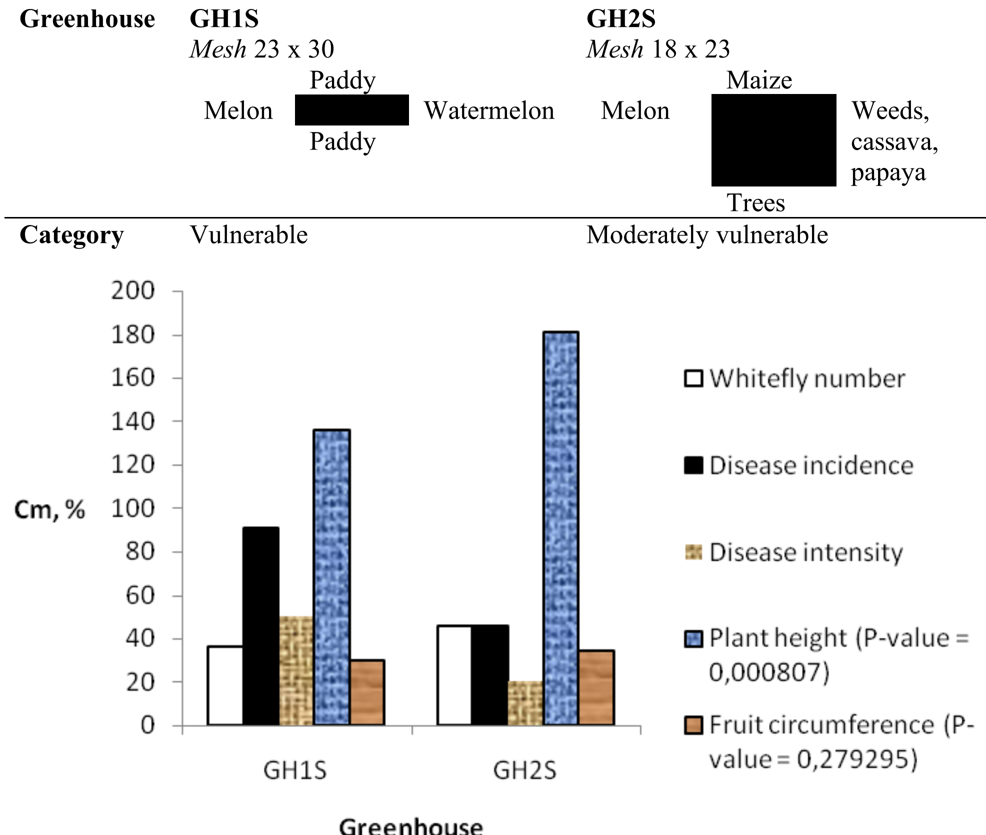
even reported the space and time dynamics of this virus in *Passiflora edulis*. This paper aimed to report begomovirus infections in melon plants that occurred in greenhouse ecosystems under local climactic conditions in Indonesian lowlands.

2. Materials and Methods

2.1. Location, Time, Infrastructure, Plants, and Sampling Procedure

Data were collected in the Prambanan area, Sleman Regency, Yogyakarta Special Province (DIY), Indonesia (7.80 S, 110.51 E; ± 282 m above sea level) from May to July 2019. The data were collected from two different greenhouses with different mesh sizes and vegetation, designed as GH1S and GH2S (Figure 1). Melon (*Cucumis melo* L.) plant seeds originated from the Laboratory of Genetics and Breeding, Faculty of Biology, UGM (Hikapel cultivar) and commercial sources (Kirani, Kinanti, and Kinanti Super cultivars). Sampling was performed using a simple random method. Sample size was calculated using Sample Size Calculator (www.abs.gov.au) with 840 population size, 99% confidence level, and 0.3 confidence interval so that minimum sampel required was n = 11 for data variables.



	Whitefly number	Disease incidence	Disease intensity	Plant height	Fruit circumference
Whitefly number	1				
Disease incidence	−1	1			
Disease intensity	−1	1	1		
Plant height	1	−1	−1	1	
Fruit circumference	1	−1	−1	1	1

Figure 1. Categories of resistance, comparison, correlation of whitefly number, disease incidence, disease intensity, plant height, and fruit circumference, based on the greenhouse, one week before harvest. GH1S: GH1 in Sleman Reg., DIY; GH2S: GH2 in Sleman Reg., DIY. Greenhouse mesh size and vegetation position are given. Plant samples were all cultivars used in this study. Whitefly number were taken from infected plant samples

2.2. Begomovirus Confirmation

Symptomatic leaves samples were taken at vegetative stage. Yellow and mosaic part of the leaves were prepared to be DNA samples using Nucleon PhytoPure (GE Healthcare) DNA extraction kit. To confirm begomovirus infection, PCR were performed on DNA samples using Krusty Homer universal primer [Krusty(Forward):5'CCNMRDGGHT GTGARGGNCC3'; Homer(Reverse):5'SVDGCRTGVGTR CANGCCAT3'] to amplify the coat protein gene from begomovirus (Subiastuti *et al.* 2019). This primer produced a DNA band of ~550 bp size (Revill *et al.* 2003). The composition of the PCR reactions used were 12.5 µL Ready Mix PCR Kit, 1.25 µL forward and reverse primers, 2 µL DNA samples, and 8 µL ddH_2O/ Aquabides, while the PCR protocol used was pre-denaturation 95°C 5 minutes, denaturation 95°C 30 seconds, annealing 52.5°C 30 seconds, elongation 72°C 45 seconds (denaturation, annealing, and elongation were carried out in 35 cycles), and post-elongation 72°C 5 minutes. These steps of PCR work referred to Subiastuti *et al.* (2019) with modification at annealing temperature. The PCR results were then visualized by 1.2% agarose gel electrophoresis using Florosafe DNA staining in a Tris Borate EDTA (TBE) buffer solution. 10,000 bp of DNA was used as a marker in electrophoresis.

2.3. Measurement of Begomovirus Infection, Plant Physiological Characteristics, and Whitefly Number

The measurement was carried out at vegetative and reproductive stage of plant growth (June and July of 2019, respectively). Begomovirus infection was measured according to the symptoms outlined by Leke *et al.* (2015) and Lapidot *et al.* (2001), namely leaf margin yellowing, leaf curling, mosaic, venation yellowing, leaf yellowing, leaf cupping, and plant stunting. Observed symptoms were recorded using a digital camera (Table 1). The incidence of disease was measured as the proportion of infected samples per total sample.

Table 1. Comparison of normal and Begomovirus-infected melon plants

Characteristics	Normal	Infected
Leaf morphology		Venation yellowing mosaic; Cupping Curling
Leaf size (cm)*	Length: 18 Width: 25	Length: 20.9 Width: 28.05
Leaf count (blades)*	20	36

Table 1. Continued

Characteristics	Normal	Infected
Fruit morphology	Seamless	Wrinkled; Scarred
Fruit circumference (cm)*	40.75	30.5
Plant height (cm)*	188	126.75
Habitus		Stunting

*quantitative data were analyzed by anova: p-value for leaf length = 0.074788; p-value for leaf width = 0.046635; p-value for leaf count = 0.109932; p-value for fruit circumference = 0.020413; p-value for plant height = 0.207857; significant differences if p-value ≤ 0.05; leaves and fruits were from Hikapel cultivar; Photos were personal documentation (2019)

Samples were also grouped on the basis of the infection severity scale proposed by Lapidot *et al.* (2001), i.e., 0 = no symptoms; 1 = very little yellowing of the leaf margins on the apical leaf; 2 = a number of leaves with minor yellowing and curling at the tips; 3 = yellowing, curling, and cupping of leaves over a wide range, with a number of reductions in plant size, but plants continued to grow; and 4 = very severe stunting and yellowing of plants, intense leaf curling and cupping, and cessation of growth. Disease intensity was measured using the formula of Dolores (1996), namely disease intensity (DI) = [Σ (number of plants included in certain symptom scale X value of certain symptom scale)] / [the number of plants observed X the highest symptom severity scale value] × 100%.

Disease intensity values were used to determine the resistance category to viruses according to the criteria given by Dolores (1996), namely: DI = 0 = Very resistant; DI ≤ 10 = Resistant; 10 < DI ≤ 20 = Moderately resistant; 20 < DI ≤ 30 = Moderately vulnerable; 30 < DI ≤ 50 = Vulnerable; and DI > 50 = Very vulnerable. Plant height and fruit circumference per sample were measured as a representative of the plant's physiological characters. The whitefly number was measured by manually counting the number of whiteflies found in infected leaves per plant sample and then totaled in each observation. Plastic clip was used to help in manually capturing the whitefly perched in infected leaves.

2.4. Statistical Analysis

The physiological characteristics of plants were analyzed using analysis of variance (ANOVA). A P value ≤0.05 indicated a significant difference. All data were also analyzed using Pearson's correlation. A positive value indicated a unidirectional trend, a negative value indicated an inverse trend, and a value close to 0 indicated the absence of a relationship between the two variables analyzed. All data analyses were performed using Microsoft Excel.

3. Results

3.1. Begomovirus Molecular Confirmation

DNA from leaf samples was extracted and the begomovirus genome was amplified using Krusty Homer's universal primers to ensure the existence of begomovirus. This primer amplified the begomovirus coat protein gene so that it could be used to detect the presence of all its species. Our data showed that sample positively infected with begomovirus (Electrophoresis showed a band measuring ~500 bp from the sample. Size 500 bp was the size of the DNA band amplified by the Krusty-Homer primer) (Figure 2).

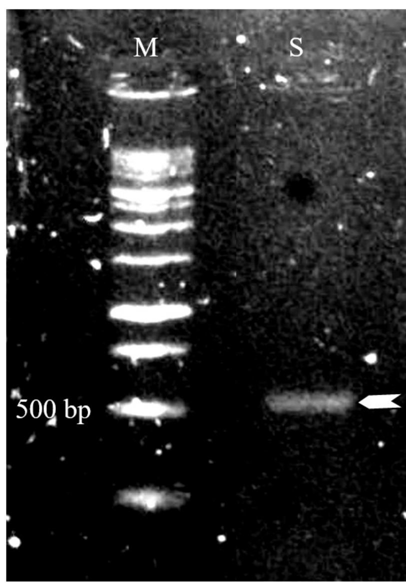


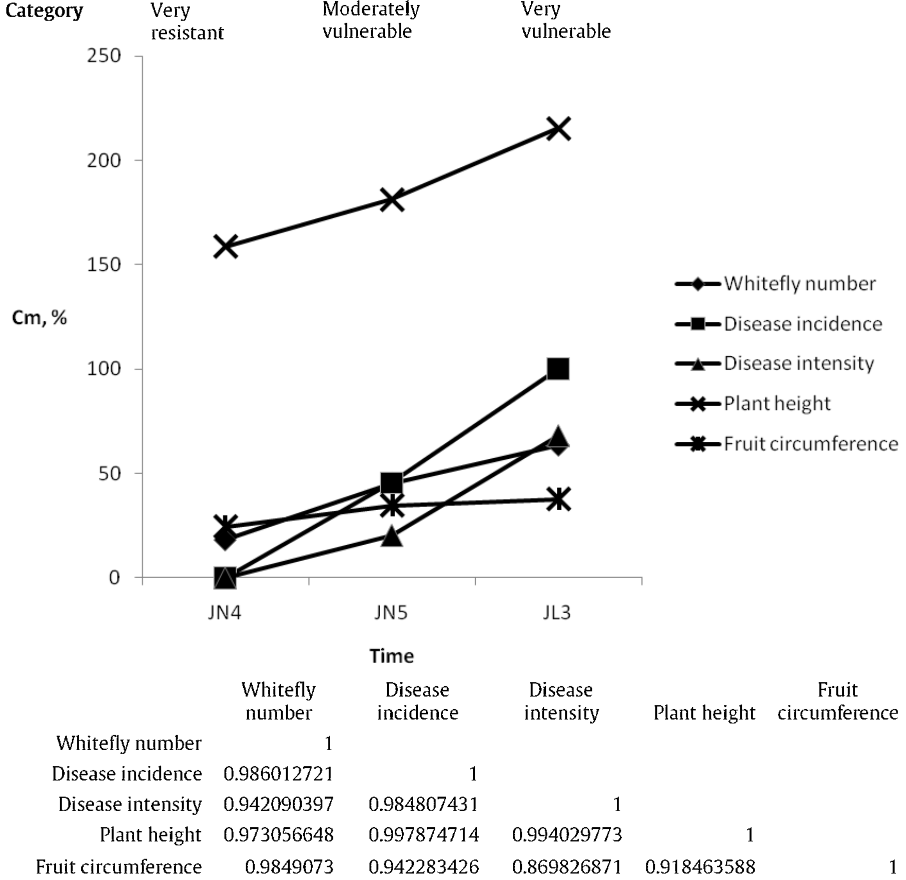
Figure 2. Electrophoresis result showed band size of ~500 bp from sample (white chevron). 500 bp is the size of DNA that was amplified by Krusty-Homer primer. M: marker, 10,000 bp (BenchTop, Promega). S: leaf sample infected with viruses. The image is combined from two of the same pictures to make it more straightforward

3.2. Begomovirus Symptoms in Melons in Indonesian Lowlands

We attempted to collect morphological symptoms of begomovirus infection and were complementing them with quantitative characters to achieve a more measurable determination of infection status (Table 1). We added symptoms in the fruit, i.e., the appearance of wrinkles and scars. We also attempted to find quantitative differences in character size between normal and virus-infected melon plants. Nevertheless, ANOVA only showed significant differences in the characters leaf width and fruit circumference.

3.3. Begomovirus Infections Showed an Escalating Pattern Over Time

Our data showed that begomovirus infections in melon plants increased from the first exposure (40 days after planting) to after the reproductive phase (80 days after planting) (Figure 3). The category of melon plant resistance to begomovirus infection increased from very resistant with low disease intensity to very vulnerable with high disease intensity. Further, the



	Whitefly number	Disease incidence	Disease intensity	Plant height	Fruit circumference
Whitefly number	1				
Disease incidence	0.986012721	1			
Disease intensity	0.942090397	0.984807431	1		
Plant height	0.973056648	0.997874714	0.994029773	1	
Fruit circumference	0.9849073	0.942283426	0.869826871	0.918463588	1

Figure 3. Categories of resistance, trends, and correlations of whitefly number, disease incidence, disease intensity, plant height, and fruit circumference, based on planting time. JN4: 4th week of June; JN5: 5th week of June; JL3: 3rd week of July. Data was taken from GH2S

positive correlation found between whitefly number and disease incidence and intensity, as well as the physiological characters of plant height and fruit circumference.

3.4. Differences in Artificial Ecosystems Had Different Effects on Begomovirus Infection

Our data showed that in different artificial ecosystems, begomovirus infection also displayed different patterns (Figure 1). The infrastructure that formed the ecosystem and vegetation that surrounded them differed in size and type, respectively.

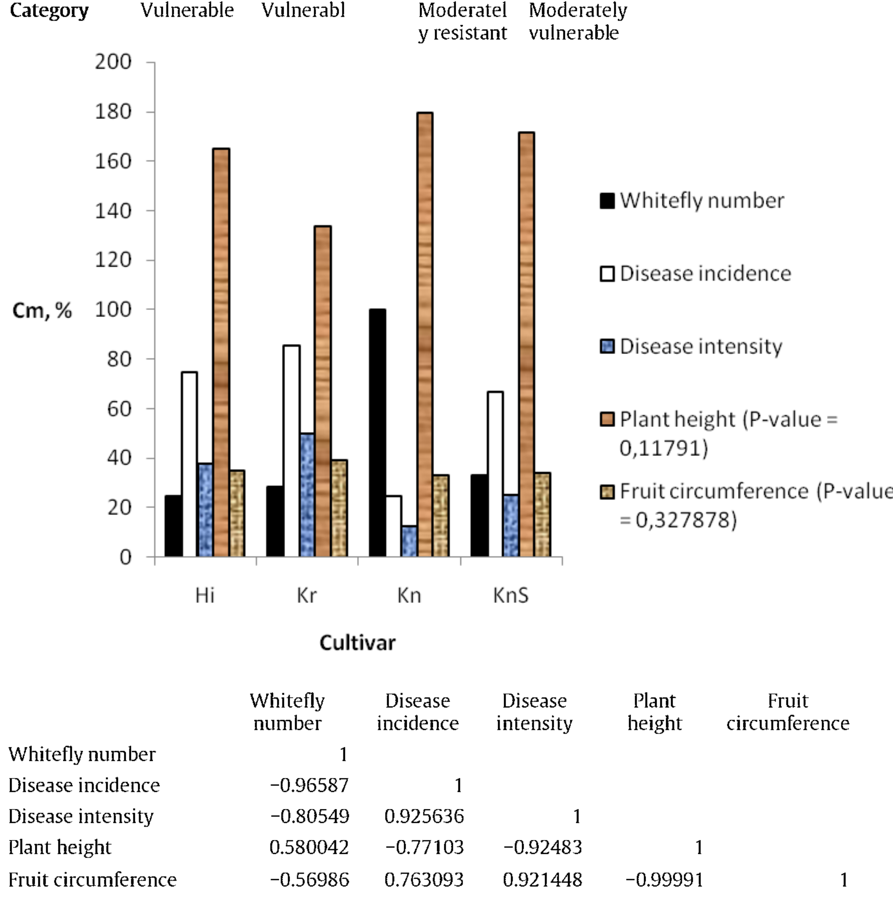
In one ecosystem (GH1S), the category of resistance was vulnerable, and the surrounding vegetation was balanced between monocotyledonous and dicotyledonous plants. In other ecosystems (GH2S), the category of resistance was moderately vulnerable, and the surrounding vegetation was dominated by dicotyledonous plants, including weeds (Figure 1).

The correlation between whitefly number and the incidence as well as intensity of the disease also showed a negative value (Figure 1). The data also showed that plant height and fruit circumference in vulnerable ecosystems were negatively correlated with the incidence and intensity of disease.

3.5. Host Genotype Differences Had Different Effects on Begomovirus Infection

Our data showed that, in melon plants infected by begomovirus, different infection conditions were found in different host cultivars (genotypes) (Figure 4). Between different genotypes, whitefly number had a negative correlation with the incidence and intensity of disease.

Physiological characteristics showed no significant differences between host genotypes. Nevertheless, plant height showed a negative correlation with the incidence and intensity of



	Whitefly number	Disease incidence	Disease intensity	Plant height	Fruit circumference
Whitefly number	1				
Disease incidence	−0.96587	1			
Disease intensity	−0.80549	0.925636	1		
Plant height	0.580042	−0.77103	−0.92483	1	
Fruit circumference	−0.56986	0.763093	0.921448	−0.99991	1

Figure 4. Categories of resistance, comparison, and correlation of whitefly number, disease incidence, disease intensity, plant height, and fruit circumference, based on cultivar. Hi: Hikapel; Kr: Kirani; Kn: Kinanti; KnS: Kinanti super

disease, whereas fruit circumference showed a positive correlation.

In terms of genotype, Kinanti was in the highest resistance category (moderately resistant) among all genotypes used in this study. Hikapel and Kirani, in contrast, were the genotypes in the most vulnerable resistance categories.

4. Discussion

One obstacle to determining the status of begomovirus infection in melons was the lack of an information database on the symptoms of this virus in melons. The fact that the symptoms of infection were like those of deficiency was one of these obstacles. Determination of begomovirus infection is generally carried out by molecular analysis in the laboratory. In this study we confirmed them using PCR analysis from all the infected leaves samples. Only one DNA sample showed the band representative of begomovirus (Figure 2), but we concluded that this one sample could be the representative of all the other samples because of the symptoms similarity found in the field. The other DNA samples showed no band at all, probably because of their impurity, contaminated with secondary metabolite from the infected leaves.

Even so, the determination of begomovirus infection also must be performed as fast as possible in the field, by both researchers and farmers alike, so that rapid decisions can be made and expedient actions taken. Efforts to document viral infection symptoms had previously been made by several researchers (Haerunisa *et al.* 2016; Inoue-Nagata *et al.* 2016; Kumar *et al.* 2017). However, to our knowledge, our documentation efforts were the first in melon plants.

Generally, begomovirus infection is identified from symptoms on leaves (mosaic yellowing, cupping, and leaf curling) and symptoms on habitus (stunted growth). In this report, we added symptoms in the fruit, i.e., the appearance of wrinkles and scars (Table 1). Other reports on symptoms of begomovirus infection in fruit showed symptoms of malformation and fruit cracking (Rodrigues *et al.* 2019). Further research on the identification of begomoviruses that damage certain fruits is also required to record viral biodiversity.

We also attempted to find quantitative differences in character size between normal and virus-infected melon plants (Table 1). Nevertheless, ANOVA only showed significant differences in the characters leaf width and fruit circumference. Thus, these two quantitative characters could be used as a reference for begomovirus infection (leaf width: 28.05 cm and fruit circumference: 30.5 cm).

Our data also showed an escalating pattern of begomovirus infection over time (Figure 3). These results agreed with those of Kyallo *et al.* (2017). and Rodrigues *et al.* (2019).

The category of melon plant resistance to begomovirus infection increased from very resistant with low disease intensity to very vulnerable with high disease intensity (Figure 3). In the vegetative phase, the plant's exposure to the virus was still low, as evidenced by the relatively low whitefly number and disease intensity (Figure 3). In the reproductive phase, the plant had experienced an energy deficit to deal with viral stress due to a relatively large physiological treatment target (Figure 3). Of course, these conjectures might be interrelated. This was evidenced by the positive correlation found between whitefly number and disease incidence and intensity, as well as the physiological characters of plant height and fruit circumference.

Begomovirus infection also displayed different patterns in different artificial ecosystems (Figure 1). In our data, although the host plants and disease vector were the same, namely melon and *B. tabaci*, respectively, the infrastructure that formed the ecosystem and vegetation that surrounded them differed in size and type, respectively (Figure 1). Both components were thought to influence the exposure of whitefly number to melon plants because the mesh size determined the size of insects that could enter from the outside ecosystem into the greenhouse ecosystem, and the type of surrounding vegetation determined the insect preference in the selection of host plants and the reservoir of whiteflies and viruses outside the greenhouse ecosystem.

The category of resistance to begomovirus between ecosystems showed differences (Figure 1). These data (vulnerable GH1S) raised questions because research on begomovirus generally shows that this viral infection only occurs in dicotyledonous plants (for example, as described by Fauquet and Stanley 2003), so that the reservoir could only be dicotyledonous. However, if we looked at the host range of the begomovirus infectious vector, *B. tabaci*, Quintela *et al.* (2016) reported that this insect also had a monocotyledonous host, namely maize. In addition, Aji *et al.* (2015) also mentioned that biological barriers did not really suppress the whitefly population increase and disease intensity.

In melon plants infected by begomovirus, different infection conditions were found in different host cultivars (genotypes), even though these genotypes were members of the same melon species (Figure 4). This demonstrated different levels of virus adaptation between genotypes. A high level of infection indicated a low level of virus adaptation in the host. Conversely, a low level of infection indicated a high level of adaptation in the host.

The data showing that Hikapel was vulnerable to begomovirus was contrary to that reported by Subiastuti *et al.* (2017). The assumption that could be made was that the vulnerable Hikapel in this study also did not have resistance to begomovirus and might be the offspring of Hikapel crosses in previous studies. The results of this study also became the first report on the resistance categories of Kinanti and Kirani melons, as comparative commercial cultivars of Hikapel, against begomovirus infection.

In conclusion, spatial and temporal information about begomovirus in specific ecosystems is needed to gain a deeper understanding of this virus. Our report described begomovirus infections that occurred in gre enhouse ecosystems with a natural climate in the lowlands. We documented symptoms of begomovirus infection in melon plants cultivated in lowlands. We also documented begomovirus infections in melon plants that increased from the first exposure to after the reproductive phase. In different artificial ecosystems, we explained different patterns of begomovirus infections in melon plants. We captured the different conditions of infection found in different genotypes of melon hosts.

Our report opened the prospect of further documentation of the spatial and temporal dynamics of begomovirus infection. Experiments carried out at different specific times and in different locations will complement the richness of existing data regarding begomovirus infections.

Conflict of Interest

The authors declare that there is no conflict of interest.

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