



## Research Article

# Evaluation of Three Cucurbitaceae Cultivars Resistant against *Begomoviruses* Based on Morphological Symptoms and Molecular Analysis<sup>(\*)</sup>

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## ABSTRACT

Cucurbitaceae is a group of annual horticultural crops with high economic prospects and benefits. The Faculty of Biology, Universitas Gadjah Mada has developed several new cultivars within the Cucurbitaceae family, such as the melon cultivar 'Hikapel', watermelon 'Citra Jingga', and butternut squash 'Citra Laga'. However, development of these cultivars has been hindered by yellow disease, typically triggered by *Begomovirus* infection. This study aimed to determine the morphological symptoms of yellow disease, to detect *Begomovirus* infection using molecular approach, and assess resistance levels of the three Cucurbitaceae cultivars. The method involved observing morphological symptoms in plants and measuring plant height. The degree of symptom severity was then converted into a vulnerability index (VI). The symptomatic leaf samples were tested using Krusty and Homer primer to confirm the symptoms caused by *Begomovirus*. The PCR results were sequenced and analyzed using Maximum Likelihood method in MEGA 11. The results showed that symptoms included curling tip of leaves, yellow mosaic on the leaves, and stunted growth in some plants, which were suggested as *Begomovirus* infection. Based on the Vulnerability Index (VI), the watermelon cultivar 'Hikapel' was considered susceptible to *Begomovirus* infection (VI = 85%), while the yellow squash 'Citra Laga' was moderately susceptible (VI = 57.5%). However, the watermelon 'Citra Jingga' was considered moderately resistant to this infection (VI = 50%). The presence of DNA the *Begomovirus* at three species Cucurbitaceae were characterized by a 550 bp band. The 'Hikapel' melon and 'Citra Laga' butternut were infected with *Tomato leafcurl New Delhi virus* (ToLCNDV), while the 'Citra Jingga' watermelon were infected with *Squash leaf curl Philippine virus* (SLCuPV).

Keywords: *Begomovirus*; Cucurbitaceae; coat protein (CP) gene; resistance

## INTRODUCTION

Some high-priority Cucurbit species cultivated in Indonesia are melons, cantaloupe, watermelons, and pumpkins (Badan Pusat Statistik, 2022). These plants have high economic prospects, therefore research on plant breeding is important to obtain superior cultivars.

The Faculty of Biology has created several superior local Cucurbit cultivar, including melon (*Cucumis melo* L.) 'Hikapel', watermelon (*Citrullus lannatus* (Thunb.) Matsum & Nakai) 'Citra Jingga', and butternut squash (*Cucurbita moschata*) 'Citra Laga'. These melon and watermelon cultivars have

been patented and obtained a Decree with SK of Ministry of Agriculture No. 038/Kpts/SR.120/D.2.7./4/2017 for 'Hikapel' melon and No. 19/PV.220/A9/01/2019 for 'Citra Jingga' watermelon. However, these cultivation face various challenges due to environmental factors, pest attacks, fungal infections, and viral diseases. Of particular concern is the response of these cultivars to viral infections, as viral resistance is an important trait that requires further development through breeding programs. Specifically, how the three cultivars respond to *Begomovirus* infection is not yet known.

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*Begomovirus* has become a threat to some plants in tropical and subtropical regions (Sufirin-Ringwald & Lapidot, 2010). Several plants infected include *Syndrella* sp., *Urena* sp., *Zinnia elegans*, papaya, melon, eggplant, okra, *Eclipta* sp., *Jatropha* sp., *Sanchus asper*, *Ageratum conyzoides*, potatoes, watermelon and *Vigna* sp. (Saxena & Tiwari, 2017).

*Begomovirus* infection was first reported in Indonesia in 1999 in West Java that then spread to Central Java in 2003 (Subiastuti *et al.*, 2019). Previous research found that *Begomovirus* species *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCVKaV) infected tomato (*Solanum lycopersicum* L.) and chili peppers (*Capsicum annum* L.) while *Squash leaf curl China virus* (SLCCNV) and *Tomato curl New Delhi virus* (ToLCNDV) had been detected to infect melon plants, and watermelon, and *Ageratum yellow vein virus* (AYVV) have been detected to infect chili plants (Santoso *et al.*, 2008; Subiastuti *et al.*, 2019). *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) and *Tomato yellow leaf curl Indonesia virus* (TYLCIDV) have been recorded infecting chili in Sumatra (Trisno *et al.*, 2009). Furthermore, *Begomovirus* infection was also found in Bali in the form of SLCCNV which has infected cucumber plants (Wiratama *et al.*, 2015).

*Begomovirus* infection can cause various morphological symptoms, including stunted growth, shriveled and curled leaves, and yellow spots on the edges of the leaves. However, the specific symptoms may vary depending on the infected plant species or cultivar. For instance, a study conducted by Kurnia *et al.* (2022) found that *Cucumis sativus* L. plants infected with *Begomovirus* exhibit curling leaves, leaf malformations, thickening of leaves, changes in leaf color and veins, and the appearance of yellow spots on leaves. Subiastuti (2015) showed that the symptoms in 'Hikapel' melon was yellow mosaics on the leaf blade, curl, and dry. In contrast, Kesumawati *et al.* (2020) reported that pumpkin and cucumber plants infected with *Begomovirus* only showed yellowing leaves

Hence, it is crucial to utilize alternative detection methods to identify viral infections. One effective approach is to detect the genes encoding viral coat proteins. *Begomoviruses* possess coat proteins (CP), which are valuable for determining genetic relationships. CP sequences exhibit significant genetic

variability, particularly in the loop section (Paul *et al.*, 2012). However, these sequences also display conserved structures, enabling the identification of variations and relationships through the CP. Furthermore, coat protein sequences are effective in the detection of *Begomoviruses* in a variety of crops, including chili, melon, watermelon, cucumber, and grass (Haerunisa *et al.*, 2016; Subiastuti *et al.*, 2019).

Several studies have provided primers for amplifying *Begomovirus* genome, including Krusty Homer (Revill *et al.*, 2003), PAR1C715/PALIV1978 (Rojas *et al.*, 1993), and AC1048/AV494 (Wyatt *et al.*, 1996). This method can identify the presence of the virus genome in a host plant even when specific symptoms are not observed. The objectives of this study were to identify the morphological symptoms of yellow disease, confirm *Begomovirus* presences and evaluate resistance levels of 'Hikapel' melon, 'Citra Jingga' watermelon, and 'Citra Laga' butternut squash based on vulnerability index (VI). We also determined the species of the virus by analysis of viral coat protein gene. The results of this study were important to develop virus-resistant local Cucurbits cultivars.

## MATERIALS AND METHODS

### Observation of Morphological Symptoms and Sample Collection

Morphological symptoms were observed from November 2021 to February 2022 in the Green House and fields at Mutihan, Madurejo, Prambanan, Sleman, Yogyakarta. The populations of 'Citra Jingga' watermelon, 'Hikapel' melons, and 'Citra Laga' butternut squash infected with *Begomovirus* were 150 (15%), 48 (25%), and 150 (15%) plants, respectively. Samples collection was carried out at 60 days after planting (DAP), with a total of 10 plants (5%) from each population.

Plant height were, measured from the base to the tip of the longest stem. Plant symptom appearances were scored on a 5-point scale ranging from 0–4 (Table 1). These severity score were utilized to calculate a vulnerability index (VI) for each plant. This index was used to assess the plant's response to infection and was calculated using the formula (Islam *et al.*, 2011):





Figure 1. Morphological symptoms showing yellowing on Cucurbits (a) '*Hikapel*' melon, (b) '*Citra Jingga*' watermelon, and (c) '*Citra Laga*' butternut squash

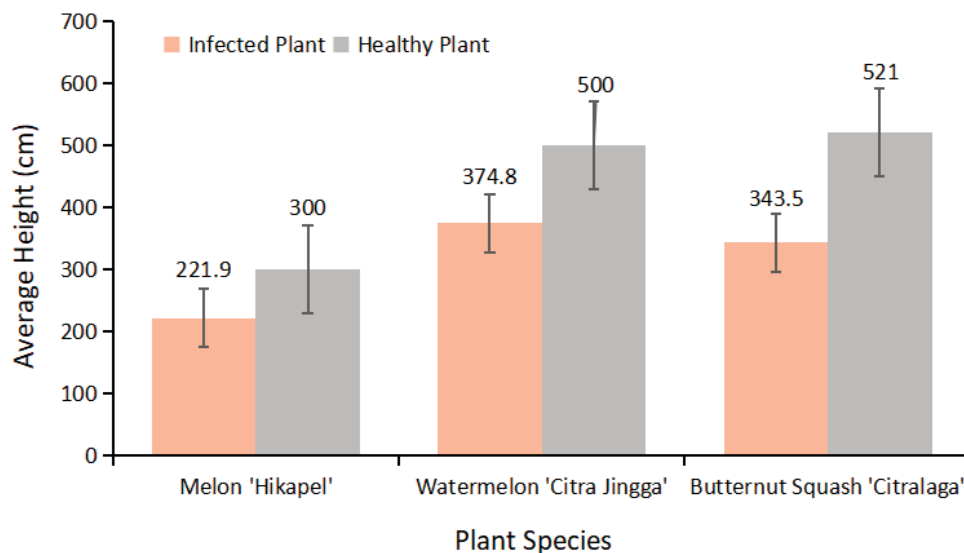


Figure 2. Average plant height of '*Hikapel*' melon, '*Citra Jingga*' watermelon, and '*Citra Laga*' butternut squash

### Plant Resistance Level

Scoring results showed that the '*Hikapel*' melon plant was susceptible by VI = 85%. The '*Citra Jingga*' watermelon plant was quite resistant by VI = 50%, and so was the '*Citra Laga*', by the value of VI = 57.5% (Table 2). Plant response to *Begomovirus* infection can be affected by virus strain and the genotype of the host. Ogbé *et al.* (2003) discovered that disease severity in plants was linked to host genotype, not virus concentration. Resistant genotypes of *African cassava mosaic virus* exhibited fewer symptoms compared to the susceptible ones, but both show similar virus concentrations. This suggests that geno-

types with mild symptoms may possess the ability to limit symptom developments.

Preventive measures and control methods should be implemented on infected plants to limit the spread of virus infection and reduce yield loss. The '*Hikapel*' melon and '*Citra Laga*' butternut squash populations were considered susceptible and moderately susceptible, respectively, as their VI values were above 50%. Therefore, in case of *Begomovirus* infection, it is crucial to promptly treat the affected plants by eliminating the source of infection to prevent crop failure. Agricultural practices used by farmers have a significant impact on the spread of viral infec-

Table 2. Resistance response of each species based on Vulnerability Index (VI)

No.	Cultivar	V1(%)	Resistance Response
1	'Hikapel' melon	85 <sup>S</sup>	susceptible
2	'Citra Jingga' watermelon	50 <sup>QR</sup>	quite resistant
3	'Citra Laga' butternut squash	57.5 <sup>QS</sup>	quite susceptible

tions in crops. Inadequate fertilizer application and the presence of weeds can stimulate viral diseases. Weeds often act as reservoirs for viruses (Hull, 2009). Consequently, it is important to employ a combination of strategies, including preventive measures, treatment, or both, to effectively suppress the spread of viruses and minimize their impact.

### Viral DNA Analysis

Plants showing symptoms of *Begomovirus* infection were further analyzed using PCR technique with *Begomovirus* primers, Krusty-Homer. The molecular analysis revealed that 12 samples from 'Hikapel' melon, 'Citra Jingga' watermelon, and 'Citra Laga' butternut squash were infected with *Begomovirus*, as indicated by the 550 bp DNA band (Figure 3). However, Kusumaningrum (2015) reported a band size of 580 bp, while Hafizh (2021) also reported a band size of 550 bp. The Krusty-Homer primer successfully amplified a part of coat protein (CP) gene, resulting in a band size of approxi-

mately 550 bp. This amplified region contains conserved segments of the CP gene (Revill *et al.*, 2003).

The phylogenetic analysis indicated that both 'Hikapel' melon and 'Citra Laga' butternut squash were infected by *Tomato leaf curl New Delhi virus* (ToLCNDV). The identified sequences showed high similarity with a cluster of Southeast Asian strains (Figure 4). These findings also align with previous report by Wilisiani *et al.* (2014) and Subiastuti *et al.* (2019), which also identified ToLCNDV as the causative agent of curl disease in melons in Indonesia. However, the discovery of *Begomovirus* species infecting butternut squash differed from previous reports. Earlier documentation only recorded *Squash leaf curl virus* (SLCV) infection in butternut squash in Bali (Selangga & Listihani, 2022). This opens up the opportunity for further investigation into the incidence rate of *Begomovirus* infection in Butternut squash.

In contrast, the 'Citra Jingga' watermelon was found to be infected by *Squash leaf curl Philippine virus* (SLCuPV). These isolates clustered with other strains from East Asia (Figure 4). However, this finding differs slightly from previous reports, which identified the *Begomovirus* species infecting watermelons in Yogyakarta was SLCCNV (Subiastuti *et al.*, 2019). Hence, further research is required to accurately identify *Begomovirus* species, as CP sequences are only used for provisional identification. Comprehensive genome characterization is necessary for precise determination (Brown *et al.*, 2015).

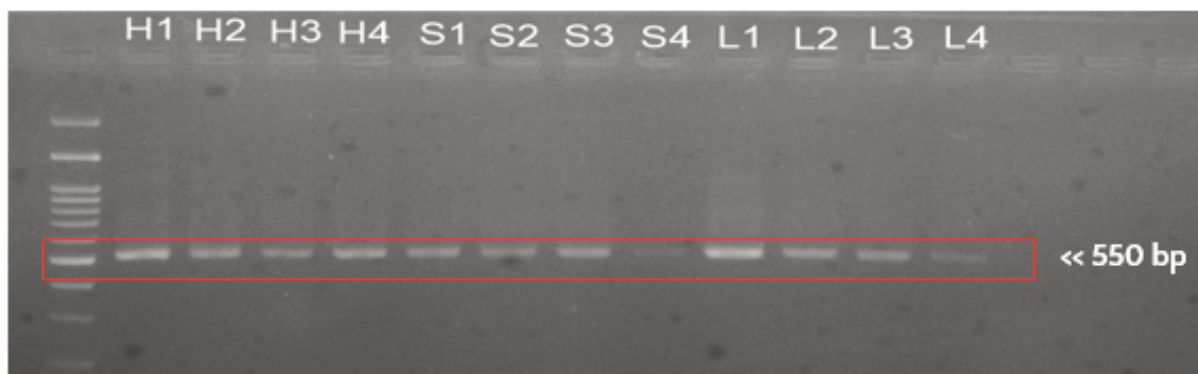


Figure 3. DNA bands from infected leaf PCR results (H = 'Hikapel' melon, S = 'Citra Jingga' watermelon, L = 'Citra Laga' butternut squash)



Figure 4. Phylogenetic tree of *Begomovirus* CP gene on 'Hikapel' melon, 'Citra Jingga' watermelon, and 'Citra Laga' butternut squash

## CONCLUSION

Melon 'Hikapel', watermelon 'Citra Jingga', and butternut squash 'Citra Laga' displayed stunting, leaf curling, and yellowing symptoms. The level of plant resistance varied among species; 'Hikapel' melon was susceptible, 'Citra Laga' butternut squash was moderately susceptible and 'Citra Jingga' watermelon was moderately resistant to *Begomovirus*. Based on molecular identification of viral coat protein gene sequences, 'Hikapel' melon and 'Citra Laga' butternut squash were infected with ToLCNDV

while 'Citra Jingga' watermelon was infected with SLCuPV.

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