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BIOLOGICAL RESPONSE AND MOLECULAR IDENTIFICATION OF THE BEGOMOVIRUS INFECTION IN THE NEW INDONESIAN MELON GENOTYPE KINAYA

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A B S T R A C T

Melon farming faces difficulties due to climate change causing unstable conditions, which make the plants more vulnerable to viruses like *Begomovirus*, leading to lower melon yields. To tackle this problem and guarantee a stable food supply, it is crucial to create melon varieties that possess resistance to these viruses by utilizing breeding techniques. Kinaya is a new genotype being developed as a *Begomovirus* tolerant variety for future prospects. Further observations are needed on the initial identification of biological responses to *Begomovirus* infection and the presence of candidate resistance genes in Kinaya. The research was conducted in open farmland in Jamusan, Yogyakarta, Indonesia, from April to August 2021. Observations and data were collected using the plant leaf infection scoring method based on the severity of symptoms in the Kinaya population. Melon and virus genomes were isolated from melon leaves exhibiting *Begomovirus* symptoms and from healthy plant leaves. We employed a set of 8 melon samples for dual objectives: identifying Begomovirus using Krusty-Homer primers and detecting Begomovirus resistance genes through SCAR primers linked to candidate resistance genes targeting *Begomovirus*. Observations showed symptoms of infection with yellow spot and leaf curling with tolerant susceptibility. Molecular identification with Krusty-Homer confirmed the presence of *Begomovirus* infection in all infected samples. Over 90% sequence similarity of the coat protein DNA virus was found in different *Begomovirus* species. *SLCCV* and *ToLCNDV* isolates from Indonesia were found to be closely related. Based on the developed SCAR markers, the DNA band of 900 bp was found in all healthy samples. Kinaya shows potential to be developed into a leading virus-resistant melon variety in Indonesia, although further research is needed to stabilize its resistant traits.

Keywords: symptoms, vulnerability index, coat protein virus, gene detection, *begomovirus*.

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the most important and most diversified cucurbit crops in the world, with a highly valued nutritional profile, and its production brings high profits to the farmers (Aldoshin *et al.*, 2020; Gómez-García *et al.*, 2020; Manchali *et al.*, 2021; Sáez *et al.*, 2022). Viral diseases, which cause loss of productivity and low profitability in the future, are one of the limiting problems in melon production. The right agronomic conditions are conducive to the spread of viral pests and diseases (Velasco *et al.*, 2020).

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The tropics regions, with their abundant rain and intense sunshine, offer great potential for melon cultivation, but also for the spread and development of virus. The uncontrolled fluctuation of environmental factors is a new challenge for melon production in unstable climates. The vector transmission of viruses is significantly affected by environmental factors such as drought (Munster *et al.*, 2017). Another factor, partly caused by anthropogenic activities, global warming caused by an increase of carbon dioxide or greenhouse gases in the atmosphere, which leads to an increase of the global temperature (Trebicki, 2020; Kesh and Kaushik, 2021). However, the high diversity and recombination rate of

these organisms allows them to easily adapt to both high and low temperatures (Jones, 2016). Unlike the adaptability of viruses, extreme weather makes plants vulnerable to viral infestations. Warmer weather due to global warming triggers changes in virus epidemiology and virus-host molecular interactions through increased multiplication, systemic movement and seed transmission. It also allows a plant's single resistance (R) gene to become inactive against virus attack due to its high temperature sensitivity (Jones, 2016; Amari *et al*., 2021; Tsai *et al*., 2022). Overall, climate change has an impact on plant production due to the susceptibility to pathogen infection and facilitates the evolution of viruses (Elad and Pertot, 2014) and has implications for food security in the future (Singh *et al*., 2023).

Viruses are difficult pests to control. Virologists do not have an array of chemicals at their disposal for the management of viral infections, as mycologists and bacteriologists do. Since these organisms are pathogenic obligate intracellular parasites, plant breeding with the development of genetic resistance is the right option for virus management (Martín-Hernández and Picó, 2021). Therefore, developing molecular markers can be an integrated solution to identify disease resistance capabilities or disease resistance genes at an early stage. Molecular markers can facilitate and accelerate conventional plant breeding (Sidiq *et al*., 2020).

SCAR (Sequence Characterized Amplified Region) is one of the molecular markers developed from RAPD (Random Amplified Polymorphic DNA) with more stable, specific and high reproducibility (Sidiq *et al*., 2020). SCAR has been used to evaluate resistance genes for powdery mildew (Ishak and Daryono, 2020), *Fusarium* spp. (Meighan *et al*., 2020) and *Podosphaera xanthii* (Unlu *et al*., 2020). One of the SCARs has been identified in association with the *Begomovirus* resistance gene. It has been developed from the purification RAPD to produce a specific amplicon linked to the resistance gene against *Begomovirus* (Subiastuti *et al*., 2019; Sidiq *et al*., 2020).

Kinaya is a new hybrid cultivar resulting from a cross between Kinanti and Sonya. Based on its morphological appearance, Kinaya is characterized by a yellow outer skin with a reticulated architecture and an oval shape. The flesh is orange with a crunchy texture and a sweetness of 9 Brix (Muhammadi and Daryono, 2022). As a parent, Sonya has a reticulated green skin. Unlike Sonya, Kinanti has a smooth golden yellow skin. Both parents are characterized by an orange coloured flesh (Yusuf and Daryono, 2021). The combination of the two parents has produced a unique variety with a golden yellow colour and reticulated architecture. This is an excellent option for farmers to ensure market demand due to the special characteristics of the skin. As a future prospect, Kinaya is being developed as a *Begomovirus* resistant melon. The current concern is that the resistance of Kinaya to pathogen infection is still unknown. Varieties vary in their ability to resist pathogens (Lambers *et al*., 2008; López *et al*., 2015). Therefore, for further breeding programmes, there is a need for earlier identification of biological responses to *Begomovirus* infection and the presence of candidate resistance genes in Kinaya.

MATERIALS AND METHODS

Research area: The observed Kinaya melon population was planted in a screen house to the north of the melon garden in Mutihan, Madurejo, Prambanan, Sleman, Special Region of Yogyakarta during April- Agustus 2021. A total of 90 plants were planted for this study and ten plants were used as samples. The plant population used was confirmed to have a minimum growth period of 2 weeks in the population.

Symptoms and plant responses observations: Symptom observation was conducted every two days since 62 until 78 days after planting (DAP). Symptom development was scored according to the leaf symptom severity scale as follows; 0 = no visible symptoms, inoculated plants showed the same growth and development as non-inoculated plants; 1 = very yellow leaf margin colour on apical leaves; 2 = some yellow and small leaf tips; 3 = some leaves turn yellow, curl and cupping with some reduction in size, but the plant continues to develop; and 4 = stunting in plants with very severe yellowing, it can be said that the leaves are cupped and curled and plant growth stops (Friedmann *et al*., 1998). The data from the observations were processed by calculating the value of the vulnerability index using the Microsoft Excel program and categorized as plants that were resistant (VI = 0%), tolerant (VI = 1 - 25%), tolerant enough (VI = 26 - 50%), susceptible (VI = 51 - 75%) or very susceptible (VI = 76 - 100%). The following equation is used to calculate the value of the plant susceptibility index (López *et al*., 2015).

$$\text{Vulnerability index (VI)} = \frac{(0\text{no} + 1\text{n1} + 2\text{n2} + 3\text{n3} + 4\text{n4})}{\text{nt}\ (\text{nc} - 1)} \times 100\%$$

Annotations: no = Number of plants on a scale of 0; n_1 = Number of plants on a scale of 1; n_2 = Number of plants on a scale of 2; n_3 = Number of plants on a scale of 3; n_4 = Number of plants on a scale of 4; n_t = The total number of plants; n_c = Number of categories

DNA isolation and quantitative measurement: DNA was extracted from fresh Kinaya leaves harvested from the screen house and stored at -20ºC. Leaves collected are the third or fourth leaf from the top, then put into plastic and coded. Then treated according to the Nucleon Phytopure extraction and purification protocol by Daryono and Natsuaki (2002). All PCR conditions are optimized and modified from the Operon Technologies Inc. protocol. DNA concentration measurements were performed using a UV-VIS spectrophotometer. Prior to use, the spectrophotometer was calibrated with a 1X buffer TE solution. The 1x TE buffer was then replaced with the diluted isolated DNA and the absorbance measured at 260 nm and 280 nm.

***Begomovirus* identification based on coat protein sequences:** *Begomovirus* identification was performed by PCR using Krusty & Homer primers (Revill *et al*., 2003). All components of the PCR reaction were cold conditioned. In the first step of DNA amplification, a PCR premix was prepared consisting of 12.5 µl Master Mix 1x (PCR kit, Bioline HS Red Mix), 1 µl (50 µM) forward primer, 1 µl reverse primer, 2 µl (200 µg/ml) template DNA and 9.5 µl ddH2O. The tube containing the components was amplified using a PCR BOECO thermal cycler instrument with the following reaction composition: pre-denaturation at 95ºC for 5 minutes, denaturation at 95ºC for 45 seconds, annealing at 47ºC for 1 minute, extension at 72ºC for 2 minutes and post-extension at 72ºC for 10 minutes. The amplicons were then visualized by gel electrophoresis. *Begomovirus*-infected samples were shown DNA bands of 550 bp in size. The sequencing data were compared with another *Begomovirus* coat protein gene sequence using BLASTn on the GenBank NCBI website, and phylogenetic analysis was performed using the neighbour-joining clustering method using the open-source software MEGA 11 with 1000 replicate bootstraps.

Table 1. Primer composition of Krusty-Homer primer

Primers	Σ nt	Sequence	Annealing Temperature
Krusty forward	20	CCNMRDGGHTGTGARGGNCC	50 ºC
Homer reverse	20	SVDGCRTGVGTRCANGCCAT	

Detection of resistance gene based on developed-SCAR marker: PCR-SCAR amplification was performed to detect a resistance gene in Kinaya. The PCR premix composition consisted of 12.5 µl Master Mix 1x (PCR kit, Bioline HS Red Mix), 1.25 µl (10 µM) forward primer, 1.25 µl reverse primer, 2 µl (250 µg/ml) template DNA and 8 µl ddH_2O. The amplification was then carried out with the following reaction composition: pre-denaturation at 95ºC for 5 minutes, denaturation at 95ºC for 45 seconds, annealing at 47ºC for 1 minute, extension at 72ºC for 2 minutes and post-extension at 72ºC for 10 minutes. The PCR products obtained were then visualized by electrophoresis using a 1% agarose gel set at 50 V and run for ± 47 minutes. At the end of the run, they were observed under a UV transilluminator for analysis. Resistance genes to *Begomovirus* was carried out using SCAR molecular markers linked to *Begomovirus* resistance genes, characterized by the appearance of DNA bands with a size of 1198 bp on the electrophoretic gel (Sidiq *et al*., 2020).

Table 2. Primer composition of developed-SCAR primer

Primers	Σ nt	Sequence	Annealing Temperature
SCAR-forward	20	TCTCGGGCTTGCTAACTGCAC	47 ºC
SCAR-reverse	20	GTCCGGACTGCCAAGAGCAT	

RESULTS AND DISCUSSION

Symptoms evaluation of Kinaya melon plants: The plant responses observed in the field were not the result of inoculation, but were natural *Begomovirus* infections that occurred in Kinaya fields. We identified the susceptibility of Kinaya during plant development up to harvest. We confirmed the presence of five distinct stages of *Begomovirus* symptoms in Kinaya melon plants. *Begomovirus* symptoms are characterized by yellow spots on the leaf surface, stunted and shriveled leaves (Figure 1). *Begomovirus* infection also affected melon fruit development (Figure 2). *Begomovirus* infection at high susceptibility levels late in harvest results in incomplete fruit and sometimes stunting (Figure 2C). On the other hand, *Begomovirus* infection early in fruit development causes fruit cracking, as seen in 'Gama Melon Parfum' and 'Hikapel' (Figure 2D).

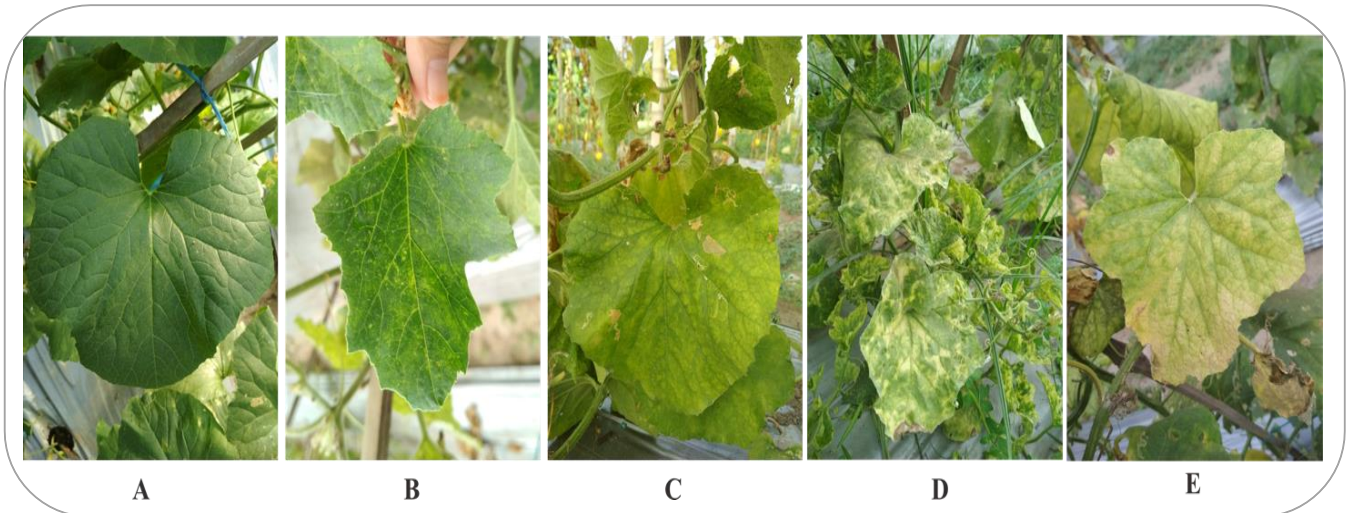

Figure 1. Development of *Begomovirus* symptoms in Kinaya melon plant. (A) absence of symptoms, (B) mild symptoms, (C) moderate symptoms, (D) severe symptoms and (E) very severe symptoms

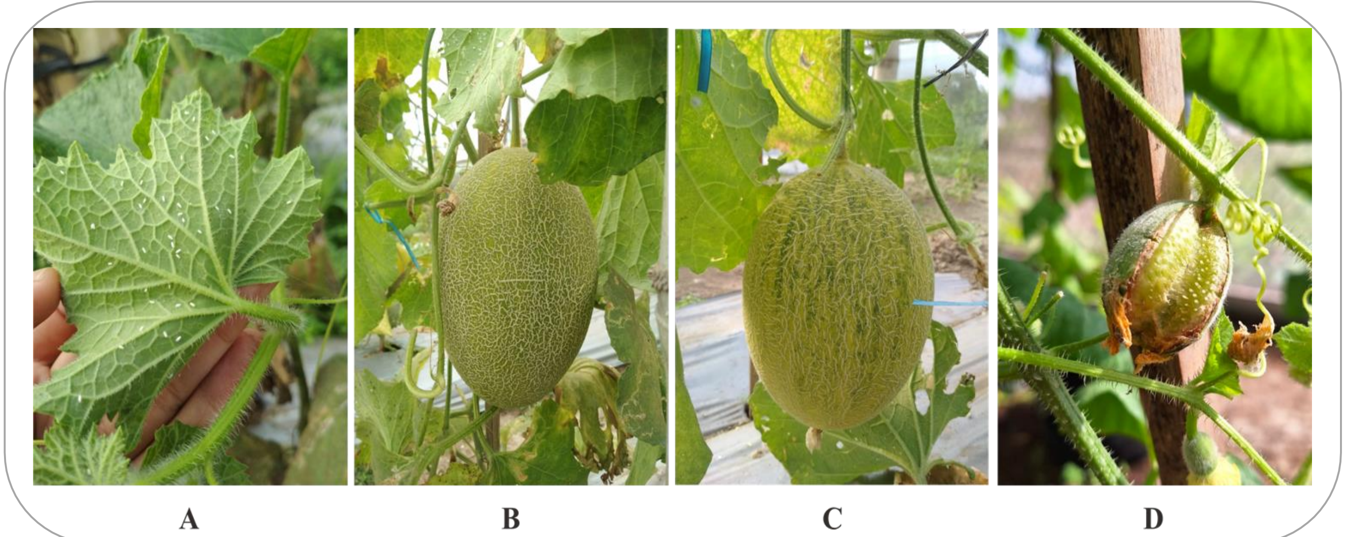

Figure 2. *Bemisa tabaci* found on the abaxial side of Kinaya leaves (A), normal Kinaya fruits (B) and the effect of Begomovirus on Kinaya fruits (C), and fruit cracking symptoms found in other cultivars such as 'Hikapel' and 'Gama Melon Parfum' (D)

Susceptibility assessment in Kinaya due to Begomovirus infection: The susceptibility index value of Kinaya from 62 to 78 DAP ranged from 14.56% to 65.66% (Table 1). At 60-68 DAP, Kinaya was considered tolerant to *Begomovirus* infection. However, susceptibility increased at the age of 70-74 DAP (Table 1). The decrease in resistance of Kinaya plants to *Begomovirus* is due to physiological decline at the end of the productive period.

Table 3. Vulnerability of Kinaya against *Begomovirus* infection during 4 weeks during observation

Observation (DAP)	Vulnerability Index (VI) (%)	Category
62	14.56	Tolerance
64	16.39	Tolerance
66	20.00	Tolerance
68	22.50	Tolerance
70	38.07	Moderately susceptible
72	39.16	Moderately susceptible
74	51.20	Susceptible
76	55.49	Susceptible
78	65.66	Susceptible

***Begomovirus* coat protein sequences analysis:** As shown in Figure 1, we used Krusty & Homer universal primers to validate *Begomovirus* symptoms on Kinaya melon plants. PCR amplification revealed a 550 bp DNA band in the leaf samples infected with *Begomovirus*. No amplification results were found with these primers in the healthy leaf samples (HK-1M, TP-3B, N1, N2 and N3) (Figure 3). Based on the electrophoresis results, preliminary screening showed that T1, T2, T3 and T4 plants were infected with *Begomovirus* with an amplicon size of 550 bp. As a positive result of amplified partial coat protein, T3 was sequenced using Sanger sequencing to provide information on percent identity and phylogenetic reconstruction to other identified organisms.

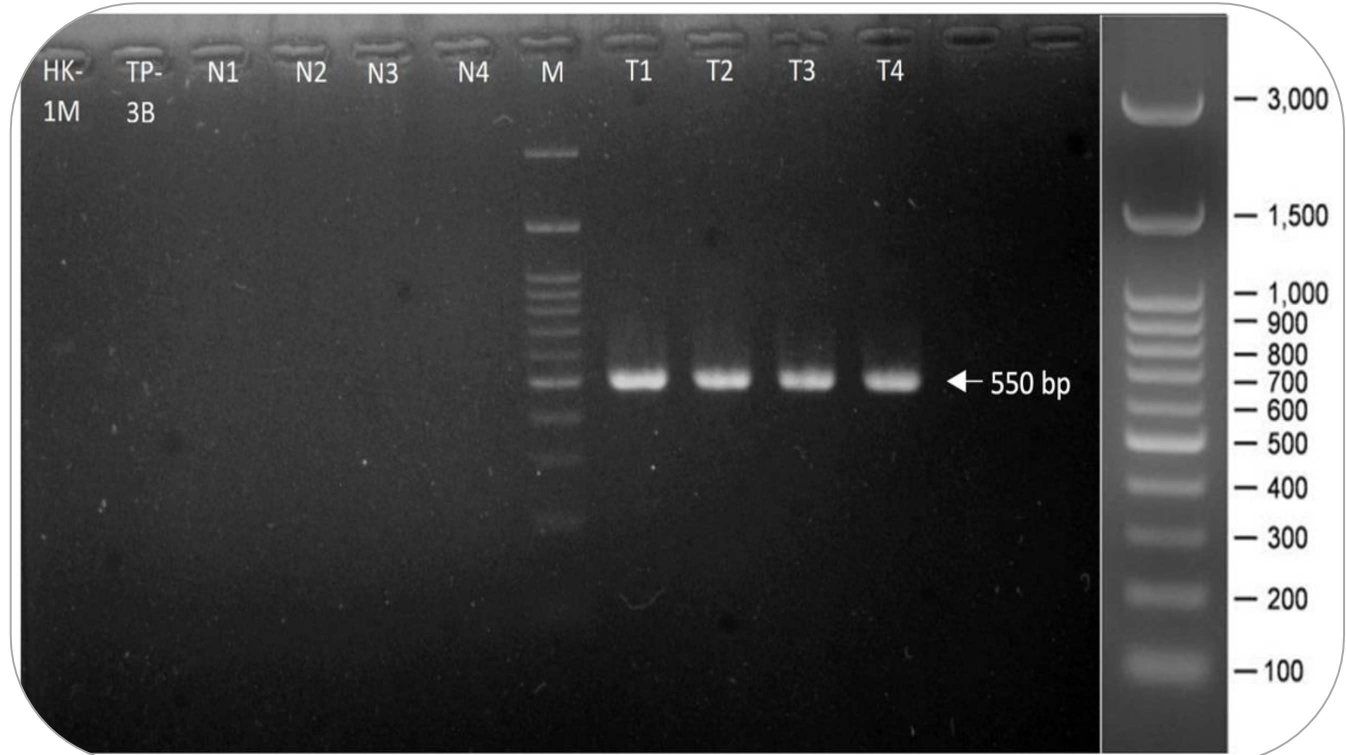

Figure 3. Amplification results by Krusty & Homer. Notes, HK-1M ('Hikapel'), TP-3B ('Tacapa Green Black'), N (healthy Kinaya), T (*Begomovirus*-infected Kinaya).

Phylogenetic evaluation of Begomovirus coat protein sequences: We identified partial sequence of *Begomovirus* coat protein from *Begomovirus*-infected Kinaya plant (T3) and compared to 20 accessions of other coat protein from different host and location. Based on Table 4, *Begomovirus* that infect Kinaya melon have a highest percentage identity to *Squash leaf curl China virus* (SLCCV) isolate from Kulon Progo, Indonesia (MZ458430 and MZ458431) with 95.67% and 95.45% respectively. Interestingly, the sequence was highly similar to another *Begomovirus* species, *ToLCNDV* (*Tomato leaf curl New Delhi virus*), from a melon leaf sample from Yogyakarta with a percentage identity of 95.02%. *Begomovirus*-infected Kinaya sequence have a high query coverage percentage of 96 - 98% to another partial *Begomovirus* coat protein from different host and location. The lowest percent identity (92.42%) was found for the *Squash leaf curl Philippines virus* (SLCPV) isolate from pumpkin.

Based on Figure 4, there is no relationship between host and *Begomovirus* type. The clade generated based on location was very high and resulted in three clades. The first clade was populated by SLCCV and LFMV isolates from China, Thailand and Vietnam with a bootstrap value of 77%. In this clade, isolates originated from different *Begomovirus* hosts, namely pumpkin, *Solanum torfum*, tomato, melon, *Siraitia grosvenorii* and luffa. The second clade consisted of PuYmMV isolates from Malaysia and SLCPV from the Philippines with pumpkin hosts. The last clade was made with *SLCCV* and *ToLCNDV* isolates from Indonesia. Interestingly, *Begomovirus* isolate Kinaya was closely related to SLCCV isolates from Kulon Progo, Yogyakarta, Indonesia with different hosts such as eggplant and melon (Figure 4). This indicates that the coat protein sequences of *Begomovirus* show a relationship based on location rather than host species.

Table 4. Identity of partial coat protein sequence of *Begomovirus*-infected Kinaya

Begomovirus	Percent identity	Query Cover	Acc Number	Location	Host
Squash leaf curl China virus (SLCCV)	95.67%	98%	MZ458430	Indonesia: Kulon Progo	Melon Field
	95.45%	98%	MZ458431	Indonesia: Kulon Progo	Eggplant field
	92,64%	98%	KF184992	China	Melon var. saccharinus
	94.58%	98%	OQ123829	Indonesia	Citrullus lanatus
	94.37%	98%	MN437659	Thailand: Nakhon Pathom	Pumpkin
	94.37%	98%	MN365019	Thailand: Nakhon Pathom	Pumpkin
	94.37%	98%	MN218675	China:Yunnan	Pumpkin
	94.37%	98%	MN218674	China:Yunnan	Pumpkin
	94.37%	98%	ON005006	China: Guangxi, Nanning	Siraitia grosvenorii
	94,16%	98%	OK429344	Thailand	Tomato
	94,16%	98%	EF197940	Malaysia: Negeri Sembilan	Cucumber
	93,94%	98%	KC857509	Viet Nam	Cucurbita moschata
	93,72%	98%	MK626673	China: Yunnan	Solanum torvum
Pumpkin yellow mosaic Malaysia virus (PuYmMV)	93,29%	98%	NC_010946	Malaysia: Negeri Sembilan	Pumpkin
Luffa yellow mosaic virus (LYMV)	92,64%	98%	NC_004824	Viet Nam	Luffa acutungula
	92,64%	98%	AF509739	Viet Nam	Luffa acutungula
Squash leaf curl Philippines virus (SLCPV)	93,16%	96%	EU487033	Philippines: Laguna	Squash
	92,42%	98%	OP771555	Philippines	Pumpkin
	92,94%	96%	OP771549	Philippines	Pumpkin
Tomato leaf curl New Delhi virus (ToLCNDV)	95.02%	98%	LC335722	Indonesia: Yogyakarta	Cucumis melo

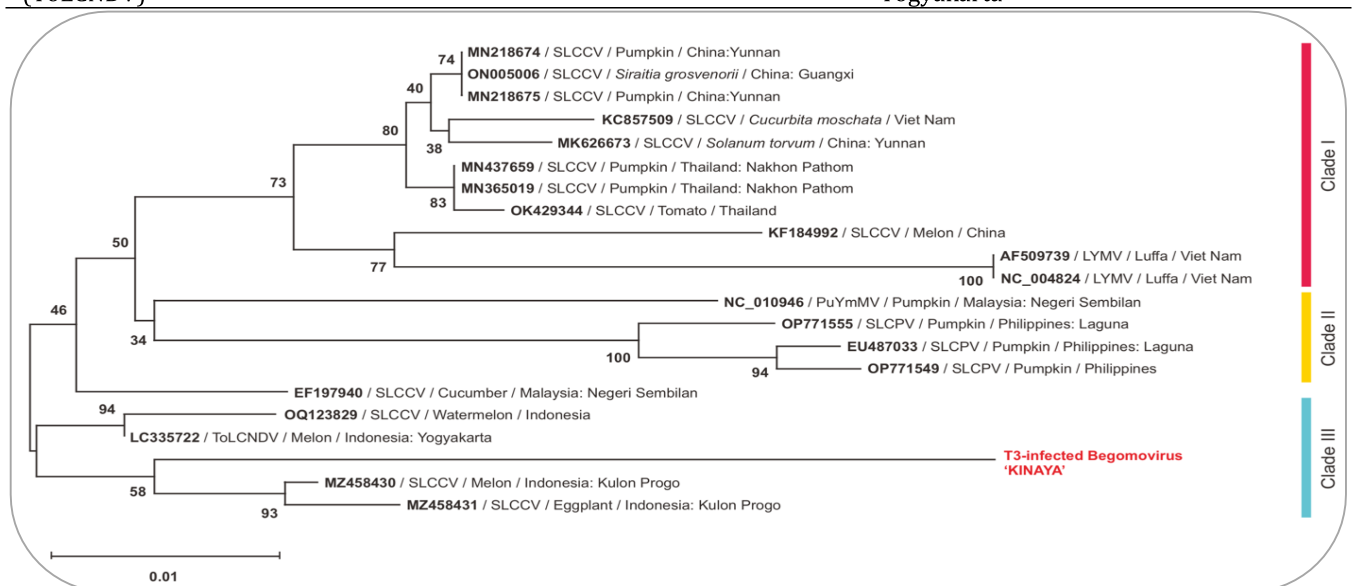

Figure 4. Phylogenetic analysis of partial coat protein of *Begomovirus* based on Neighbour-Joining (NJ) topology with Bootstrap values from 1000 replicates for each clade. Bold letter represent accession number. *Begomovirus* organism; *SLCCV* (*Squash leaf curl China virus*), *LFMV* (*Luffa yellow mosaic virus*), *PuYmMV* (*Pumpkin yellow mosaic Malaysia virus*), *ToLCNDV* (*Tomato leaf curl New Delhi virus*), *SLCPV* (*Squash leaf curl Philippines virus*).

Resistance gene identification based on developed-SCAR marker: Based on Figure 5, amplification based on the developed SCAR marker produced amplicons of 900 bp in length. The specific band was found in the healthy Kinaya plants (N1, N2, N3 and N4) and also in the other cultivars such as 'Hikapel' (HK-1M) and 'Tacapa Green Black' (TP-3B). This band was not found in *Begomovirus*-infected Kinaya.

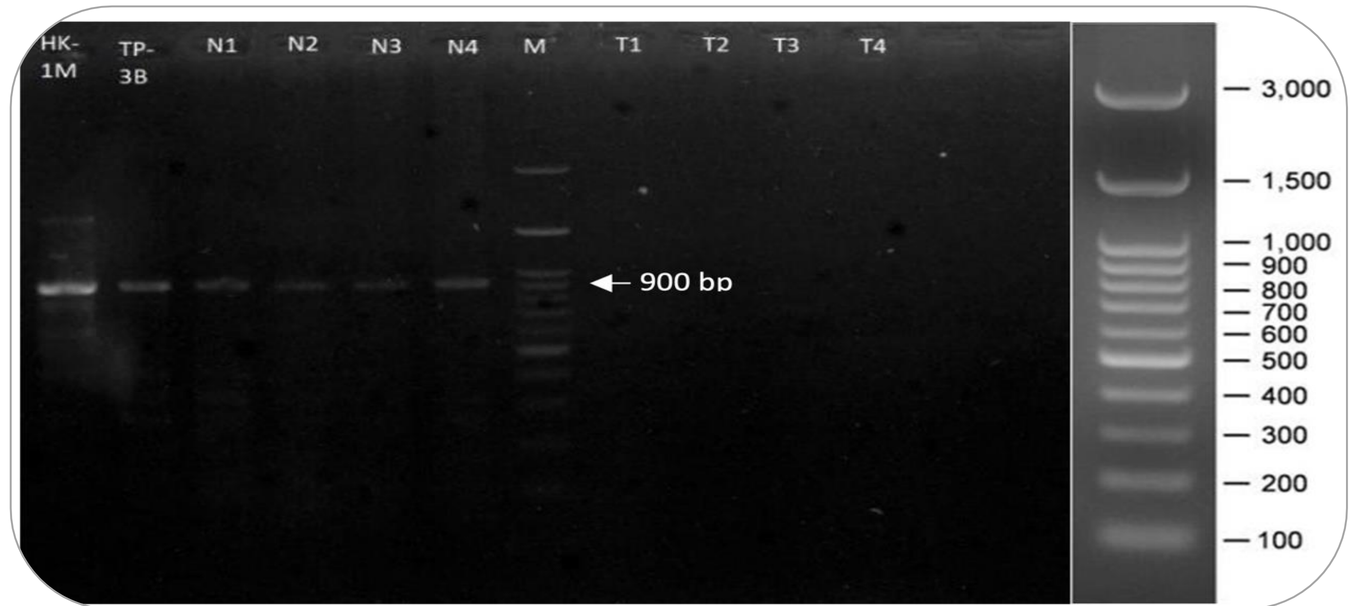

Figure 5. DNA amplification result of *Begomovirus* resistance gene based on developed-SCAR markers

DISCUSSION

Begomovirus is a member of the family *Geminiviridae*, specifically transmitted by *Bemisia tabaci* or whitefly. The whitefly is a complex cryptic species of small insects with piercing and sucking mouthparts of the family Aleyrodidae and order Homoptera. These insects are found worldwide in tropical, subtropical and cold climates. Whiteflies have a wide range of food sources, with more than 600 crops including cotton, tomato, tobacco, cassava, sweet potato and many other flowering and vegetable crops being their food sources. The whitefly is also an important disease vector for more than 200 plant species (Wang *et al*., 2018). *Bemisia tabaci* is one of the most widespread whitefly species in the world and the most virulent among other whitefly species (Delatte, 2005).

Viruses are transmitted in a circulative (persistent) and non-propagative manner (Götz and Winter, 2016). Persistent transmission means that the vector can inoculate the virus over an extended period (days/weeks) and transmit the virus after moulting and often throughout the life of the vector. Circulative viruses are viruses that move from the vector gut to the haemolymph and other vector tissues, and generally circulatory viruses are non-propagative, i.e. viruses are not transmitted to vector offspring by infection of embryos or germ cells in female insects (Hogenhout *et al*., 2008), but from host plants to other vectors. Whiteflies attack plants at three growth stages: seedling, flowering, and fruit development. In the nymph and imago stages, whiteflies suck plant sap through their mouthparts. The whitefly penetrates the plant tissue intercellularly and inserts its fluid into the phloem, inhibiting the photosynthetic process and affecting the condition of the fruit (Mohamed, 2012). Unfortunately, control of the whitefly vector using pesticides is not effective due to resistance and long-term environmental impacts (Mascarin *et al*., 2013; Wang *et al*., 2018).

Plant diseases can develop when several conditions are met, such as; virulent pathogens, susceptible host plants and appropriate environmental conditions, known as the plant disease triangle. Disease attack is influenced by host characteristics such as; plant type, growth form, population structure and density, health status and plant resistance. Presence, population size, infectivity, adaptability, dispersal, survival and reproductive ability affect the ability of pathogens to infect plants (Gaur *et al*., 2021). Based on the susceptibility analysis, Kinaya was categorised as tolerance genotypes at 68 DAP. The harvest time of Kinaya is usually up to 80 DAP (Muhammadi and Daryono, 2022), the susceptible level was observed from 72 DAP in the last production time

and it affected the fruit product of Kinaya inappropriately (Figure 2C). In another melon genotype, *Begomovirus* infection caused stunted growth, yellow leaf mosaic and extremely poor fruit quality in susceptible melon 'Melona' (Setiyobudi *et al*., 2020). 'Melona' is an Indonesian hybrid variety with fruit weights normally up to 1000 grams (Yusuf *et al*., 2023), but *Begomovirus* infection caused an average weight of only about 300 grams (Setiyobudi *et al*., 2020). Kinaya demonstrates the potential to be developed as a *Begomovirus*-resistant melon variety, considering its relatively good resistance response up to 68 DAP. However, Kinaya's tolerance potential to *Begomovirus* needs to be complemented with intensive fertilization during cultivation to enhance plant performance against virus infections and improve the quality of harvested fruits. Although plant disease resistance and tolerance are primarily determined by genetics, environmental factors, such as nutrient deficiencies and toxicities, can also have a significant impact on these traits. This means that even plants with a high genetic potential for disease resistance and tolerance can be affected by suboptimal growing conditions (Dordas, 2008).

Early detection is required to succeed in controlling plant viruses. Techniques for detecting plant diseases include visual observation of plants or image segmentation using genetic algorithms (Singh and Misra, 2017), spectroscopy (Khaled *et al*., 2018), molecularly using PCR-based methods (RAPD, ISSR, SCAR, SSR, etc.) or isothermal amplification (Rubio *et al*., 2020), serological methods (ELISA), high throughput sequencing (Rubio *et al*., 2020), and using multispectral imaging and spatial-spectral machine learning (Peng *et al*., 2022). Diagnosis of Begomovirus using PCR-based methods has been widely conducted, including in Indonesia. This method has become one of the frequently used approaches due to its speed, reliability, and sensitivity. There are several degenerate primers that can be utilized for diagnosing Begomovirus infections in plants, such as the Krusty Homer primer (Revill *et al*., 2003), PAR IC715/PAL IV978 (Rojas, 1993), UPV1/UPC2 (Briddon *et al*., 1994), and others. However, the selection of techniques for diagnosing plant virus infections should take into consideration sensitivity, speed, cost, instrument availability, and disease severity (Subiastuti *et al*., 2017).

The identification of *Begomovirus* in Yogyakarta and Central Java, Purworejo was confirmed by (Subiastuti *et al*., 2019). Based on four different plant species, the identified *Begomovirus* species were *Squash leaf curl China virus* (*SLCCV*), *Pepper yellow leaf curl Indonesia virus* (*PYLCInV*), *Tomato yellow leaf curl Kanchanaburi virus* (*TYLCKaV*) and *Tomato leaf curl New Delhi virus* (*ToLCNDV*). The begomovirus species identified in the melon population are very similar to *SLCCV* and *ToLCNDV* based on partial coat protein sequences (Subiastuti *et al*., 2019), which are similar to this study.

The identified melon-infecting *Begomovirus* are closely related to the Squash leaf curl China virus (*SLCCV* or *SLCCNV*) isolated from Kulon Progo, Indonesia (MZ458430 and MZ458431) with melon and eggplant hosts. Depending on the region and host of infection, SLCCNV has been found on many squash crops such as chayote, wax gourd, ash gourd and yellow gourd. *SLCCNV* was first reported on many squash crops and cucurbits in several countries, including pumpkin in Bangladesh (Vo *et al*., 2022), zucchini in Shandong Province, China (Shi *et al*., 2022), tomato in China (Qiu *et al*., 2022), *Benincasa hispida* in India (Riyaz *et al*., 2013)r, and *Cucurbita pepo* in Pakistan and India (Tahir *et al*., 2010; Saritha *et al*., 2011). The first *SLCCNV* reported on melon plants in China was the SLCCNV-HN strain, which caused yellowing, leaf curling and stunted plants (Wu *et al*., 2020). Due to tolerance in early plant development, these infection characteristics were not fully observed in Kinaya. *Begomovirus* infection in Kinaya caused rough fruit skin (Figure 2C) and severe yellowing of leaves (Figures 1E and 2C). The inhibition of optimal fruit development in Kinaya was the final effect of infection.

Another close phylogeny was identified with *Tomato leaf curl New Delhi virus* (*ToLCNDV*). *ToLCNDV* is a bipartite *Begomovirus* with two double-stranded DNA genomes, DNA-A and DNA-B. The coat protein on DNA-A plays a role in *Begomovirus* infectivity in tomato (Vo *et al*., 2023). *ToLCNDV* was first reported to cause an outbreak of disease in tomato plants in the Indian subcontinent. More recently, it has been found in Southeast Asia, East Asia, the Middle East, the Mediterranean and Europe (Mnari-Hattab *et al*., 2015; Fortes *et al*., 2016; Panno *et al*., 2016; Yazdani-Khameneh *et al*., 2016; Moriones *et al*., 2017; Zaidi *et al*., 2017), with recombination leading to the evolution of new strains of *ToLCNDV* (Mastrochirico *et al*., 2023). Characteristics of infection in Kinaya include leaf curling, leaf mosaic (Figure 1) and roughened fruit skin (Figure 2C). The symptoms are similar to the disease syndrome reported in zucchini (*Cucurbita pepo* L.), including leaf curling, swollen veins, severe leaf mosaic,

short internodes and roughened fruit skin caused by *ToLCNDV* (Juárez *et al*., 2014). Similarities were also shown by close phenetic relationships (Figure 4) and partial sequence identity (Table 4).

Viral pathogens are a main problem affecting negatively on plant growth and development (Saleem *et al*., 2019). The mechanisms involved in the resistance response to *Begomovirus* infection remain unknown. Gene ontology (GO) classification based on RNA-seq transcriptomes of resistant and susceptible cultivars (WM-7, kachri group and Piñonet Piel de Sapo ibericus group, respectively) revealed promising candidate resistance genes. Transcription, DNA replication and helicase activity are categories of genes that are up-regulated in resistant cultivars and enhance their resistance by reducing *ToLCNDV* replication and intercellular spread (Sáez *et al*., 2022). The SCAR markers have been developed for the detection of diseased melon plants by ensuring a diagnostic protocol that is specific, reliable, sensitive and rapid (López-Mondéjar *et al*., 2012). Several studies have developed SCAR markers linked to plant disease resistance traits, such as resistance to *Mungbean yellow mosaic virus* in mungbean (Dhole and Reddy, 2012), *Fusarium oxysporum* in banana (Cunha *et al*., 2015), *Begomovirus* in tomato (Garcia-Andrez *et al*., 2007), powdery mildew in pea (Srivasthava *et al*., 2012), etc. *Begomovirus* resistance genes were identified using SCAR primers resulting from the development of RAPD OPA-4. Unfortunately, the size of the DNA band does not match the previous studies findings, which reported it as 1198 bp (Sidiq *et al*., 2020). However, the consistent appearance of the DNA band in uninfected samples should be considered, prompting the need for sequencing of the target DNA band or checking primer stability.

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