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Begomovirus diversity and distribution on melon plants in Bali, Indonesia

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Abstract. *Temaja IGRM, Sudiarta IP, Wirya GNAS, Selangga DGW, Listihani L, Ambarawati IGAA, Kasim NN, Sapanca PLY, Pandawani NP. 2024. Begomovirus diversity and distribution on melon plants in Bali, Indonesia. Biodiversitas 25: 572-582.* Melon crops in Bali, Indonesia often exhibit curling and yellowing symptoms in young plants, the cause of which remains unidentified. This research aimed to determine the presence of Begomovirus isolates from Bali and their distribution among melon plants. The research methods involved a comprehensive survey, meticulous sampling, precise virus detection with PCR, thorough sequencing analysis, and accurate calculation of viral disease incidence based on DNA sequencing results. Sampling and symptom observation were conducted in seven Bali regencies: Denpasar City, Gianyar, Badung, Tabanan, Buleleng, Jembrana, and Bangli. The PCR method with Begomovirus universal primers SPG1/SPG2 was used for virus identification, followed by nucleotide sequencing. The research identified three viruses infecting melon plants in Bali: *Squash leaf curl virus* (SLCuV), *Squash leaf curl China virus* (SLCCNV), and *Squash leaf curl Philippines virus* (SLCuPV). The highest disease incidence for SLCuV, SLCCNV, and SLCuPV was found in Denpasar, Buleleng, and Badung, at 40%, 30%, and 30%, respectively. SLCCNV has spread to seven regencies in Bali, namely Denpasar City, Gianyar, Tabanan, Buleleng, Bangli, and Jembrana. SLCuV has spread to six regencies in Bali, all except Badung Regency. SLCuPV was only found in Denpasar City, Badung, and Buleleng. The molecular characteristic of SLCuV Bali isolate has the closest nucleotide and amino acid homology with East Timor isolate (KY652743) which is 98.4% (99.0%), SLCCNV Bali isolate is closest with Malaysia isolate (EF197940) which is 98.8% (99.8%), and SLCuPV is closest with Taiwan isolate (JF746195) which was 98.8% (99.5%). The novelty of this research lies in the first report of SLCuPV infection in Curcubitaceae plants in Indonesia, a finding that could significantly impact our understanding of viral infections in crops.

Keywords: Cucurbiaceae, leaf curl disease, SLCCNV, SLCuPV, SLCuV

Abbreviations: PCR: Polymerase Chain Reaction, SLCCNV: *Squash leaf curl China virus*, SLCuPV: *Squash leaf curl Philippines virus*, SLCuV: *Squash leaf curl virus*

INTRODUCTION

The Begomovirus genus is comprised of viruses that infect dicotyledone plants. This genus consists of viruses with bipartite genomes with genes located in two different singular single-stranded DNA molecules (DNA A and DNA B 2,6-2,8 kb in size, respectively) or monopartite with all genes contained in one singular single-stranded DNA (2,8 kb) (Gong et al. 2021). Whiteflies transmit Begomovirus from the *Bemisia* genus in a persistent, non-propagative manner. The DNA A and DNA B components contain genes that code protein by the virus's sense strand (v-sense) and complementary sense strand (c-sense). The DNA A component contains one gene (AV1) in the v-sense and three genes (AC1, AC2, and AC3) in the c-sense. The DNA B component has one gene (BV1) in the v-sense and one gene (BC1) in the c-sense (Breves et al. 2023).

The research on Begomovirus is urgent as it causes varied symptoms in plants from different families. The symptoms of *Squash leaf curl virus* (SLCuV) infection in *Cucurbita moschata*, *C. pepo*, and *C. maxima* are the curling of leaves and even stunting. The tissue between the leaf blades turns mottled and green in color. Flowers cannot grow or form fruits, or the formed fruit is smaller than normal and shrinks. This virus induces green mosaic symptoms by destroying the leaf blade, causing the leaf to twist (Selangga et al. 2018; Listihani et al. 2019a; Listihani and Selangga 2021; Selangga and Listihani 2021; Listihani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2023).

Begomovirus has now received serious attention. Several reasons for this are that Begomovirus causes socially and economically impactful diseases (Selangga and Listihani 2022; Widodo et al. 2023), its usage as the vector and inducer of gene silencing (Mubin et al. 2019; Zhai et al. 2022), and its contribution as the model in learning the mechanism of intracellular and intercellular macromolecule movements (Happle et al. 2021; Breves et al. 2023).

Several researchers have studied Begomovirus genetic diversity. Among them are Begomovirus diversity infecting soy, legumes, and grasses (Chatzivassiliou 2021; Fiallo-Olivé et al. 2021), the genetic diversity in Begomovirus mixed infection of tomatoes, chilies, and cucumbers (Listihani et al. 2019; Sohrab 2020; Selangga and Listihani 2021; Selangga et al. 2022b; Temaja et al. 2022), and cassava (Aimone et al. 2021). Immediate action is needed to understand and mitigate these symptoms.

Begomovirus, which has been found in several Cucurbitaceae plants, has been reported to be able to infect several plants. *Squash leaf curl virus* (SLCuV) is found to infect *Cucumis sativus*, *C. melo* (Selangga and Listihani 2022), *Cucurbita moschata*, *C. pepo*, all cultivars of *C. maxima*, and *Phaseolus vulgaris* (Farrag et al. 2014). Another Begomovirus species that infects Cucurbitaceae plants is the *Tomato leaf curl virus* (TLCV), which infects *Lagenaria leucantha*, *Luffa acutangula*, *C. melo*, *C. sativus* (Listihani et al. 2019a; Fontenele et al. 2021; Krishnan et al. 2023), *C. melo* var. *reticulatus* and *Benincasa hispida* (Vignesh et al. 2023). The *Melon leaf curl virus* in Arizona is reported to have a wide host range with an average 75% disease incidence which consists of *Cucumis sativus*, *C. melo*, *Citrullus lanatus*, *Cucurbita pepo*, *C. foetidissima*, *C. maxima*, *C. moschata*, *P. vulgaris*, and *Nicotiana benthamiana* (Maliano et al. 2021), *Squash leaf curl China virus* (SLCCNV) in melon (Hui-Jie et al. 2020), *Squash leaf curl Philippines virus* (SLCuPV) first infected chayote (*Sechium edule*) plants in Taiwan (Tsai et al. 2011). Young shoots and leaves of chayote (*Sechium edule*) are commonly consumed as vegetables in Taiwan. In Hualien County, a major chayote-producing area in Taiwan, as many as 15% of the chayote crop was unmarketable due to mosaic symptoms on leaves infected with SLCuPV (Tsai et al. 2011). In addition, SLCPHV has been detected in naturally infected melons, pumpkins, and squash (Tsai et al. 2011). Thus, this research's objective is to detect and determine the diversity and distribution of Begomoviruses infecting melon crops in Bali, Indonesia.

MATERIALS AND METHODS

Survey and sampling

The melon cultivation survey was performed in nine districts in Bali Province, Indonesia, i.e.: Denpasar, Gianyar, Badung, Karangasem, Bangli, Klungkung, Tabanan, Buleleng, and Jembrana. Twenty samples of plants exhibiting virus infection symptoms were collected from each regency. The total number of samples detected by PCR is 140.

Begomovirus detection and identification in melon plants by Polymerase Chain Reaction (PCR) method

The presence of Begomovirus in melon plants with yellow curl symptoms was detected via PCR using Begomovirus universal primers SPG1/SPG2 (Li et al. 2004). The primer pairs were constructed based on the highly conserved genome region of Begomovirus, the AC1 and AC2 genome coding regions (Rojas et al. 1993). The PCR method stages consist of DNA extraction, DNA amplification, and amplification product visualization.

DNA extraction and amplification

The total DNA was extracted from melon plants by Doyle and Doyle's (1987) method. Extracted DNA was used as a template in the PCR amplification, which used the primer pairs SPG1 (5'-CCCCKGTGCGWRAATCCAT-3')/ SPG2 (5'-ATCCVAAYWTYCAGGGAGCTAA-3') with the DNA band size ±912 bp. The amplification was conducted on a thermal cycler (GeneAmp PCR System 9700) with an amplification reaction consisting of 12.5 µL Dream Taq Green Master Mix (Thermo Scientific, US), 9.5 µL ultra-pure water, 1.0 µL of primer SPG 1, and SPG 2 each, and 1.0 µL template DNA that totaled into 25 µL. The amplification program for SPG1/SPG2 was predenaturation at 94ºC for 5 minutes, denaturation at 94ºC for one minute, annealing at 50ºC for 1 minute, elongation at 72ºC for 1 minute, extension for 7 minutes at 72ºC, and storage at 4ºC (Li et al. 2004).

Visualization of DNA amplification

A nucleic acid staining dye, FluoroVue™ (Smobio, Taiwan), was added to agarose 1% in electrophoresis Tris borate-EDTA 0.5x (40 mM Tris, 20 mM sodium acetate, and 1 mM EDTA, pH 7.0). Electrophoresis was performed at 100 volts for 30 minutes, and the DNA was visualized under a UV transilluminator; a digital camera documented DNA strands.

Nucleotide sequencing and genetic relationship analysis

Nucleotide sequencing of DNA fragment amplification product was conducted through the FirstBase (Malaysia) analysis service. The nucleotide sequences were analyzed for similarities with nucleotide sequences available in GenBank by utilizing the basic local alignment search tool (BLASTN) program in the National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) site. The nucleotide homology was analyzed in BioEdit software, and the phylogenetic tree was analyzed by molecular evolutionary genetics analysis (MEGA v6.0) software (Tamura et al. 2013) under the neighbor-joining method with 1,000 bootstraps.

The disease incidence for each Begomovirus species

As many as 10 samples from each regency found positive in PCR using the Begomovirus universal primer with thick DNA strands were used for sequencing analysis. The disease incidence for Begomovirus species based on the sequencing analysis was determined by the following equation by Listihani et al. 2019a:

$$DI = \frac{n}{N} x\ 100\%$$

Where:
DI : Disease incidence percentage (%)
n : The number of plants infected by Begomovirus species
N : The number of samples used in sequencing analysis

RESULTS AND DISCUSSION

Variations of disease symptoms in melon plants

The symptoms of melon plants in Bali vary greatly (Figure 1). The disease symptoms found in the field are green mosaic, mottled with curly leaf, vein clearing, cupping downward, cupping upward, vein banding, leaf tissue thinning, yellow leaf, and yellow mosaic (Table 1). Generally, the symptoms often found in the field are mosaic, mottling, vein banding, and stunting.

Green mosaic, vein banding, and dwarf symptoms are found in all sampling locations, while the rarely found symptoms are cupping downward and leaf tissue thinning. Other than viral infection on the leaves, viral infection was also found in the melon fruits in the form of fruit malformation. The varied symptoms found in the field were probably because sampling was performed on different melon varieties and ages. Several factors influence the variety of symptoms in the field, such as the plant age, which affects the plant's immune system; cultivar, which determines the plant's susceptibility to certain viruses; plant genotype, which can either resist or be susceptible to viral infections; plant growth phase, which affects the plant's ability to recover from infections; and the virus strain, which determines the severity of the infection (Listihani et al. 2019b; Listihani et al. 2020; Pandawani et al. 2022; Selangga et al. 2022a; Edula et al. 2023; Selangga et al. 2023). Symptoms variety may also be influenced by environmental factors such as the climate around the plants (Tsai et al. 2022; Jiang and Zhou 2023). However, the melon sampling in this research was performed within a short period, so environmental factors in several areas were not too different and did not affect the symptom variability.

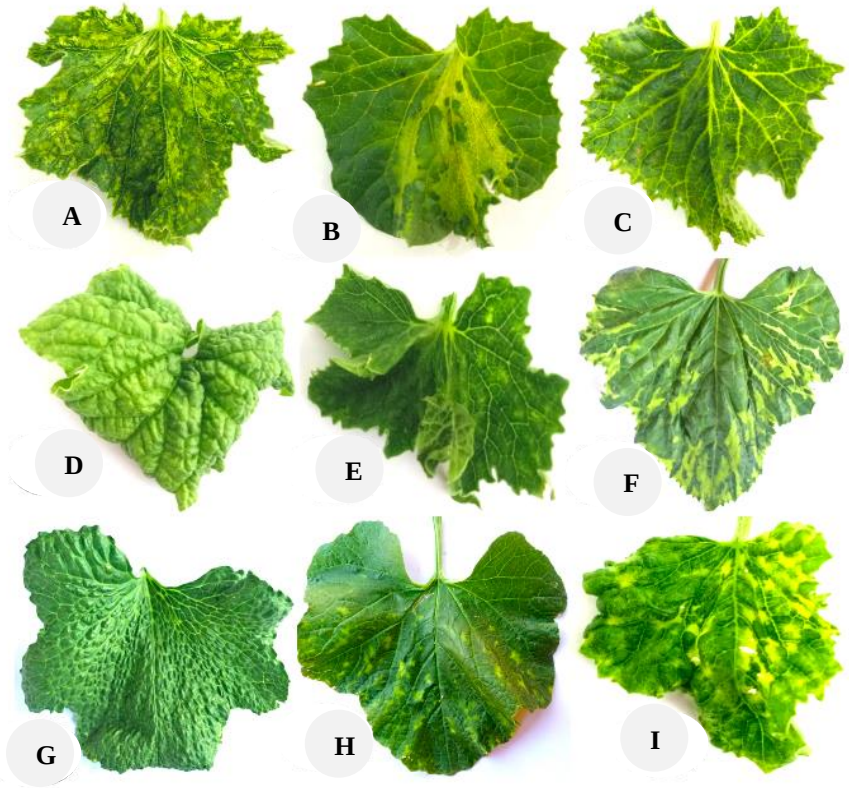

Figure 1. Dominant symptoms associated with virus infection in melon plants in Bali: A. Green mosaic; B. Stripes accompanied by leaf curling; C. Vein clearing; D. Downward cupping; E. Upward cupping; F. Vein banding; G. Narrowing of leaf tissue; H. Yellow leaves; I. Yellow mosaic

Table 1. Variations in symptoms of virus infection in melon plants in Bali

Locations	Symptom variations										Variety
	Mh	Mk	Bl	Cw	Cu	Vc	Vb	Pj	K	kd	
Denpasar	√	√	√	-	√	√	√	√	√	√	Alisa F1
Gianyar	√	√	√	-	√	√	√	√	√	√	Okasa
Badung	√	√	√	√	√	√	√	-	-	√	Alisa F1
Tabanan	√	-	√	-	-	-	√	-	√	√	Action 434
Buleleng	√	√	√	√	√	√	√	√	√	√	Mai 119
Jembrana	√	√	-	-	-	-	√	-	-	√	Action 434
Bangli	√	√	√	-	√	-	√	-	-	√	Alisa F1

Notes: Mh: Green mosaic, Mk: Yellow mosaic, Bl: Striped, Cw: Downward cupping, Cu: Upward cupping, Vc: Vein clearing, Vb: Vein banding, Pj: Tissue narrowing, K: Yellow, kd: Dwarf

All variants planted in the field showed vulnerability to certain viruses. The virus infection symptoms on melon plants that started at the start of growth are hard to distinguish from other viral infections due to their varied symptoms (Hadi et al. 2024). The differences in virus symptom variation are not only caused by different plant varieties but also by environmental factors. Recent studies have revealed interactions between light or temperature and virus infection, indicating that light or temperature can modulate the severity of virus symptoms (Chung et al. 2018; Jiang et al. 2023). This highlights the need for a thorough understanding of the complex interactions between virus factors, host responses, and environmental cues. By synthesizing these recent advances, this comprehensive review aims to provide valuable insights into the complex mechanisms underlying virus-induced symptoms in plants. Therefore, it is crucial to understand these mechanisms to develop effective strategies to detect, prevent, and control virus infections, ultimately safeguarding global crop production and ensuring food security in the face of emerging viral diseases.

Molecular characterization of Begomovirus in melon plants in Bali

The PCR amplification utilizing Begomovirus universal primers SPG1/SPG2 gene AC1 and AC2 Geminivirus, which were 912 bp in size, managed to confirm Geminivirus infection in melon plants from seven sampling locations (Data not shown). The Begomovirus universal primers have often been used to detect Begomovirus, namely *Pepper yellow leaf curl* Indonesia virus in chili pepper, *Sweet potato leaf curl virus* in sweet potato, *Squash leaf curl China virus* in melon, and *Tobacco mottle leaf curl virus* in tobacco (*Nicotiana tabacum*) (Selangga and Listihani 2021; Selangga et al. 2021; Listihani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2022b; Sutrawati et al. 2022; Temaja et al. 2022; Listihani et al. 2023; Selangga et al. 2023).

Samples from several locations in Bali that SLCuV infected exhibited generally found characteristic symptoms in the form of yellow spots on the leaf, yellow mosaic with thickening of the leaf blade, and curly leaf (Figure 1.F, 1.H, and 1.I). Those symptoms have been reported by Vargas-Salinas et al. (2020) as SLCuV infection symptoms in the Cucurbitaceae group in the Baja California Peninsula, Mexico. The characteristic symptoms of SLCCNV in melon plants in Bali are curly leaves alongside vein clearing (Figure 1.A, 1.B, and 1.C). The SLCCNV infection symptoms in melon in Bali are the same as the symptoms in the cucumber plants in Bali (Wiratama et al. 2015). The characteristic SLCuPV infection symptom is green curly leaf (Figure 1.D and 1.E).

Detections through PCR managed to find positive reactions in 140 melon leaf samples. The result showed three types of viruses infecting melon plants in Bali, namely *Squash leaf curl virus* (SLCuV), *Squash leaf curl China virus* (SLCCNV), and *Squash leaf curl Philippines virus* (SLCuPV) (Figure 1). The highest disease incidence for SLCuV, SLCCNV, and SLCuPV could be found in Denpasar, Buleleng, and Badung, respectively, with values of 40%, 30%, and 30% (Figure 2).

The melon plants in Denpasar City and Buleleng showed the presence of SLCuV, SLCCNV, and SLCuPV infections. SLCuV and SLCCNV infected melon in Gianyar, Tabanan, Bangli, and Jembrana Regency, while SLCCNV and SLCuPV infected the melon plants in Badung Regency. This showed that SLCuV and SLCCNV spread to almost all regencies in Bali, while SLCuPV was only found in several areas of Bali (Denpasar, Badung, and Buleleng).

DNA samples from the amplification process were used in the sequencing stage to determine the virus species. Sequencing analysis confirmed that the viruses infecting plants with curly and yellow symptoms are *Squash leaf curl virus* (SLCuV), *Squash leaf curl China virus* (SLCCNV), and *Squash leaf curl Philippines virus* (SLCuPV). The nucleotide and amino acid sequence analysis of SLCuV isolate from Bali showed low to high homology, 88.8-98.4%, and 89.9-99.0% consecutively, with SLCuV sequences available in GenBank (Table 2). The sequence of SLCuV from Bali showed the highest homology with East Timor isolate (KY652743) from pumpkin plants, which were 98.4% and 99.0% consecutively (Table 2). This research result is the first report of SLCuV infection on melon plants in Indonesia.

The phylogenetic analysis grouped the SLCuV into two groups: I and II (Figure 3). SLCuV subgroups I and II form separate groups. The SLCuV subgroup I has several hosts: pumpkin, tomatoes, and cotton. The SLCuV subgroup II comprises pumpkin and melon. The SLCuV isolate from Bali has closer relationships with East Timor and Taiwan isolates compared to Oman, Lebanon, Pakistan, Egypt, Jordan, the United States, Palestine, and Israel isolates.

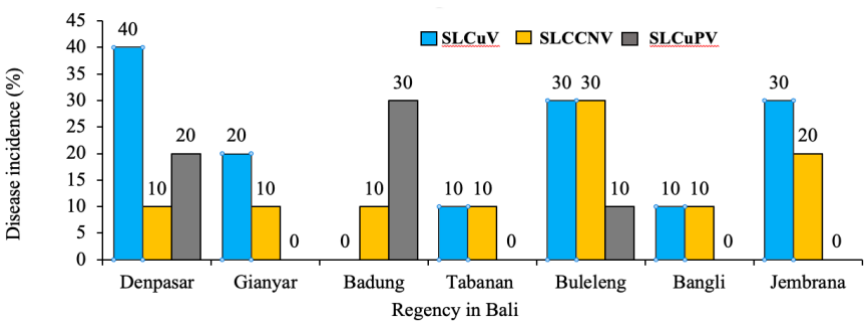

Figure 2. Disease incidence for *Squash leaf curl virus* (SLCuV), *Squash leaf curl China virus* (SLCCNV), and *Squash leaf curl Philippines virus* (SLCuPV) that infect melon plants in Bali based on the results of sequencing analysis (10 samples were subjected to sequencing analysis in each regency)

Table 2. Nucleotide (nt) and amino acid (aa) homology of SLCuV isolates from Bali to isolates from other countries deposited in GenBank

Isolate	Source	Host	Homology nt (aa) (%)	Accession number
SLCuV-T4D	East Timor	*Cucurbita moschata*	98.4(99.0)	KY652743
-YL	Taiwan	*Cucurbita* sp.	94.9 (98.3)	EU479710
-PAL	Palestina	*Cucurbita* sp.	90.1 (93.9)	KC441465
-Sq-5A	Oman	*Cucurbita pepo*	90.1 (93.9)	MT032114
-Homra	Jordan	*Lycopersicum esculentum*	90.3 (94.2)	JX444577
-LB2	Lebanon	*Cucurbita pepo*	90.2 (94.0)	HM368373
-Cairo	Egypt	*Cucurbita pepo*	90.0 (92.8)	DQ285019
-S8	Oman	*Cucurbita* sp.	90.1 (93.9)	HG969277
-IsSq3	Israel	*Cucurbita* sp.	90.1 (93.9)	KT099131
-LB3-7-44	Lebanon	*Cucurbita* sp.	90.2 (94.0)	KM595138
-JO3-330	Jordan	*Cucurbita* sp.	90.2 (94.0)	KM595213
-Bani Suef	Egypt	*Cucurbita pepo*	89.8 (91.8)	MK284931
-Kaha	Egypt	*Lycopersicum esculentum*	89.7 (91.7)	MG763920
-504-Pak	Pakistan	*Gossypium hirsutum*	89.0(90.5)	MF504011
-396-Pak	Pakistan	*Gossypium hirsutum*	89.7 (91.7)	MF504010
-Jor	Jordan	*Malva parviflora*	88.8 (89.9)	EF532620
-NC	USA	*Cucurbita* sp.	89.4 (91.3)	NC_001936
-CASq-C2	USA	*Cucurbita* sp.	89.8 (91.8)	KT099117
TYLCV-Masan	South Korea	*Lycopersicum esculentum*	70.2 (72.3)	HM130912

Note: *TYLCV: *Tomato yellow leaf curl virus* is an isolate outside the group; nt (nucleotide) and aa (amino acid)

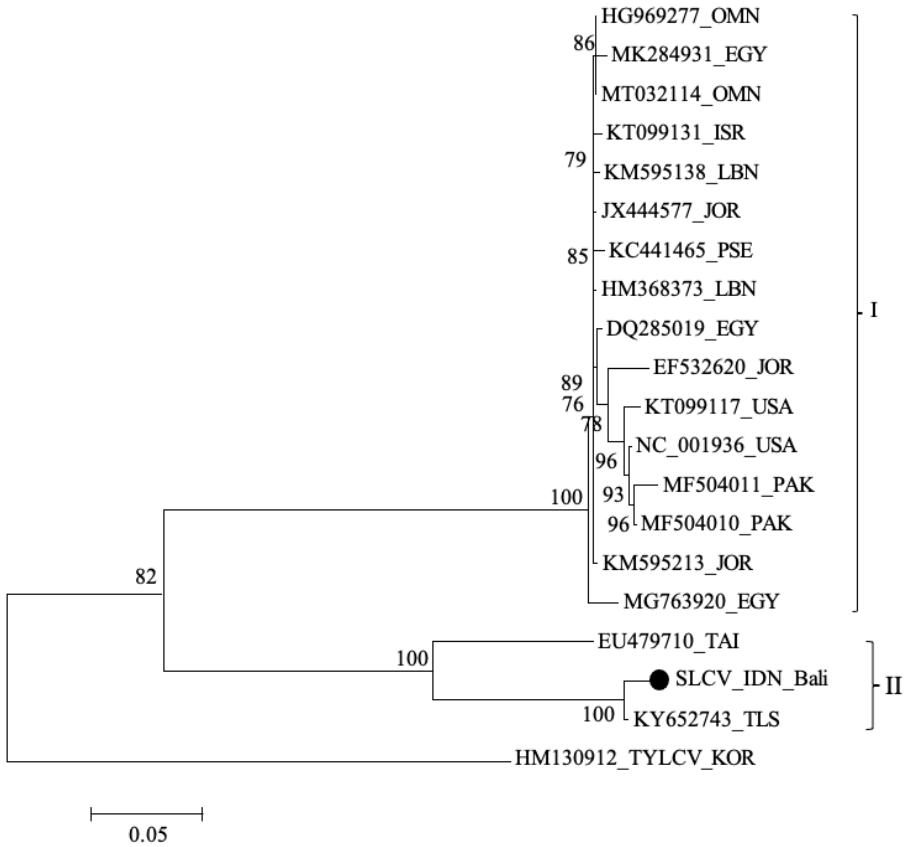

Figure 3. Phylogenetic tree based on the AC1 dan AC2 gene from SLCuV Bali isolates. *Tomato yellow leaf curl virus* (TYLCV) was used as an outgroup. Isolates marked with gray highlights are Bali isolates. The dot is a melon isolate from Bali. Oman (OMN), Egypt (EGY), Israel (ISR), Lebanon (LBN), Jordan (JOR), Palestine (PSE), Amerika (USA), Pakistan (PAK), Taiwan (TAI), East Timor (TLS), Indonesia (IDN), South Korea (KOR). Bootstraps values greater than 70% based on 1000 replicates are shown on tree branches. The scale bar below the tree indicates 0.05 nucleotide substitutions per site

Table 3. Nucleotide (nt) and amino acid (aa) homology of Bali isolate SLCCNV to isolates from other countries deposited in GenBank

Isolate	Country	Host	Homology nt (aa) (%)		Accession number
			9	6	
SLCCNV-9	Indonesia	*Cucumis melo*		97.2 (99.4)	-
-6	Indonesia	*Cucumis melo*	97.2 (99.4)		-
-MC1	Malaysia	*Cucumis sativus*	98.3 (99.6)	98.8 (99.8)	EF197940
-T4D	East Timor	*Cucurbita moschata*	95.6 (97.9)	96.3 (98.9)	KY652743
-BASq-17	Indonesia	*Cucurbita maxima*	95.0 (97.2)	95.6 (98.4)	LC511776
-KP1	India	*Benincasa hispida*	92.2 (94.0)	92.6 (94.8)	KF188433
-Sq-1	India	*Cucurbita pepo*	92.6 (94.8)	93.3 (94.8)	MH836313
-J1	India	*Jasminum sambac*	92.1 (93.9)	92.5 (94.3)	MF102264
-CPoAL4	Pakistan	*Cucurbita pepo*	91.2 (93.9)	92.5 (94.3)	AM286794
-CRI136	Thailand	*Cucurbita moschata*	92.5 (94.0)	93.0 (94.4)	MN437662
-Hanoi	Vietnam	*Cucurbita moschata*	93.3 (94.9)	93.7 (94.8)	KC857509
-Tha	Thailand	*Cucurbita moschata*	92.5 (94.3)	93.0 (94.4)	AB330078
-Guangki	Cina	*Cucurbita moschata*	93.9 (94.7)	94.4 (94.7)	MG525551
-YN1803	Cina	*Cucurbita moschata*	92.4 (94.0)	92.9 (94.3)	MN218675
-GZ01	Cina	*Cucurbita moschata*	93.0 (94.7)	93.3 (94.7)	KC171648
-HA3	Cina	*Cucumis melo*	92.1 (94.0)	92.5 (94.3)	KF184993
-K	Vietnam	*Cucurbita* sp.	91.7 (94.0)	92.1 (94.0)	AF509741
TYLCV-Masan	South Korea	*Lycopersicum esculentum*	70.3 (72.3)	70.9 (73.1)	HM130912

Note: *TYLCV: *Tomato yellow leaf curl virus* is an isolate outside the group; nt (nucleotide) and aa (amino acid)

The SLCCNV nucleotide and amino acid homology between melon isolates in Bali were 97.2% and 99.4%, respectively (Table 3). Genome-wide pairwise identities of 91% and 94% for Begomoviruses are proposed as the demarcation threshold belonging to different species and strains (Brown et al. 2015). This means the samples from Bali aligned with sequences from Genbank are SLCCNV isolates. The SLCCNV isolates from Bali have nucleotide homology (98.3-98.8%) and amino acid homology (99.6-99.8%), the highest with a close relationship with Malaysia isolate, which infects cucumber plants (EF197940) and has lower homology with isolates from other countries.

The relationship between isolates based on phylogenetic analysis showed that SLCCNV isolates from 6 and 9 were in one phylogeny line with SLCCNV isolates from Malaysia and were separated from SLCCNV isolates infecting cucurbit plants in other Asian countries (Figure 4). The DNA-A of SLCCNV from Bali is classified as one of the SLCCNV strains, called SLCCNV-Malaysia. The SLCCNV isolate from Bali, which infects melon, was in the same group as the Malaysia isolate, while the SLCCNV isolate from Northern Sumatra was in another group along with the East Timor isolate. Based on host pants observation, the SLCCNV isolates could not be grouped based on their hosts. The phylogenetic tree profile indicated different SLCCNV strains based on the origin area.

The nucleotide and amino acid sequence analysis of the SLCuPV isolate from Bali showed high homology, respectively 94.0-98.8% and 96.0-99.5%, with SLCuPV sequences in GenBank basis data (Table 4). The SLCuPV sequence from Bali showed the highest homology with the Taiwan isolate (JF746195) originating from chayote in 98.8% and 99.5% (Table 4).

The high homology between SLCuPV Bali isolates with other countries means the SLCuPV genetic diversity is low. SLCuPV has been reported in the Philippines and Taiwan and was first found in Indonesia by this research. Neoh et al. (2023) stated that the highest amino acid and nucleic acid diversity of SLCuPV is between SLCuPV Taiwan isolates. Thus, the SLCuPV infecting melon, chayote, honey pumpkin, and wax gourd in Indonesia and Taiwan are from the same SLCuPV strain.

The phylogenetic analysis grouped the SLCuPV into two groups: group I Taiwan isolates and group II Philippines isolates (Figure 5). The SLCuPV isolates from Bali, Indonesia has a closer relationship with Taiwan isolates compared to the Philippines isolates. The differences in geographical location and host species contribute less to the SLCuPV genetic diversity. This is assumed due to the SLCuPV host adaptation with the melon plants that the SLCuPV forms a group within the phylogenetic despite originating from different geographical areas. The SLCuPV infects plants from the Cucurbitaceae and Solanaceae family. The SLCuPV is only found in Bali in melon plants and has not been found in other regions in Indonesia. This research is the first report of SLCuPV infection on melon plants in Bali.

The SLCuV and SLCCNV have long since 2015 been infecting Cucurbitaceae plants in Indonesia (Wiratama et al. 2015; Haerunisa et al. 2016; Selangga and Listihani 2022). The SLCuPV is first reported to infect the Cucurbitaceae plant in Indonesia in this research. The SLCuV, SLCCNV, and SLCuPV are effectively transmitted by *Bemisia tabaci* whitefly (Hemiptera: Aleyrodidae) in a persistent circulative nonpropagative manner. According to the information provided by this single-gene genotyping approach, *B. tabaci* is considered a complex of 11 well-defined high-level groups containing at least 44 distinct species (Krause-Sakate et al. 2020). Among them, the Middle East-Asia Minor 1 (MEAM1, former B biotype), Mediterranean (MED, former Q biotype), and New World (NW, former A biotype) are found in South America (Krause-Sakate et al. 2020; Gautam et al. 2022; Qureshi et al. 2022; Temaja et al. 2022).

Table 4. Nucleotide (nt) and amino acid (aa) homology of SLCuPV Bali isolates to isolates from other countries deposited in GenBank

Isolate	Source	Host	Homology nt (aa) (%)	Accession number
SLCuPV-1-1	Taiwan	*Sechium edule*	98.8 (99.5)	JF746195
-PA1	Taiwan	*Cucurbita moschata*	97.5 (98.8)	DQ866135
-AFPK5slv	Taiwan	*Cucurbita moschata*	97.7 (98.8)	EF199774
-Wg1	Taiwan	*Benincasa hispida*	96.9 (98.2)	EU310406
-Phi	Filipina	*Cucurbita moschata*	94.7 (96.3)	AB085793
-P133	Filipina	*Cucurbita moschata*	94.4 (96.0)	EU487041
-P88	Filipina	*Cucurbita pepo*	94.0 (96.0)	EU487033
-PRJ	Filipina	*Cucurbita moschata*	94.7 (96.3)	NC_005845
TYLCV-Masan	Korea	*Lycopersicum esculentum*	70.6 (71.7)	HM130912

Note: *TYLCV: *Tomato yellow leaf curl virus* is an isolate outside the group; nt (nucleotide) and aa (amino acid)

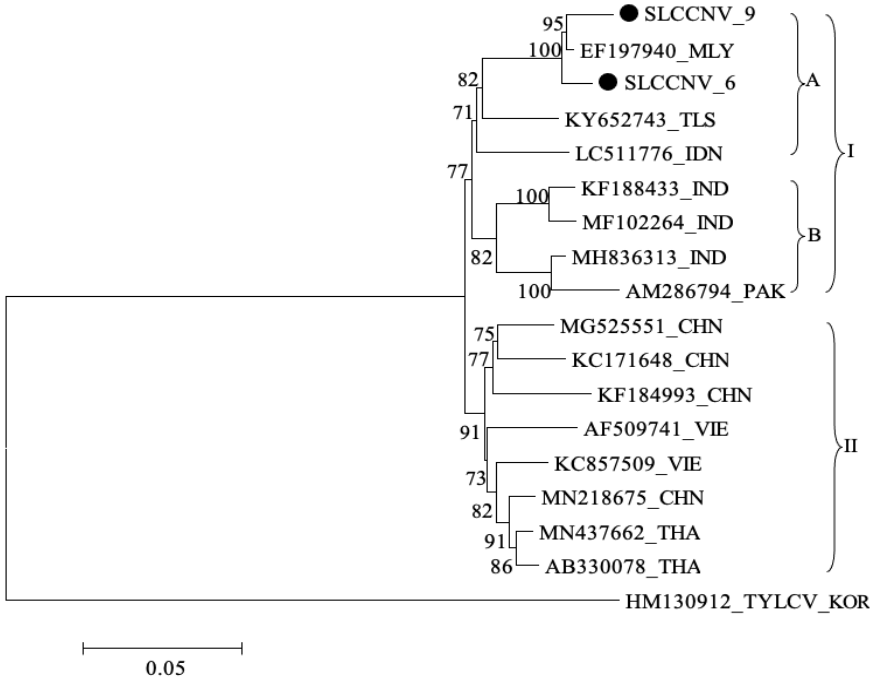

Figure 4. Phylogenetic tree based on the AC1 dan AC2 gene from SLCCNV Bali isolates. *Tomato yellow leaf curl virus* (TYLCV) was used as an outgroup. Isolates marked with gray highlights are Bali isolates. The dot is a melon isolate from Bali. Malaysia (MLY), East Timor (TLS), Indonesia (IDN), India (IDN), Pakistan (PAK), China (CHN), Vietnam (VIE), Thailand (THA), South Korea (KOR). Bootstraps values greater than 70% based on 1000 replicates are shown on tree branches. The scale bar below the tree indicates 0.05 nucleotide substitutions per site

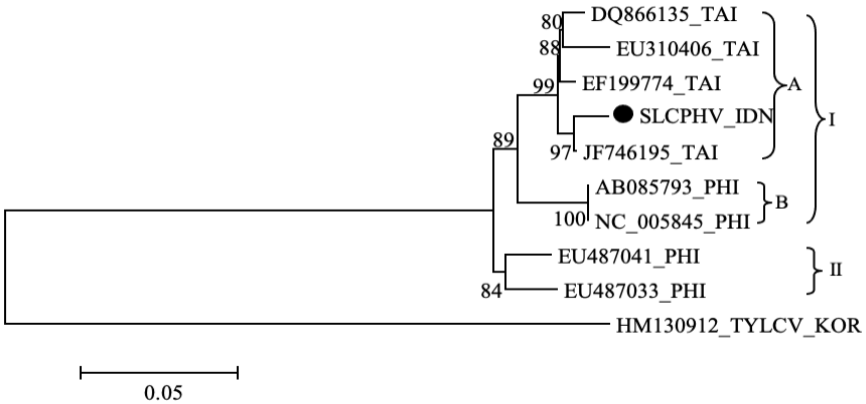

Figure 5. Phylogenetic tree based on the AC1 dan AC2 gene from SLCuPV Bali isolates. *Tomato yellow leaf curl virus* (TYLCV) was used as an outgroup. Isolates marked with gray highlights are Bali isolates. The dot is a melon isolate from Bali. Philippines (PHI), Taiwan (TAI), Indonesia (IDN). Bootstraps values greater than 70% based on 1000 replicates are shown on tree branches. The scale bar below the tree indicates 0.05 nucleotide substitutions per site

The SLCuV, SLCCNV, and SLCuPV have a wide host range within the Cucurbitaceae, such as melon, pumpkin, cucumber, bitter gourd, watermelon, wax gourd, and Chinese okra (Wiratama et al. 2015; Haerunisa et al. 2016; Listihani et al. 2019a). Other than Cucurbitaceae, the SLCCNV was also reported to be able to naturally infect *Chenopodium murale*, *Convolvulus* sp., *Prosporis farcta*, and *Malva parviflora* (Al-Musa et al. 2008). Weeds are known as reservoir plants and play a role in the epidemiology of diseases caused by Begomovirus. Weeds conserve the presence of viruses and their vector insects and cause outbreaks in the next planting season. *A. conyzoides* is known as the natural host of Begomovirus. This weed is often found in various horticultural cultivation and generally shows leaf blade paleness and yellow mosaic symptoms (Khan et al. 2014).

Despite the new SLCuPV has only been reported to infect melon plants in Bali and is not a main concern, this issue needs further attention. The extant inoculum source in the field is feared to spread to other regions with the help of the whitefly and Cucurbitaceae seedling market. The Cucurbitaceae nursery in Sekaan Village, Bangli, often distributes seedlings to several regions in Bali. This becomes a factor in the spread of plant pathogen distribution in Bali. The presence of new viruses in a region can cause disease epidemics if proper control and spread prevention to other regions are not in place.

Of the three viruses, SLCuV has the highest disease incidence while infecting melon plantations in Bali compared to the other viruses. The success in identifying the dominant virus in the melon plant transmitted by whiteflies shows the importance of observing factors associated with the epidemic of that virus. The generally cultivated vulnerable cultivars, the presence of weed as an inoculum source and alternative host in the field, and the high population of viruliferous insect vectors are the main factors in the emergence of Begomovirus in the field. Moreover, in similar research, the causes of the virus infection epidemic on cucumber plants in Java are the type of variant cultivated, the weed coverage, mulch usage, insect vector population, and inoculum source.

In conclusion, three Begomovirus species have infected melon plants in Bali, namely SLCuV, SLCCNV, and SLCuPV. The SLCCNV has spread to seven regencies in Bali, namely Denpasar City, Gianyar, Tabanan, Buleleng, Bangli, and Jembrana. In comparison, SLCuV has spread to six regencies in Bali, all except Badung Regency. The SLCuPV is only found in Denpasar City, Badung, and Buleleng. The molecular characteristic of SLCuV Bali isolate has the closest nucleotide and amino acid homology with the isolate East Timor isolate (KY652743), the SLCCNV Bali isolate is closest with the Malaysia isolate (EF197940), and the SLCUPV is closest with the Taiwan isolate (JF746195).

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