DISEASE NOTE

First report of *Sweet potato leaf curl virus* (SPLCV) on *Ipomoea batatas* in Bali, Indonesia

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Total text pages: 8

The numbers of tables: 1

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Abstract

During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. The vein yellowing symptoms were validated by extracting total DNA, performing PCR, subcloning with TA cloning, and sequencing the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia.

Key words: *Sweet potato leaf curl virus*·Sweetpotato·*Begomovirus*·Yellowing·

Sweetpotato (Ipomoea batatas) many producted in Asia, particularly in the Far East Asia and Southeast Asia countries. In Indonesia, sweetpotato becomes staples food particularly in Eastern Indonesia. Food diversification is a solution to the problem of food needs in Indonesia. Sweetpotato production in Indonesia in 2015, 2016 and 2017 amounted to 2298, 2169, and 2023 tons (Central Bureau of Statistics 2017). According to this data, annual production has reduced. In 2019, a field assessment of sweet potato production in nine regions of Bali Province discovered yellowing symptoms on plants in the Badung and Gianyar regions (Fig. 1). The leading cause of sweet potato virus disease in Bali is the sweet potato leaf curl virus (SPLCV). The same viral infection was detected in China, resulting in a 20% decline in sweet potato yield (Feng et al. 2000).

SPLCV, which belongs to the Begomovirus genus, causes leaf curl in several types of sweet potato (Kim et al. 2015). The SPLCV contains two ORFs, V1 and V2, in the virion sense, and four ORFs (C1, C2, C3, and C4) in the complementary sense, separated by an IR (intergenic region) containing a conserved stem-loop motif within a 2.8 kb circular single-stranded DNA (Kim et al. 2015). The full genomic sequence of SLCV from Korea was characterized, as well as its phylogenetic relationship to other sweepovirus species (Choi et al. 2012).

According to Moyer and Salazar (1989), the SPLCV virus was discovered for the first time in Japan and Taiwan in 1980. Moreover, in several countries such as the United States of America, Brazil, Italy, Spain, Peru, Kenya, Uganda, India, China and Korea, similar viruses have been found (Briddon et al. 2006; Kwak et al. 2006; Parotka et al. 2010; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). Yellow veins and upward curling leaves are symptoms of SPLCV infection in

young sweet potato plants (Kim et al. 2015). The host range of SLCV such as Ipomoea purpurea, I. nil, I. batatas, I setosa, I aquatica, and Nicotiana benthamiana (Valverde *et al.* 2007; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). Clark and Hoy (2006) observed a more than 30% reduction in sweet potato yield due to SPLCV infection. SPLCV can be transmitted persistently by the whitefly vector *Bemisia tabaci* (Simmons et al. 2009) and grafting. There have been no transmission reports through mechanical or seeds transmission (Loebenstein et al., 2003 However, currently, SPLVC transmission has been found through seeds. SPLVC DNA can replicate in seedlings. This incident has occurred in Korea. The proof is SPLCV detected in endosperm and embryos by PCR. Previously, SPLCV have not been reported in Indonesia.

In this study, the disease incidence of these viruses based on observations of symptoms in the fields. The incidence of viral diseases in the field showed that the disease incidence is more than 50% occurs in Badung and Gianyar Regions (data not shown). One hundred eighty samples of sweet potato leaves exhibiting symptoms consistent with SPLCV infection were collected from nine regions throughout Bali Province (Badung, Bangli, Buleleng, Denpasar, Gianyar, Karangasem, Klungkung, Jembrana and Tabanan). The purpose of this collection was to determine the presence of SPLCV in sweet potatoes.

To confirm the vein yellowing symptoms, the total DNA was extracted, and PCR was performed. Then, the PCR products were subcloned using TA cloning and followed by plasmid DNA sequencing. PCR amplification was done by using universal primer of Begomovirus SPG1 (5'-CCCCKGTGCGWRAATCCAT-3') and SPG2 (5'-ATCCVAAYWTYCAGGGAGCT AA-3') for an amplicon of ± 900 bp (Li et al. 2004). Dream Taq Green Master Mix (2X) was utilized for the Amplification reactions. (Thermo

Fisher Scientific, Waltham, MA, USA). Material composition in the amplification reaction it was 14.5–17.3 µL H_2O, 2.5 µL buffer containing 10x Mg^{2+}, 2 µL dNTP 2.5 mM, 1 µL SPG1 and SPG2 primers respectively, 0.2–0.3 µL Dream Taq Polymerase, and 0.2 µL MgCl 25 mM. Following that, the amplified DNA bands were observed on a 1% agarose gel in 0.5x TBE buffer (Tris-borate EDTA). The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar (Fig. 2). The result of PCR from plants not showing symptoms and that no amplification was observed. The disease symptoms have the ability to transmit its diseases from the symptomatic plants to healthy plants through grafting to *I. setosa*. The results of this detection prove the presence of Begomovirus infection in sweetpotato plants. DNA fragments of Begomovirus were cloned toward pTZ57R/T vector plasmid (InsTAclone PCR Cloning Kit, Thermo Scientific, USA) and injected into competent cells of E. coli DH5α. Sequence analysis was performed on the recombinant plasmid DNA extract. Using Clustal W, the partial genes' nucleotide and amino acid sequences were matched to the SPLCV sequences in the GenBank database (Thompson et al. 1994). The sequence identity matrix options in BioEdit version 7.05 software were used to calculate the sequence identities (Hall 1999). Phylogenetic trees were generated from the aligned sequences employing a bootstrap procedure, and Neighbor-Joining algorithms, which estimated using 1000 repeats (Kumar et al. 2016; Ameri and Ayazpour 2021)

The nucleotide and amino acid sequence homology of the SPLCV Bali isolate varied between 97.8% and 98.8%. This indicates a low degree of variation. The homology of SPLCV Bali isolates to that of isolates from other countries was ranged 86.5-97.2% and 88.7-98.4%, respectively. Two clones from Badung (LC586169) and Gianyar (LC586170) shared a maximum identity of 96.2-97.2% and 97.3-98.4% at nucleotide and

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The phylogenetic tree analysis showed that SPLCV divided into II groups. Group I consists of isolates from Asia, II are isolates from America and Europe. SPLCV Bali isolates were in the same group with other Asia isolates. This study is the first report on SPLCV infection in sweet potatoes in Bali. Since the province has been leading the sweet potato production in Indonesia such result finding will help strengthen plant health certification standards in order to provide virus-tested propagative materials and bulbs for domestic growers and export to other countries.

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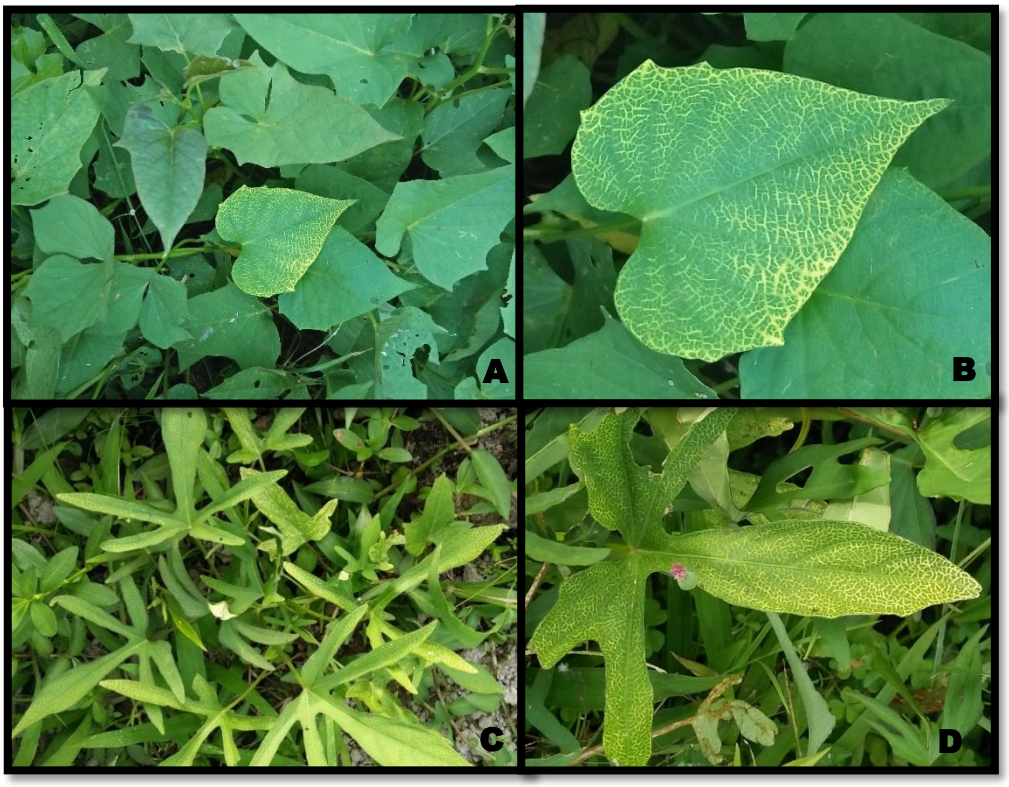

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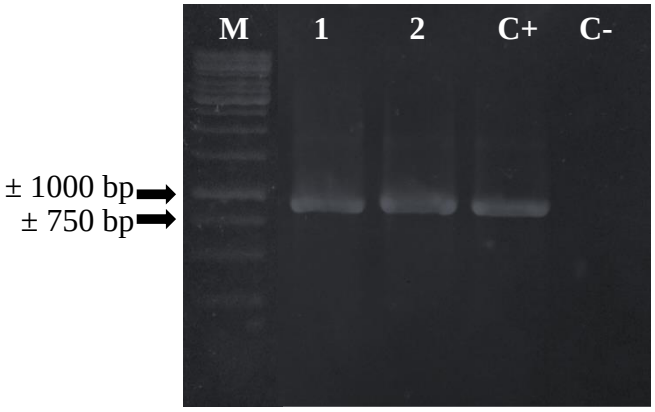

Figure 2 Visualization of PCR products using universal primer of *Begomovirus* SPG1 / SPG2: melon leaves from the Gianyar (1), Badung (2), positive control (C+), negative control (C-), DNA ladder 1 kb (M) (Thermo Fisher Scientific, USA)

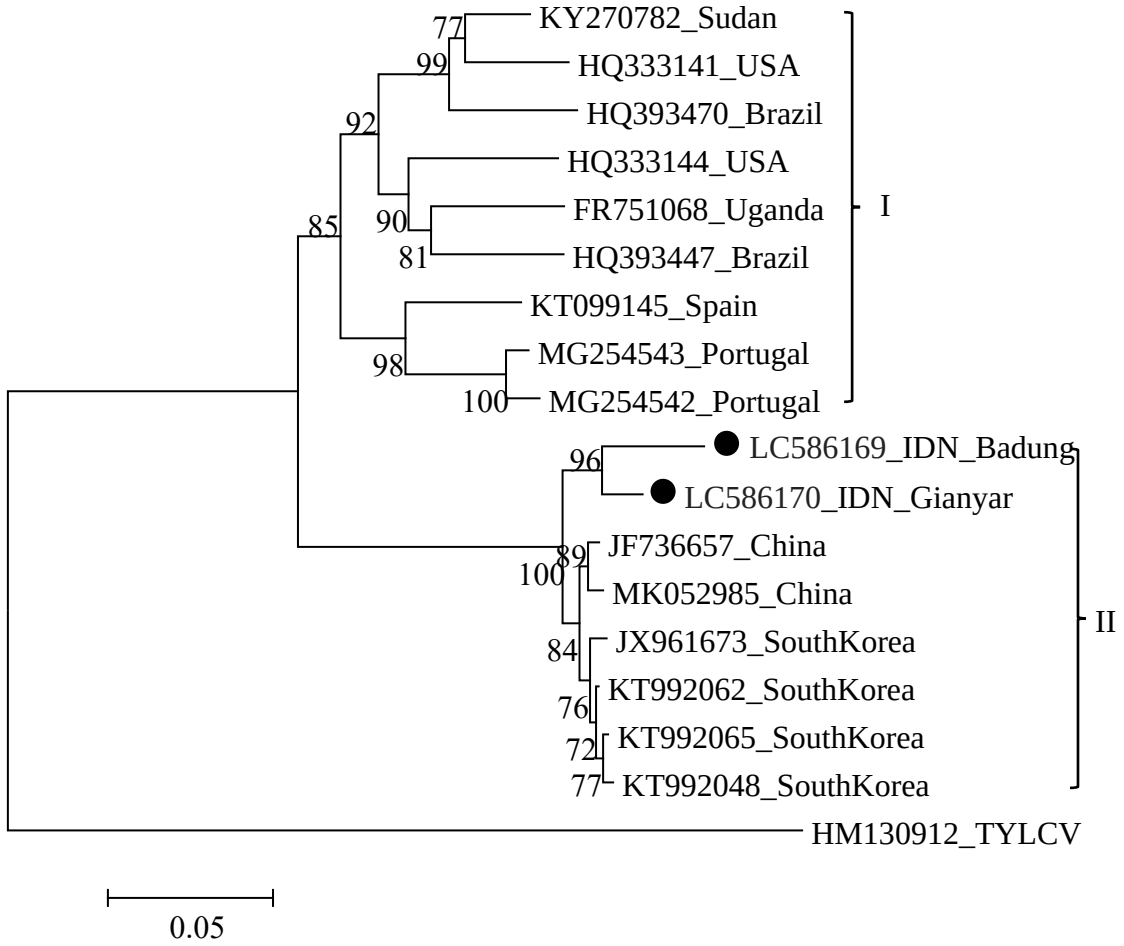

Figure 3 Phylogeny tree of the AC1 and AC2 gene of SPLCV Bali isolate; nucleotide sequences of SPLCV using *Tomato yellows leaf curl virus* (TYLCV) as out group. IDN-Indonesia

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Table 1 Comparison of the Bali SPLCV isolate's nucleotide (nt) and amino acid (aa) sequences to isolates from other countries found in GenBank.

Isolate	Geographical origin	Host	Symptoms	Homology (%)				Accession number
				Badung		Gianyar		
				nt	aa	nt	aa	
Abs-1	Badung, Bali, Indonesia	Ipomoea batatas	Vein clearing			97.8	98.8	LC586169
Ubud-1	Gianyar, Bali, Indonesia	Ipomoea batatas	Vein clearing	97.8	98.8			LC586170
Hu-194	Hunan, China	Ipomoea batatas	Unknown	97.0	98.2	97.2	98.4	MK052985
ZJ	Zhejiang, China	Ipomoea setosa	Leaf curling	94.3	96.2	96.5	97.4	JF736657
202	South Korea	Ipomoea batatas	Leaf curling	94.0	96.2	96.1	97.1	KT992065
169	South Korea	Ipomoea batatas	Leaf curling	96.2	97.3	96.4	97.6	KT992062
GE-21	Muan, South Korea	Ipomoea batatas	Unknown	94.0	96.2	96.0	97.1	JX961673
7	South Korea	Ipomoea batatas	Leaf curling	93.6	95.7	95.7	96.9	KT992048
Sp3-2	Spain	Unknown	Unknown	87.4	89.8	89.0	90.9	KT099145
P213-11	Southern Portugal	Ipomoea indica	Vein clearing	87.0	89.2	88.6	90.2	MG254543
P213-8	Southern Portugal	Ipomoea indica	Vein clearing	86.9	88.4	88.3	90.0	MG254542
409	Khartoum, Sudan	Ipomoea batatas	Lef curling	87.6	89.6	88.8	90.4	KY270782
Uk-2008	Kampala, Uganda	Ipomoea setosa	Leaf curling	87.3	89.6	88.8	90.4	FR751068
648B-9	South Carolina, USA	Ipomoea batatas	Leaf curling	87.0	89.2	88.2	90.0	HQ333144
BR-Uti-08	Bahia, Brazil	Ipomoea batatas	Leaf curling	86.5	88.7	88.2	90.0	HQ393447
WS1-4	South Carolina, USA	Ipomoea setosa	Leaf curling	87.5	89.8	88.5	90.2	HQ333141
MP3-09	Pernambuco, Brazil	Ipomoea batatas	Leaf curling	86.8	87.6	87.8	89.7	HQ393470
*TYLCV	Masan, South Korea	Lycopersicon esculentum	Leaf curling	63.8	67.2	66.5	69.6	HM130912

*TYLCV: *Tomato yellows leaf curl virus* as *out group*; nt (nucleotide) and aa (amino acid)

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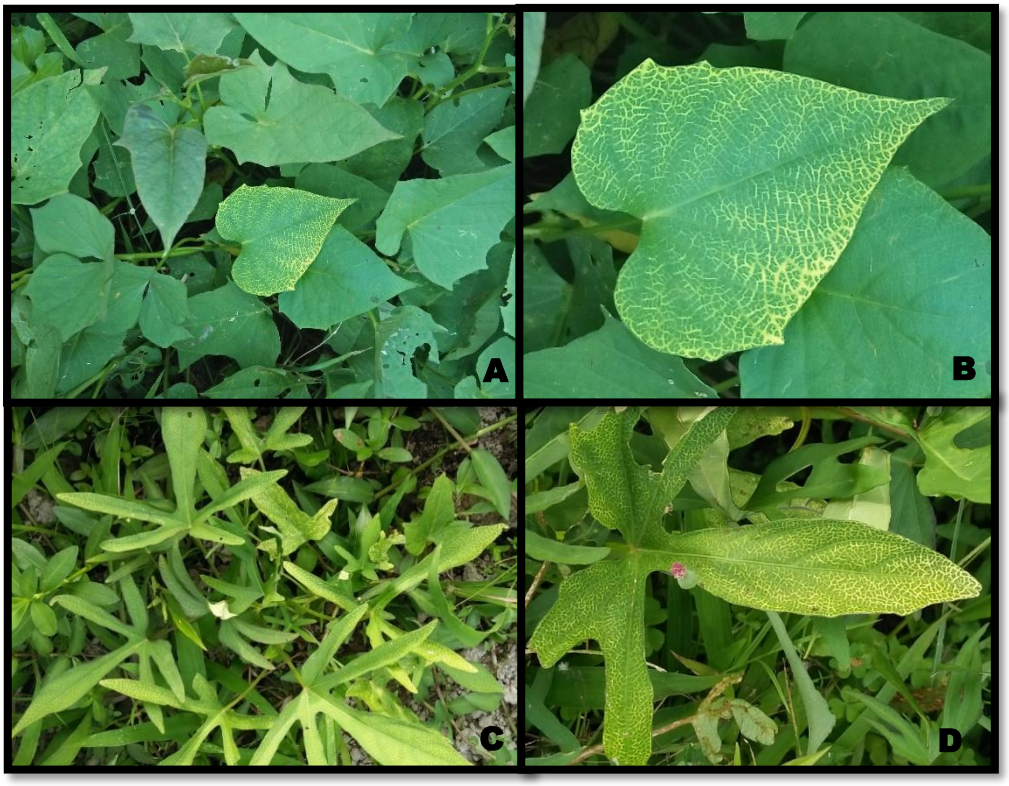

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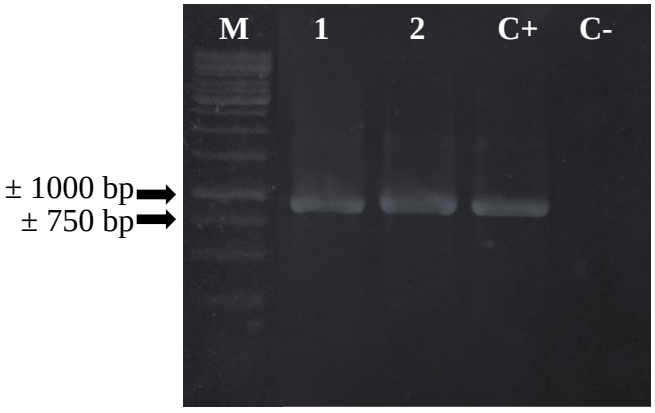

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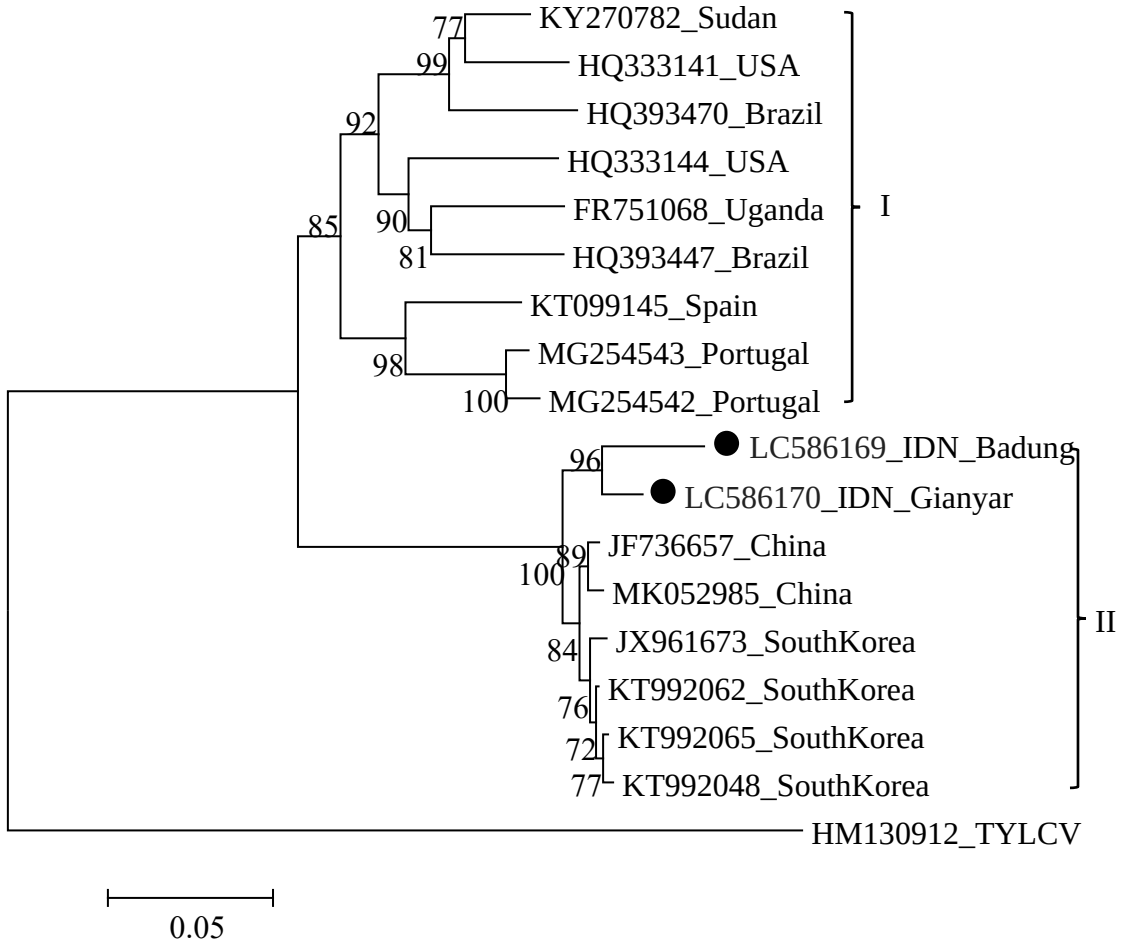

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Table 1 Comparison of the Bali SPLCV isolate's nucleotide (nt) and amino acid (aa) sequences to isolates from other countries found in GenBank.

Isolate	Geographical origin	Host	Symptoms	Homology (%)				Accession number
				Badung		Gianyar		
				nt	aa	nt	aa	
Abs-1	Badung, Bali, Indonesia	Ipomoea batatas	Vein clearing			97.8	98.8	LC586169
Ubud-1	Gianyar, Bali, Indonesia	Ipomoea batatas	Vein clearing	97.8	98.8			LC586170
Hu-194	Hunan, China	Ipomoea batatas	Unknown	97.0	98.2	97.2	98.4	MK052985
ZJ	Zhejiang, China	Ipomoea setosa	Leaf curling	94.3	96.2	96.5	97.4	JF736657
202	South Korea	Ipomoea batatas	Leaf curling	94.0	96.2	96.1	97.1	KT992065
169	South Korea	Ipomoea batatas	Leaf curling	96.2	97.3	96.4	97.6	KT992062
GE-21	Muan, South Korea	Ipomoea batatas	Unknown	94.0	96.2	96.0	97.1	JX961673
7	South Korea	Ipomoea batatas	Leaf curling	93.6	95.7	95.7	96.9	KT992048
Sp3-2	Spain	Unknown	Unknown	87.4	89.8	89.0	90.9	KT099145
P213-11	Southern Portugal	Ipomoea indica	Vein clearing	87.0	89.2	88.6	90.2	MG254543
P213-8	Southern Portugal	Ipomoea indica	Vein clearing	86.9	88.4	88.3	90.0	MG254542
409	Khartoum, Sudan	Ipomoea batatas	Lef curling	87.6	89.6	88.8	90.4	KY270782
Uk-2008	Kampala, Uganda	Ipomoea setosa	Leaf curling	87.3	89.6	88.8	90.4	FR751068
648B-9	South Carolina, USA	Ipomoea batatas	Leaf curling	87.0	89.2	88.2	90.0	HQ333144
BR-Uti-08	Bahia, Brazil	Ipomoea batatas	Leaf curling	86.5	88.7	88.2	90.0	HQ393447
WS1-4	South Carolina, USA	Ipomoea setosa	Leaf curling	87.5	89.8	88.5	90.2	HQ333141
MP3-09	Pernambuco, Brazil	Ipomoea batatas	Leaf curling	86.8	87.6	87.8	89.7	HQ393470
*TYLCV	Masan, South Korea	Lycopersicon esculentum	Leaf curling	63.8	67.2	66.5	69.6	HM130912

*TYLCV: *Tomato yellows leaf curl virus* as *out group*; nt (nucleotide) and aa (amino acid)

COVERING LETTER

Dear **Editor-in-Chief,**

I am here with enclosed a research article,

Title:

The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Population dynamics and damage intensity of brown planthopper (BPH) and *Nilaparvata lugens* (Stal.) provide information about the economic threshold status of *N. lugens* in Bali. This study enriched information on the genetic diversity of *N. lugens* isolates from eastern Indonesia, particularly Bali. It is the first report of *N. lugens* biotype Y in Indonesia. In addition, the latest information in this study is that rice varieties Situbagendit and Inpari 32, which were previously resistant to BPH, are now found to be susceptible to BPH. This information is critical as a basis for controlling *N. lugens* in Indonesia

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Denpasar July 2022

Sincerely yours,

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Listihani Listihani

The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

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Abstract. The brown planthopper is an important pest on rice crops in Indonesia. The genetic diversity of BPH isolates in western Indonesia has been extensively reported, whereas eastern Indonesia isolates have not been reported. This research aims to analyze genetic diversity and evaluate the BPH attack's intensity on Bali rice plants. The research method used was an observation of attack percentage, population dynamics, attack intensity, and genetic diversity of BPH in 9 districts in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). Molecular identification was carried out on *N. lugens* DNA in the mtCOI fragment. BPH attacks of >50% were found in the districts of Gianyar, Bangli, Jembrana, and Badung. The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill. In general, rice varieties grown in all observation locations were susceptible to BPH, such as Ciherang, IR-64, Inpari 32, and Situbagendit. In the Ciherang and IR-64 varieties, the highest attack intensity average value reached 30%. The sequence of *N. lugens* isolate from Bali Jembrana showed the highest nucleotide and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y of 99.5 -99.74% and 100%, respectively. This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. This study reported that Rice varieties Situbagendit and Inpari 32, previously resistant to BPH, are reported as susceptible to BPH.

Keywords: attack intensity, genetic diversity, Inpari 32, Situbagendit, susceptible variety

Abbreviations (if any): The BPH, Rice Plants

Running title: The Brown Planthopper (*Nilaparvata lugens* Stal.) Attack and Its Genetic Diversity on Rice in Bali, Indonesia

INTRODUCTION

The brown planthopper (BPH) (*Nilaparvata lugens* Stal, Hemiptera: Delphacidae) is the most destructive rice pest in Indonesia. Repeated outbreaks of BPH in Indonesia are caused by continuous rice cultivation, extensive use, and over-application of insecticides (Baehaki 2012). This pest is vascular monophagous in rice (Cheng et al. 2013; Ferrater et al. 2013). Feeding by nymphs and imago at the base of the plant causes rapid wilting and drying of the plant (Bottrell and Schoenly 2012; Cheng et al. 2013; Bao and Zhang 2019). In addition, BPH is also a vector of *Rice grassy stunt virus* and *Rice ragged stunt virus* (Bao and Zhang 2019). At high population levels of *N. lugens* can cause significant losses in rice production (Cheng et al. 2013; Zheng et al. 2013; Bao and Zhang 2019).

The BPH cannot tolerate winter in northern Asia, including Japan, Korea, and northern China (He et al. 2012; Fu et al. 2012; Fu et al. 2014). The population originally came from subtropical and tropical areas by flying long distances during the summer (Fu et al. 2014; Hu et al. 2014). BPH infestation in temperate climates originated from annual migrations from tropical Asia and China (He et al. 2012). During autumn, BPH re-migrates (north-to-south) and BPH populations have been studied in China and India (Bottrell and Schoenly 2012). Such return migration may help explain how long-distance migration is maintained in the winter.

The intensification of rice production triggered the BPH outbreak in Tropical Asia during the green revolution era in the 1970s and 1980s (Bottrell and Schoenly 2012). Until now, *N. lugens* is the main problem causing rice harvest failure in several countries. Inaccurate identification and prolonged identification of *N. lugens* are obstacles to its field management strategy.

Traditionally, BPH has been identified at the species level by morphological features using anatomical characteristics, namely, wings, front, and external genitalia (Lv et al. 2015). Accurate identification requires extensive expertise and experience and yet sometimes can lead to errors. Morphological identification by an entomologist can reduce the potential for errors. Practical morphological identification is only possible when dealing with small sample sizes and

well-preserved specimens. Therefore, it is crucial to utilize a new identification method that is accurate, fast, time-saving, and suitable for large numbers of specimens.

Molecular techniques with high reproducibility and fast results offer an excellent alternative to traditional morphological classification. Several mitochondrial and nuclear genes have been used as genetic markers to differentiate related species. These include the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, nuclear 12S-16S-18S ribosomal RNA genes, and ITS1 and ITS2 internal transcription spacers (Yu et al. 2014; Brengues et al. 2014; Gomez-Polo et al. 2014; Wang et al. 2016; Zheng et al. 2021). ITS1 and ITS2 are nonfunctional spacers that separate the 18S-5.8S and 5.8S-28S rRNA genes, respectively (Wang et al. 2016; Zheng et al. 2021). As ITS sequences have low intra-species variation but high variation between species, they are helpful for species classification and phylogenetic analysis for morphologically similar organisms, both in prokaryotes and eukaryotes (Zheng et al. 2021). Finally, from the molecular identification of the combined mitochondrial COI-COII and ten microsatellite marker loci (Winnie et al. 2020).

The genetic diversity of *N. lugens* has been reported in several countries such as China, South Korea, Pakistan, India, and Malaysia (Jing et al. 2012; Zheng et al. 2021; Anant et al. 2021; Latif et al. 2012). The genetic diversity of *N. lugens* in Indonesia is widely reported in western Indonesia (Java Island) (Winnie et al. 2020; Chaerani et al. 2021). Reports on the genetic diversity of *N. lugens* in eastern Indonesia have not been found. Therefore, this study aims to analyze genetic diversity and determine the intensity of BPH attacks on rice plants in eastern Indonesia, especially Bali.

MATERIALS AND METHODS

Brown Planthopper Sampling from Rice Dwarf Disease Endemic Areas

Samples were taken from nine locations in Bali Province (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana). The brown planthopper samples taken from rice plants were nymphs and imagos. Nymphs and imagos were used for total DNA extraction. After arriving at the laboratory, the nymphs and imago were stored dry at -20ºC.

Observation of BPH Attack Symptoms and Quantity of BPH Population/rice hill

Observation of symptoms of BPH attack was carried out by observing symptoms of damage to rice plants due to BPH attack. The abundance of the BPH/rice hill population was obtained by counting all nymphs and imagoes obtained. Data on the population per cluster from 20 samples at each observation location were then averaged.

BPH Attack Percentage

The percentage of BPH attacks is calculated using the following formula:

$$P = \frac{a}{b} x\ 100\%$$

Note:
P = Attack percentage (%)
a = Number of rice hills affected by BPH
b = Number of rice hills observed

Damage Intensity

Determination of scoring on symptoms of rice damage due to BPH attack is based on Table 1. The intensity of damage due to a BPH attack is determined using the formula (Erdiansyah and Damanhuri 2018):

$$I = \sum_{i=1}^{i} \frac{Ni\ x\ Vi}{N\ x\ Z} x\ 100\%$$

Note:
I = Damage intensity
Ni = The number of affected rice hills on the score i
Vi = Score i
N = The number of rice hills observed
Z = Highest score

Total DNA Extraction from Brown Planthopper

Total DNA extraction of brown planthopper was obtained from one individual imago or one individual nymph based on the modified method of Goodwin et al. (1994). One individual imago was put into a microtube and then added

with 100 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl, 100 mM Tris-HCI, 20 mM EDTA, and 1% PVP (-40 °C)). Next, 1 µl of proteinase K was added, then the insects were crushed using a micro-pistil, vortexed, and incubated in a water bath of 65°C for 3 minutes. After that, the tube was added with 100 µl CI (chloroform: isoamyl alcohol) in a ratio of 24:1. The tube was then vortexed for 3 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant formed was transferred to a new microtube (60 µl) and then added with 3 M NaOAc (pH 5.2), as much as 1/10 of the total volume of the supernatant. Isopropanol was added up to 2/3 of the total volume of the supernatant, then incubated at -20°C for one night. The tube was centrifuged at 10,000 rpm for 10 min, and the supernatant was discarded. The pellets were washed with 100 µl of 80% ethanol (cold) and centrifuged at 8000 rpm for 5 minutes. In the final step, the supernatant was removed, and the pellet was dried for approximately 1 hour. It was then added with a solution of 20 µl TE and stored at -20°C until used.

Amplification of mtCOI Fragments Using the PCR Method

PCR reactants were manufactured with a total volume of 25 µl consisting of 12.5 µl Go Tag Green Master Mix (Promega, US) and 9.5 µl ddH2O. DNA amplification of the mtCOI fragment was carried out using a pair of universal primers mtCOI LCO 1490 (3'-GGTCAACAAATCATAAAGATATTGG-5') and HCO 2198 (5'TAAACTTCA GGGTGACCA AAAAATCA-3') (Folmer et al. 1994) each 1 µl, and 1 µl DNA template. PCR reactions were carried out with a Perkin Elmer 480 Thermocycler (Applied Biosystem, US). The PCR reaction was initiated by initial denaturation for 5 min at 94°C. The PCR was continued for 35 cycles in the following order: 94°C for 1 minute, 52°C for 35 seconds, 72°C for 1 minute 30 seconds, and a final extension of 72°C for 7 minutes. The PCR results were then analyzed in 1% agarose gel. The DNA fragments of mtCOI were visualized using a UV transilluminator after being immersed in a 2% ethidium bromide solution for 15 minutes and photographed with a digital camera. The result of amplification by PCR technique was in the form of mtCOI DNA fragments with a size of ± 710 base pairs (pb).

Analysis of DNA Sequence Results

Nucleotide Sequencing DNA fragment purification and mtCOI nucleotide sequencing were performed at PT. 1st Base, Malaysia. The results were then registered in the NCBI gene bank (http://www.ncbi.nlm.nih.gov). Analysis of mtCOI DNA sequence data ChromasPro program was used to combine forward and reverse nucleotide sequences to obtain the mtCOI gene (ChromasPro version 2.01. 2006). The Bioedit program was used to compare mtCOI fragments between samples (Multiple alignments). The phylogenetic relationship was built by comparing the mtCOI sample fragments from the brown planthopper from Indonesia with the mtCOI fragments already stored in the NCBI GenBank (http://www.ncbi.nlm.nih.gov). The criteria for retrieving mtCOI fragments at GenBank were fragments with a nucleotide base length of ± 800 bp (Boykin et al. 2007) (Table 1). The phylogenetic tree was constructed using the PAUP 4.0b10 program with the maximum parsimony cladistic quantitative method. The cladogram was compiled using the Heuristic method. The cladogram used results from the strick consensus with the statistical bootstrap test to obtain a 100% probability.

RESULTS AND DISCUSSION

The brown planthopper causes direct and indirect damage to rice plants. Direct damage was in the form of stunted and uneven growth of rice plants (Figure 1A and 1B), yellow plants (Figure 1C), and hopperburn caused by fluid in rice plant cells sucked by BPH nymphs, brachiptera (Fig. short wings), and macroptera (long wings) (Figures 1E and 1F). Indirect damage was caused by BPH, which acts as a vector of grass dwarf virus and empty dwarf virus, causing stunted rice plants (Figure 1D). Besides Bali or other parts of Indonesia, BPH attacks on rice crops were also reported in China, where hopperburn affected 60% of all examined crops (Hu et al. 2014).

A percentage of BPH attacks of more than 50% was found in Gianyar, Bangli, Jembrana, and Badung Regencies (Table 2). The BPH population was primarily found in Ciherang and IR-64 varieties of rice in the Badung Regency, with 43.67 BPH per rice hill (Table 2). Baehaki (2012) added that the economic threshold could be measured through the number or population of pests and planting age. BPH is said to have reached the economic threshold when the population of this pest was found in the field, as many as nine BPH per rice hill when the rice age was less than 40 DAP or 18 BPH when the rice was more than 40 DAP (Baehaki 2012). In general, rice varieties grown in all observation locations in Bali were BPH susceptible varieties, such as Ciherang, IR-64, Inpari 32, and Situbagendit.

The dynamics of BPH development in the field can be influenced by several factors, including host plant factors and natural enemies (Ferrater et al. 2015; Horgan et al. 2015; Kobayashi 2016). The host plant factors that affect the BPH population are related to the age of the rice plant. When the observations were made, the rice plants were still in the vegetative phase, aged 4-6 WAP. According to Jing et al. (2014), naturally, BPH usually comes to young rice fields, and insects usually come in the first two weeks after planting. Thus, the brown planthopper in rice cultivation might be the first generation of planthoppers that have not yet reproduced because one BPH life cycle takes between 3-4 weeks (IRRI 2009).

BPH observations in Denpasar, Tabanan, Karangasem, and Klungkung cities were dominated by macroptera imago (Table 2). According to Horgan et al. (2017), the planthopper that first came to the plantation was the macroptera

planthopper as a winged immigrant planthopper. Meanwhile, in Badung, Gianyar, Buleleng, Bangli, and Jembrana regencies, nymphal BPH was dominated by BPH, and several individuals were in the imago phase of brachiptera and macroptera. The dominance of the nymph phase caused the population of BPH in Badung, Gianyar, Buleleng, Bangli, and Jembrana districts to be the highest when compared to the cities of Denpasar, Tabanan, Karangasem, and Klungkung. The presence of the brachiptera planthopper might be contributed to the increase in the nymph population (Baehaki 2012). According to Horgan et al. (2015), rapid population growth usually occurs in groups with many young individuals.

The average intensity of BPH attack on Ciherang and IR-64 varieties of rice was higher than in other varieties. In the Ciherang and IR-64 varieties of rice, the average value of the highest attack intensity was 30% (Figure 2). It is because farmers grow rice varieties Ciherang and IR-64 from year to year without any replacement of other varieties. Furthermore, rice varieties Ciherang and IR-64 became very susceptible to BPH attacks. In addition, BPH is a pest that begins to attack rice plants from a young age, even when the rice is still in the nursery.

According to Vu et al. (2014), fluctuations in BPH pest attacks are more influenced by the growth phase of the rice plant that is the host in the field. BPH pests are often found when rice plants are in the vegetative and generative stages (Bottrell and Schoenly 2012). Horgan et al. (2017) added that BPH pests could damage rice plants at all stages of growth and act as vectors for grass and dwarf viruses. BPH attack was higher when rice was in the vegetative phase than in the generative phase (Horgan et al. 2015). It happens because the pests attack the young rice stalks. Considering the type of mouth of BPH, which is included in the suction, BPH can suck the liquid from the rice stems and cause the plant leaves to turn yellow (Anant et al. 2021). According to Choi et al. (2019), during the vegetative phase, food availability in the form of nitrogen is abundant in rice plants. Rice plants need nitrogen to form plant organs. Food is one of the factors that affect the life of insects. Horgan (2018) continued that the N element absorbed by plants also serves as a source of nutrition for BPH. If food is available with good quality (suitable for pests), then the insect pest population will increase, and vice versa (Horgan 2018).

The mtCOI DNA band was only successfully amplified from the total DNA extraction of one imago or nymph and not more than one BPH imago. The mtCOI fragment that was successfully amplified corresponds to a size of ±710 bp in all samples from nine districts in Bali, namely Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana (Figure 3). Nucleotide and amino acid sequence analysis showed high homology with *N. lugens* sequences in the database at GenBank, 94.2 – 99.7% and 95.8 - 100%, respectively (Table 3). *N. lugens* sequences from Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana showed the highest nucleotide, and amino acid homology with *N. lugens* isolate FSD-034 from Pakistan (MK301229) biotype Y, respectively. 99.5 -99.74% and 100% (Table 3). The results of the molecular detection of *N. lugens* using the PCR method in Bali, Indonesia, are the first reports of the molecular character of *N. lugens* in Indonesia.

Samples from Indonesia formed a group with *N. lugens* biotype Y fragment mtCOI from Pakistan, India, South Korea, and China (Figure 4). This study found *N. lugens* biotype Y in rice plants for the first time in Indonesia. The Indonesian sample did not form separate groups according to the proximity of the district locations but formed a polytomy cladogram (Figure 4). This polytomy cladogram shows that the *N. lugens* between regencies (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) were observed to have the same ancestry. These results indicate high locomotion ability with genetic mixing between *N. lugens* in Bali isolates. Similar conditions were also demonstrated in *N. lugens* among Asian isolates using mitochondrial sequences showing genetic mixing. It can also be correlated with the theory of long-distance migration of *N. lugens*, which migrates from the tropics (northern Vietnam) in April-May to temperate regions (China, Korea, and Japan) in June-July as shown based on meteorological studies (Otuka et al. 2008). The population of *N. lugens* is a long-distance migratory flight from the tropics to temperate Asia before modern pesticides are widely used in tropical rice. Due to the infrequent use of insecticides prior to the 1960s in the tropics, factors other than insecticides may have triggered long-wing movements to form *N. lugens* populations (Bottrell and Schoenly 2012).

In previous studies in Indonesia, BPH biotypes 1, 2, 3, and 4 have been found. Kobayashi et al. (2014) reported that the brown planthopper is a highly adaptive insect because it can form new biotypes. In early 1975 the IR-26 rice variety from IRRI Philippines was introduced. The IR-26 variety was unique because it contained a Bph1 resistant gene to anticipate fluctuations in the brown planthopper population. However, in 1976 there was a great population explosion in several rice production centers due to changes in the brown planthopper population from biotype 1 to biotype 2. As an anticipatory measure against brown planthopper biotype 2, in 1980, the IR-42 rice variety (containing the bph2 resistant gene) was introduced from IRRI Philippines. Unfortunately, in 1981 there was another explosion in the brown planthopper population in Simalungun, North Sumatra, and several other areas due to changes in the brown planthopper population from biotype 2 to biotype 3. To deal with the brown planthopper biotype 3, rice variety IR-56 was introduced (containing the gene bph3 resistance) in 1983 and IR-64 (containing the bph1+ resistance gene) in 1986. The introduction process continues. In 1991, the IR-74 variety (containing the bph3 resistant gene) was introduced. In 2006, the resistance gene IR-64 was broken because the brown planthopper population changed to biotype 4. The stability of the biotype zero brown planthoppers persisted for 41 years before becoming brown planthopper biotype 1. The change of brown planthopper biotype 1 to biotype 2 only took 4 years, and the change of biotype 2 brown planthopper to biotype 3 within 5 years. Until 2005, the brown planthopper biotype 3 was still dominated by biotype 3, and in 2006 the biotype 4 brown planthopper began to develop. The long existence of the biotype 3 brown planthopper was caused by the development of the IR-64

(bph1+) variety over a long period. IR-64 is a resistant variety (durable resistance) that can withstand changes in brown planthoppers to a more virulent biotype.

The continuous cultivation of IR-64 rice varieties by farmers in Bali led to the emergence of a new biotype BPH, namely Y. Insects of biotype Y originated from biotype 1 by eating YHY15 resistant varieties for more than two years for 33 generations (Jing et al. 2012). Rice varieties YHY15 carry the Bph15 resistance gene (Jing et al. 2012).

This study shows great potential in the population of *N. lugens* to adapt to previously resistant rice varieties. This study reported that rice varieties Situbagendit and Inpari 32, previously resistant to BPH, were susceptible to BPH. This research can provide information to farmers not to continuously plant susceptible varieties, which could cause BPH epidemics in the field, as well as the emergence of new, more virulent BPH biotypes. Thus a new control strategy based on a forecasting system can be developed for the regional management of this insect.

CONCLUTIONS

N. lugens that attacks rice plants in Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) belongs to biotype Y. Symptoms of damage to rice plants are most severe in Badung Regency. Apart from Ciherang and IR-64 varieties, Situbagendit and Inpari 32 varieties are susceptible to BPH attack.

ACKNOWLEDGEMENTS

This research was funded by PDKN program provided by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for Listihani and team through PDKN Scheme with contract no. 160/E5/PG.02.00.PT/2022.

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TABLES LIST

Table 1. The damage score of rice plants due to BPH attack

Score	Appearance	Description
0	Healthy	No planthopper was found in any rice hill.
1	Very light damage	The rice hills occupied by the planthoppers did not show dead midribs, few exuviae, and the rice stalks had not yet overgrown with Dematium and Cladosporium fungi that followed the brown planthopper attack.
3	Slightly damaged	The rice hills occupied by the planthoppers have shown dead midribs, many exuviae, and the rice stems are overgrown with Dematium and Cladosporium fungi that follow the brown planthopper attack.
5	Heavily damaged	Rice hills inhabited by planthoppers showed damage marked by many dead midribs, many exuviae, stunted and black-looking tillers, and overgrown with Dematium and Cladosporium fungi.
7	Partially dead	Some of the stems in the rice hill die, or the rice hill withers due to planthoppers attack.
9	Hopperburn	Rice hills die from hopperburn

Sumber: Baehaki (2012)

Table 2. Population and symptoms of BPH attack on rice plants in Bali

Location	**Rice varieties**	**Rice plant age (DAP)**	**BPH attack percentage (%)**	**BPH population abundance (individues/rice hills)**
Denpasar City	Situbagendit, Inpari 32	35	35.43	7.41
Badung	Ciherang, IR-64	42	73.61	43.67
Gianyar	Ciherang, Inpari 32	45	52.26	12.49
Tabanan	Inpari 32	41	37.94	9.26
Buleleng	Ciherang, IR-64	33	46.82	11.28
Karangasem	Situbagendit	30	32.73	7.92
Klungkung	Inpari 32	43	35.89	8.53
Bangli	Ciherang, IR-64	42	52.80	14.83
Jembrana	Ciherang, Inpari 32	36	57.32	11.95

Note: DAP= day after planting

Table 3. Nucleotide (nt) and amino acid (aa) homology of *N. lugens* in rice from Bali, Indonesia, compared with *N. lugens* from other countries in GenBank

Isolate	Origin of isolate	Biotype	Accession number	Homology nt (aa) (%) *N. lugens*_IDN_								
				Denpasar	Badung	Gianyar	Tabanan	Buleleng	Karangasem	Klungkung	Bangli	Jembrana
FSD-034	PAK	Y	MK301229	99.5 (100)	99.6 (100)	99.5 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.7 (100)	99.5 (100)	99.6 (100)
HZZ55	IND	Y	MK032794	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.6 (100)	99.5 (100)	99.6 (100)
SAEVG_Morph0111	IND	Y	MN520923	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.4 (100)	99.5 (100)	99.5 (100)	99.5 (100)	99.6 (100)
KBPH	KOR	Y	MK590088	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
KOREA_BPH	KOR	Y	LC461184	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.4 (100)	99.5 (100)
WUHAN-Y	CHN	Y	KC333653	99.3 (100)	99.5 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)	99.4 (100)	99.3 (100)	99.4 (100)
WUHAN-3	CHN	3	JN563997	97.8 (98.1)	97.2 (97.8)	97.5 (98.9)	97.5 (98.9)	97.4 (97.9)	97.8 (98.1)	97.5 (98.9)	97.2 (98.8)	97.6 (98.0)
WUHAN-2	CHN	2	JN563996	96.3 (97.5)	96.3 (97.5)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.4 (97.5)	96.3 (97.5)	96.2 (97.4)	96.3 (97.5)
WUHAN-1	CHN	1	JN563995	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)	95.4 (96.7)	95.6 (96.8)	95.6 (96.8)	95.3 (96.7)	95.4 (96.7)	95.3 (96.7)
GX	CHN	1	LC461186	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.4 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
Gangavathi	IND	1	OL451531	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)	95.5 (96.8)	95.5 (96.8)	95.3 (96.7)	95.3 (96.7)	95.3 (96.7)
WUHAN-L	CHN	L	KC333654	94.2 (95.8)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.3 (96.0)	94.4 (96.2)	94.2 (95.8)	94.4 (96.2)	94.4 (96.2)
N. bakeri	CHN	-	JX266790	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	84.8 (85.9)	84.6 (85.6)	85.2 (86.1)	84.8 (85.9)	85.2 (86.1)	85.2 (86.1)
Sogatella furcifera	CHN	-	HM160123	75.6 (76.9)	75.6 (76.9)	76.2 (77.8)	77.6 (78.4)	77.4 (78.4)	76.8 (77.8)	75.6 (76.9)	76.8 (77.8)	77.6 (78.4)

Notes: nt (nucleotide), aa (amino acid), IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), CHN (China), *N. bakeri* and *Sogatella furcifera* from China was used as outgroups

FIGURES LIST

Figure 1. Symptoms of BPH attack on rice plants in Bali: A. rice plant growth is stunted; B. uneven plant growth (spots); C. yellow plant; D. dwarf rice plants; E. plants die like burning (hopperburn); F. BPH brachiptera and macroptera were found on rice stalks.

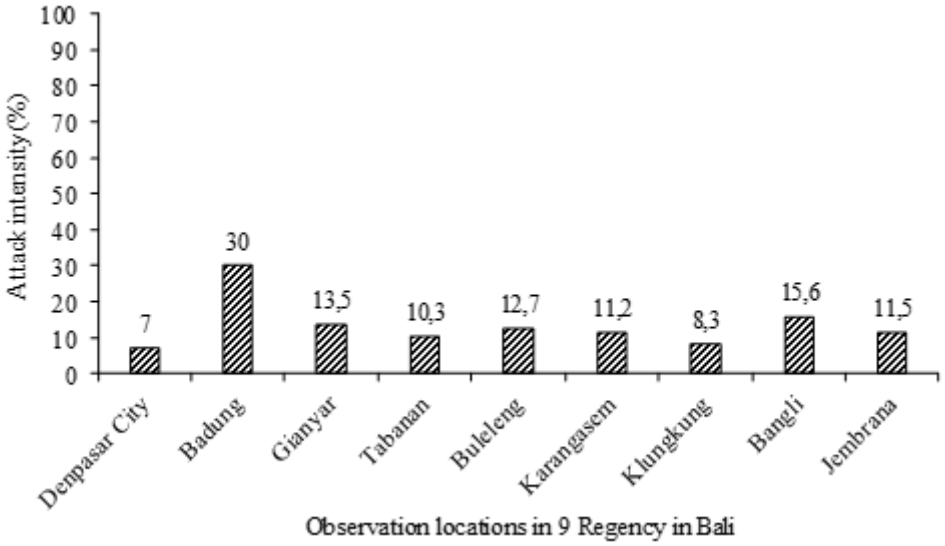

Figure 2. The attack intensity of *N. lugens* on rice in Bali Province

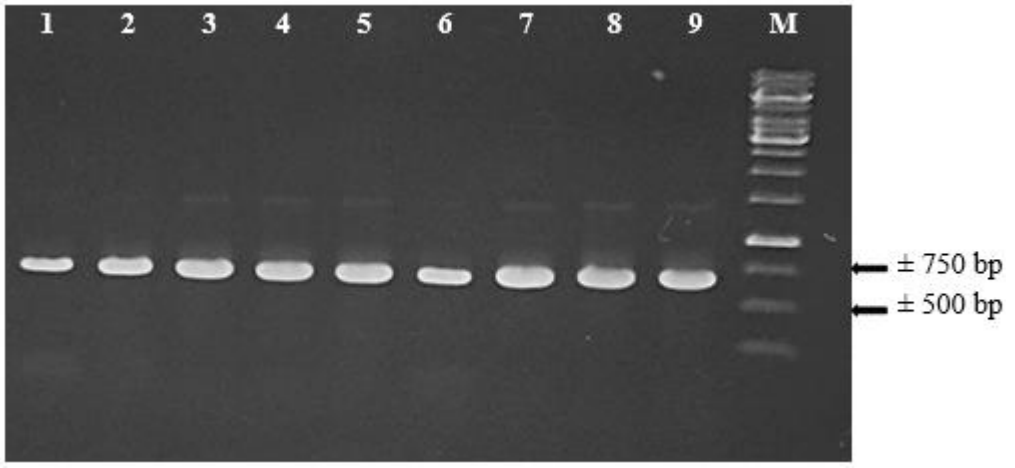

Figure 3. DNA amplification of *N. lugens* in rice plants in Bali using primers LCO 1490/HCO 2198. 1. Denpasar City, 2. Bagung, 3. Gianyar, 4. Tabanan, 5. Buleleng, 6. Karangasemt, 7. Klungkung, 8. Bangli, 9. Jembrana, and M. DNA marker 1 kb (Thermo Scientific)

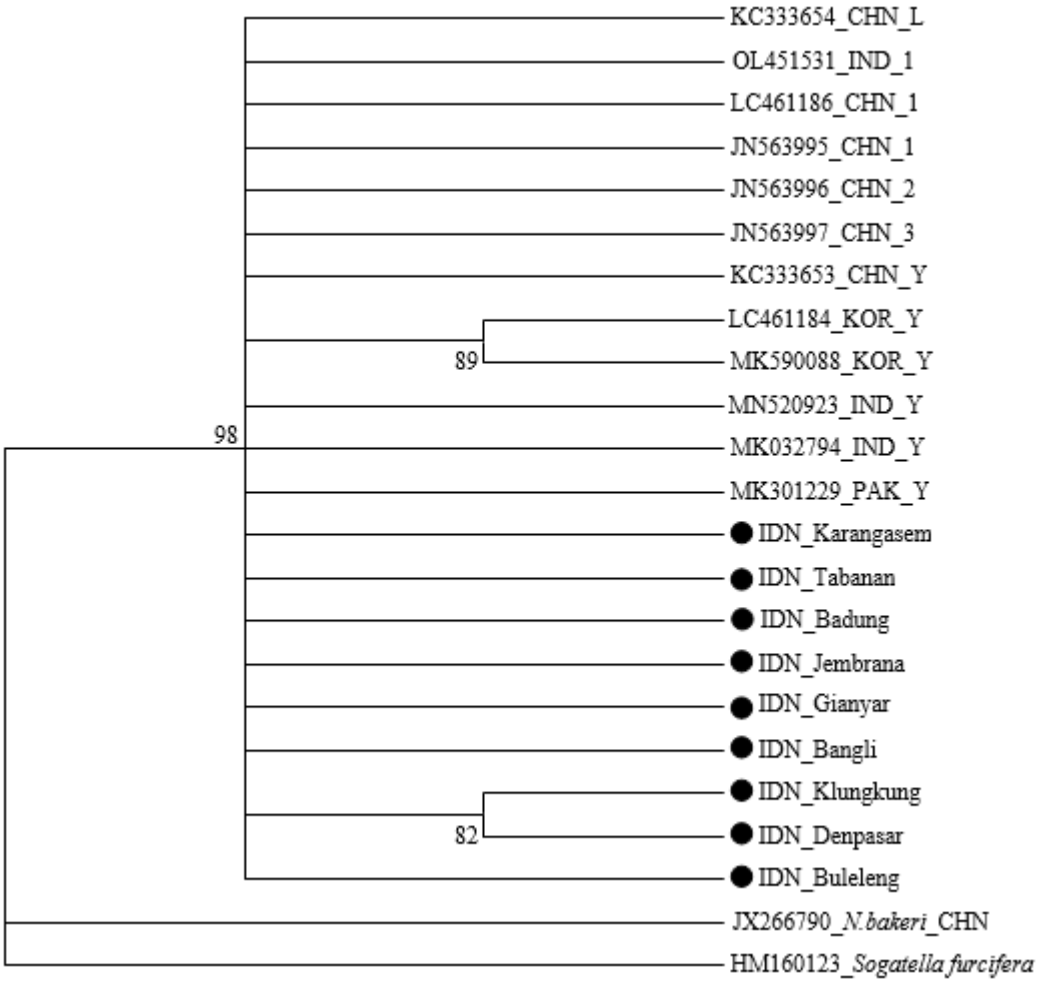

Figure 4. The cladogram of the mtCOI fragment of *N. lugens* from eastern Indonesia, Bali (Badung, Gianyar, Klungkung, Bangli, Karangasem, Tabanan, Denpasar City, Buleleng, and Jembrana) was compared with mtCOI fragments from several regions of the world that had been deposited on the NCBI website. *N. bakeri* and *Sogatella furcifera* from China were used as outgroups. The numbers on the branching cladograms represent bootstrap values with 100% probability. IDN (Indonesia), PAK (Pakistan), IND (India), KOR (South Korea), and CHN (China), isolates marked with black dots are Bali isolates.

SUBMISSION CHECKLIST

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Indian Phytopathology

First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia

--Manuscript Draft--

Manuscript Number:	
Full Title:	First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia
Article Type:	Short Communication
Manuscript Classifications:	400: Virology
Funding Information:	Lembaga Penelitian dan Pengabdian Kepada Masyarakat (K.100/B.01.01/LPPM-UNMAS/V/2021) — Dr. Listihani Listihani
Abstract:	During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. To confirm the vein yellowing symptoms, we extracted total DNA, PCR, subcloned the PCR products using TA cloning and sequence analyzed the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia. Key words: Sweet potato leaf curl virus · Sweetpotato · Begomovirus · Yellowing ·
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DISEASE NOTE

First report of *Sweet potato leaf curl virus* (SPLCV) on *Ipomoea batatas* in Bali, Indonesia

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Total text pages: 8

The numbers of tables: 1

The numbers of figures: 3

The nucleotide sequence reported is available in the DDBJ/EMBL/GenBank databases under accession number LC586169 and LC586170

Abstract

During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. To confirm the vein yellowing symptoms, we extracted total DNA, PCR, subcloned the PCR products using TA cloning and sequence analyzed the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia.

Key words: *Sweet potato leaf curl virus*·Sweetpotato·*Begomovirus*·Yellowing·

Sweetpotato (Ipomoea batatas) many producted in Asia, particularly in the Far East Asia and Southeast Asia countries. In Indonesia, sweetpotato becomes staples food particularly in Eastern Indonesia. Food diversification is a solution to the problem of food needs in Indonesia. Sweetpotato production in Indonesia in 2015, 2016 and 2017 amounted to 2298, 2169, and 2023 tons (Central Bureau of Statistics 2017). Based on these data, production tends to decline every year. In 2019, during a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves in Badung and Gianyar regions (Fig.1). Here we provide the first report that a major cause of sweetpotato virual diseases in Bali is Sweetpotato leaf curl virus (SPLCV). Virus infections have been reported can cause up to 20% decrease in sweet potato yield in China more than 20% (Feng et al. 2000).

The SPLCV (Sweet potato leaf curl virus) is a member of genus Begomovirus causing leaf curl in several members of sweetpotato (Kim et al. 2015). The SPLCV has two ORF (open reading frames), i.e. V1 and V2 in the virion sense and four ORFs C1, C2, C3, and C4 in the complementary sense, disjuncted by an IR (intergenic region) containing a conserved stem-loop motif, in a 2.8 kb single stranded circular DNA (Kim et al. 2015). Complete genome of SLCV from Korea have been characterized, and their phylogenetic analysis with other sweepovirus species have been determined (Choi et al. 2012).

SPLCV virus was first reported from Japan and Taiwan in 1998 (Moyer and Salazar 1989). SPLCV and related sweepoviruses have been found several countries, i.e. China, Korea, India, Brazil, Italy, Kenya, Peru, Spain, Uganda, and United States (Briddon et al. 2006; Kwak et al. 2006; Paprotka et al. 2010; Albuquerque et al. 2011;

Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). SPLCV causes disease symptoms in young sweetpotato plants such as vein yellowing and upward curling of the leaves (Kim et al. 2015). The host range of SLCV such as Ipomoea purpurea, I. nil, I. batatas, I setosa, I aquatica, and Nicotiana benthamiana (Valverde *et al.* 2007; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). SPLCV infections have been reported to decrease sweetpotato yields by more than 30% (Clark and Hoy 2006). SPLCV can be transmitted persistently by the whitefly vector Bemisia tabaci (Simmons et al. 2009), as well as by grafting, but not previous report on mechanical or seed transmission is available (Loebenstein et al. 2003). Presently, SPLCV can be transmitted via seeds more than 70% in sweetpotato in Korea and SPLCV DNA can replicate in developing seedlings. The proof is SPLCV detected in endosperm and embryos by PCR. Previously, SPLCV have not been reported in Indonesia.

In this study, the disease incidence of these viruses based on observations of symptoms in the fields. The incidence of viral diseases in the field showed that the disease incidence is more than 50% occurs in Badung and Gianyar Regions (data not shown). The 180 older leaves of sweetpotato with symptoms like those caused by SPLCV infection were collected from Bali Province (Denpasar, Badung, Gianyar, Buleleng, Bangli, Tabanan, Karangasem, Klungkung, and Jembrana). These were detected for the presence of viruses known to infect sweetpotato.

To confirm the vein yellowing symptoms, we extracted total DNA, PCR, subcloned the PCR products using TA cloning and sequence analyzed the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus SPG1 (5'-CCCCKGTGCGWRAATCCAT-3') and SPG2 (5'-ATCCVAAYWTYCAGGGAGCT AA-3') for an amplicon of ± 900 bp (Li et al. 2004). Amplification reactions were

prepared using Dream Taq Green Master Mix (2X) (Thermo Fisher Scientific, Waltham, MA, USA). Material composition in the amplification reaction it was 14.5–17.3 µL H_2O, 2.5 µL buffer containing 10x Mg^{2+}, 2 µL dNTP 2.5 mM, 1 µL SPG1 and SPG2 primers respectively, 0.2–0.3 µL Dream Taq Polymerase, and 0.2 µL MgCl 25 mM. Resulting DNA bands amplification was visualized on 1% agarose gel in 0.5x TBE (Tris-borate EDTA) buffer. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar (Fig. 2). The result of PCR from plants not showing symptoms and that no amplification was observed. The disease symptoms have the ability to transmit its diseases from the symptomatic plants to healthy plants through grafting to *I. setosa*. The results of this detection prove the presence of Begomovirus infection in sweetpotato plants. DNA fragments of Begomovirus were cloned into pTZ57R/T vector plasmid (InsTAclone PCR Cloning Kit, Thermo Scientific, USA) and inserted into competent cells of E. coli DH5α. Recombinant plasmid DNAs were extracted and sequence analyzed. The nucleotide and amino acid sequences of the partial gene were aligned with those of corresponding SPLCV sequences deposited in GenBank database, using Clustal W (Thompson et al. 1994). Sequence identities are calculated using sequence identity matrix options in BioEdit version 7.05 software (Hall 1999). Phylogenetic trees are constructed of sequences aligned using Clustal W and MEGA 7.0 software with bootstrap and neighbor-joining algorithms support, estimated using 1000 replicates (Kumar et al. 2016).

The homology of nucleotide and amino acid sequences of the SPLCV among Bali isolates was ranged 97.8% and 98.8%, indicating low variation among them. The homology of SPLCV Bali isolates to that of isolates from other countries was ranged 86.5-97.2% and 88.7-98.4%, respectively. Two clones from Badung (LC586169) and

Gianyar (LC586170) shared a maximum identity of 96.2-97.2% and 97.3-98.4% at nucleotide and amino acid level, respectively towards the SPLCV isolate reported from China (MK052985) and South Korea (KT992062), confirming the association of SPLCV with vein yellowing symptoms on sweetpotato in Bali, so we designated the isolate as SPLCV-IDN (Indonesia).

The phylogenetic tree analysis showed that SPLCV divided into II groups. Group I consists of isolates from Asia, II are isolates from America and Europe. SPLCV Bali isolates were in the same group with other Asia isolates. Based on previous reports, (Albuquerque et al. 2011; Wasswa et al. 2011; Bi and Zhang 2011; Kim et al. 2015), this study represents the first report of SPLCV infecting sweetpotato in Bali. Since the province has been leading the sweet potato production in Indonesia such result finding will help strengthen plant health certification standards in order to provide virus-tested propagative materials and bulbs for domestic growers and export to other countries.

Acknowledgements

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This study did not include experiments with human participants or animals performed by any of the authors.

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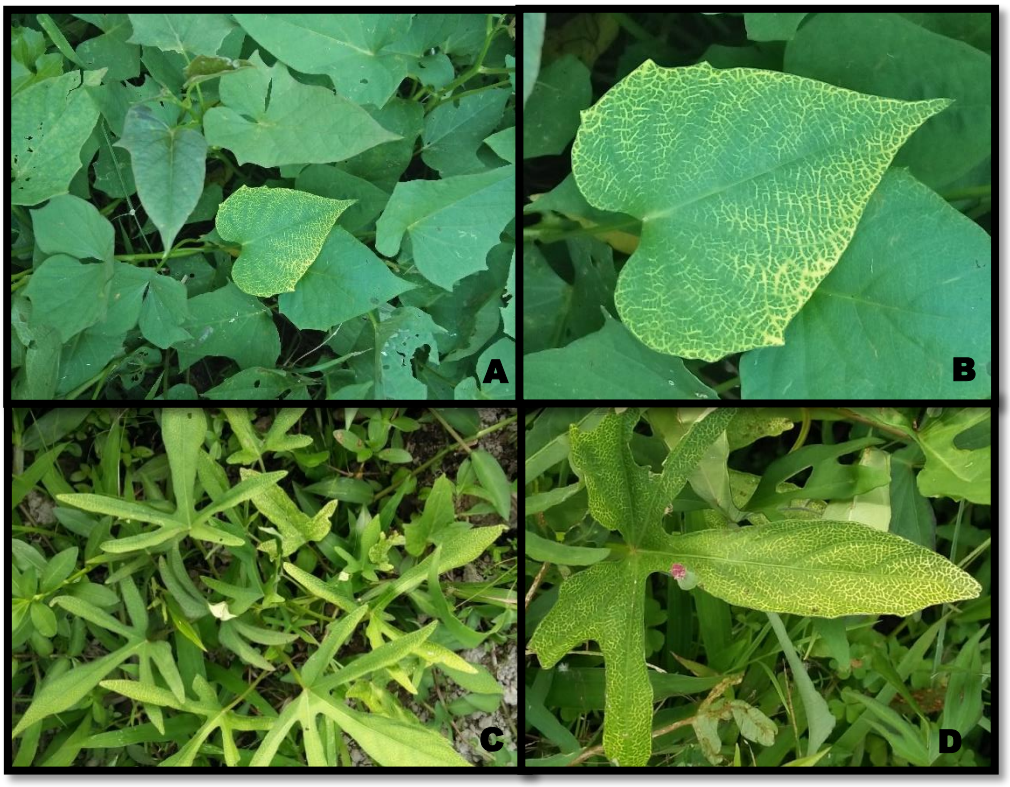

Figure 1 Typical symptoms on infected sweet potato crops in Bali: vein clearing (A-D)

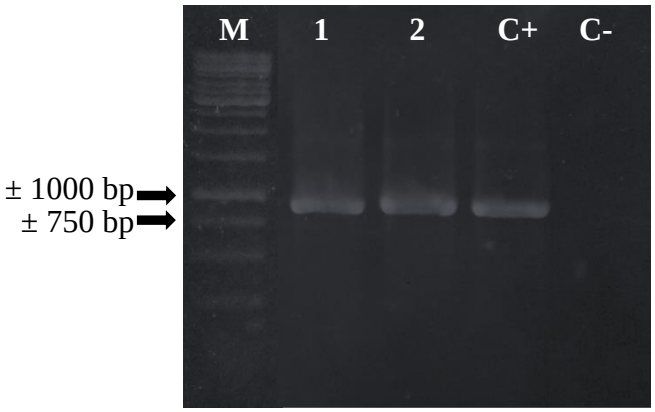

Figure 2 Visualization of PCR products using universal primer of *Begomovirus* SPG1 / SPG2: melon leaves from the Gianyar (1), Badung (2), positive control (C+), negative control (C-), DNA ladder 1 kb (M) (Thermo Fisher Scientific, USA)

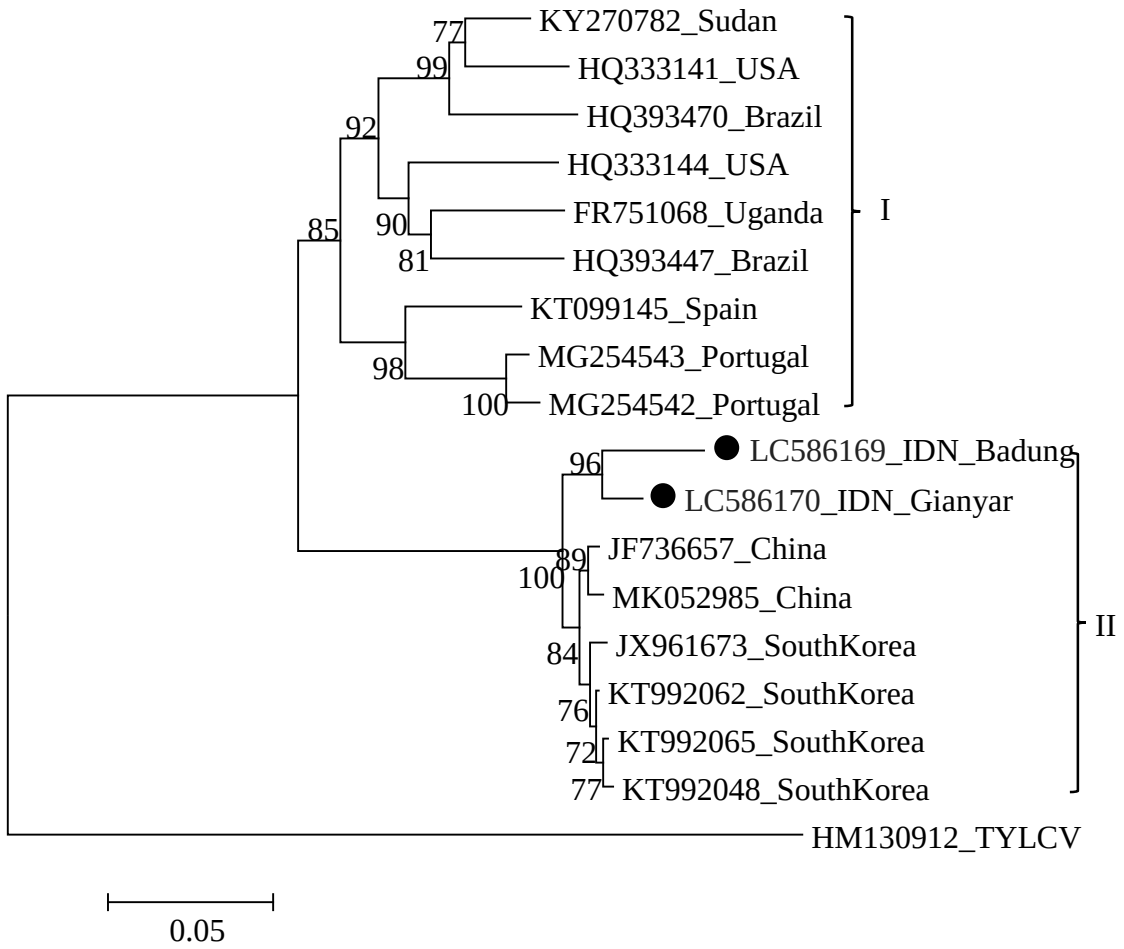

Figure 3 Phylogeny tree of the AC1 and AC2 gene of SPLCV Bali isolate; nucleotide sequences of SPLCV using *Tomato yellows leaf curl virus* (TYLCV) as out group. IDN-Indonesia

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Table 1 Homology of nucleotide (nt) and amino acid (aa) of SPLCV Bali Isolates with those of other isolates from other country established in GenBank

Isolate	Geographical origin	Host	Symptoms	Homology (%)				Accession number
				Badung		Gianyar		
				nt	aa	nt	aa	
Abs-1	Badung, Bali, Indonesia	Ipomoea batatas	Vein clearing			97.8	98.8	LC586169
Ubud-1	Gianyar, Bali, Indonesia	Ipomoea batatas	Vein clearing	97.8	98.8			LC586170
Hu-194	Hunan, China	Ipomoea batatas	Unknown	97.0	98.2	97.2	98.4	MK052985
ZJ	Zhejiang, China	Ipomoea setosa	Leaf curling	94.3	96.2	96.5	97.4	JF736657
202	South Korea	Ipomoea batatas	Leaf curling	94.0	96.2	96.1	97.1	KT992065
169	South Korea	Ipomoea batatas	Leaf curling	96.2	97.3	96.4	97.6	KT992062
GE-21	Muan, South Korea	Ipomoea batatas	Unknown	94.0	96.2	96.0	97.1	JX961673
7	South Korea	Ipomoea batatas	Leaf curling	93.6	95.7	95.7	96.9	KT992048
Sp3-2	Spain	Unknown	Unknown	87.4	89.8	89.0	90.9	KT099145
P213-11	Southern Portugal	Ipomoea indica	Vein clearing	87.0	89.2	88.6	90.2	MG254543
P213-8	Southern Portugal	Ipomoea indica	Vein clearing	86.9	88.4	88.3	90.0	MG254542
409	Khartoum, Sudan	Ipomoea batatas	Lef curling	87.6	89.6	88.8	90.4	KY270782
Uk-2008	Kampala, Uganda	Ipomoea setosa	Leaf curling	87.3	89.6	88.8	90.4	FR751068
648B-9	South Carolina, USA	Ipomoea batatas	Leaf curling	87.0	89.2	88.2	90.0	HQ333144
BR-Uti-08	Bahia, Brazil	Ipomoea batatas	Leaf curling	86.5	88.7	88.2	90.0	HQ393447
WS1-4	South Carolina, USA	Ipomoea setosa	Leaf curling	87.5	89.8	88.5	90.2	HQ333141
MP3-09	Pernambuco, Brazil	Ipomoea batatas	Leaf curling	86.8	87.6	87.8	89.7	HQ393470
*TYLCV	Masan, South Korea	Lycopersicon esculentum	Leaf curling	63.8	67.2	66.5	69.6	HM130912

*TYLCV: *Tomato yellows leaf curl virus* as *out group*; nt (nucleotide) and aa (amino acid)

Indian Phytopathology

First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia

--Manuscript Draft--

Manuscript Number:	IPPJ-D-21-00315R2
Full Title:	First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia
Article Type:	Short Communication
Abstract:	During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. The vein yellowing symptoms were validated by extracting total DNA, performing PCR, subcloning with TA cloning, and sequencing the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia. Key words: Sweet potato leaf curl virus · Sweetpotato · Begomovirus · Yellowing ·
Response to Reviewers:	Dear Palash Deb Nath Editor Indian Phytopathology Thank you for for review. I have revised the article according to the advice of the editor team 1. I have corrected the format of the reference section according to the Indian Phytopathology format. 2. I have included 1 reference from Indian Phytopathology Thank you very much Best regards, Listihani

DISEASE NOTE

First report of *Sweet potato leaf curl virus* (SPLCV) on *Ipomoea batatas* in Bali, Indonesia

Listihani Listihani* · I Gusti Ayu Diah Yuniti · Putu Fajar Kartika Lestari · Putu Eka Pasmidi Ariati

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Total text pages: 8

The numbers of tables: 1

The numbers of figures: 3

The reported nucleotide sequence can be found in the DDBJ/EMBL/GenBank databases under the accession numbers LC586169 and LC586170.

Abstract

During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. The vein yellowing symptoms were validated by extracting total DNA, performing PCR, subcloning with TA cloning, and sequencing the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia.

Key words: *Sweet potato leaf curl virus*·Sweetpotato·*Begomovirus*·Yellowing·

Sweetpotato (Ipomoea batatas) many producted in Asia, particularly in the Far East Asia and Southeast Asia countries. In Indonesia, sweetpotato becomes staples food particularly in Eastern Indonesia. Food diversification is a solution to the problem of food needs in Indonesia. Sweetpotato production in Indonesia in 2015, 2016 and 2017 amounted to 2298, 2169, and 2023 tons (Central Bureau of Statistics 2017). According to this data, annual production has reduced. In 2019, a field assessment of sweet potato production in nine regions of Bali Province discovered yellowing symptoms on plants in the Badung and Gianyar regions (Fig. 1). The leading cause of sweet potato virus disease in Bali is the sweet potato leaf curl virus (SPLCV). The same viral infection was detected in China, resulting in a 20% decline in sweet potato yield (Feng et al. 2000).

SPLCV, which belongs to the Begomovirus genus, causes leaf curl in several types of sweet potato (Kim et al. 2015). The SPLCV contains two ORFs, V1 and V2, in the virion sense, and four ORFs (C1, C2, C3, and C4) in the complementary sense, separated by an IR (intergenic region) containing a conserved stem-loop motif within a 2.8 kb circular single-stranded DNA (Kim et al. 2015). The full genomic sequence of SLCV from Korea was characterized, as well as its phylogenetic relationship to other sweepovirus species (Choi et al. 2012).

According to Moyer and Salazar (1989), the SPLCV virus was discovered for the first time in Japan and Taiwan in 1980. Moreover, in several countries such as the United States of America, Brazil, Italy, Spain, Peru, Kenya, Uganda, India, China and Korea, similar viruses have been found (Briddon et al. 2006; Kwak et al. 2006; Parotka et al. 2010; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). Yellow veins and upward curling leaves are symptoms of SPLCV infection in

young sweet potato plants (Kim et al. 2015). The host range of SLCV such as Ipomoea purpurea, I. nil, I. batatas, I setosa, I aquatica, and Nicotiana benthamiana (Valverde *et al.* 2007; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). Clark and Hoy (2006) observed a more than 30% reduction in sweet potato yield due to SPLCV infection. SPLCV can be transmitted persistently by the whitefly vector *Bemisia tabaci* (Simmons et al. 2009) and grafting. There have been no transmission reports through mechanical or seeds transmission (Loebenstein et al., 2003 However, currently, SPLVC transmission has been found through seeds. SPLVC DNA can replicate in seedlings. This incident has occurred in Korea. The proof is SPLCV detected in endosperm and embryos by PCR. Previously, SPLCV have not been reported in Indonesia.

In this study, the disease incidence of these viruses based on observations of symptoms in the fields. The incidence of viral diseases in the field showed that the disease incidence is more than 50% occurs in Badung and Gianyar Regions (data not shown). One hundred eighty samples of sweet potato leaves exhibiting symptoms consistent with SPLCV infection were collected from nine regions throughout Bali Province (Badung, Bangli, Buleleng, Denpasar, Gianyar, Karangasem, Klungkung, Jembrana and Tabanan). The purpose of this collection was to determine the presence of SPLCV in sweet potatoes.

To confirm the vein yellowing symptoms, the total DNA was extracted, and PCR was performed. Then, the PCR products were subcloned using TA cloning and followed by plasmid DNA sequencing. PCR amplification was done by using universal primer of Begomovirus SPG1 (5'-CCCCKGTGCGWRAATCCAT-3') and SPG2 (5'-ATCCVAAYWTYCAGGGAGCT AA-3') for an amplicon of ± 900 bp (Li et al. 2004). Dream Taq Green Master Mix (2X) was utilized for the Amplification reactions. (Thermo

Fisher Scientific, Waltham, MA, USA). Material composition in the amplification reaction it was 14.5–17.3 µL H_2O, 2.5 µL buffer containing 10x Mg^{2+}, 2 µL dNTP 2.5 mM, 1 µL SPG1 and SPG2 primers respectively, 0.2–0.3 µL Dream Taq Polymerase, and 0.2 µL MgCl 25 mM. Following that, the amplified DNA bands were observed on a 1% agarose gel in 0.5x TBE buffer (Tris-borate EDTA). The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar (Fig. 2). The result of PCR from plants not showing symptoms and that no amplification was observed. The disease symptoms have the ability to transmit its diseases from the symptomatic plants to healthy plants through grafting to *I. setosa*. The results of this detection prove the presence of Begomovirus infection in sweetpotato plants. DNA fragments of Begomovirus were cloned toward pTZ57R/T vector plasmid (InsTAclone PCR Cloning Kit, Thermo Scientific, USA) and injected into competent cells of E. coli DH5α. Sequence analysis was performed on the recombinant plasmid DNA extract. Using Clustal W, the partial genes' nucleotide and amino acid sequences were matched to the SPLCV sequences in the GenBank database (Thompson et al. 1994). The sequence identity matrix options in BioEdit version 7.05 software were used to calculate the sequence identities (Hall 1999). Phylogenetic trees were generated from the aligned sequences employing a bootstrap procedure, and Neighbor-Joining algorithms, which estimated using 1000 repeats (Kumar et al. 2016; Ameri and Ayazpour 2021)

The nucleotide and amino acid sequence homology of the SPLCV Bali isolate varied between 97.8% and 98.8%. This indicates a low degree of variation. The homology of SPLCV Bali isolates to that of isolates from other countries was ranged 86.5-97.2% and 88.7-98.4%, respectively. Two clones from Badung (LC586169) and Gianyar (LC586170) shared a maximum identity of 96.2-97.2% and 97.3-98.4% at nucleotide and

amino acid level, respectively towards the SPLCV isolate reported from China (MK052985) and South Korea (KT992062), confirming the association of SPLCV with vein yellowing symptoms on sweetpotato in Bali, so we designated the isolate as SPLCV-IDN (Indonesia).

The phylogenetic tree analysis showed that SPLCV divided into II groups. Group I consists of isolates from Asia, II are isolates from America and Europe. SPLCV Bali isolates were in the same group with other Asia isolates. This study is the first report on SPLCV infection in sweet potatoes in Bali. Since the province has been leading the sweet potato production in Indonesia such result finding will help strengthen plant health certification standards in order to provide virus-tested propagative materials and bulbs for domestic growers and export to other countries.

Acknowledgements

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Compliance with ethical standards

The authors state that they are not implicated in any conflict of interest.

This study did not involve human or animal subjects. Hence, ethical standards were not required.

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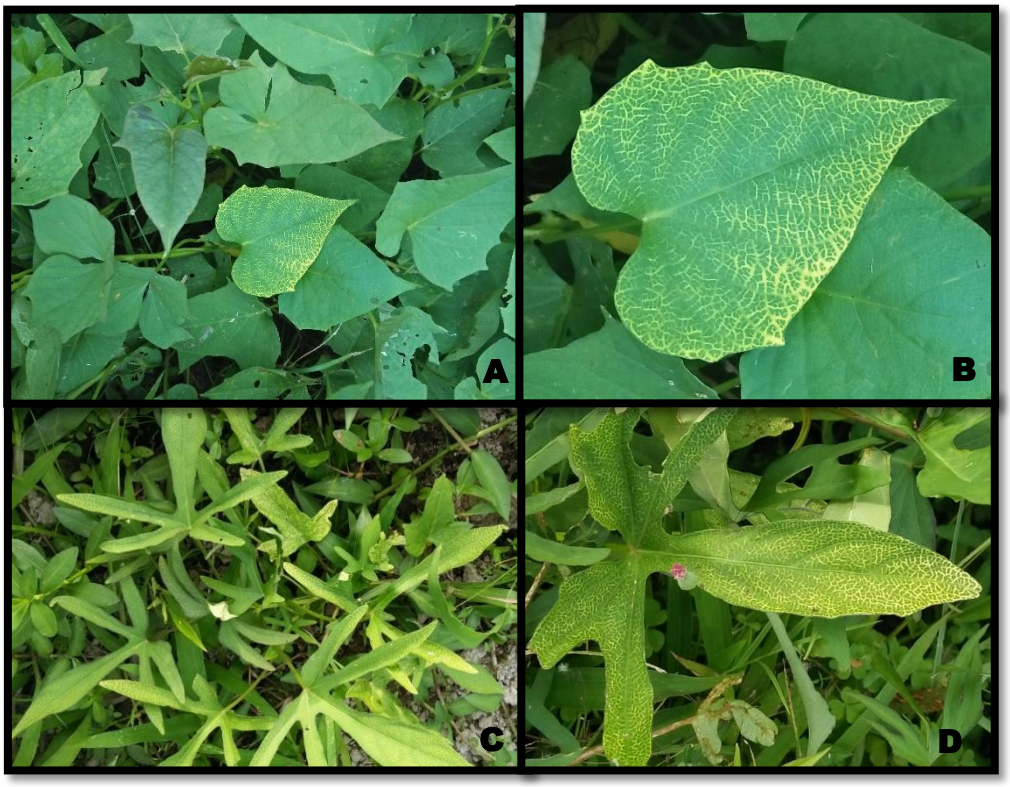


Figure 1 Typical symptoms on infected sweet potato crops in Bali: vein clearing (A-D)

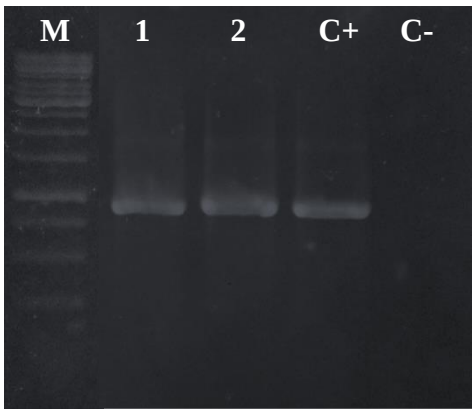
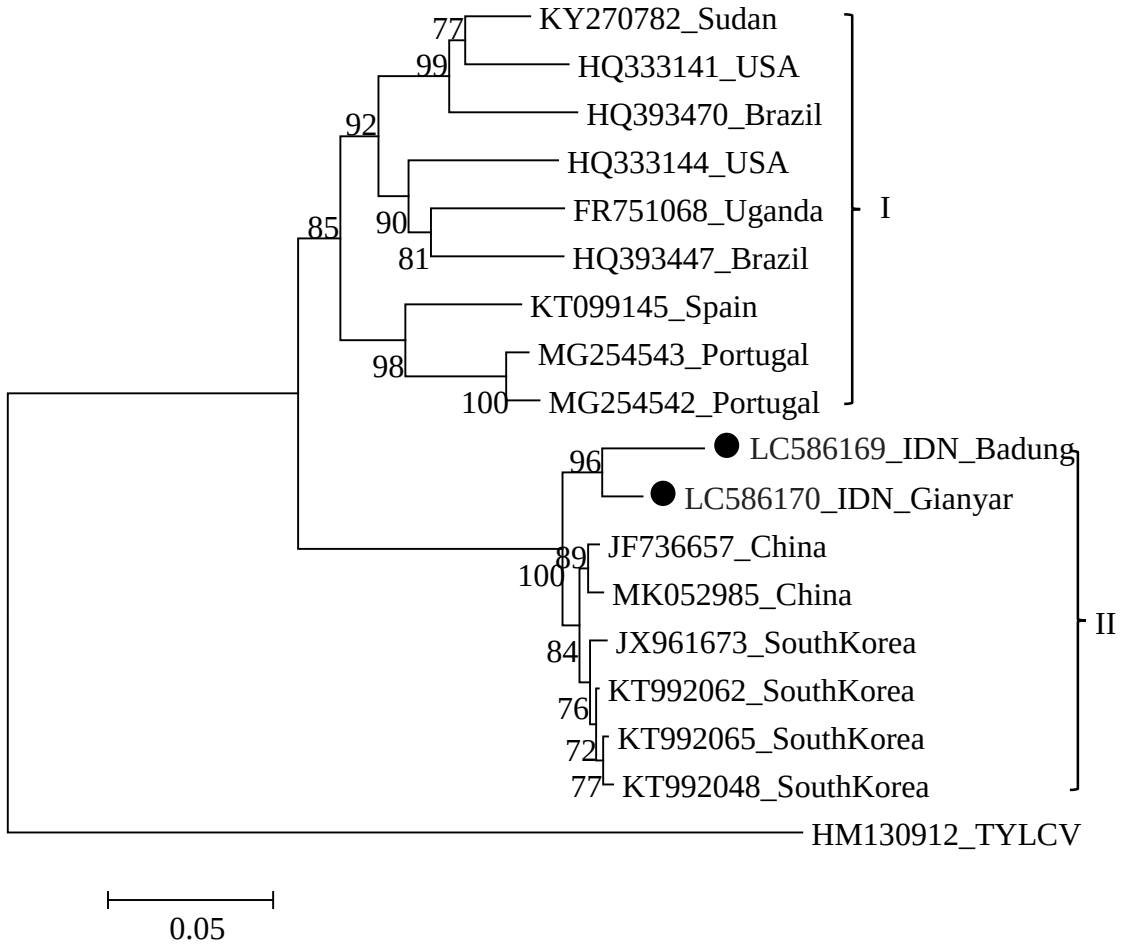


Figure 2 Visualization of PCR products using universal primer of *Begomovirus* SPG1 / SPG2: melon leaves from the Gianyar (1), Badung (2), positive control (C+), negative control (C-), DNA ladder 1 kb (M) (Thermo Fisher Scientific, USA)



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*TYLCV: *Tomato yellows leaf curl virus* as *out group*; nt (nucleotide) and aa (amino acid)

Indian Phytopathology

First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia

--Manuscript Draft--

Manuscript Number:	IPPJ-D-21-00315R3
Full Title:	First report of Sweet potato leaf curl virus (SPLCV) on Ipomoea batatas in Bali, Indonesia
Article Type:	Short Communication
Funding Information:	Lembaga Penelitian dan Pengabdian Kepada Masyarakat (K.100/B.01.01/LPPM-UNMAS/V/2021) — Dr. Listihani Listihani
Abstract:	During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. The vein yellowing symptoms were validated by extracting total DNA, performing PCR, subcloning with TA cloning, and sequencing the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia. Key words: Sweet potato leaf curl virus · Sweetpotato · Begomovirus · Yellowing ·
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Author Comments:	During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. To confirm the vein yellowing symptoms, we extracted total DNA, PCR, subcloned the PCR products using TA cloning and sequence analyzed the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of ± 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia.
Response to Reviewers:	Dear Mr. Palash Deb Nath Editor Indian Phytopathology We will respond to comments from reviewers

	Reviewer #1: We have followed the format of writing a short communication article in Indian Phytopathology. we have corrected write of the introduction and the method that is too long and corrected the another sentence according to your command Reviewer #2: We have corrected the writing according to your suggestions. We inform to you that sweetpotato production data in Indonesia is only up to 2018. For production data for 2019 and 2020, the data is not yet available on the Indonesian government website. So, we write of sweetpotato production data from 2015 to 2018 in our article. Best regards, Listihani

DISEASE NOTE

First report of *Sweet potato leaf curl virus* (SPLCV) on *Ipomoea batatas* in Bali, Indonesia

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Total text pages: 8

The numbers of tables: 1

The numbers of figures: 3

The reported nucleotide sequence can be found in the DDBJ/EMBL/GenBank databases under the accession numbers LC586169 and LC586170.

Abstract

During a field survey at 9 regions in Bali Province, we found plants with vein yellowing symptoms on young leaves. The vein yellowing symptoms were validated by extracting total DNA, performing PCR, subcloning with TA cloning, and sequencing the plasmid DNA. PCR amplification was done by using universal primer of Begomovirus. The SPG1 / SPG2 primers amplified DNA bands of 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar. The nucleotide and amino acid sequences of two isolates from sweetpotato in Bali had highest identity with that of SPLCV isolates China and South Korea. This is the first report of SPLCV infection on sweetpotato in Indonesia.

Key words: *Sweet potato leaf curl virus*·Sweetpotato·*Begomovirus*·Yellowing·

Sweetpotato (*Ipomoea batatas*) many producted in Asia, particularly in the Far East Asia and Southeast Asia countries. In Indonesia, sweetpotato becomes staples food particularly in Eastern Indonesia. Food diversification is a solution to the problem of food needs in Indonesia. Sweetpotato production in Indonesia in 2015, 2016, 2017, and 2018 amounted to 2298, 2169, 2023, and 1914 tons (Central Bureau of Statistics 2019). According to this data, annual production has reduced. In 2019, a field assessment of sweet potato production in nine regions of Bali Province discovered yellowing symptoms on plants in the Badung and Gianyar regions (Fig. 1). The leading cause of sweetpotato virus disease in Bali is the *Sweet potato leaf curl virus* (SPLCV). The same viral infection was detected in China, resulting in a 20% decline in sweetpotato yield (Feng et al. 2000).

According to Moyer and Salazar (1989), the SPLCV virus was discovered for the first time in Japan and Taiwan in 1980. Moreover, in several countries such as the United States of America, Brazil, Italy, Spain, Peru, Kenya, Uganda, India, China and Korea, similar viruses have been found (Briddon et al. 2006; Kwak et al. 2006; Parotka et al. 2010; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Kim et al. 2015). Yellow veins and upward curling leaves are symptoms of SPLCV infection in young sweetpotato plants (Kim et al. 2015). The host range of SLCV such as *Ipomoea purpurea, I. nil, I. batatas, I setosa, I aquatica,* and *Nicotiana benthamiana* (Clark and Hoy 2006; Albuquerque et al. 2011; Wasswa et al. 2011; Zhang and Ling 2011; Choi et al. 2012; Kim et al. 2015). SPLCV can be transmitted persistently by the whitefly vector *Bemisia tabaci* (Simmons et al. 2009) and grafting. There have been no transmission reports through mechanical or seeds transmission. However, currently, SPLVC transmission has been found through seeds (Kim et al. 2015). SPLVC DNA can replicate in seedlings. This incident has occurred in Korea. The proof is SPLCV detected in

endosperm and embryos by PCR. Previously, SPLCV have not been reported in Indonesia.

In this study, the disease incidence of these viruses based on observations of symptoms in the fields. The incidence of viral diseases in the field showed that the disease incidence is more than 50% occurs in Badung and Gianyar Regions (data not shown). One hundred eighty samples of sweet potato leaves exhibiting symptoms consistent with SPLCV infection were collected from nine regions throughout Bali Province (Badung, Bangli, Buleleng, Denpasar, Gianyar, Karangasem, Klungkung, Jembrana and Tabanan). The purpose of this collection was to determine the presence of SPLCV in sweet potatoes.

To confirm the vein yellowing symptoms, the total DNA was extracted, and PCR was performed. Then, the PCR products were subcloned using TA cloning and followed by plasmid DNA sequencing. PCR amplification was done by using universal primer of Begomovirus SPG1 and SPG2 (Li et al. 2004). Dream Taq Green Master Mix (2X) was utilized for the Amplification reactions (Thermo Fisher Scientific, Waltham, MA, USA). The SPG1 / SPG2 primers amplified DNA bands of 900 bp from two leaf samples showing symptoms of vein yellowing from Badung and Gianyar (Fig. 2). The result of PCR from plants not showing symptoms and that no amplification was observed. The disease symptoms have the ability to transmit its diseases from the symptomatic plants to healthy plants through grafting to *I. setosa*. The results of this detection prove the presence of Begomovirus infection in sweetpotato plants. DNA fragments of Begomovirus were cloned toward pTZ57R/T vector plasmid (InsTAclone PCR Cloning Kit, Thermo Scientific, USA) and injected into competent cells of E. coli DH5α. Sequence analysis was performed on the recombinant plasmid DNA extract. Using

Clustal W, the partial genes' nucleotide and amino acid sequences were matched to the SPLCV sequences in the GenBank database (Ameri and Ayazpour 2021).

The nucleotide and amino acid sequence homology of the SPLCV Bali isolate varied between 97.8% and 98.8%. This indicates a low degree of variation. The homology of SPLCV Bali isolates to that of isolates from other countries was ranged 86.5-97.2% and 88.7-98.4%, respectively. Two clones from Badung (LC586169) and Gianyar (LC586170) shared a maximum identity of 96.2-97.2% and 97.3-98.4% at nucleotide and amino acid level, respectively towards the SPLCV isolate reported from China (MK052985) and South Korea (KT992062), confirming the association of SPLCV with vein yellowing symptoms on sweetpotato in Bali, so we designated the isolate as SPLCV-IDN (Indonesia).

The phylogenetic tree analysis showed that SPLCV divided into II groups. Group I consists of isolates from Asia, II are isolates from America and Europe. SPLCV Bali isolates were in the same group with other Asia isolates. This study is the first report on SPLCV infection in sweet potatoes in Bali. Since the province has been leading the sweet potato production in Indonesia such result finding will help strengthen plant health certification standards in order to provide virus-tested propagative materials and bulbs for domestic growers and export to other countries.

Acknowledgements

The research funded by Research Institute and Community Service from University of Mahasaraswati Denpasar with contract No. K.100/B.01.01/LPPM-UNMAS/V/2021.

Compliance with ethical standards

The authors state that they are not implicated in any conflict of interest.

This study did not involve human or animal subjects. Hence, ethical standards were not required.

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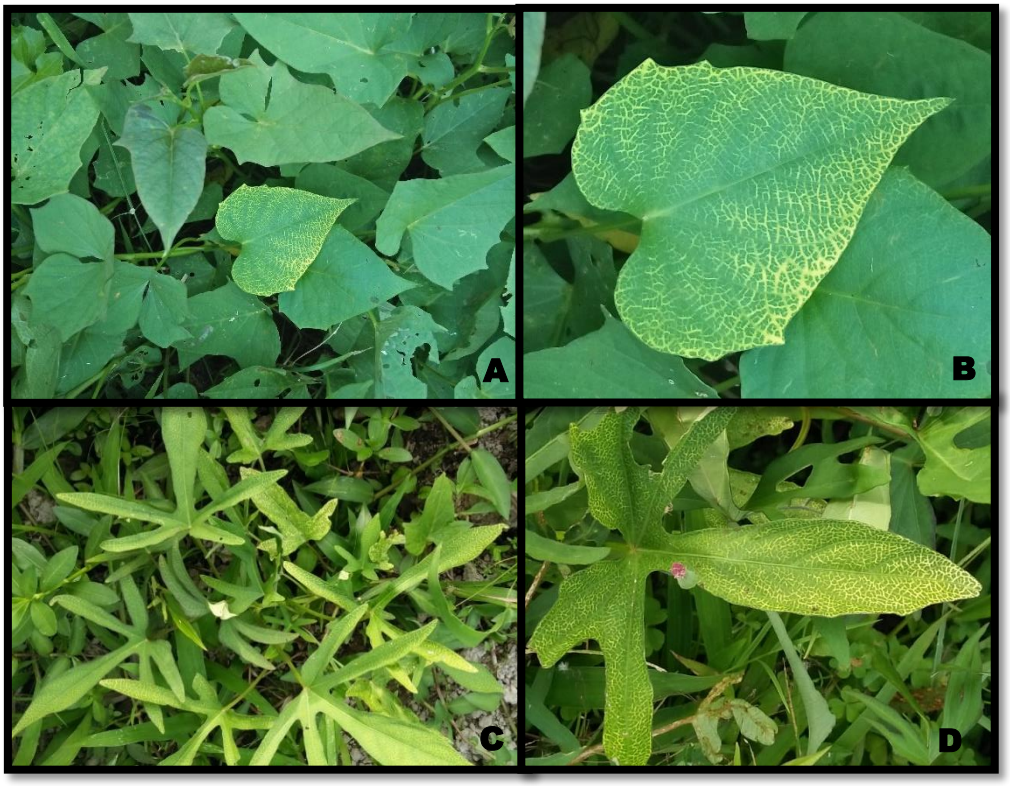

Figure 1 Typical symptoms on infected sweet potato crops in Bali: vein clearing (A-D)

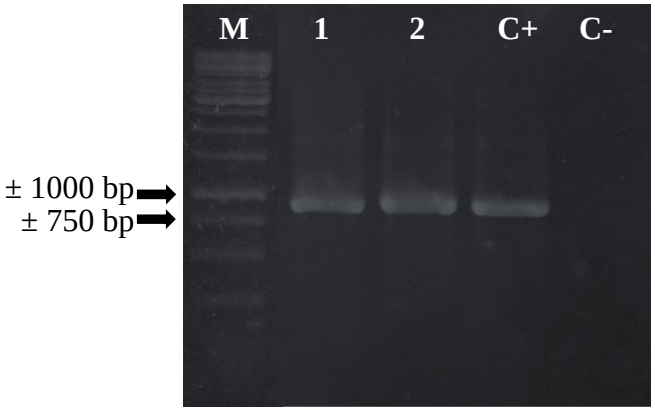

Figure 2 Visualization of PCR products using universal primer of *Begomovirus* SPG1 / SPG2: melon leaves from the Gianyar (1), Badung (2), positive control (C+), negative control (C-), DNA ladder 1 kb (M) (Thermo Fisher Scientific, USA)

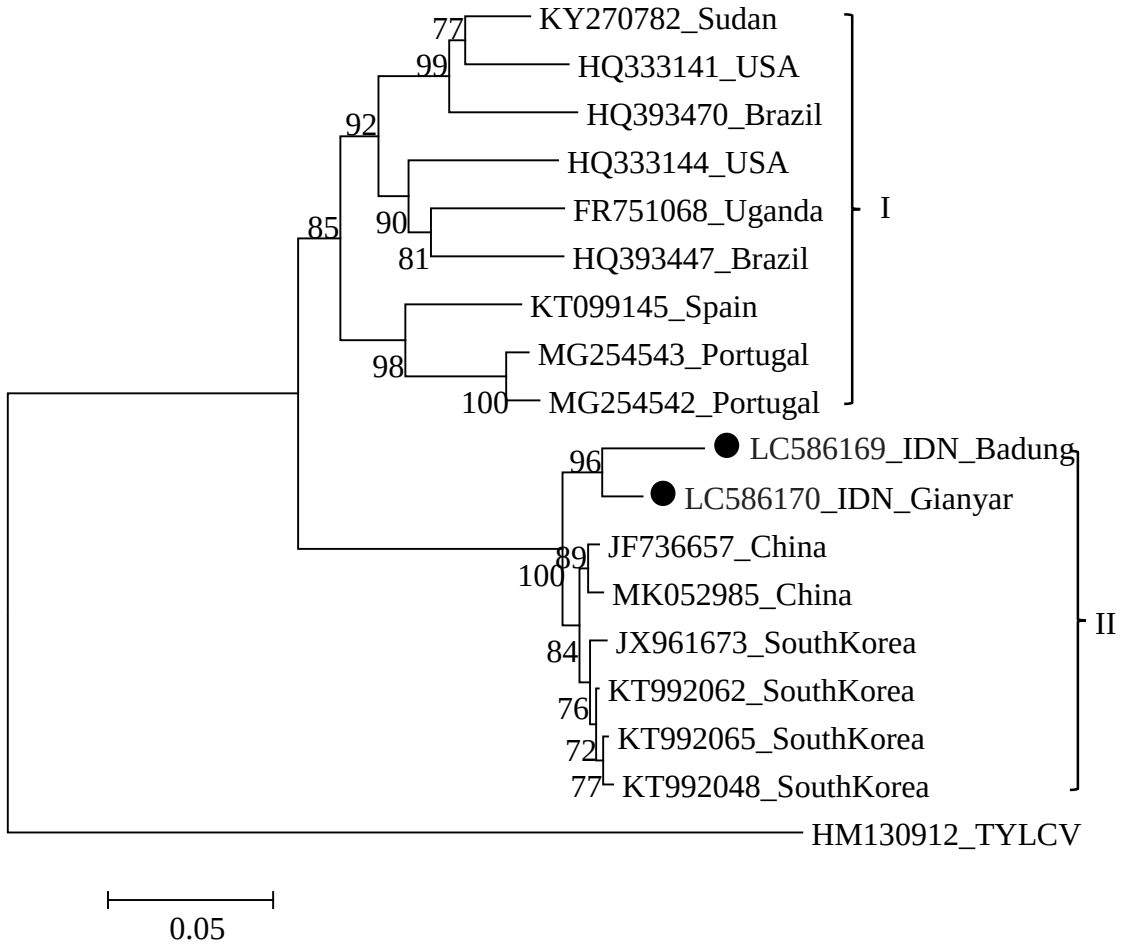

Figure 3 Phylogeny tree of the AC1 and AC2 gene of SPLCV Bali isolate; nucleotide sequences of SPLCV using *Tomato yellows leaf curl virus* (TYLCV) as out group. IDN-Indonesia

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Table 1 Comparison of the Bali SPLCV isolate's nucleotide (nt) and amino acid (aa) sequences to isolates from other countries found in GenBank.

Isolate	Geographical origin	Host	Symptoms	Homology (%)				Accession number
				Badung		Gianyar		
				nt	aa	nt	aa	
Abs-1	Badung, Bali, Indonesia	Ipomoea batatas	Vein clearing			97.8	98.8	LC586169
Ubud-1	Gianyar, Bali, Indonesia	Ipomoea batatas	Vein clearing	97.8	98.8			LC586170
Hu-194	Hunan, China	Ipomoea batatas	Unknown	97.0	98.2	97.2	98.4	MK052985
ZJ	Zhejiang, China	Ipomoea setosa	Leaf curling	94.3	96.2	96.5	97.4	JF736657
202	South Korea	Ipomoea batatas	Leaf curling	94.0	96.2	96.1	97.1	KT992065
169	South Korea	Ipomoea batatas	Leaf curling	96.2	97.3	96.4	97.6	KT992062
GE-21	Muan, South Korea	Ipomoea batatas	Unknown	94.0	96.2	96.0	97.1	JX961673
7	South Korea	Ipomoea batatas	Leaf curling	93.6	95.7	95.7	96.9	KT992048
Sp3-2	Spain	Unknown	Unknown	87.4	89.8	89.0	90.9	KT099145
P213-11	Southern Portugal	Ipomoea indica	Vein clearing	87.0	89.2	88.6	90.2	MG254543
P213-8	Southern Portugal	Ipomoea indica	Vein clearing	86.9	88.4	88.3	90.0	MG254542
409	Khartoum, Sudan	Ipomoea batatas	Lef curling	87.6	89.6	88.8	90.4	KY270782
Uk-2008	Kampala, Uganda	Ipomoea setosa	Leaf curling	87.3	89.6	88.8	90.4	FR751068
648B-9	South Carolina, USA	Ipomoea batatas	Leaf curling	87.0	89.2	88.2	90.0	HQ333144
BR-Uti-08	Bahia, Brazil	Ipomoea batatas	Leaf curling	86.5	88.7	88.2	90.0	HQ393447
WS1-4	South Carolina, USA	Ipomoea setosa	Leaf curling	87.5	89.8	88.5	90.2	HQ333141
MP3-09	Pernambuco, Brazil	Ipomoea batatas	Leaf curling	86.8	87.6	87.8	89.7	HQ393470
*TYLCV	Masan, South Korea	Lycopersicon esculentum	Leaf curling	63.8	67.2	66.5	69.6	HM130912

*TYLCV: *Tomato yellows leaf curl virus* as *out group*; nt (nucleotide) and aa (amino acid)