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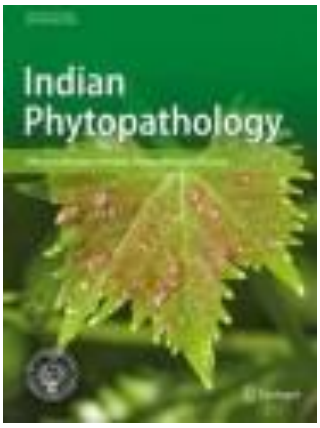

Dr. Ganga D Sinniah
Principal Research Officer
Plant Pathology Division
Tea Research Institute of Sri Lanka
Talawakelle, Sri Lanka

Dr Frank Kwekucher Ackah
Mycologist/Plant Pathologist
Department of Crop Science
College of Agriculture and Natural Sciences
University of Cape Coast, Ghana

Dr. Hebert-Zair Barrels Cureno
Natural Processes Division
Community Forest Engineering
Intercultural University of the State of Puebla
Calle Principal a Lipuntahuaca S/N.,
Lipuntahuaca, Huehuetla, Puebla,
C.P, Mexico

Prof. Jing Lan
Inner Mongolia Agricultural University
Plant Pathology
306 Xinjian east street
Hohhot, Inner Mongolia, China

Bacteriology
Dr. A. Kumar
Principal Scientist
Division of Plant Pathology,
ICAR-Indian Agricultural Research Institute
New Delhi, Delhi

Dr. Jagjeet Singh Lore
Plant Pathologist
Department of Plant Breeding & Genetics
Punjab Agricultural University
Ludhiana, Punjab

Dr. R. Ramesh
Principal Scientist (Plant Pathology)
ICAR-Central Coastal Agricultural Research Institute
Old Goa, Paneji, Goa

Mycology
Dr. Udai Bhan Singh
Scientist
ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM)
Kushmaur, Post Box. No. 6,
Mau Nath Bhanjan
Uttar Pradesh

Dr. N.K. Dubey
Professor and Co-ordinator (Applied Microbiology)
Centre of Advanced Study in Botany,
Banaras Hindu University,
Varanasi, Uttar Pradesh

Dr. Rashmi Dubey
Scientist 'C'
Botanical Survey of India,
Western Regional Centre,
7-Koregaon Road, Pune
Maharashtra

Dr. Laszlo Kredics
Department of Microbiology, Faculty of Science and
Informatics, University of Szeged
Közép fasor, Szeged,
Hungary

Dr. Amirreza Amirmijani,
Assistant Professor in Mycology
Department of Plant Protection
Faculty of Agriculture
University of Jiroft
Jiroft - IRAN

Dr. Palash Deb Nath
Professor
Department of Plant Pathology,
Assam Agricultural University,
Jorhat, Assam

Dr. T. Makeshkumar
Principal Scientist (Plant Pathology)
ICAR-Central Tuber Crops Research Institute,
Thiruvanthapuram, Kerala

Dr. Naimuddin
Principal Scientist (Plant Pathology)
Division of Crop Protection
ICAR-Indian Institute of Pulses Research
Kanpur, Uttar Pradesh

Dr. Raj Verma
Principal Scientist
I.A.R.I Regional Station,
Survey No. 125A, Baner Phata,
I.T.I Road, Aundh,
Pune, Maharashtra

Dr. Md Shamim Akhter
Senior Scientist
Plant Pathology Division
Bangladesh Agricultural Research Institute (BARI) BANGLADESH

Prof. Elvis Asare-Bediako
Professor of Plant Virology
Dean, School of Agriculture
College of Agriculture and Natural Sciences
University of Cape Coast, Ghana, West Africa

Dr. L.M. Suresh
Maize Pathology Lead – Sub Saharan Africa
International Maize and Wheat Improvement Center (CIMMYT)
World Agroforestry Centre (ICRAF)
United Nations Avenue
Gigiri, Nairobi, Kenya

Dr. Mujeebur Rahman Khan
Professor, Department of Plant Protection
Dean, Faculty of Agricultural Sciences,
Aligarh Muslim University
Aligarh, UP, India

Dr. Vishal Singh Somvanshi
Principal Scientist
Division of Nematology
LBS Centre,
ICAR-IARI, New Delhi

Dr Sukhjeet Kaur Randhawa
Nematologist
Department of Vegetable Science
Punjab Agricultural University,
Ludhiana

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SHORT COMMUNICATION

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

Listihani Listihani[1] · I. Gusti Ayu Diah Yuniti[1] · Putu Fajar Kartika Lestari[1] · Putu Eka Pasmidi Ariati[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.

Keywords *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

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

✉ Listihani Listihani
listihani9@gmail.com

[1] Study Program of Agrotechnology, Faculty of Agriculture and Business, Universitas Mahasaraswati Denpasar, Denpasar 80233, Indonesia

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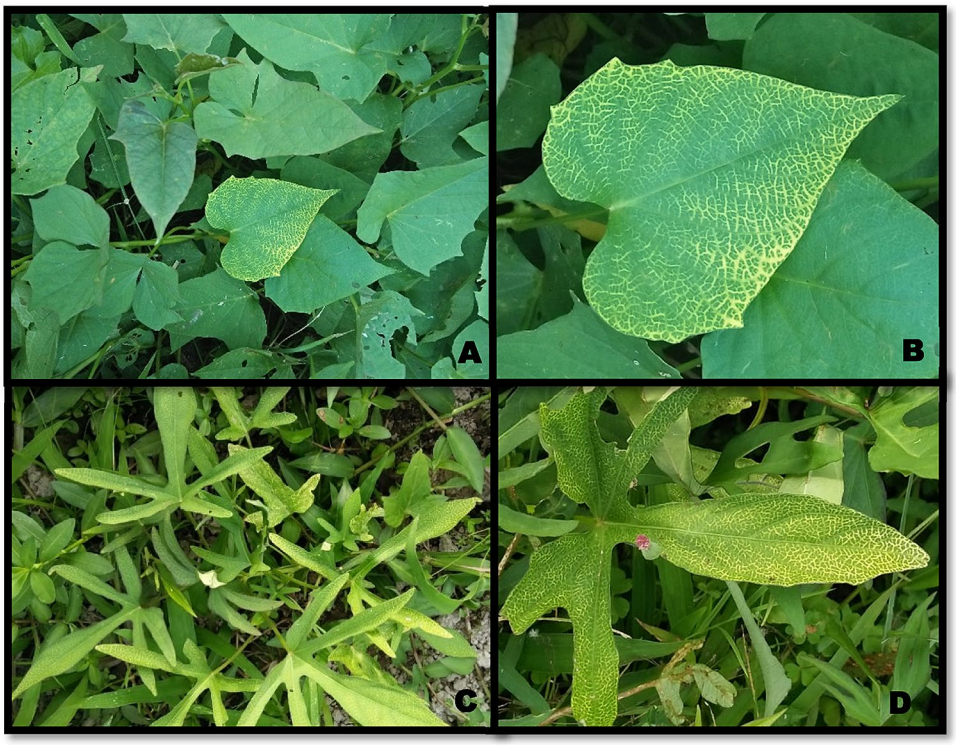

Fig. 1 Typical symptoms on infected sweet potato crops in Bali: vein clearing (**A**–**D**)

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

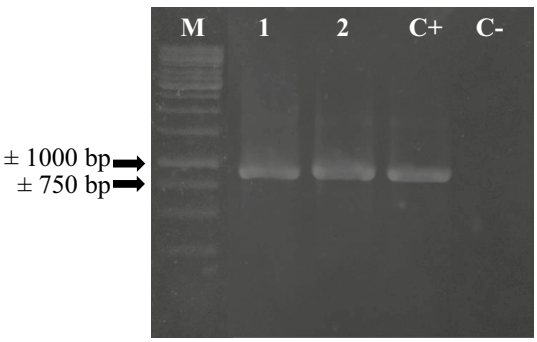

Fig. 2 Visualization of PCR products using universal primer of *Begomovirus* SPG1/SPG2: melon leaves from the Gianyar (1), Badung (2), positive control (C+), negative control (C−), DNA ladder 1 kb (M) (Thermo Fisher Scientific, USA)

SPLCV sequences in the GenBank database (Ameri and Ayazpour 2021) (Fig. 3).

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)

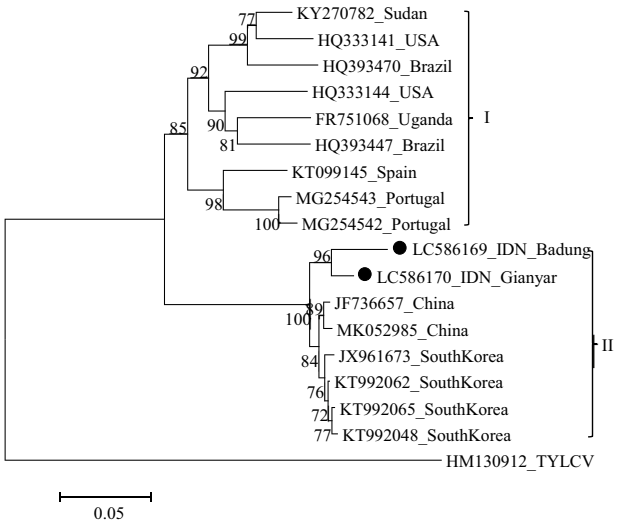

Fig. 3 Phylogeny tree of the AC1 and AC2 gene of SPLCV Bali isolate; nucleotide sequences of SPLCV using *Tomato yellows leaf curl virus* (TYLCV) as out group. IDN-Indonesia

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 (Table 1).

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	
ZJ Abs-1	Badung, Bali, Indonesia	Ipomoea batatas	Vein clearing			97.8	98.8	LC586169
U Ubud-1	Gianyar, Bali, Indonesia	Ipomoea batatas	Vein clearing	97.8	98.8			LC586170
hu194 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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Declarations

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

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

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