Jurnal Perlindungan Tanaman Indonesia, Vol. 25, No. 1, 2021: 56–63
DOI: 10.22146/jpti.64545
Available online at http://jurnal.ugm.ac.id/jpti
ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Research Article

Molecular Identification of *Sweet potato virus C* on Sweetpotato in Bali, Indonesia

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Received March 7, 2021; revised March 23, 2021; accepted June 21, 2021

ABSTRACT

A survey was conducted in several sweet potato cultivations in Bali Province. Survey found that many plants exhibited potyvirus symptom, such as chlorosis blotches. This study was to determine disease incidence, detection and identification of the virus causing these symptoms on sweet potato plants in Bali. Samples were collected by purposive sampling of 10 plants from each location in Bali (Denpasar, Gianyar, Badung, Buleleng, Tabanan, Klungkung, Karangasem, Jembrana, Bangli). Disease insidence was observed based on viral symptoms in the field. Identification of nucleic acids was done using Potyvirus universal primer and DNA sequencing. Disease incidence in Bangli, Buleleng, and Denpasar Regencies was > 50%. RT-PCR and CiFor/CiRev Potyvirus universal primers successfully amplified ± 700 bp of CI genes from all samples from Bangli, while samples from 8 other districts were not amplified using the same primers. The SPVC isolate of sweet potato showed nucleotide and amino acid homology similarities with the sweet potato isolate from East Timor (MF572066), 96.8% and 97.4%, respectively and these were referred to the "Asian" strain. This indicates that SPVC has spread in East Java and Bali.

Keywords: chlorosis blot symptoms; identification; *Potyvirus*; SPCV Bali isolates

INTRODUCTION

Sweet Potatoes (*Ipomoea batatas*) are one of the staple foods of Balinese people. Apart from being used as a source of carbohydrates to replace rice, sweet potatoes also contain many vitamins that are beneficial for human health. Food diversification is one of may solutions for food problems faced by Indonesia (*Pusat Data dan Informasi Pertanian*, 2016). Indonesia's sweet potato production in 2013, 2014, 2015, 2016 and 2017 amounted to 2387, 2383, 2298, 2169, and 2023 tonnes, respectively (*Badan Pusat Statistik,* 2017). Based on these data, sweet potatoes production tends to decline every year. In addition, sweet potato in the field always shows unhealthy symptoms due to infection by many types of pathogens, including viruses.

The important viruses on sweet potato reported in several countries include *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato feathery mottle virus* strain internal cork (SPFMV-IC), *Sweet potato feathery mottle virus* strain russet crack (SPFMV-RC), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato chlorotic stunt virus* (SPCSV), and *Sweet potato virus G* (SPVG) (Abad *et al*.. 2007; Kwak *et al*., 2007; Rannali *et al*., 2008; Souto *et al*. 2003; Yinghong *et al*. 2006). Begomovirus infection on sweet potatoes were reported from Kenya, United States and Brazil that include *Sweet potato leaf curl virus* (SPLCV), SPLCV-US (isolate United States) and SPLCV-SP (isolate Sao Paolo) (Miano *et al*. 2006; Albuquerque *et al*. 2011). Several viral infections in sweet potatoes have been

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reported to cause yield losses such as SPFMV 80–90%, SPVG 30–40%, and SPFMV + SPCSV 50–98%. (Valverde *et al.* 2007).

In Indonesia, *Sweet potato potyvirus G* (SPVG) was reported to infect sweet potatoes in Tana Toraja, South Sulawesi, *Sweet potato feathery mottle virus* (SPFMV) in Bogor, and *Pepper Yellow Leaf Curl Virus* (PYLCV) and *Sweet potato virus C* in Malang. (Anjarsari *et al.*, 2013; Hondo *et al.*, 2018; Damayanti *et al.*, 2019). SPVC was reported to infect sweet potatoes in Malang with symptoms of chlorosis blotches and had nucleotide homology ranging from 89.5% –98.0% (Damayanti *et al.*, 2019).

Recently, based on field observations, symptoms of chlorosis blotches were found on sweet potato leaves in Gianyar (Figure 1) with incidence less than 20%. Chlorotic spots found resembled those of sweet potato plants infected with SPVC (Damayanti *et al.*, 2019). The species causing the symptom of chlorosis blotches in Bali is not known yet, therefore, detection of nucleic acids was carried out using Potyvirus universal primers. Previous research has reported that CMV (Valverde *et al.*, 2007), PYLCV, SPVG and SPFMV (Anjarsari *et al.*, 2013; Hondo *et al.*, 2018; Damayanti *et al.* 2019) were already found in Indonesia, therefore detection was done with CMV-specific primers, and Begomovirus and Potyvirus universal primers.

MATERIALS AND METHODS

Survey and Sampling

The survey and sampling of sweet potato plants were carried out in Bali (Denpasar, Gianyar, Badung, Buleleng, Tabanan, Klungkung, Karangasem, Jembrana, Bangli). Sampling was carried out by purposive sampling, ten symptomatic samples were taken from each location. Total samples taken were 90 and used as material for virus detection. The incidence of disease (ID) was calculated by:

$$ID = \frac{\text{The number of symptomatic samples}}{\text{Total samples in the field}} \times 100\%$$

RNA Extraction

Total RNA was extracted from symptomatic leaf tissue using the CTAB method. Total viral RNA was isolated from infected leaf as described by Doyle & Doyle (1987). Fresh tissue (0.1 gram) was grinded with liquid nitrogen and 500 µL of 10% CTAB buffer (cetil-trimethyl-ammonium bromide, 0.1 M Tris-HCl pH 8, 0.05 M EDTA, 0.5 M NaCl, 1% β-mercaptoethanol) was added. Mixtures were than transferred to 1.5 ml micro tubes and incubated in a water bath at 65°C for 1 hour (30 minutes for total RNA extraction). Micro tubes were shaked every 10 minutes to separate the lipids and proteins. After 60 minutes (30 minutes for total RNA extraction), micro tubes containing mixtures were taken from the water bath and allowed to stand for 2 minutes at room temperature, then 500 µL of the chloroform:isoamylalcohol mixtures with a ratio of 24: 1 (v/v) were added. The mixture was homogenized for 5 minutes, then centrifuged at 14,000 rpm for 15 minutes. A total of 450 µL of supernatant was taken and transferred into a new micro tube, then 3 M of sodium acetate was added from the volume of the supernatant. The mixture was homogenized and incubated at -80 °C for 2 hours or -20 °C overnight. After incubation, the nucleic acid mixture was centrifuged at 12,000 rpm for 10 minutes to precipitate the nucleic acids. The pellets were washed using 500 µL of 70% ethanol, then centrifuged again at 8000 rpm for 5 minutes, pellets were dried. After drying, pellets containing total RNA were dissolved in 50 to 100 µL of nucleic free water or TE buffer (pH 8) and it was stored at -20 °C until further processing.

cDNA Synthesis

The total RNA was used as a template for cDNA synthesis. The reverse transcription (RT) consisted of 1 µl oligo dNTP 10 mM, 2 µl total RNA, and 3.75 µl dH_2O. All reagents were homoegenized gently and incubated at 65°C for 5 minutes, then immediately chilled on ice. After that, reactants consisting of 2 µl of RT buffer, 1 µl dNTP 10 mM, 0.5 µl DTT 50 mM, 0.5 µl RNAse inhibitor (RiboLock RNase Inhibitor 20 units/µl, Thermo scientific), 0.5 µl MmuLV (Revertaid 200 units/µl, Thermo Scientific) to a total volume of 10 µl were added. The reverse transcription reaction was carried out at 42°C for 60 minutes followed by 70°C for 10 minutes in order to deactivate the enzymes. The cDNA product was used as a template for amplification.

Amplification of RNA by RT-PCR

The nucleotide pair used to amplify Potyvirus was CiFor (5'- GGI VVI GTI GGI WSI GGI AAR TCI AC-3') / CiRev (5'- ACI CCR TTY TCD ATD ATR TTI GTI GC-3'), with target amplicon size of ± 683 bp (Ha *et al.* 2008). cDNA amplification started from predenaturation at 94°C for 3 minutes for 1 cycle and continued for 40 cycles consisting of denaturation at 94°C for 1 minute, sticking at 40°C for 30 seconds, and DNA synthesis at 68°C for 1 minute. The last cycle is added synthesis stage at 68°C for 5 minutes. The composition of amplification reaction for a total volume of 25 µl was 12.5 µl Go Taq green 2× (Thermo scientific), 1 µl 10 µM reverse primer, 1 µl 10 µM forward primer, 9.5 µl nuclease-free water, and 1 µl cDNA.

DNA Visualization

DNA was electophoresis on 1% agarose gel (0.3 gram of agarose dissolved in 0.5x TBE buffer 30 ml). The agarose gel solution was cooled to 50ºC for 15 minutes, then fluoroVuo TM nucleic acid dye was added (Smobio, Taiwan). Electrophoresis was carried out at 100 V for 20 minutes. The results of the electrophoresis were then visualized under ultraviolet transilluminator and documented with a digital camera.

DNA Analysis

The amplified DNA fragments were sent to First Base Malaysia for the nucleotide tracing process. Results were then traced using BLAST to determine level of similarity to the nucleotide sequences found in GenBank (Basic Local Alignment Search Tool) program. Matrix homology identities were analyzed using BioEdit software, and phylogenetic trees were analyzed using MEGA v6.0 software using bootstrap 1000 times.

RESULTS AND DISCUSSION

Disease Symptoms in the Field

The sweet potato plants found in the field showed symptoms of chlorotic blotches (Figure 1). This symptom has been reported in sweet potato plants in Indonesia, Timor Leste, and Australia (Damayanti *et al.*, 2019; Maina *et al.*, 2018). The results showed that the incidence of disease caused by SPVC was more than 50% in Bangli, Buleleng, and Denpasar Regencies (Table 1). This shows that of the nine districts, only three districts have high incidence of the virus. The Ayamurasaki cultivar showed the lowest incidence of disease compared to other cultivars.

Amplification of Potyvirus DNA by RT-PCR

PCR results showed DNA with amplicon size ± 700 bp were successfully detected in samples from Bangli (Figure 2). Meanwhile, samples from eight other districts were not amplified with Potyvirus primers.

Results of molecular identification showed that SPVC was not detected in sweet potato plants in Central Java and West Java; thus, this implies that until now SPVC still limited in East Java and has not been found in other areas. SPVC infection in sweet potato plants in

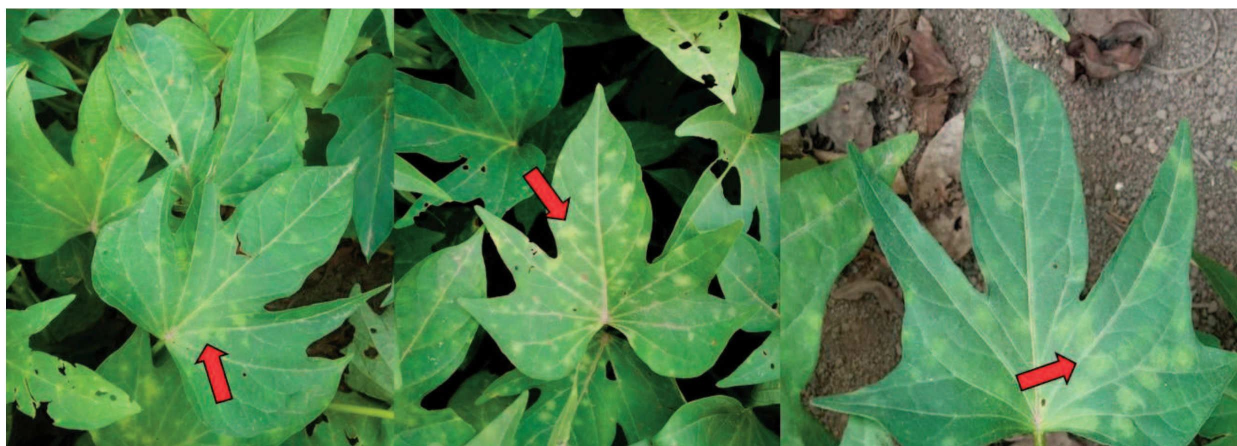

Figure 1. Symptoms of viral infection found in sweet potato plants in Bali are chlorosis blotches

ISSN 1410-1637 (print), ISSN 2548-4788 (online)

Table 1. Disease incidence of virus infections on sweet potato plants in Bali is based on field observation

Regions	Variety	Plant age (DAP)*	Disease incidence (%)
Denpasar	Ase Kuning	35	55.66
Badung	IR Melati	37	42.53
Bangli	Sukuh	30	66.43
Buleleng	IR Melati	44	62.36
Tabanan	Ayamurasaki	40	24.24
Karangasem	Sukuh	34	47.89
Klungkung	Sukuh	28	38.96
Gianyar	Ayamurasaki	37	27.27
Jembrana	Ase Kuning	43	35.78

*DAP= day after planting

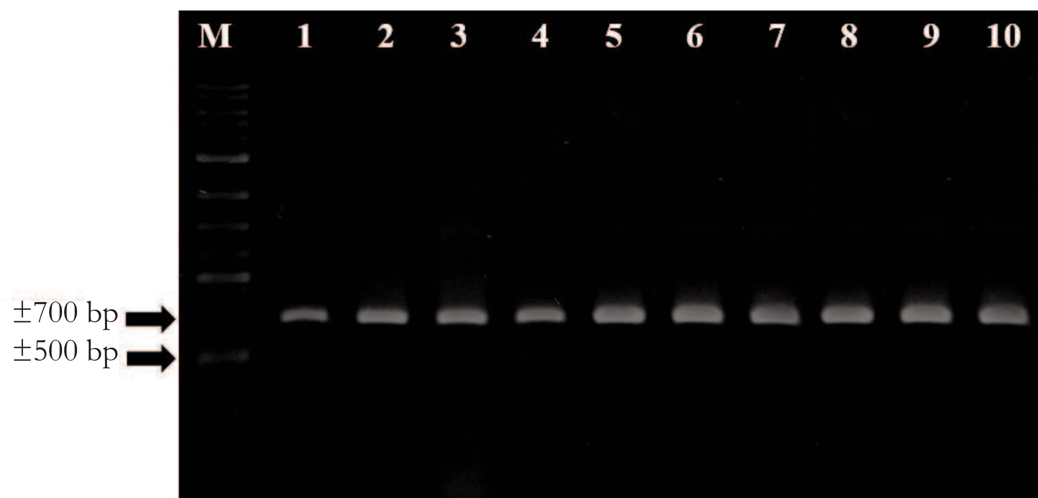

Figure 2. Visualization of DNA amplification with primers SPVC CIFOR/CiRev, samples from Gianyar (1–10); M, DNA Marker 1 kb (Smobio)

Bali is the first report in this study. The incidence of SPVC was very high in Bangli, presumably because of its high vector population, high temperatures ranging from 25–40 °C, and there no sweet potato varieties that are resistant to SPVC. The high disease incidence was influenced by the use of hybrid seeds throughout the growing season and monoculture cultivation system (Maruthi *et al.*, 2007), some of factors to cause this disease in Bali. Most fields are monoculture causing high disease incidence. In addition, another factor that causes the relatively fast spread of SPVC is the discovery of many vectors of aphids in the field. Maina *et al.*, (2018) also reported that the spread of SPVC in sweet potato in Australia was caused by insect vectors.

Molecular Characterization of SPVC

The homology of SPVC nucleotides and amino acids similarities sweet potato isolates from Bali with other countries (East Timor, China, South Korea, USA, Argentina, Australia, South Africa, Spain, Israel, Kenya, and Peru) were 89.6–96.8% and 90.4–97.4%, respectively (Table 2). These data indicate samples from Bali which were aligned with the sequence of SPVC isolates from GenBank. The SPVC isolates from Bali had the nucleotides homology at 96.8% and amino acids 97.4% and these were closely related to the Timor Leste isolate which infected sweet potato plants (MF572066), and had nucleotides homology than other countries. According to Adams *et al.* ,(2005),

Table 2. Homology of nucleotide (nt) and amino acid (aa) of SPVC Bali isolates with isolates from other country established in GenBank

Isolate	Geographical origin	Host	Symptoms	Homology (%) to Bangli Isolates		Accession number
				nt	aa	
TM66A	East Timor	*Ipomoea setosa*	Chlorosis blotches	96.8	97.4	MF572066
TM33A	East Timor	*Ipomoea setosa*	Chlorosis blotches	96.6	97.2	MF572064
Yu-17-47	China	*Ipomoea batatas*	Chlorosis blotches	96.5	97.2	MK778821
China1	China	*Ipomoea batatas*	Chlorosis blotches	96.3	97.0	KU877879
UN202	South Korea	*Ipomoea batatas*	Chlorosis blotches	96.3	97.0	KP115622
11-5	USA	*Ipomoea nil*	Chlorosis blotches	95.9	96.3	MH782222
Arg	Argentina	*Ipomoea batatas*	Chlorosis blotches	91.8	92.2	KF386015
A2-4CA	Australia	*Ipomoea setosa*	Chlorosis blotches	91.3	91.9	MG656433
SS-Bless	South Africa	*Ipomoea batatas*	Chlorosis blotches	90.7	91.5	MH023309
AM-MB2	Spain	*Ipomoea batatas*	Chlorosis blotches	89.7	90.6	KU511269
IL	Israel	*Ipomoea batatas*	Chlorosis blotches	90.6	91.2	JX489166
SRF109a	Kenya	*Ipomoea batatas*	Chlorosis blotches	89.7	90.6	MH264531
C1	Peru	*Ipomoea batatas*	Chlorosis blotches	89.6	90.4	GU207957
*ZYMV	East Timor	*Cucurbita pepo*	Mosaic	62.8	63.2	KY225545

**Zucchini yellow mosaic virus* (ZYMV) from East Timor was used as out group

the demarcation value of coat protein genes in Potyvirus can be identified as the same species if nucleotide homology above 76% and the amino acid sequence homology was more than 82%. The relationship between isolates based on the phylogenetic analysis showed that SPVC isolates from Bangli were in the same group with SPVC isolates from Timor Leste, China, Japan, South Korea and different from SPVC isolates that infected sweet potatoes from other countries (Australia, South Africa, America, Argentina, Spain, Israel, Kenya, and Peru) (Figure 3). In the phylogenetic tree, SPVC isolates were divided into three groups, namely groups I, II, and III. When viewed based on geographic location, SPVC group I included groups from Australia, Africa, and America. Group II included the Asia group, and group III included groups from Africa, Asia, and America.

From November to April, strong northwest monsoons each year brings heavy rain from East Timor and Indonesia across the sea to northern Australia. These monsoons can carry insect vectors that transmit viruses (Eagles *et al*., 2013; Maina *et al*., 2017). This case has occurred for *Zucchini yellow mosaic virus* (ZYMV; genus Potyvirus) in Australia, because symptoms of SPVC are similar to those found from ZYMV, which both a group of potyviruses that are transmitted nonpersistently by aphids (Clark *et al*., 2012; Loebenstein *et al*., 2009). However, due to the geographical conditions of Bali which has sea boundaries with other islands, it is possible that viruliferous vector insects cannot cross the vast ocean borders.

In addition, importation of infected tubers to Indonesia and East Timor may come from the same source, or may even be imported from East Timor. Moreover, in line with global climate changes, increasingly extreme weather conditions are predicted to occur in Indonesia. This includes more severe tropical cyclones in Southeast Asia and the annual monsoons that blow from northern Australia (Jones, 2016; Jones & Barbetti, 2012). Weather conditions will increase the likelihood of vector insects reaching areas in Indonesia and transmit SPVC to other sweet potatoes. The expected increase in insect vector arrivals from Southeast Asia (East Timor) arising from more intense cyclones and wind currents requires increased surveillance of viral pathogens in crops grown in Indonesia. Increased vector surveillance followed by rapid action to contain or eradicate new viruses will help prevent the establisment of destructive viruses such as SPVC in sweet potatoes, this statement according to Maina *et al*. (2017).

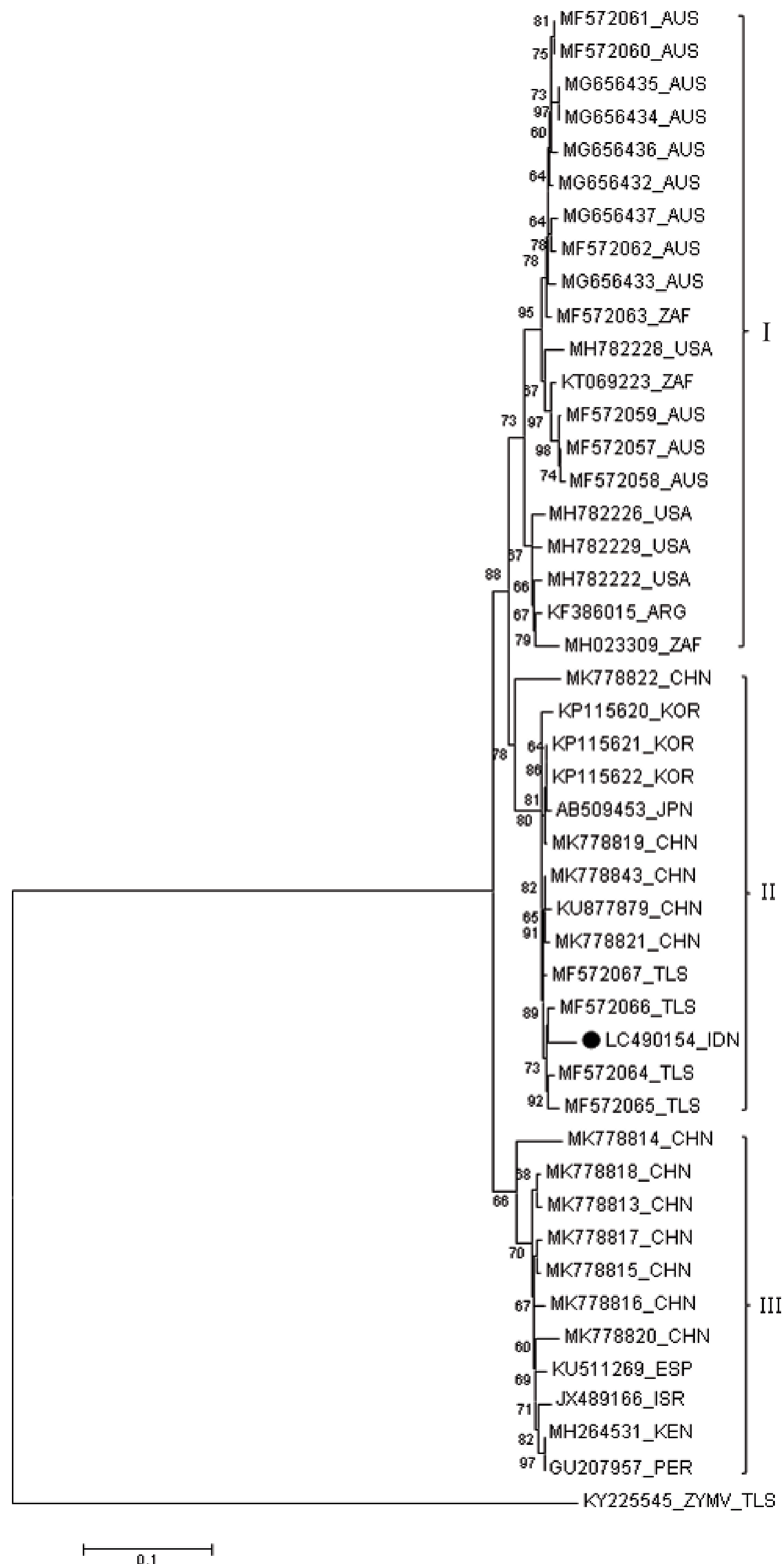

Figure 3. The phylogenetic tree of CI SPVC gene from Balinese isolates against 44 SPVC isolates in Genbank, *Zucchini yellow mosaic virus* (ZYMV) from East Timor was used as a comparison outside the group, Isolates marked with a dot were isolates from Bali. IDN-Indonesia, AUS-Australia, ZAF-South Africa, USA-America, ARG-Argentina, CHN-China, KOR-South Korea, JPN-Japan, TLS-East Timor, ESP-Spain, ISR-Israel, KEN-Kenya, PER-Peru

CONCLUSION

SPVC was successfully detected and identified from sweetpotato cultivation in Bali with homologous nucleotide and amino acid homology similarities with East Timor isolates. The SPVC has spread in East Java and Bali, therefore this SPVC report on the Bali Island is the first while second report after East Java.

ACKNOWLEDGEMENT

Thanks are conveyed to the coordinator of the Udayana University Plant Disease Laboratory for providing a virus detection tool for this research.

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