Molecular Characterization of *Sweet potato feathery mottle virus* (SPFMV) Isolates from Easter Island, French Polynesia, New Zealand, and Southern Africa

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ABSTRACT

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Strains of *Sweet potato feathery mottle virus* (SPFMV; *Potyvirus*; Potyviridae) infecting sweetpotato (*Ipomoea batatas*) in Oceania, one of the worlds' earliest sweetpotato-growing areas, and in southern Africa were isolated and characterized phylogenetically by analysis of the coat protein (CP) encoding sequences. Sweetpotato plants from Easter Island were co-infected with SPFMV strains C and EA. The EA strain isolates from this isolated location were related phylogenetically to those from Peru and East Africa. Sweetpotato plants from French Polynesia (Tahiti, Tubuai, and Moorea) were co-infected with SPFMV strains C, O, and RC in different combinations, whereas strains C and RC were detected in New Zealand. Sweetpotato plants from Zimbabwe were infected with strains C and EA and those from Cape Town, South Africa, with strains C, O, and RC. Co-infections with SPFMV strains and *Sweet potato virus G* (*Potyvirus*) were common and, additionally, *Sweet potato chlorotic fleck virus* (*Carlavirus*) was detected in a sample from Tahiti. Taken together, occurrence of different SPFMV strains was established for the first time in Easter Island, French Polynesia, and New Zealand, and new strains were detected in Zimbabwe and the southernmost part of South Africa. These results from the Southern hemisphere reflect the anticipated global distribution of strains C, O, and RC but reveal a wider distribution of strain EA than was known previously.

Sweetpotato (*Ipomoea batatas* Lam.) is an important subsistence and food security crop in many tropical parts of the world and also widely cultivated in subtropical regions. It is the third most important root crop grown globally after potato and cassava (11). It is believed to have originated in a crop domestication center located in southern Mexico and Central America (46). However, cultivated forms of sweetpotato may also have been selected from wild ones in the Andean crop domestication center in South America (26). It is also believed that migrating Polynesians were distributing sweetpotato plants within the islands of the Pacific Ocean long before European colonization of the region (9,26).

Viruses infecting sweetpotato have been identified and partially characterized at the

molecular level, especially in East Africa, Australasia, Peru, and the United States (1,3,5,22,29,34,35,39-41,43). However, their molecular characterization has received much less attention in other important sweetpotato production areas, such as Asia (3,5,10,15,27,28,36,37), Oceania (35), and southern (2,35) and western parts of Africa. *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*, family *Potyviridae*) is considered the most widely distributed of the approximately 20 known sweetpotato viruses (18,25,41,45). Many SPFMV isolates have been partially characterized at the molecular level (40,41,43).

SPFMV consists of four strains: common (C), East-African (EA), ordinary (O), and russet crack (RC) which are readily distinguished by phylogenetic analysis of the coat protein (CP)-encoding sequences (22). Strain C is distant phylogenetically from the other three strains, and its classification as a separate virus has been suggested (41). Three SPFMV strains (C, O, and RC) seem to be distributed globally and occur in sweetpotato production areas of North and South America, northern Africa, Asia, and Australia, where SPFMV

has been studied at the molecular level (1,10,15-17,22,27,28,36,37,40,41,43). Strain RC is not reported from East Africa, whereas strain EA was considered previously to be restricted to East Africa (22,31,41). However, recent studies revealed that strain EA is also found in Peru in South America (43) and occurs in Vietnam in Southeast Asia (15). Apart from Australia (see above) and the Northland region of New Zealand (12), SPFMV has also been detected in parts of Oceania, including the Solomon Islands (6). SPFMV also appears in listings of viruses in Fiji, Tonga, Indonesia, the Philippines, and Papua New Guinea (33,38) but the original reports and details of detection methods used were not cited. Similarly, little is known about the strains of SPFMV infecting sweetpotato in southern parts of Africa (2). The CP sequences of an EA strain and a C strain from Zambia and South Africa, respectively, are available on the sequence databank (Table 1), and SPFMV seems to be present also in Zimbabwe (8). This article reports a study in which sweetpotato plants were sampled in Oceania, Zimbabwe, and South Africa so that the strains of SPFMV that occur in these parts of the Southern hemisphere could be identified.

MATERIALS AND METHODS

Plant material. Collection of samples of sweetpotato plant material from different parts of Oceania (Fig. 1), South Africa, and Zimbabwe was described previously (35). In French Polynesia, storage roots of unknown, purple-skinned landraces were collected from commercial plantings in Toa hotu, Tahiti, and Mata ura, Tubuai, whereas orange-fleshed landraces were collected in Opunohu, Moorea, in 2004 (Fig. 1). In Dargaville in the Northland region of New Zealand, cuttings were taken from sweetpotato cultivars or landraces growing in home gardens in 2004. Storage roots from Easter Island of different skin colors and shapes were purchased from an open farmers' market at Hanga Roa. In South Africa, storage roots of cv. Borie were purchased in Cape Town. In

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Table 1. *Sweet potato feathery mottle virus* isolates whose complete coat protein encoding sequences were included in the study

Isolate	Strain ^a	Geographic origin	Accession number	Reference
3817-2	C	Tahiti	EU809495	This study
CTB3-1	C	South Africa	EU809485	This study
NZ1-1	C	New Zealand	EU809483	This study
NZ1-2	C	New Zealand	EU809493	This study
NZ4-4	C	New Zealand	EU809508	This study
OR1-2	C	Moorea	EU809486	This study
PS1B-4	C	Easter Island	EU809499	This study
PS3A-3	C	Easter island	EU809502	This study
ZIMB1-2	C	Zimbabwe	EU809510	This study
6	C	Argentina	U96625	Alvarez 1997, <i>unpublished</i>
25-4A	C	Kenya	AY523543	Ateka et al. 2004, <i>unpublished</i>
97-5S	C	Kenya	AY459594	Ateka et al. 2003, <i>unpublished</i>
Aus4c	C	Australia	AJ781778	41
Aus5c	C	Australia	AJ781779	41
Aus142-AC	C	Australia	AM050891	41
Aus55-4C	C	Australia	AM050892	40
C	C	United States	S43451	1
CY1	C	Taiwan	EF492048	Wang et al. 2007, <i>unpublished</i>
Ita1	C	Italy	AM076411	32
Md1-1	C	South Africa	AY459601	Ateka et al. 2003, <i>unpublished</i>
Nam12	C	Uganda	AY459596	Ateka et al. 2003, <i>unpublished</i>
Sor	C	Uganda	AJ539129	31
PS1B-1	EA	Easter Island	EU809496	This study
PS1B-2	EA	Easter Island	EU809497	This study
PS1B-3	EA	Easter Island	EU809498	This study
PS3A-1	EA	Easter Island	EU809500	This study
PS3A-2	EA	Easter Island	EU809501	This study
ZIMB1-1	EA	Zimbabwe	EU809509	This study
Ch2 (Pe)	EA	Peru	EU021067	43
Fe	EA	Peru	EU021070	43
Mis1	EA	Tanzania	AJ781783	41
M2-44	EA	Peru	EU021069	43
Nam1	EA	Uganda	AJ010704	22
Nam3	EA	Uganda	AJ010702	22
Nam6	EA	Uganda	AJ010703	22
Piu	EA	Peru	EU021072	43
Putisrabe	EA	Madagascar	AY459597	Ateka et al. 2003, <i>unpublished</i>
Ruk	EA	Uganda	AJ010707	22
Ruk55-2	EA	Uganda	AY523549S2	Ateka et al. 2004, <i>unpublished</i>
SP-33	EA	Peru	EU021068	43
Tar1	EA	Tanzania	AJ781784	41
Tar2	EA	Tanzania	AJ781785	41
VN-SP1	EA	Vietnam	DQ925467	16
Zam1	EA	Zambia	AY523552S4	Ateka et al. 2004, <i>unpublished</i>
3900	O	Tubuai	EU809484	This study
3817-1	O	Tahiti	EU809494	This study
CTB3-3	O	South Africa	EU809504	This study
OR1-4	O	Moorea	EU809488	This study
OR2-2	O	Moorea	EU809490	This study
OR2-4	O	Moorea	EU809492	This study
5	O	Argentina	U96624	Alvarez 1997, <i>unpublished</i>
115-1s	O	Kenya	AY523540	Ateka et al. 2004, <i>unpublished</i>
Arua10	O	Uganda	AY459595	Ateka et al. 2003, <i>unpublished</i>
Bau	O	Niger	AJ010699	22
CH	O	China	Z98942	10
CY2	O	Taiwan	EF492049	Wang et al. 2007, <i>unpublished</i>
K2	O	Korea	AF015541	36
Nig3	O	Niger	AJ010705	22
O	O	Japan	D16664	28
OR1-1	RC	Moorea	EU809482	This study
OR1-3	RC	Moorea	EU809487	This study
OR2-1	RC	Moorea	EU809489	This study
OR2-3	RC	Moorea	EU809491	This study
CTB3-2	RC	South Africa	EU809503	This study
NZ4-1	RC	New Zealand	EU809505	This study
NZ4-2	RC	New Zealand	EU809506	This study
NZ4-3	RC	New Zealand	EU809507	This study
Aus2	RC	Australia	AJ781775	41
Aus5RC	RC	Australia	AJ781776	41
Aus6	RC	Australia	AJ781777	41
Aus120-7	RC	Australia	AM050889	40
Aus142-ARC	RC	Australia	AM050890	40
C (RC)	RC	United States	S43450	1
Fio	RC	Peru	EU021065	43
K1	RC	Korea	AF015540	36
KmtMil	RC	Peru	EU021066	43
M2-41	RC	Peru	EU021064	43
S	RC	Japan	D86371	37

^a Common (C), East-African (EA), ordinary (O), and russet crack (RC) strains.

Zimbabwe, storage roots of cv. Chena were collected from fields close to the Victoria Falls airport. The samples from Easter Island and Africa were collected in 2005.

The samples were sent to the University of Helsinki, Finland. Plants were grown from them in an insect-proof greenhouse under the conditions stipulated in the import permit provided by the Finnish plant quarantine authorities. Plants of the virus indicator host *I. setosa* were grown from seed. Scions of *I. setosa* were top grafted on sweetpotato plants. Natural daylight was extended and supplemented when needed with illumination from high-pressure sodium lamps (OSRAM E40/ES, 400 W) to maintain a 16-h photoperiod (light intensity $250 \mu\text{E s}^{-1} \text{m}^{-2}$) at a temperature of 20 and 24°C (night and day).

Serological detection of SPFMV. SPFMV was detected in the leaves of sweetpotato plants or the leaves of scions of *I. setosa* grafted on sweetpotato 4 weeks after grafting by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) or double-antibody sandwich (DAS) ELISA with polyclonal anti-SPFMV rabbit antibodies and the corresponding alkaline phosphatase (AP) conjugated anti-rabbit mouse antibodies or AP-conjugated anti-SPFMV rabbit antibodies, respectively, using procedures described previously (13,40). The samples in this study were also tested serologically for *Sweet potato virus 2* (SPV2, genus *Potyvirus*), which was also called Sweet potato virus Y (4) or *Ipomoea vein mosaic virus* (21,39) in some studies, and *Sweet potato virus G* (SPVG, genus *Potyvirus*), *Sweet potato mild mottle virus* (SPMMV, genus *Ipomovirus*), *Sweet potato chlorotic fleck virus* (SPCFV, genus *Carlavirus*), and *Sweet potato chlorotic stunt virus* (SPCSV; genus *Crinivirus*). The antiviral CP antibodies used were kindly provided by S. Fuentes and L. F. Salazar (International Potato Center-CIP, Lima, Peru) or H. J. Vetten (Institute for Plant Virology, Braunschweig, Germany). Detection of SPVG in the same samples has been published elsewhere (35).

RNA extraction, cDNA synthesis, and PCR amplification. For molecular detection and further characterization of SPFMV isolates, leaf samples were collected from scions of *I. setosa* grafted onto the sweetpotato plants. Leaf tissue (0.5 g) was frozen in liquid nitrogen and ground with mortar and pestle. Total RNA was extracted using a Trizol-like reagent (7). RNA was diluted to 200 μl with nuclease-free water (Sigma-Aldrich, St. Louis) and quantified using a spectrophotometer (Agilent 8452 A; Agilent Technologies, Waldbronn, Germany). cDNA synthesis was carried out in a 25- μl reaction volume with 3 μg of total RNA, polyT(25) primer, and *Moloney murine leukemia virus* (MMLV) reverse transcriptase (Promega

Corp., Madison, WI) according to the manufacturer's instructions. The cDNA was diluted 1:10 and used as a template in polymerase chain reaction (PCR) amplification.

The forward primer FM1360-F was used to amplify SPFMV strains RC, O, and EA, whereas the forward primer SPVC474-F was used for strain C (40). The reverse primer 10820-R (41) was used with both forward primers. The amplified fragments (including primer sequences) were expected to be 1,356 nucleotides (nt) in length for strains RC, O, and EA and 1,335 nt for strain C. The amplicons consisted of 187 nt (strains RC, O, and EA) and 172 nt (strain C) of the RNA-dependent RNA polymerase (NIB)-encoding region, the entire CP-encoding region (945 nt in strains RC, O, and EA and 939 nt in strain C), and 224 nt of the 3'-nontranslated (3'NTR) region. With a few samples, PCR amplification was also done with the *Potyvirus*-specific degenerate forward primer PVD2-F (14) and the reverse primer 10820-R (41), which resulted in a product of approximately 1.8 kb.

The PCR reaction (25 μl) consisted of 5 μl of 5 $\times$ Phusion GC reaction buffer (containing 7.5 mM MgCl_2), 0.5 μl of MgCl_2 (50 mM), 0.5 μl of 10 mM dNTPs, 2 μl of 10 μM primers, 0.25 μl (0.5 U) of Phusion

high-fidelity DNA polymerase (Finnzymes, Espoo, Finland), and 2.5 μl of cDNA. The cycling parameters were 98°C for 1 min; followed by 40 cycles of 98°C for 10 s, 59°C (primer FM1360-F), 57°C (SPVC474-F), or 56°C (PVD2-F) for 20 s, and 72°C for 45 s; and final extension at 72°C for 10 min. Amplification products were analyzed by agarose gel electrophoresis and visualized under UV light.

Restriction fragment length polymorphism analysis, cloning, and sequencing. PCR amplicons were subjected to restriction analysis without cloning to detect possible mixed infections of SPFMV strains or variants. An aliquot of 5 μl of the PCR products was used for digestion with the restriction endonuclease *AccI* (Promega Corp.) at 37°C for 2 h according to the manufacturer's instructions. Restriction fragments were separated by electrophoresis at 130 V for 45 to 60 min in a 1.5% agarose gel supplemented with ethidium bromide and visualized under UV light.

The PCR amplification products were purified using E.Z.N.A Gel Extraction Kit (Omega Bio-Tek, Lilburn, GA). Prior to ligation, an A-tailing reaction was carried out on the PCR products. The reaction mix (10 μl) contained 1 μl of 10 mM dATPs, 1 μl of 10 $\times$ optimized DyNAzyme PCR buffer (containing MgCl_2), and 1 U of DyNAzyme II DNA polymerase (Finn-

Fig. 1. Areas of Oceania and Australasia which were sampled for strains of *Sweet potato feathery mottle virus* (SPFMV): Northland, New Zealand (NZ), Tahiti (TH), Tubuai (TB), and Easter Island (EI). A few sweetpotato samples (35) were also tested from Java (JA) and Hawaii (HI) but no SPFMV was detected. For SPFMV strains detected in Australia (AU), East Asia, the United States, and Peru, see Table 2. Note that, in the map, the distances do not represent actual distances. For example, the distance between Tahiti and Tubuai is approximately 700 km, whereas the distance from Easter Island to Tahiti and New Zealand is approximately 4,000 and 6,000 km, respectively.

zymes). The reaction was done at 70°C for 30 min and terminated by putting the tube on ice. Subsequently, PCR products were ligated to the pGEM-T or pGEM-T Easy Vector (Promega Corp.) according to the manufacturer's instructions and cloned in *Escherichia coli* (strain DH5 α).

Subsequently, clones were selected for sequencing based on differences in restriction fragment length polymorphism (RFLP) patterns. Bacterial cells were picked from colonies of *E. coli* on petri dishes and screened by colony PCR and restriction analysis of PCR products. The colony PCR reaction (20 μ l) consisted of 1 $\times$ (NH $_4$)SO $_4$ buffer, 1 μ l of MgCl $_2$ (25 mM), 0.4 μ l of dNTPs (10 mM), 0.4 μ l of primers (10 μ M each), and 0.5 U of *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania). The primer pairs used for amplification of the viral genome fragments were also used for colony PCR. The cycling conditions were 94°C for 2 min; 30 cycles of 94°C for 30 s, 52°C for 20 s, and 72°C for 2 min; and final extension at 72°C for 10 min. An aliquot of 1 to 5 μ l of

the PCR amplification product was digested and analyzed by electrophoresis as described above.

The plasmids were purified from *E. coli* with GenElute Plasmid Miniprep Kit (Sigma-Aldrich) and sequenced using M13 primers (Promega Corp.) and, subsequently, using nested primers designed according to the viral sequences. Both strands of at least two independent cDNA clones from each SPFMV isolate were sequenced. All sequencing was done with an Applied Biosystems Dye Terminator (v.3.1) sequencing kit and a capillary sequence analyzer at the Haartman Institute, Sequencing Core Facility, University of Helsinki, Finland. Sequences were edited and assembled using the ContigExpress program of the Vector NTI software package (Invitrogen, Carlsbad, CA). Phylogenetic analyses were done on the complete CP-encoding sequences (Table 1). Sequence alignments and phylogenetic analysis using the neighbor-joining method were done using the MEGA software package (version 3.1; 23).

RESULTS

Initially, SPFMV was detected in sweetpotato primarily by serological testing of *I. setosa* tissue samples from the scions top-grafted on sweetpotato plants. Next, reverse-transcription PCR using SPFMV-specific primers that amplified a 1.35-kb portion of the 3' end of the viral genome was used. The amplicons obtained were subjected to RFLP analysis using *AccI*, which provided preliminary information about the SPFMV strain present or whether there was mixed infection with different strains (Fig. 2). The RFLP patterns readily distinguished strains RC and C from each other and also distinguished these two strains from strains O and EA. However, the patterns for strains O and EA were too similar to each other to be readily distinguishable (Fig. 2A), as predicted based on a computer-assisted virtual RFLP analysis of the SPFMV sequences available in databases (*data not shown*). The strains of SPFMV present were identified by phylogenetic analysis of the CP-encoding sequences (Table 1; Fig. 3). The results revealed frequent co-infections with different strains of SPFMV in sweetpotato plants (Tables 1 and 2).

The storage roots of cv. Borie sampled in Cape Town, South Africa, were co-infected with strains C (isolate CTB3-1), O (isolate CTB3-3), and RC (isolate CTB3-2) (Tables 1 and 2; Fig. 3). Storage roots of cv. Chena from Victoria Falls, Zimbabwe, were co-infected with strains EA (isolate ZIMB1-1) and C (isolate ZIMB1-2). In addition, these sweetpotato plants from South Africa and Zimbabwe were also infected with SPVG (35).

Co-infection with strain C (isolate NZ4-4) and variants of strain RC (isolates NZ4-1, NZ4-2, and NZ4-3) were detected in plants of cv. Owairaka Red collected from Dargaville, in the Northland region of New Zealand (Tables 1 and 2). These same plants were also infected with SPVG (35). Plants of cv. Romanau collected from the same area contained only SPFMV strain C (isolates NZ1-1 and NZ1-2) (Tables 1 and 2; Fig. 3).

Three strains of SPFMV were detected in sweetpotato sampled in French Polynesia. The unknown cultivar from Toa hotu, Tahiti, was co-infected with SPFMV strains C (isolate 3817-2) and O (isolate 3817-1) (Tables 1 and 2; Fig. 3). Furthermore, it was infected with SPVG (35) and also SPCFV that was unambiguously detected based on high absorbance values in repeated tests by DAS-ELISA. The storage roots of an unknown cultivar collected from Oponohu, Moorea, located 17 km northwest from Tahiti, were co-infected with strains C (isolate OR1-2), O (isolates OR1-4, OR2-2, and OR2-4), and RC (isolates OR1-1, OR1-3, OR2-1, and OR2-3) (Fig. 3). Strain O (isolate 3900) alone was detected in storage roots of an unknown cultivar sampled in Mata ura, Tubuai, lo-

Fig. 2. Restriction fragment length polymorphism (RFLP) of amplicons obtained by reverse-transcription polymerase chain reaction (PCR) from *Sweet potato feathery mottle virus* (SPFMV)-infected plants were digested with *AccI*. **A**, Typical restriction patterns of amplicons covering the 3' terminus of the viral genome (1.35 kb, amplified with primers FM1360 and 10820) for the four strains of SPFMV. Cloned PCR products were amplified from plasmids and digested with *AccI*. **B**, RFLP analysis of the PCR amplicons obtained from infected plants. The RFLP patterns in OR1 and PS1B indicate a mixed infection with SPFMV strains, whereas strains O, EA, and C are detectable in plants 3817, ZIMB1, and NZ1, respectively. M = Gene Ruler DNA Ladder Mix (MBI Fermentas) in which the sizes of a few marker bands have been indicated in nucleotides.

cated 640 km south of Tahiti (Tables 1 and 2; Fig. 3). These plants were also infected with SPVG (35).

Storage roots of cvs. Tukuamei and Cara Chica from Easter Island were co-infected with strain C (isolates PS1B-4 and PS3A-3, respectively) and strain EA (isolates PS1B-1, PS1B-2, and PS1B-3 in Tukuamei and isolates PS3A-1 and PS3A-2 in Cara Chica) (Tables 1 and 2; Fig. 3). The plants of cv. Tukumehai were also infected with SPVG (35). Four of the five SPFMV isolates of the EA strain from Easter Island clustered together in the phylogenetic tree and were closest to EA strain isolates from Peru [isolates M2-44, Piu, SP-33, Ch2(Pe), and Fe] (43) (Fig. 3). In addition, the previously characterized EA strain isolate from Zambia, Africa (isolate Zam1) was closely related to EA strain isolates from Easter Island and Peru. In contrast, the EA strain isolates PS1B-3 characterized from Easter Island and ZIMB1-1 characterized from Zimbabwe in this study and the large number of EA strain isolates previously characterized from East Africa were more closely related to each other (Table 1; Fig. 3).

Apart from the unknown cultivar from Tahiti, no other sample tested in this study was positive for SPCFV, and no sample infected with SPV2, SPMV, or SPCSV was detected.

DISCUSSION

Although strains C, O, and RC of SPFMV are considered to be widely distributed in sweetpotato-growing areas worldwide (41,43,45), the occurrence of these strains has been little studied in most regions of and bordering the Pacific. There have been no such studies in the islands of Oceania, one of the world's earliest sweetpotato-growing regions (9,26). The results of this study in the Southern Hemisphere support the conclusion about the worldwide distribution of these three strains (Table 2). The combined presence of strains C and RC was revealed in three widely dispersed locations—South Africa, French Polynesia, and New Zealand—as had been reported previously in Peru and the United States. Strain C was also found in two other widely dispersed locations, Zimbabwe and Easter Island, whereas strain RC was reported previously in Japan, China, Peru, and the United States (Table 2). Strain O was found in South Africa and French Polynesia in this study and was described previously from Japan, China, Southeast Asia, and East Africa (Table 2). In addition, strains C, O, and RC have all been documented from sweetpotato in Australia (17,40,41).

Detection of SPFMV strain EA in Easter Island was somewhat unexpected. Strain EA was formerly considered to be largely restricted to East Africa, which constitutes a center of evolution and diversity for it (41,42). However, strain EA was recently

found in sweetpotato plants in Peru (43), which is within the Andean domestication center, one of the two global domestication centers of sweetpotato (9,26), and has been

found in Vietnam in Southeast Asia (15). It was proposed that sweetpotato plants were originally taken to Oceania several millennia ago from the coastline of South Amer-

Fig. 3. Phylogenetic analysis of the *Sweet potato feathery mottle virus* (SPFMV) coat protein encoding sequences (945 nucleotides). The clusters containing virus isolates belonging to the four strains of SPFMV are indicated. The scale bar indicates as Kimura units (20). The bootstrap values of 1,000 replicates are shown.

Table 2. Occurrence of strains of *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato virus G* (SPVG) in sweetpotato in different geographical areas of the Pacific region and eastern Africa

Location ^b	Strain of SPFMV ^a				Reference	SPVG ^c
	C	RC	O	EA		
South America						
Peru	+	+	–	+	43,44	+
Oceania						
Australia	+	+	+	–	17,40,41	(+)
Easter Island	+	–	–	+	This study	+
Moorea	+	+	+	–	This study	–
New Zealand	+	+	–	–	This study	+
Tahiti	+	–	+	–	This study	+
Tubai	–	–	+	–	This study	+
North America:						
United States	+	+	–	–	1,21,39	+
East Asia						
China and Taiwan	+	+	+	–	10, AJ310201, AJ310202	+
Japan	–	+	+	–	27,28,37	–
Korea	–	+	+	–	36	–
Vietnam	–	–	–	+	16	–
Africa						
East Africa	+	–	+	+	2,22,41	+
Zimbabwe	+	–	–	+	This study	+
South Africa	+	+	+	–	This study, AY459601	+

^a Common (C), russet crack (RC), ordinary (O), and East-African (EA) strains.

^b Locations listed in Table 2 are detailed in Table 1, including isolate names and genebank accession numbers.

^c Reported or reviewed in previous reports (2,35,43). Parentheses for SPVG in Australia indicate that the virus was detected in newly imported sweetpotato in post-entry quarantine and then destroyed (35). In East Africa, SPVG was detected in Ethiopia (35).

ica that now includes Ecuador, Peru, and northern Chile (9,26). This scenario seems plausible because sweetpotato plants were grown in Oceania long before the arrival of European colonizers and were introduced to widely dispersed locations in the Pacific. The most recent example of these introductions was to New Zealand about 800 years ago by settlers from Polynesia (24). Because SPFMV strains C, O, and RC but not EA were found in sweetpotato landraces in several islands of French Polynesia, and strains C and RC but not EA were detected in sweetpotato plants grown in New Zealand, it seems that strain EA might not have reached these locations yet despite cultivation of sweetpotato in them long before Europeans colonizers arrived. Taken together, these findings suggest that strain EA originates in East Africa and has been distributed from there to other locations relatively recently.

Molecular detection procedures have revealed infection with SPVG in sweetpotato in many parts of the Pacific region (35). In contrast, SPV2 was not found in the samples tested in this study. Moreover, the only parts of the Pacific region in which it has been detected are Taiwan, where it was originally described (4); China (5); and Australia and New Zealand (17,34,40). This suggests that SPVG is more widely distributed than SPV2 in the Pacific region. Co-infection with SPVG and several strains of SPFMV was found frequently in many areas, which was a noteworthy feature of this study (Table 2). Furthermore, one cultivar tested from Tahiti was co-infected with SPVG, three strains (C, O, and RC) of SPFMV, and also SPCFV, a virus which has not been reported previ-

ously from French Polynesia or other parts of Oceania. SPCFV is listed among viruses occurring in Indonesia and the Philippines but detection methods have not been specified (38). In Australia, SPCFV was detected and partially sequence characterized from sweetpotato also infected with SPV2 and SPFMV strain RC (17).

It is not known how severe the yield losses caused by mixed infections with different strains of SPFMV, other potyviruses, or the *Carlavirus* SPCFV may be in sweetpotato cultivars and landraces grown in the Pacific region. Studies on the economic implications of such mixed infections on sweetpotato production are lacking. In cv. Beauregard grown in the United States (21) and Australia (40), mixed infections with SPFMV strain RC, SPVG, and SPV2 did not greatly alter expression of foliar symptoms or individual virus titers compared with single-virus infections (21); however, such mixed infections resulted in premature death of *I. setosa* scions. Moreover, different sweetpotato cultivars are likely to differ in sensitivity to infection and studies on the implications of multiple infections on yield of tuberous roots are needed. Similar studies are also warranted in South Africa, where cv. Borie was co-infected not only by strains C, O, and RC of SPFMV but also by SPVG. In Africa, co-infections of SPCSV with SPFMV or SPMMV cause synergistic interactions that induce the most severe diseases which often result in nearly complete yield loss in infected sweetpotato plants (19,30). SPCSV also synergizes SPVG, SPV2, and SPCFV as well as the different strains of SPFMV (21,44). Fortunately, SPCSV and SPMMV were not

found in Australia and Oceania in this or previous studies (35,41). The main vector of SPCSV (*Bemisia tabaci* Gennadius) (25) is present in these areas and, therefore, a key biosecurity issue is to prevent SPCSV from spreading to the Pacific region.

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