Molecular variability of sweetpotato feathery mottle virus and other potyviruses infecting sweetpotato in Peru

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Abstract

Several potyviruses are found infecting sweetpotato (*Ipomoea batatas*) in Peru, of which sweetpotato feathery mottle virus (SPFMV, genus *Potyvirus*) is the most common. However, sequence data for these viruses are not available from Peru. In this study, the 3'-terminal ~1,800 nucleotide sequences of 17 potyvirus samples collected from the six main sweetpotato-producing areas of Peru over the past 20 years were determined and analyzed. Results of sequence comparisons and phylogenetic analysis showed that three of the four recognized SPFMV strain groups, including the East African strain, are established in Peru as well as two other potyviruses: sweetpotato virus G (SPVG) and sweetpotato virus 2 (SPV2). The analysis further revealed that SPFMV, SPVG and SPV2 are related and form an *Ipomoea*-specific phylogenetic lineage within the genus *Potyvirus* and identified for the first time recombination events between viruses from different strain groups of SPFMV.

Keywords: sweetpotato virus II, ipomoea vein mosaic virus, Sweetpotato virus Y.

Introduction

Sweetpotato virus disease (SPVD) is probably the most devastating disease constraint of sweetpotato [*Ipomoea batatas* (L.) Lam] worldwide [9]. It is caused by co-infection of the aphid-transmitted sweetpotato feathery mottle virus (SPFMV, family *Potyviridae*, genus *Potyvirus*) and the whitefly transmitted sweetpotato chlorotic stunt virus (SPCSV; genus *Crinivirus*; family *Closteroviridae*) [17, 20, 45].]. Single infections of SPFMV usually show mild or no symptoms, and no appreciable yield reduction can be observed [12, 20, 33]. However, co-infection with SPCSV causes SPVD, which is characterized by very severe symptoms such as general chlorosis, stunting, leaf strapping, leaf crinkling and even plant death [17, 23, 45], and yield losses ranging from 70 to100% [20, 33, 38, 40]. Molecular studies have shown that co-infection of SPCSV enhances SPFMV RNA viral titers by at least 600-fold, whereas SPCSV titers remain equal or are reduced as compared to single infection [23, 24, 38]. The severity of SPVD, and the degree of SPFMV titer increase, depends on the strain of SPFMV involved in the double infection [20, 24]. Besides SPFMV, several other potyviruses, as well as other, unrelated viruses, can cause synergistic diseases when co-infecting with SPCSV [24, 38, 51], although the importance of these interactions for yield losses in the field are not well known.

SPFMV is one of the most widespread viruses infecting sweet potato [36]. The CP genomic region of SPFMV has been used in previous studies to establish phylogenetic relationships among SPFMV samples. It can be divided into four phylogenetic lineages [5, 21, 25, 38, 49, 50]: East Africa (EA), constituted by East African samples; Russet Crack (RC), comprising samples from Australia, Africa, Asia and North America; Ordinary (O) containing samples from Japan, China, Korea, Niger, Nigeria and Argentina; and Common (C) including samples from USA, China, Australia, East Africa and Argentina. Unlike the geographically unrestricted C, RC and O strains, the EA strain has almost exclusively been detected in the East African countries, the possible exceptions being two sequences available from the GenBank from Spain and Portugal [53] (Table 1). SPFMV has been reported from sweet potato in Peruvian fields since 1987 [30], but it was only after 1998 that the prevalence of SPVD emerged, possibly due to the increase of whitefly populations during the exceptionally strong El Niño phenomenon of that year [20]. Although the impact of SPVD on the yield of Peruvian sweet potato cultivars has been assessed [20], no molecular studies have been carried out concerning variability of its two causal agents. Cedano et al. [10] reported that some SPFMV isolates differed in the severity of symptoms induced in *Ipomoea nil*. SPFMV-C1, was collected during the 1980s [39] and shown to be closely related to the C strain [22, 42], whereas three other isolates, M2-41, M2-44 and C-18, were obtained from SPVD-infected sweet potato plants and differed in their symptomatology and serological reaction with monoclonal and polyclonal antibodies [20].

Table 1. SPFMV isolates and samples used in this study

Isolate/ sample	Strain	Origin/ collection date	Acc number		Isolate/ sample	Strain	Origin/ collection date	Acc number
Fe	EA[a]	Ferreñafe, Peru/2006	EU021070		Bkb 1	EA	Tanzania	AJ781781
C14	EA[a]	Cañete, Peru/2006	EU021071		Bkb 2	EA	Tanzania	AJ781782
Ch2	EA[a]	Chimbote, Peru/2006	EU021067		Mis1	EA	Tanzania	AJ781783
M2-44	EA[a]	Cañete, Peru/2003	EU021069		Tz1	EA	Tanzania	AJ539131
Piu	EA[a]	Piura, Peru/2006	EU021072		Tz2	EA	Tanzania	AJ539132
SP-33	EA[a]	Huaral, Peru/1987	EU021068		Tar1	EA	Tanzania	AJ781784
Fio	RC[a]	Cañete, Peru/2005	EU021065		Tar2	EA	Tanzania	AJ781785
KmtMil	RC[a]	Cañete, Peru/2005	EU021066		Kby 1	EA	Uganda	AJ781791
M2-41	RC[a]	Cañete, Peru/1999	EU021064		Kby 2	EA	Uganda	AJ781792
C1	C[a]	Lima, Peru/1987	EU021057		Mbl2	EA	Uganda	AJ781788
Ch4	C[a]	Chimbote, Peru/2006	EU021062		Bny	EA	Uganda	AJ539130
C18	C[a]	Cañete, Peru/1999	EU021059		Nak	EA	Uganda	AJ781790
M2-63	C[a]	Cañete, Peru/1999	EU021060		Apa	EA	Uganda	AJ781787
C21	C[a]	Cañete, Peru/1999	EU021061		Mpg2	EA	Uganda	AJ781789
SR	C[a]	San Ramón, Peru/2005	EU021063		Rak6e	EA	Uganda	AJ010706
YV	C[a]	USA	EU021058		85-7S	EA	Kenya	AY459593
Aus5c	C	Australia	AJ781779		54-9S	EA	Kenya	AY459592
Aus4c	C	Australia	AJ781778		Canar3	EA	Spain	AY459600
C	C	USA	S43450		Port	EA	Portugal	AY459599
SOR	C	Uganda	AJ539129		Zam 1	EA	Zambia	AY523552S4
25-4[a]	C	Kenya	AY523543		Unj1	EA	Tanzania	AJ781786
51-9S	C	Kenya	AY459591		O	O	Japan	D16664
Nam 12	C	Uganda	AY459596		TZ4	O	Tanzania	AY459598
Aus6	RC	Australia	AJ781777		Strain 5	O	Argentina	U96624
Aus5	RC	Australia	AJ781776		Nig 3	O	Nigeria	AJ010705
Aus2	RC	Australia	AJ781775		Arua10	O	Uganda	AY459595
Eg1	RC	Egypt	AJ515378		CH	O	China	Z98942
Eg9	RC	Egypt	AJ515379		Bau	O	Nigeria	AJ010699
S	RC	Japan	D86371		115-1S	O	Kenya	AY523538S3
Bag	EA	Tanzania	AJ781780					

[a] Sequence determined in this study

Studies on other potyviruses infecting sweet potato are less abundant. These include sweet potato latent virus (SPLV), found in Asia, Africa and Peru [20, 29], sweet potato mild speckling virus (SPMSV) from Argentina and Peru [15, 20], sweet potato virus G (SPVG), identified in China, Egypt, Ethiopia, Europe and the United States [3, 6, 12, 13, 21, 47] and a potyvirus first reported as sweet potato virus II [43], and later named ipomoea vein mosaic virus [47], sweet potato virus Y [4] or sweet potato virus 2 (SPV2) [49], has been identified from the United States [47], Africa, Taiwan, China, Portugal [49] and Australia [6]. Since the proposal to refer to this new potyvirus

species as SPV2 has been favorably considered by the International Committee on the Taxonomy of Viruses, this name is used here. SPLV and SPMSV have been reported in Peru at low frequency [20].

Understanding the molecular variation of viruses is essential to design knowledge-based strategies to control them. In the present study, we determine the nucleotide sequence of the region encompassing the 3'-terminal ~1,800 nucleotides (nts) of 17 potyvirus samples mostly collected from SPVD-affected plants from the major sweetpotato-producing areas in Peru. Most of the viruses were identified as SPFMV, but we also report for the first time the occurrence of SPV2 and SPVG in Peru and South America. Phylogenetic analysis of SPFMV sequences indicates a variable population of SPFMV in Peru, including EA, C and RC strain groups, and provides evidence for the existence of recombinants between strains.

Materials and methods

Virus-infected plant samples and virus isolates

One symptomless sweetpotato plant and 14 with SPVD-like symptoms were collected at random from six main sweetpotato-producing areas in Peru (Fig. 1). Plants with SPVD-like symptoms were found in all locations except for the Chira Valley in Piura, where only symptomless plants were collected. Details of the samples and isolates, their names, province of origin and year of collection are shown in Table 1 (Fig. 4 for SPV2 and SPVG). Stem cuttings of collected plants were maintained in an insect-proof greenhouse at CIP headquarters, Lima, Peru, for at least 3 weeks before analysis. Asymptomatic plants were grafted onto the indicator plant *I. setosa*, which was observed for symptom development and analyzed by serological means. The presence and identity of sweetpotato viruses were confirmed using antisera included in the NCM-ELISA sweetpotato virus detection kit from CIP, Lima, Peru [51], according to the manufacturer's protocol. A number of SPFMV isolates kept in desiccated *I. nil* leaves for as long as 20 years, as well as the Peruvian isolate of SPFMV, C1 [39, 42], and the North American isolate, YV [35], maintained in *Nicotiana benthamiana* and *I. nil*, respectively, as part of the CIP virus collection, were also included in the study. Isolation of these viruses was done by three consecutive single-lesion transfers on *Chenopodium amaranticolor* [10, 30, 35, 39].

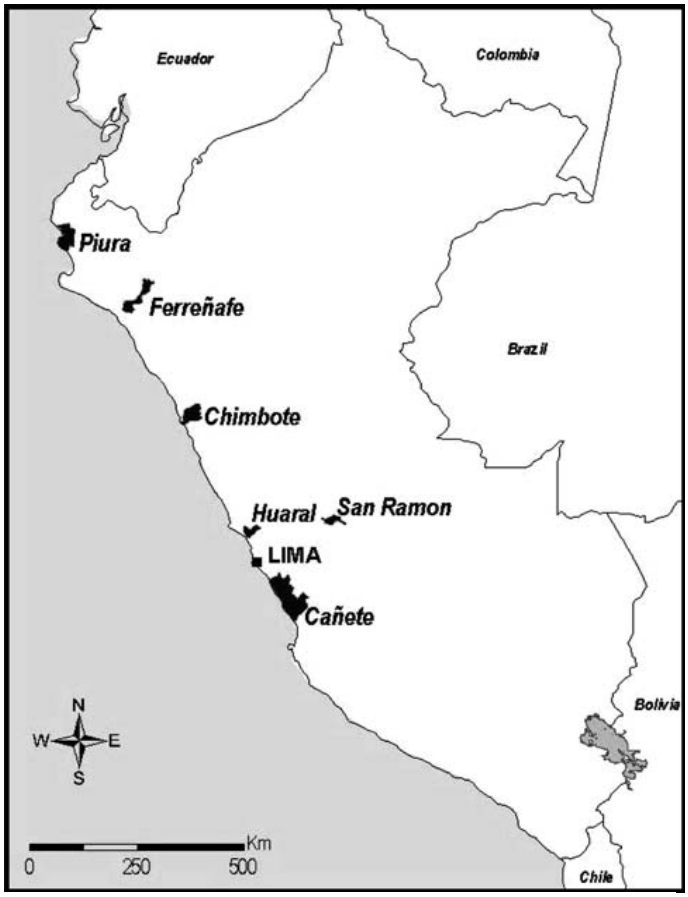

Figure 1. Locations of the sweet potato fields surveyed in Peru

RNA extraction, RT-PCR and cloning

Total RNAs were extracted from approximately 0.2 g leaves of SPVD-infected sweetpotato or SPFMV-infected indicator plants using TRIZOL reagent (Invitrogen, CA, USA) according to the manufacturer's procedure. For lyophilized samples, the initial amount of tissue was 0.02 g. Although this provided sufficient amounts of total RNA, a further purification of high-molecular-weight RNA with 4M LiCl4 significantly improved the subsequent RT-PCR reaction. The integrity of the isolated RNA was visually verified after electrophoresis in a standard formaldehyde agarose gel and staining with ethidium bromide. Reverse transcription was performed on extracted RNA using AMV reverse transcriptase (Promega, WI, USA) according to the manufacturer's

recommendations, with the primer FMV10820 (Table 2), corresponding to the last 20 nts of the virus genome excluding the poly A tail of the 'SPFMV' subgroup (see Discussion) of potyviruses [50]. A fragment comprising the 3'-terminal ~1,800 nts of the potyvirus genome including the 3'-terminal part of the NIb gene, the complete CP gene and the 30 UTR was amplified by PCR using the potyvirus-specific forward primer PVD-2 (Table 2) [16] and the reverse primer FMV 10820. The PCR cycling conditions were set as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 15 s, 52°C for 20 s and 72°C for 90 s, and then one final elongation at 72°C for 10 min. PCR products were separated on 1% agarose gels and fragments of interest recovered by using the Wizard SV gel extraction kit (Promega) according to the manufacturer's recommendation. The eluted DNA was ligated into plasmid vector pCR 2.1 (Invitrogen) according to the manufacturers' instructions and cloned in Escherichia coli strain DH5*a*.

Table 2. List of synthetic oligonucleotides primers used in this work

Virus	Primer name	Sequences	Reference
SPFMV, SPV2, SPVG (cloning)	FMV 10820	5'-GGCTCGATCACGAACCAA-3'	Tairo et al. (50)
	PVD-2	5'-GGBAAYAAYAGYGGDCARCC-3'	Gibbs and Mackenzie (16)
SPFMV (sequencing)	FMV 9675 F	5'-AGATGCIGGWGCRRACCCWCCAG-3'	This study
	FMV 9675 R	5'-CTGGWGGGTYYGCWCCNGCATCT-3'	This study
	FMV 10244 F	5'-CATGCAGTGCCTACTTTTAGGC-3'	This study
	FMV 10244 R	5'-GCCTAAAAGTAGGCACTGCATG-3'	This study
SPVG (sequencing)	SPVG – F	5'-GGATGAAACCTGGGCAAACAC-3'	This study
	SPVG – R	5'-CGATACACCACACCATGAGACC-3'	This study
SPV2 (sequencing)	SPV2 – F	5'-GGCAGCTTCAAAGAGTTAGGGC-3'	This study
	SPV2 – R	5'-TGTGTGTTATCTGGAGACGTGGC-3'	This study

DNA sequencing, sequence analysis and phylogenetic relationships

Plasmids containing the amplified viral sequences were sequenced in both directions (Macrogen, Seoul, Korea). Internal primers were designed for sequencing as shown in Table 2. At least two individual clones per sample were sequenced, and if inconsistencies were detected, then further clones were sequenced. In one case in which an unexpected gap was identified, the fragment was reamplified from a new RNA extraction and re-sequenced for confirmation. Sequences were assembled using ContigExpress, included in the Vector NTI software package (Invitrogen). The alignments and phylogenetic analyses were performed with the MEGA 4 software package [27] and included a number of sequences downloaded from the GenBank database (Table 1, Fig.4). Distances were calculated using the Kimura 2-parameter model, and trees were assembled using neighbour joining with 1,000 bootstrap replicates.

Recombinant analysis

Putative recombination events were predicted by the Recombination Detection Program (RDP) 2.0 [31]. For further confirmation, sequence alignments were cut at the predicted recombination junctions, and phylogenetic analysis of the aligned sequences corresponding to each side of the junction was performed. Viruses grouping in different strain groups with strong (>90%) bootstrap support in the different phylogenic trees were considered true recombinants.

Results

Nucleotide (nt) sequences encompassing the 3'-terminal part of the NIb gene, the complete CP gene and the 3'UTR of 14 Peruvian samples infected with SPFMV, the SPFMV isolate C1 from Peru, and the isolate YV from USA were determined in this study, as well as those of one sample of SPV2 and SPVG. RNA was also obtained from 12 desiccated leaf samples of SPFMV-infected plants collected in Peru as far back as 20 years ago. Although this yielded apparently intact RNA as assessed by gel electrophoresis, RT-PCR amplicons of the correct size were obtained from only three of these samples, and only one of these amplified products could be cloned (SP-33) and proved to be an SPFMV sequence.

Analyses of SPFMV sequences

Analysis of SPFMV included more than 40 sequences taken from the GenBank database in addition to the ones determined in this study. The deduced CP sequences of most SPFMV-infected samples were 315 aa in length with 155 aa showing variability (36.5%), 60 of which were located in the N-terminal region. With the exception of the type isolate C, all isolates belonging genetically to strain group C lacked two amino acid residues at positions 62 and 63, as reported previously by Tairo et al. [50]. In addition, however, a deletion of 14 aa was found in the CP N-terminal region (at aa position 42–55) of the Peruvian EA isolate M2-44. Nucleotide and amino acid sequence identities for different regions are presented in Fig. 2.

a NIb C-terminal (last 208 aa)

	C	RC	O	EA
C	93.2 ± 0. 6 **95.9 ± 0. 9**			
RC	75.9 ± 1.7 **85.3 ± 2.2**	98.4 ± 0.3 **98.9 ± 0.4**		
O	75.5 ± 1.7 **84 ± 2.3**	87.5 ± 1.2 **92.3 ± 1.6**	95.4 ± 0.9 **96.6 ± 1.2**	
EA	75.3 ± 1.7 **84.6 ± 2.3**	87.6 ± 1.2 **92.9 ± 1.5**	88.5 ± 1.2 **94.2 ± 1.3**	96.5 ± 0.3 **98.3 ± 0.3**

b CP

	C	RC	O	EA
C	96.1 ± 0.3 **96.4 ± 0.5**			
RC	77.5 ± 1.1 **83.3 ± 1.9**	98.5 ± 0.2 **98.5 ± 0.4**		
O	76.8 ± 1.1 **81.4 ± 1.9**	91 ± 0.7 **95.1 ± 0.9**	95.4 ± 0.3 **96.2 ± 0.6**	
EA	77.7 ± 1.1 **82.7 ± 1.9**	93.2 ± 0.6 **96 ± 0.9**	92.3 ± 0.6 **94.8 ± 0.9**	96.3 ± 0.3 **97.1 ± 0.5**

c CP N-terminal

	C	RC	O	EA
C	93.5 ± 0.8 **91.4 ± 1.5**			
RC	62.7 ± 3 **64.3 ± 5.2**	97 ± 0.6 **95.4 ± 1.4**		
O	59.1 ± 3 **57.9 ± 5.3**	83.6 ± 2 **86.1 ± 3.2**	93.5 ± 0.8 **92.1 ± 1.7**	
EA	60.8 ± 2.9 **62.1 ± 5.3**	87.5 ± 1.8 **87 ± 2.9**	86.1 ± 1.8 **85.4 ± 3.2**	94.3 ± 0.7 **92 ± 1.6**

d 3'UTR

	C	RC	O	EA
C	92.9 ± 0.9			
RC	85.2 ± 2.	99 ± 0.3		
O	84.4 ± 2	97 ± 0.7	96.7 ± 0.7	
EA	87.47 ± 2	98 ± 0.5	97.4 ± 0.5	98.1 ± 0.4

Figure 2. Mean nucleotide and amino acid (*bold*) inter- and intra-group identities (%) calculated for the C-terminal 208 aa of the NIb (a); the entire coat protein, CP (b); the N-terminal aa of the CP (c) and 3'UTR (d). Recombinant sequences were excluded

Phylogenetic analysis of the aligned sequences split the SPFMV samples into four strain groups: C, RC, O and EA. Peruvian SPFMVs were found to correspond to groups EA, RC and C (Figs. 3, 4). Visual inspection of the alignments of the complete ~1,800 nt fragments suggested that some SPFMV samples might be the result of recombination between strain groups; i.e. in isolates C and YV the CP encoding region and 3'UTR seemed to be derived both from a C strain and a non-C strain virus, respectively, and in both of the Egyptian samples, Eg1 and Eg9, the central part of the NIb gene and the remaining 3' part of the genome appeared to originate from viruses of strain groups EA and RC, respectively. To confirm this, recombination analysis was performed using the various algorithms of the RDP2.0 program, which all predicted, with high, but varying probability, the suspected recombination events in the strains in question. The algorithm Max-Chi Squared [32] provided by the RDP program predicted the following breakpoints, in accordance with the suspected points identified in the sequence alignments: the first one, located at nt position 9,597 (strain S [44]; nt 570 in our fragment) within the 3'-terminal part of the NIb gene was shared by SPFMV Eg-9 and SPFMV Eg-1, whereas the second one, located at nt position 10,500 (nt 1473 in our fragment) within the 3' terminal region of the CP was present in SPFMV-YV and -C (Fig. 3a). Further confirmation of recombination was obtained by construction of phylogenetic trees using the segments corresponding to each side of the predicted recombination breakpoints (Fig. 3b–e). The topologies of the four trees produced were similar, distinguishing the four strain groups, except for the tree created using the sequences encompassing nts 1473-3'end, in which only two well-supported clades could be discriminated; one comprising viruses of the O, RC and EA strains, and one comprising C strain viruses (Fig. 3c). The conflicting affinity of the Egyptian viruses between the trees produced using the different genome regions clearly confirms the predicted recombination events; i.e. Eg1 and Eg9 are members of strain group RC when alignments comprising the nt positions 570-3'-end are analyzed (Fig. 3e), but belong to strain group EA on the basis of the nt positions 0–570 (Fig. 3d). Similarly, isolates C and YV belong to the strain group C when the region encompassing nts 0–1452 is analyzed (Fig. 3b) but group together with RC, EA and O when 30 nts are analyzed (Fig. 3c).

Analyses of SPV2 and SPVG sequences

A Blast search indicated that sequences closely related to SPVG and SPV2 had been amplified from two plants from Huaral showing typical symptoms of SPVD. Infection by these two viruses was confirmed by NCM-ELISA. Phylogenetic analysis of the SPV2 sequence showed it was closely related to sample Thomas 16A from South Africa, and confirmed the four genetically distinct lineages suggested previously by Ateka et al. [6, Fig. 4). The SPVG sequence determined here, however, was quite distinct from other reported SPVG samples, suggesting that SPVGHua2 might represent a novel strain. Thus, SPVG also can be divided into at least three genetically distinct lineages represented by the CH2, Hua2 and the remaining samples, respectively (Fig. 4). Inclusion of additional potyvirus CP sequences into the analysis further revealed that SPV2, SPVG, SPFMV and a potyvirus isolated from sweetpotato in Zimbabwe are related, forming a well-supported separate phylogenetic lineage within the genus Potyvirus (Fig. 4).

Discussion

Our study, the first attempt to classify Peruvian sweetpotato potyviruses at the molecular level, demonstrated the presence of SPFMV strains C, RC and EA, as well as SPV2 and SPVG in the main sweetpotato-producing regions. This is the first time that SPFMV of strain group EA has been reported from the Americas and SPV2 or SPVG from South America. Two of the isolates, C1 and SP-33, corresponding to strain group C and EA, respectively, were collected in 1987, indicating that these viruses had been present in Peru before SPVD occurred at a high incidence in 1997.

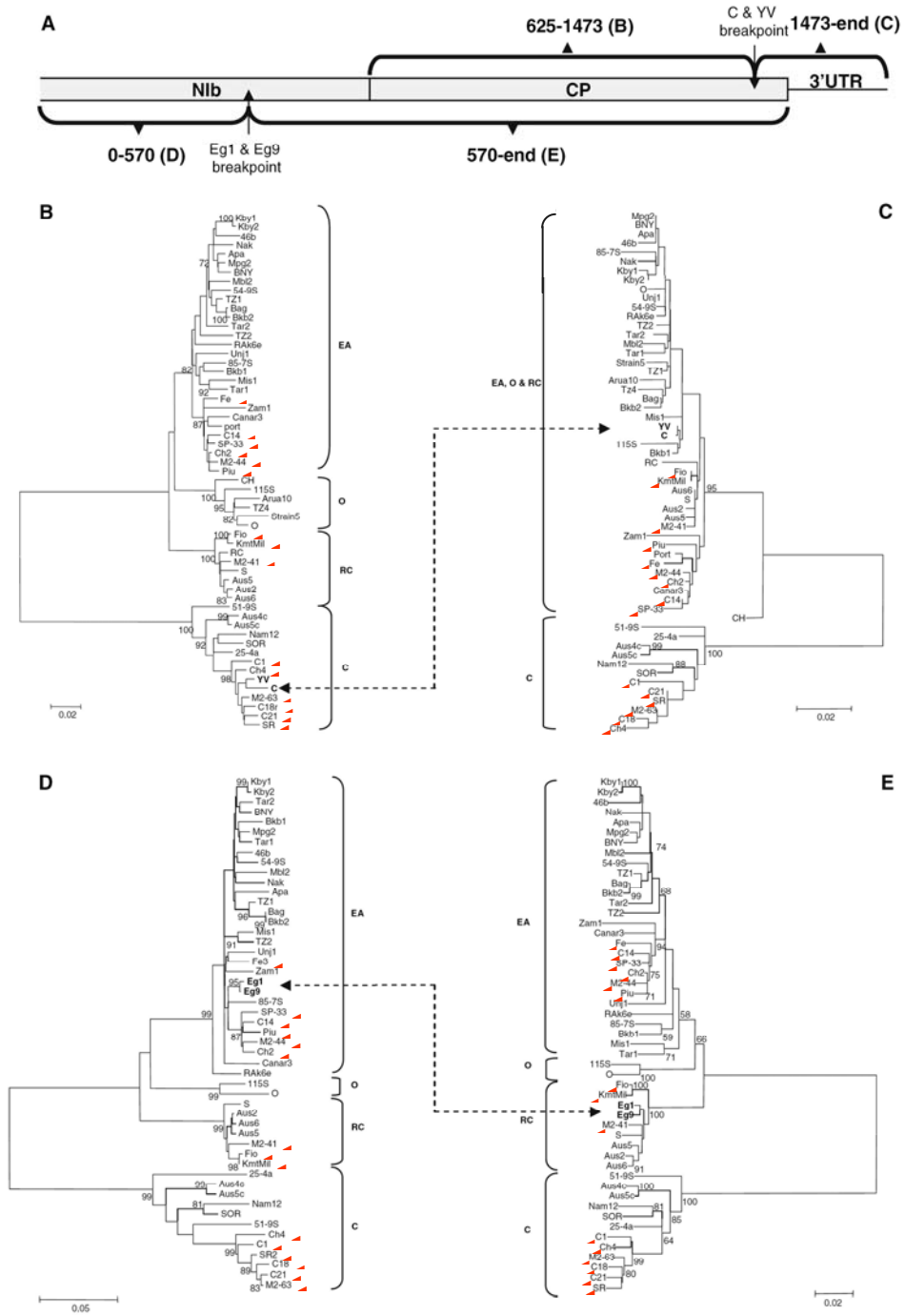

Figure 3. Phylogenetic trees calculated from alignments of nucleotide sequences on either side of recombination breakpoints identified with the RDP program. **A,** schematic representation of the 3' region of SPFMV used for analysis indicating the regions used and the corresponding trees matching to predicted recombination breakpoints in isolates C and YV, and Eg1 and Eg9. **B–E,** phylogenetic trees of SPFMV sequences encompassing the regions indicated in **A**, *arrowheads* indicate the conflicting groupings of sequences in which recombination events were predicted. The *scale bar* in each figure indicates Kimura nucleotide distances. In **B** the sequences corresponding to the NIb genes were not included because they are not available for isolate C. Similarly, the sequence of the 3'UTR is not included in the analysis in (**E**) as this region is not available for the isolates Eg1 and Eg9. The percentage of bootstrap support out of 1,000 replicates is given at each of the major nodes in the trees. Red arrowheads indicate the samples/isolates analyzed in this study.

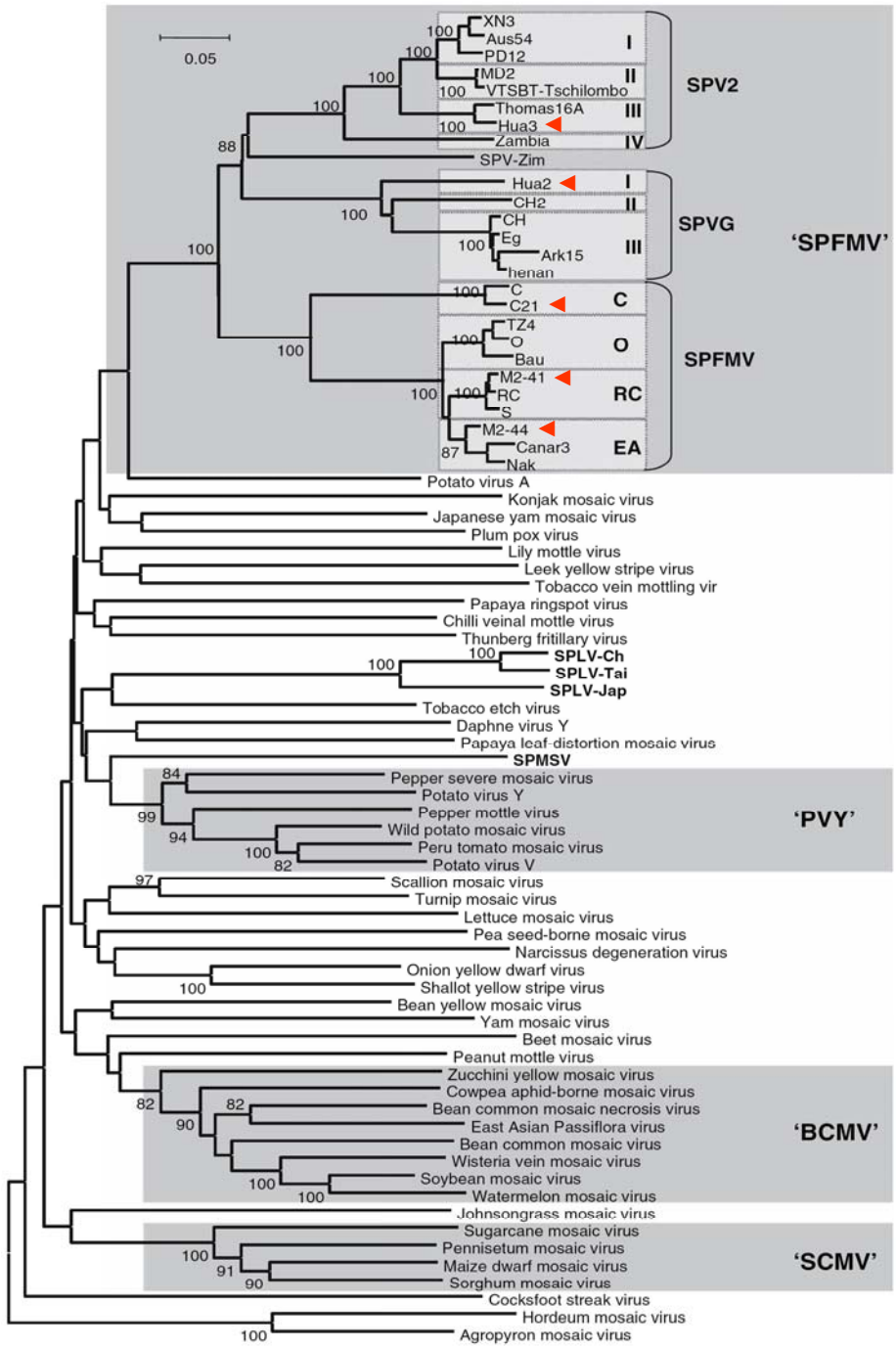

Figure 4. Phylogentic tree of CP nucleotide sequences of potyviruses. The *Ipomoea*-specific 'SPFMV' subgroup, as well as previously identified subgroups are shaded in *grey*. Sweet potato-infecting viruses are in *bold*, proposed virus strain groups are shaded in *light grey* with roman numerals, except for SPFMV, which is according to (25). The *scale bar* indicates Kimura nucleotide distance. The percentage of bootstrap support out of 1,000 replicates is given at the major nodes in the trees where they exceeded 80%. GenBank accession numbers: *SPFMV* sweet potato feathery mottle virus (see Table 1); SPV-Zim: (AF016366); *SPLV* sweet potato latent virus (Ch: X84011, Jap: E15420, Tai: X84012); *SPMSV* sweet potato mild speckling (U61228); *SPVG* sweet potato virus G (Ark15: Ref 3, CH2: X76944, CH: Z83314, Eg: AJ515380, Henan: DQ399861, Hua2: EU218528); *SPV2* sweet potato virus 2 (Aus54: AM050884, Hua3: EU218529, MD2: AY459606, PD12: AY459607, Thomas16A: AY459608, VTSBT-Tschilombo: AY459609, XN3: AY459611, Zambia: AY459610); agropyron mosaic virus

(NC_005903); bean common mosaic virus (NC_003397); bean common mosaic necrosis virus (NC_004047); bean yellow mosaic virus (NC_003492); beet mosaic virus (NC_005304); chilli veinal mottle virus (NC_005778); cocksfoot streak virus (NC_003742); cowpea aphid-borne mosaic virus (NC_004013); daphne virus Y (NC_008028); East Asian passiflora virus (NC_007728); hordeum mosaic virus (NC_005904); Japanese yam mosaic virus (AB027007); johnsongrass mosaic virus (NC_003606); konjac mosaic virus (NC_007913); leek yellow stripe virus (NC_004011); lettuce mosaic virus (NC_003605); lily mottle virus (NC_005288); maize dwarf mosaic virus (NC_003377); narcissus degeneration virus (NC_008824); onion yellow dwarf virus (NC_005029); papaya leaf-distortion mosaic virus (NC_005028); papaya ringspot virus (NC_001785); pea seed-borne mosaic virus (NC_001671); peanut mottle virus (NC_002600); pennisetum mosaic virus (NC_007147); pepper mottle virus (NC_001517); pepper severe mosaic virus (NC_008393); Peru tomato mosaic virus (NC_004573); plum pox virus (NC_001445); potato virus A (NC004039); potato virus V (NC_004010); potato virus Y (NC_001616); scallion mosaic virus (NC_003399); shallot yellow stripe virus (NC_007433); sorghum mosaic virus (NC_004035); soybean mosaic virus (NC_002634); sugarcane mosaic virus (NC_003398); Thunberg fritillary virus (NC_007180); tobacco etch virus (NC_001555); tobacco vein mottling virus (NC_001768); turnip mosaic virus (NC_002509); watermelon mosaic virus (NC_006262); wild potato mosaic virus (NC_004426); wisteria vein mosaic virus (NC_007216); yam mosaic virus (NC_004752); and zucchini yellow mosaic virus (NC_003224). Red arrowheads indicate the samples/isolates analyzed in this study.

Comparison of the SPFMV sequences with those available from the database enabled us to identify novel variations amongst SPFMV strains. The CP aa sequence of the EA sample M2-44, which, coincidentally, was reported as one of the most detrimental isolates identified in Peru [20], lacks 14 amino acids. Although deletions seem rare in the CP of SPFMV, deletions of 12 aa in the CP region are common in isolates of yam mosaic virus [2]. Besides the characteristic 42-nt (14-aa) deletion found in the C terminus of the CP of strain M2-44, we also obtained the first evidence for recombinants of SPFMV. Four samples containing recombinant segments were detected visually, and their occurrence was confirmed using specialized software and phylogenetic analysis (Fig. 3). The 3'-terminal sequences of the recombinant isolate YV were amplified and cloned from two individual RNA extractions to be sure that the recombination event detected was not an artifact of the PCR reaction. The fact that the same recombination is found in isolate C from the USA, which was cloned and sequenced using separate primers by a different laboratory, corroborates that this is unlikely to be a PCR artifact. The tentative recombinant viruses Eg1 and Eg9 [21] share the same recombination breakpoint in the NIb-encoding region and originate from the same geographic region in Egypt. It is therefore likely that they share a common evolutionary ancestor. The same can be argued for the two recombinant North American strains of SPFMV identified in this study. Recombination in evolutionary history of potyviruses is not a novelty and has been reported for a number of potyvirus species, such as *Yam mosaic virus* [8], *Yam mild mosaic virus* [7], *Potato virus Y* (PVY [18]), *Plum pox virus* [19], *Turnip mosaic virus* [41], *Lettuce mosaic virus* [26] and *Sugarcane mosaic virus* (SCMV [54]), but also between isolates of closely related species such as *Bean common mosaic virus* (BCMV), and *Soybean mosaic virus* [14], *Bean common mosaic necrosis virus* and BCMV [28] and those of other related viruses [52]. These reports are evidence for an important role for recombination in the evolution of potyviruses, although their frequency may vary significantly between virus species. Such recombination events may lead to more virulent virus strains [18, 54] and even new species [14, 52] or genera [52].

In a previous study by Gutiérrez et al. [20], the SPFMV samples M2-44, M2-41 and C18, here shown to correspond to strains EA, RC and C, respectively, were compared and found to vary in reaction to different antisera, as well as the severity of symptoms induced in *I. nil* and sweetpotato. In both hosts, the EA isolate produced the most severe symptoms, whereas the RC strain produced the mildest symptoms. However, Moyer et al. [37] reported that isolate C (strain group C) caused milder symptoms than isolate RC (strain group RC), and in Japan an RC isolate (SPFMV-S) was reported to be the most severe [34]. Although an EA strain group isolate was not included in these studies, the contradictory results obtained for severity of C and RC isolates in the study of Gutiérrez et al. [20], and that of Moyer et al. [37], suggest that symptom severity may not necessarily be a characteristic of the strains, but rather that of individual isolates. Similarly, the ability to infect *N. benthamiana* appears to be an isolate-specific rather than a strain-specific characteristic, as only some isolates from different strain groups (EA and C) are able to infect this host [20, 37]. Nevertheless, the isolates belonging to strain group C are genetically distinct from the other strain groups of SPFMV. Therefore, biological differences are expected. It has been suggested that strain group C may represent a separate virus species [50]. Our extended analysis agrees with the percentage of CP nt and aa sequence identities previously reported for samples/isolates of strain group C (Fig. 2b: 75.6–78.7% nt, 79.5–85.2 aa) [25, 50]. However, these identities do not provide a clue for classifying the C strain as another viral species, because they are very close to the CP nt and aa sequence identities of 76–77 and 82%, respectively, used currently as threshold values for potyvirus species demarcation [1]. Similarly, the partial NIb sequences analyzed in this study are at the edge of the recommended species demarcation criterion of 75%

identity (Fig. 2a). In contrast, the variability found in the 3'UTR (80.0–85.4% identity, Fig. 2c) appears well above that recommended to distinguish different potyvirus species (76% identity [1]). The definitive reclassification of SPFMV-C as a new species is something that can only be resolved with the help of the entire genome sequence of a C isolate. Preliminary data from the Peruvian C1 isolate indeed indicate that variability in other parts of the genome significantly exceeds those found for strains of the same species (our unpublished data). This and the fact that polyclonal or monoclonal antibodies to SPFMV may not recognize all isolates of strain group C [50] underlines the need to develop appropriate diagnostic methods for detection of these viruses. A PCR-RFLP-based method described by Tairo et al. [49] may be too expensive for routine detection purposes, especially in developing countries, which often lack facilities for using molecular techniques.

The identification of two of the potyvirus-specific amplicons as pertaining to SPV2 and SPVG presents the first report of the occurrence of these viruses in Peru and South America. Hence, all currently recognized sweetpotato potyviruses are endemic to Peru. In routine testing from CIP's germplasm collection and from seed production in Huaral and Can˜ete using specific antibodies, SPVG is frequently detected (c. 30% of symptomatic samples) and appears to be more prevalent than SPV2 (c. 3%). Despite this, SPFMV is by far the most common virus found (c. 90% of symptomatic samples), and consequently, the other potyviruses may contribute little to yield losses caused by SPVD. Although it has been shown that the titers of all these viruses increase upon co-infection with SPCSV, titers of SPFMV increase more than those of the other potyviruses [24, 47, 51]. These higher replication rates upon co-infection with SPCSV may provide a possible explanation for the prevalence of SPFMV over the other potyviruses, which are out-competed. This also implies that if cultivars with resistance to only SPFMV were deployed, the other potyviruses could rapidly replace it, causing similar synergistic virus diseases. Because available antisera to SPFMV show a weak serological cross-reaction in NCM-ELISA with SPVG as well as SPV2, infection with these viruses may previously in many cases have been attributed to SPFMV, and their prevalence worldwide may be greater than previously known. The availability of SPVG- and SPV2-specific antibodies is now facilitating the detection of both viruses in samples from different countries, discriminating them from the presence of SPFMV.

Phylogenetic analysis of various samples of the three viruses identified in this study, together with a representative repertoire of other potyviruses, enabled us to show that these viruses form a well-supported phylogenetic subgroup within the genus *Potyvirus* (Fig. 4), together with an unknown virus reported from Zimbabwe [11]. Besides notable sequence similarity (including identical last 20 nts), this 'SPFMV' subgroup distinguishes itself by having a narrow host range, mainly confined to the Convolvulaceae, indicating a likely common evolutionary ancestor adapted to this family of hosts. On the other hand, SPLV and SPMSV, the two sweetpotato-infecting potyviruses not belonging to the 'SPFMV' subgroup, are phylogenetically distantly related and have broader host ranges including Chenopodiaceae and Solanaceae [29]. Other potyvirus subgroups also have certain host specificities (Fig. 4), such as the 'SCMV', 'BCMV' and 'PVY' subgroups predominantly infecting gramineous, leguminous, and solanaceous plants, respectively [46, 48], suggesting a significant role for virus host co-evolution in potyvirus speciation. Further sequencing of SPV2, SPVG and SPFMV-C genomes may enable us to shed some light onto the specific characteristics required for adaptation to convolvulaceous hosts, although the identification of a highly conserved region in the P1 protein of SPFMV and the ipomovirus sweetpotato mild mottle virus [52] already alludes to an important role for that protein in host specificity.

Acknowledgments The study was partially supported by a joint project with the University of Helsinki (Prof. J.P.T. Valkonen; grant #1110797, Academy of Finland) aiming to develop durable resistance to SPVD. We are grateful to Jari Valkonen for critical reading of the manuscript, and to Jim Moyer for providing the YV isolate of SPFMV.

References

Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family Potyviridae. Arch Virol 150:459–479

Aleman-Verdaguer M-E, Goudou-Urbino C, Dubern J, Beachy RN, Fauquet C (1997) Analysis of the sequence diversity of the P1, HC, P3, NIb and CP genomic regions of several yam mosaic potyvirus isolates: implications for the intraspecies molecular diversity of potyviruses. J Gen Virol 78:1253–1264

Alemu T (2004) Characterisation of viruses of pepper (Capsicum spp.) and sweet potato (Ipomoea batatas) from Ethiopia (in German). PhD thesis, University of Bonn, p 126

Ateka EM, Barg E, Njeru RW, Lesemann DE, Vetten HJ (2004) Further characterization of 'sweet potato virus 2': A distinct species in the genus Potyvirus. Arch Virol 149:225–239

Ateka EM, Njeru RW, Kibaru AG, Kimenju JW, Barg E, Gibson RW, Vetten HJ (2004) Identification and distribution of viruses infecting sweet potato in Kenya. Ann Appl Biol 144:371–379

Ateka EM, Barg E, Njeru RW, Thompson G, Vetten HJ (2007) Biological and molecular variability among geographically diverse isolates of sweet potato virus 2. Arch Virol 152:479–488

Bousalem M, Dallot S, Fuji S, Natsuaki KT (2003) Origin, worldwide dispersion, bio-geographical diversification, radiation and recombination: an evolutionary history of Yam mild mosaic virus (YMMV). Infect Genet Evol 3:189–206

Bousalem M, Douzery EJ, Fargette D (2000) High genetic diversity, distant phylogenetic relationships and intraspecies recombination events among natural populations of Yam mosaic virus: a contribution to understanding potyvirus evolution. J Gen Virol 81:243–255

Carey EE, Gibson RW, Fuentes S, Machmud M, Mwanga ROM, Turyamureeba G, Zhang L, Ma D, Abo El-Abbas F, El-Bedewy R, Salazar LF (1999) The causes and control of virus diseases of sweet potato in developing countries: is sweetpotato virus disease the main problem?. In: Impact on a changing world, Program Report 1997–1998. The International Potato Center, Lima, Peru, pp 241–248

Cedano C, Fuentes S, Salazar LF (1989) Identification and some characteristics of sweet potato (Ipomoea batatas L.) feathery mottle virus isolated in Peru. Fitotatología 24:43–44 (abstract in Spanish)

Chavi F, Robertson AI, Verduin BJM (1997) Survey and characterization of viruses in sweetpotato from Zimbabwe. Plant Dis 81:1115–1122

Clark CA, Hoy MW (2006) Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. Plant Dis 90:83–88

Colinet D, Nguyen M, Kummert J, Lepoivre P (1998) Differentiation among potyviruses infecting sweet potato based on genusand virus-specific reverse transcription polymerase chain reaction. Plant Dis 82:223–229

Desbiez C, Lecoq H (2004) The nucleotide sequence of Watermelon mosaic virus (WMV, Potyvirus) reveals interspecific recombination between two related potyviruses in the 5' part of the genome. Arch Virol 149:1619–1632

Di Feo L, Nome SF, Biderbost E, Fuentes S, Salazar LF (2000) Etiology of sweet potato chlorotic dwarf disease in Argentina. Plant Dis 84:35–39

Gibbs A, Mackenzie A (1997) A primer pair for amplifying part of the genome of all potyvirids by RT-PCR. J Virol Methods 63:9–16

Gibson RW, Mpembe I, Alicai T, Carey EE, Mwanga ROM, Seal SE, Vetten HJ (1998) Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. Plant Pathol 47:95–102

Glais L, Tribodet M, Kerlan C (2002) Genomic variability in Potato potyvirus Y (PVY): evidence that PVYNW and PVYNTN variants are single to multiple recombinants between PVYO and PVYN isolates. Arch Virol 147:363–378

Glasa M, Palkovics L, Kominek P, Labonne G, Pittnerova S, Kudela O, Candresse T, Subr Z (2004) Geographically and temporally distant natural recombinant isolates of Plum pox virus (PPV) are genetically very similar and form a unique PPV subgroup. J Gen Virol 85:2671–2681

Gutiérrez D, Fuentes S, Salazar LF (2003) Sweet potato virus disease (SPVD): Distribution, incidence, and effect on sweetpotato yield in Peru. Plant Dis 87:297–302

IsHak JA, Kreuze JF, Johansson A, Mukasa SB, Tairo F, Abo El-Abbas FM, Valkonen JPT (2003) Some molecular characteristics of three viruses from SPVD-affected sweet potato plants in Egypt. Arch Virol 148:2449–2460

Jordan R, Hamond J (1991) Comparison and differentiation of potyviruses isolates and identification of strain-virus-subgroupspecific and potyvirus group-common epitopes using monoclonal antibodies. J Gen Virol 72:25–36

Karyeija RF, Kreuze JF, Gibson RW, Valkonen JPT (2000) Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. Virology 269:26–36

Kokkinos CD, Clark CA (2006) Interactions among Sweet potato chlorotic stunt virus and different potyviruses and potyvirus strains infecting sweet potato in the United States. Plant Dis 90:1347–1352

Kreuze JF, Karyeija RF, Gibson RW, Valkonen JPT (2000) Comparison of coat protein gene sequences show that East African isolates of Sweet potato feathery mottle virus form a genetically distinct group. Arch Virol 145:567–574

Krause-Sakate R, Fakhfakh H, Peypelut M, Pavan MA, Zerbini FM, Marrakchi M, Candresse T, Le Gall O (2004) A naturally occurring recombinant isolate of Lettuce mosaic virus. Arch Virol 149:191–197

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Larsen RC, Miklas PN, Druffel KL, Wyatt SD (2005) NL-3 K strain is a stable and naturally occurring interspecific recombinant derived from Bean common mosaic necrosis virus and Bean common mosaic virus. Phytopathology 95:1037–1042

Loebenstein G, Fuentes S, Cohen J, Salazar LF (2003) Sweet potato. In: Loebenstein G, Thottappilly (eds) Virus and virus-like diseases of major crops in developing countries. Kluwer, Dordrecht, pp 223–248

López D, Salazar LF (1987) Studies on Sweet potato feathery mottle virus (SPFMV) in Peru. Fitopatología 22:40 (abstract in Spanish)

Martin DP, Williamson C, Posada D (2005) RDP2: recombination detection and analysis from sequence alignments. Bioinformatics 21:260–262

Maynard-Smith J (1992) Analyzing the mosaic structure of genes. J Mol Evol 34:126–129

Milgram M, Cohen J, Loebenstein G (1996) Effects of Sweet potato feathery mottle virus and Sweet potato sunken vein virus on sweet potato yields and rates of reinfection of virus-free planting material in Israel. Phytoparasitica 24:189–193

Mori M, Sakai J, Kimura T, Usugi T, Hayashi T, Hanada K, Nishiguchi M (1995) Nucleotide sequence analysis of two nuclear inclusion body and coat protein genes of a sweet potato feathery mottle virus severe strain (SPFMV-S) genomic RNA.Arch Virol 140:1473–1482

Moyer JW (1986) Variability among strains of sweet potato feathery mottle virus. Phytopathology 76:1126 (abstract)

Moyer JW, Abad JA (2000) Sweet potato virus isolation, indentification and detection: 20 years later. In: Nakasawa Y, Ishiguro K (eds) International workshop on sweet potato cultivar decline study. Miyakonojo, Japan, pp 90–98

Moyer JW, Kennedy GG, Abou-Ghadir MF (1980) Identification of two sweet potato feathery mottle virus strains in North Carolina. Plant Dis 64:762–764

Mukasa SB, Rubaihayo PR, Valkonen JPT (2006) Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfected sweetpotato plants. Plant Pathol 55:458–467

Nakashima JT, Salazar LF, Wood KR (1993) Sweet potato feathery mottle potyvirus (C1 isolate) virion and RNA purification. J Virol Methods 44:109–116

Njeru RW, Mburu MWK, Cheramgoi E, Gibson RG, Kiburi ZM, Obudho E, Yobera D (2004) Studies on the physiological effects of viruses on sweet potato yield in Kenya. Ann Appl Biol 145:71–76

Ohshima K, Tomitaka Y, Wood JT, Minematsu Y, Kajiyama H, Tomimura K, Gibbs AJ (2007) Patterns of recombination in turnip mosaic virus genomic sequences indicate hotspots of recombination. J Gen Virol 88:298–315

Querci M, Fuentes S, Salazar LF (1992) Construction, cloning and use of radioactive RNA probes for the detection of the Peruvian strain C1 of sweet potato feathery mottle virus. Fitopatología 27:93–97

Rossel HW, Thottappilly G (1988) Complex virus diseases of sweet potato. In: Exploration, maintenance and utilisation of sweet potato genetic resources. Report of first sweet potato planning conference 1987. International Potato Center, Lima, Peru, pp 291–302

Sakai J, Mori M, Morishita T, Tanaka M, Hanada K, Usugi T, Nishiguchi M (1997) Complete nucleotide sequence and genome organization of sweet potato feathery mottle virus (S strain) genomic RNA: the large coding region of the P1 gene. Arch Virol 142:1553–1562

Schaefers GA, Terry ER (1976) Insect transmission of sweet potato disease agents in Nigeria. Phytopathology 66:642–645

Shukla DD, Ward CW, Brunt AA (1994) The Potyviridae. CAB international, Littlehampton, UK, p 516

Souto E, Sim J, Chen J, Valverde R, Clark C (2003) Properties of strains of Sweet potato feathery mottle virus and two newly recognized potyviruses infecting sweet potato in the United States. Plant Dis 87:1226–1232

Spetz C, Taboada AM, Darwich S, Ramsell J, Salazar LF, Valkonen JPT (2003) Molecular resolution of a complex of potyviruses infecting solanaceous crops at the centre of origin in Peru. J Gen Virol 84:2565–2578

Tairo F, Jones RAC, Valkonen JPT (2006) Potyvirus complexes in sweet potato: occurrence in Australia, serological and molecular resolution, and analysis of the Sweet potato virus 2 (SPV2) component. Plant Dis 90:1120–1128

Tairo F, Mukasa SB, Jones RAC, Kullaya A, Rubaihayo PB, Valkonen JPT (2005) Unraveling the genetic diversity of the three main viruses involved in Sweet Potato Virus Disease (SPVD), and its practical implications. Mol Plant Pathol 6:199–211

Untiveros M, Fuentes S, Salazar LF (2007) Synergistic interaction of Sweet potato chlorotic stunt virus (Crinivirus) with carla-, cucumo-, ipomo- and potyviruses infecting sweet potato. Plant Dis 91:669–676

Valli A, Lopez-Moya JJ, Garcıa JA (2007) Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae, J Gen Virol 88:1016–1028

Valverde RA, Lozano G, Navas-Castillo J, Ramos A, Valde´s F (2004) First report of Sweet potato chlorotic stunt virus and Sweet potato feathery mottle virus infecting sweet potato in Spain. Plant Dis 88:428

Zhong Y, Guo A, Li C, Zhuang B, Lai M, Wei C, Luo J, Li Y (2005) Identificaction of natural occurring recombinant isolate of Sugarcane mosaic virus causing maize dwarf mosaic disease. Virus Genes 30:75–83