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Biological and molecular variability among geographically diverse isolates of sweet potato virus 2

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Summary

Sweet potato virus 2 (SPV2) is a tentative member of the genus *Potyvirus*, family *Potyviridae*. In addition to the type isolate of SPV2 recently characterised in greater detail, twelve additional isolates of this virus were obtained from sweet potato clones originating from China, Portugal, South Africa and Zambia. Sequences of the coat protein (CP) gene and 3′ non-translated region (NTR) were determined. Comparisons of the CP gene sequences of these isolates revealed nucleotide and amino acid sequence identities ranging from 81 to 99% and from 86 to 99%, respectively. Phylogenetic analysis of sequences distinguished several groups, which partially correlated with the geographic origin of the isolates, and indicated that some isolates from South Africa and a Zambian isolate are most distinct both in CP and 3′NTR sequences. Host range studies of a selected number of isolates revealed some differences in test plant reactions, which appeared to correlate to some extent with the geographic origin and molecular distinctness of the SPV2 isolates. The results strongly suggest the occurrence of biologically and genetically diverse strains of SPV2.

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Introduction

Sweet potato virus 2 (SPV2) is a tentative new member of the genus *Potyvirus*, family *Potyviridae* [4]. The virus, also referred to as sweet potato virus Y [4] and Ipomoea vein mosaic virus [28], was first isolated from sweet potato plants from Taiwan and Nigeria [26]. Only recently, an SPV2 isolate originating from Taiwan or Nigeria was characterised in greater detail [4]. Electron microscopy revealed that SPV2 has filamentous particles of 850 nm in length and induces cytoplasmic cylindrical inclusions consisting of pinwheels and scrolls. The virus was non-persistently transmitted by *Myzus persicae* and mechanically transmitted to several species of the genera *Chenopodium*, *Datura*, *Nicotiana*, and *Ipomoea*. Based on serological and molecular data, SPV2 is related to other potyviruses infecting sweet potato but appears to be a distinct member of the genus *Potyvirus* [4]. Although SPV2 has been isolated from sweet potato plants showing mild symptoms consisting of leaf mottle, vein yellowing

and/or ringspots [26, 29], the significance of this virus to sweet potato production is not clear as similar symptoms may be caused by other viruses, and sweet potato cultivars inoculated with SPV2 under controlled (greenhouse) conditions failed to produce obvious symptoms [4, 28]. However, there is experimental evidence that, similar to sweet potato feathery mottle virus (SPFMV) [17], SPV2 is strongly synergised by the crinivirus sweet potato chlorotic stunt virus (SPCSV) in sweet potato (*Ipomoea batatas*) and the test plant *I. setosa* [28; our unpublished data], suggesting that SPV2 might be economically important in areas where SPCSV occurs.

Several of the viruses that naturally occur in sweet potato are members of the genus *Potyvirus* [20]. The most common worldwide is SPFMV, which has also been well studied and of which several strains occur [1, 16, 18, 22, 23, 30]. There is much less information on the other sweet potato potyviruses, which, besides SPV2, include members of the species *Sweet potato latent virus* (SPLV) found in Asia [20], *Sweet potato mild speckling virus* (SPMSV) from Argentina [11], *Sweet potato virus G* (SPVG) from Egypt [14], China [9, 10], Europe [our unpublished data], and the United States [28] as well as a few additional, potentially distinct potyviruses [20].

As part of a virus survey of sweet potato crops in South Africa in 2001, several SPV2 isolates were established in herbaceous test plants following mechanical inoculation of leaf extracts from sweet potato plants. In addition, SPV2 isolates were obtained also from individual sweet potato plants originating from China, Portugal and Zambia. The availability of this geographically diverse range of SPV2 isolates and our observation that some of these isolates appeared to induce different symptoms in test plants prompted us to generate information on the molecular and biological variability of SPV2.

Materials and methods

Origin, isolation and maintenance of SPV2 isolates

Cuttings of SPV2-infected sweet potato plants were collected from farmers' fields in different regions of South Africa. R. W. Gibson, Chatham Maritime, Kent, UK, kindly sent us a sweet potato cutting, which had been collected in a farmer's field near Silowezi, Zambia, and was infected with SPCSV, SPFMV and SPV2. A sweet potato sample (XN3) from Guangdong Province, China, infected with SPFMV, SPVG and SPV2 was kindly provided by P. Detrixhe, Gembloux, Belgium, and cuttings from several SPV2-infected sweet potato plants from Portugal were kindly provided by D. Louro, Oeiras, Portugal. Further details of the samples and isolates are given in Table 1.

In attempts to obtain pure SPV2 isolates, single local lesions were serially transferred using sap inoculation techniques (see below) onto fully developed leaves of *Chenopodium quinoa* L. Purity and identity of the local lesion isolates of SPV2 were confirmed by DAS- and TAS-ELISA using polyclonal and monoclonal antibodies (from the stock of BBA Braunschweig) to common, mechanically transmissible sweet potato viruses, namely SPV2, SPFMV, SPLV, SPMSV, sweet potato mild mottle virus, and sweet potato chlorotic fleck virus. All the SPV2 isolates were subsequently maintained in *Nicotiana benthamiana* Domin by sap inoculation.

Host range studies

The experimental host range of six isolates of SPV2, namely SPV2-type, J51, Thomas16A, PD12, M521 and Zambia, which were available as pure isolates at the start of this experiment, was determined by sap inoculation using a range of commonly used indicator plant species (Table 2). Infected *N. benthamiana* leaves were macerated in a mortar containing 0.1 M potassium phosphate buffer, pH 7.8, and rubbed onto carborundum-dusted leaves of young seedlings. Non-inoculated plants of each species were included as controls. The plants were observed for symptom development under glasshouse conditions and analysed by DAS-ELISA using SPV2 antiserum. The inoculated and top leaves of the indicator plants were tested separately to distinguish between local and systemic infections.

RNA extraction, RT-PCR, cloning and sequencing

For cloning and sequencing of the 3′ end of the genomic RNA of each SPV2 isolate, the total RNA of the 12 isolates was extracted from 100 mg infected leaf tissue of *N. benthamiana* using the Nucleospin® Plant kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. Synthesis of complementary DNA (cDNA) was done using an oligo(dT) primer and moloney murine leukemia virus reverse transcriptase (MMLV-RT) (Invitrogen/Gibco). For the South African isolates, the resulting cDNA was amplified by PCR using the primers PV1/SP6 and PV2I/T7 described by Mackenzie et al. [21]. PCR conditions were as follows: denaturation at 94 °C for 3 min followed by 30 cycles of 30 s at 94 °C, primer annealing at 58 °C for 1 min, extension at 72 °C for 3 min, and a final

Table 1. Designation, geographic origin, GenBank accession number, number of 3′-terminal nucleotides (nt) determined and 3′ nontranslated region (NTR) size of the SPV2 isolates and of the other virus isolates used for phylogenetic analysis

Isolate	Location	Country	GenBank accession no.	Nucleotides determined	NTR size
XN3	Guangdong Province	China	AY459611*	1212	213
260702/1	–	Portugal	AY459614*	1210	211
300902/31	–	Portugal	AY459613*	996	n.d.[c]
Louro1	–	Portugal	AY459604*	1411	214
J51	KwaZulu Natal	South Africa	AY459603*	1378	213
MD2	KwaZulu Natal	South Africa	AY459606*	1835	213
M521	KwaZulu Natal	South Africa	AY459605*	1839	213
PD12	KwaZulu Natal	South Africa	AY459607*	1856	213
Thomas16A	Mpumalanga	South Africa	AY459608*	1839	213
TshilomboIV1-1	Tshilombo, Northern	South Africa	AY459612*	1809	212
VTSB-Tshilombo	Tshilombo, Northern	South Africa	AY459609*	1840	214
Zambia	Silowezi	Zambia	AY459610*	1835	211
SPV2-type	–	Nigeria or Taiwan	AY232437 [4]	2006	213
Pink1[a]	Perth, SW	Australia	AM 050887 [29]	1857	214
LSU-2[b]	Louisiana	USA	AY178992 [28]	1186	n.d.[c]
SPV-Zw	–	Zimbabwe	AF016366 [8]	2054	215
Sweet potato virus G (SPVG)	–	China	X76944 [10]	1288	221

* These isolates were sequenced in this study.

[a] Pink1 represents six very similar isolates from Australia studied by Tairo et al. [29]

[b] LSU-2 and LSU-5, two very similar isolates from the U.S.A. referred to as isolates of Ipomoea vein mosaic virus by Souto et al. [28], were not included in the phylogenetic trees as the nucleotide sequences of their 3′NTR have not been determined and only partial CP sequences are available

[c] Not determined (*n.d.*)

Table 2. Reactions of *Chenopodium*, *Datura*, *Ipomoea* and *Nicotiana* species to six selected SPV2 isolates

Test plant	SPV2 isolate					
	SPV2-type	J51	M521	PD12	Thomas16A	Zambia
C. amaranticolor	cLL	cLL	cLL	cLL	cLL	–
C. murale	cLL	cLL	cLL	–	cLL	cLL
C. quinoa	cLL	cLL	cLL	cLL	cLL	cLL
D. metel	–	–	–	–	cLL	–
I. nil	VMo	VMo	VMo	VMo	VMo	VMo
I. setosa	VMo	VMo	VMo	VMo	VMo	VMo
N. benthamiana	LD, VC	LD, VC	LD, VC	LD, VC	LD, VC	LD, VC
N. clevelandii	Mo	Mo	Mo	Mo	Mo	Mo
N. hesperis	Chl	–	Chl	–	Chl, LD, St	Chl, LD, St
N. occidentalis P1	Chl, NRS	Chl	Chl, NRS	Chl	Chl, NRS	Chl, NRS
N. occid. ssp. *obliqua*	Chl	–	Chl	–	Chl	Chl

Symptoms observed were chlorosis (*Chl*), chlorotic local lesions (*cLL*), leaf distortion (*LD*), mosaic (*Mo*), systemic vein mosaic (*VMo*), no symptoms and no infection (–), necrotic ringspots (*NRS*), stunting (*St*) and vein clearing (*VC*)

extension step at 72 °C for 8 min. A degenerate primer (5′-GCATGGRTTYTKGATCAGGC-3′) was derived from the nucleotide sequences obtained from the South African isolates and was subsequently used in combination with the oligo(dT) primer to amplify the entire CP and 3′NTR of the SPV2 isolates from China, Portugal and Zambia. Amplification products were analysed by electrophoresis in a 1% agarose gel and were cloned into pGEM®-T vector

(Promega, Madison, WI, USA), and subsequently introduced into *Escherichia coli* strain DH5α competent cells (Promega) by transformation according to the manufacturer's instructions. Plasmids were isolated from recombinant *E. coli* using the Macherey-Nagel kits. Three clones from each virus isolate were sequenced in forward and reverse directions by a commercial company (MWG-Biotech AG, Ebersberg, Germany).

Sequence comparisons and phylogenetic analysis

Phylogenetic analyses of the CP and 3′NTR nucleotide sequences were done using the default settings of either the DNAMAN software (Lynnon Biosoft, Quebec, Canada) or the ClustalX software [15] in combination with the Treeview programme [24]. One thousand bootstrapped data sets were generated to estimate the statistical significance of the branching. The accession numbers of the isolates used for comparisons are given in Table 1.

Results

Isolation and identification of SPV2 isolates

Sweet potato plants from which SPV2 isolates were obtained most often contained several viruses. When no virus other than SPV2 was detected, symptoms in sweet potato were very mild, consisting of a pale vein yellowing and/or faint chlorotic spots. Since SPV2 was not readily detected directly in sweet potato by serological means, strong DAS-ELISA reactions with the SPV2 antiserum were only observed upon graft inoculation of infected sweet potato onto *I. setosa*. Following sap inoculation from *I. setosa* onto *C. quinoa* followed by two to three transfers of a single local lesion in *C. quinoa*, ten pure isolates of SPV2 (300902/31, J51, M521, MD2, PD12, Thomas16A, TshilomboIV1-1, VTSB-Tshilombo, Zambia and XN3) were obtained. They were subsequently maintained in *N. benthamiana*, a systemic host of SPV2.

Host range and symptoms

To study the host range and possible phenotypic differences among SPV2 isolates, six isolates that were available as pure isolates at the beginning of this study were used for sap inoculation onto different test plant species (Table 2). All six isolates induced a systemic vein mosaic in *Ipomoea nil* and *I. setosa*, chlorotic local lesions in *Chenopodium quinoa*, vein clearing and leaf distortion in *N. benthamiana*, and mosaic in *N. clevelandii*. If a plant species was susceptible to an SPV2 isolate, infection of the *Nicotiana* species by the various SPV2 isolates invariably led to systemic infections, whereas the *Chenopodium* species and *Datura metel* only formed chlorotic local lesions. Since *D. stramonium*, *Gomphrena globosa*, *N. glutinosa*, and *N. debneyi* did not show symptoms upon inoculation with any of the six isolates and tested negative for SPV2 in DAS-ELISA, they appeared immune to SPV2 under our experimental conditions.

Some of the six isolates differed from one another in test plant reactions (Table 2). J51 and PD12 only caused a systemic chlorosis in *N. occidentalis* P1, whereas the four other isolates produced more severe systemic symptoms by additionally causing necrotic ringspots. J52 and PD12 differed from the other isolates also by not infecting *N. hesperis* and *N. occidentalis* ssp. *obliqua*. However, PD12 and J51 differed from each other in that PD12 did not infect *C. murale*. Only Zambia failed to infect *C. amaranticolor*, whereas Thomas16A was the only isolate that infected *D. metel*. These two latter isolates caused more severe symptoms on virtually all the susceptible test plants and especially in *N. hesperis*, which showed leaf chlorosis and distortion as well as severe stunting. Conversely, comparatively mild symptoms were induced by SPV2-type and M521, which only caused chlorosis in *N. hesperis*.

Comparison of the nucleotide sequences of the coat protein (CP) gene and 3′ nontranslated region (NTR)

To test whether biologically and geographically diverse isolates of SPV2 show corresponding genetic variations, the CP gene and the 3′NTR nucleotide (nt) sequences of the six biologically characterised isolates and further six isolates from different geographical regions were determined. The number of 3′-terminal nucleotides determined ranged from 996 to 1856 for the individual isolates (Table 1). All isolates possessed a CP gene consisting of 996 nt and encoding 332 amino acid (aa) residues.

Whereas we failed to determine the 3′NTR of the isolate 300902/31 (despite repeated attempts), the 3′NTR sizes of the 11 other isolates ranged from 211 to 214 nt (w/o stop codon; Table 1).

Alignment of the deduced CP amino acid (aa) sequences determined in this study and including the CP sequence of a representative SPV2 isolate from Australia (Pink1; acc. no. AM050887) [29] revealed several motifs highly conserved in the potyviral CPs, namely the WCIENG and the AFDF motifs [25] as well as the aa triplet Asp-Ala-Gly (DAG) required for aphid transmission of potyviruses [6, 7], which was located eight aa downstream of the NIb/CP cleavage site.

Pairwise comparisons of the CP nt and aa sequences gave identities ranging from 81 to 99% and from 86 to 99%, respectively (Table 3), indicating many synonymous nt changes. There was considerable variation in the N terminus of the SPV2 CPs. In contrast to the highly conserved aa sequence of the C-terminal two-thirds (235 aa) of the SPV2 CP (93–99%), the aa sequence identities

Table 3. Pairwise percent coat protein amino acid sequence identity (below diagonal) and nucleotide sequence identity (above diagonal) among 13 isolates of SPV2 as determined using the DNAMAN software

SPV2 isolate	1	2	3	4	5	6	7	8	9	10	11	12	13
1. 260702/1		97.7	94.7	97.2	97.5	97.9	82.1	89.1	98.2	94.6	98.4	98.5	99.2
2. M521	96.4		93.6	99.1	98.8	96.8	81.9	89.4	97.1	93.7	97.3	97.2	97.7
3. MD2	95.5	94.0		93.2	93.8	94.8	81.3	88.1	94.8	99.2	94.8	94.6	94.9
4. PD12	95.8	98.8	93.7		98.3	96.5	81.9	89.3	96.6	93.2	96.8	96.7	97.2
5. J51	95.8	98.2	93.4	97.6		98.8	82.0	89.1	97.1	93.8	97.3	97.2	97.7
6. SPV2-type	97.9	96.1	95.8	95.5	95.5		81.5	89.1	98.0	94.9	97.9	97.6	98.1
7. Zambia	87.0	86.4	86.4	86.1	86.4	87.0		82.4	81.7	80.9	81.5	81.4	81.9
8. Thomas16A	94.3	95.8	93.4	95.2	94.9	94.0	86.1		88.9	87.9	89.1	88.7	89.4
9. TshilomboIV1-1	98.2	96.4	97.0	95.8	95.8	97.9	87.3	94.3		94.8	98.0	97.9	98.4
10. VTSB-Tshilombo	95.2	93.4	98.5	92.8	92.8	95.2	85.8	92.8	96.4		94.9	94.5	94.9
11. XN3	97.6	95.8	95.2	95.2	95.2	97.3	86.1	94.0	97.6	94.6		98.1	98.6
12. Louro1	98.5	96.1	95.2	95.5	95.2	97.6	86.7	94.0	97.9	94.9	97.3		98.7
13. 300902/31	98.8	96.4	95.5	95.8	95.8	97.9	87.0	94.3	98.2	95.2	97.6	98.5	

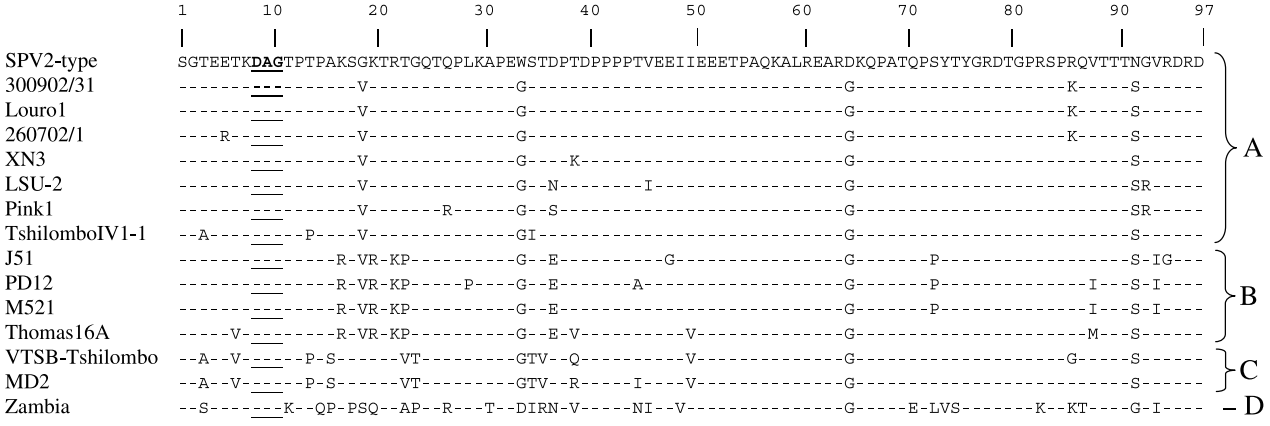

Fig. 1. Multiple alignments of CP N-terminal amino acid sequences of SPV2 isolates from China, Portugal, South Africa, and Zambia, including a representative isolate each from the U.S.A. (*LSU-2*) and Australia (*Pink1*) (for isolate details see Table 1). Only amino acids that differ from those of the type isolate SPV2-type are shown, with identical amino acids indicated by dashes. The motif DAG involved in aphid transmissibility is underlined (and in bold in the SPV2-type sequence)

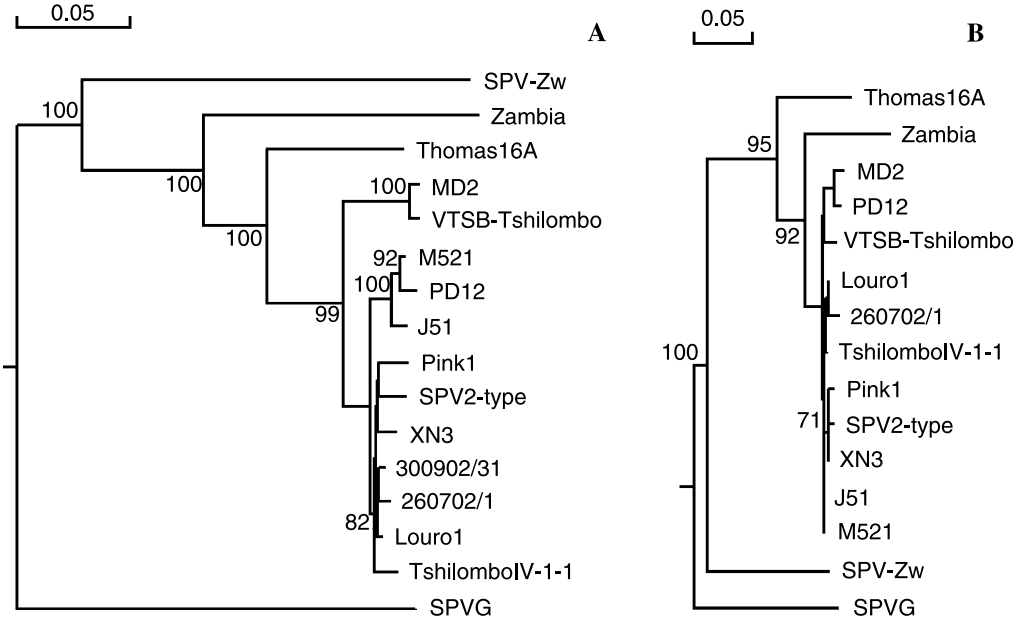

Fig. 2. Neighbour-joining relationship dendrograms of the coat protein nucleotide (**A**) and 3′NTR (**B**) sequences of geographically diverse SPV2 isolates, using SPV-Zw and sweet potato virus G (SPVG) as outgroup sequences. Trees were generated using the default parameters of DNAMAN. Numbers at nodes indicate bootstrap scores higher than 80%. The scale bar represents a genetic distance of 0.05 for the horizontal branch lengths. For isolate designations see Table 1

in the CP N-terminal portion ranged from 67 to 99% (data not shown). Based on differences in CP N-terminal aa sequences (Fig. 1), four groups of isolates could be visually distinguished: (A) a large clade consisting of the type isolate, the Chinese isolate XN3, the three Portuguese isolates (Louro1, 300902/31, 260702/1), a South African isolate (TshilomboIV-1-1) and isolates from Australia (Pink1) and USA (LSU-2); (B) four South African isolates (J51, PD12, M521, Thomas16A); (C) two isolates from South Africa (MD2, VTSB-Tshilombo); and (D) the single isolate from Zambia. This grouping was largely confirmed by the phylogenetic analysis of the entire CP nt sequences of the SPV2 isolates (Fig. 2A). Here, however, Thomas16A was strikingly different from group B and appeared to be an SPV2 isolate intermediate between, and clearly distinct from, groups C and D.

The percent identities of the 3′NTR sequences between the SPV2 isolates ranged from 85 to 100% (data not shown) and were thus higher than those of the CP nt sequences. The phylogenetic relationships among the isolates on the basis of the 3′NTR sequences followed a pattern similar to that observed for the CP nt sequences, as the topologies of the two trees were very similar (Fig. 2A, B). However, the most striking difference between the two trees was that isolate Zambia had the most divergent CP sequence, whereas the most divergent 3′NTR sequence of the SPV2 isolates was that of Thomas16A. This difference was mainly due to the considerable variation in the CP N-terminus of the Zambian isolate. Both Thomas16A and Zambia consistently took a position clearly distinct from all the other isolates. Apart from Thomas16A, all isolates from South Africa were closely related (aa sequence identities of 93–99%) to one another and to isolates from China, Portugal and the type isolate of SPV2.

To compare the molecular variation of the SPV2 isolates with that of other sweet potato potyviruses closely related to SPV2 [4], a further phylogenetic analysis of the CP aa sequences of the SPV2 isolates and those of the common (C) and russet crack (RC) strains of SPFMV [1], a virus isolate from Zimbabwe (SPV-Zw) [8] and a representative SPVG isolate [10] was done. Here, the majority

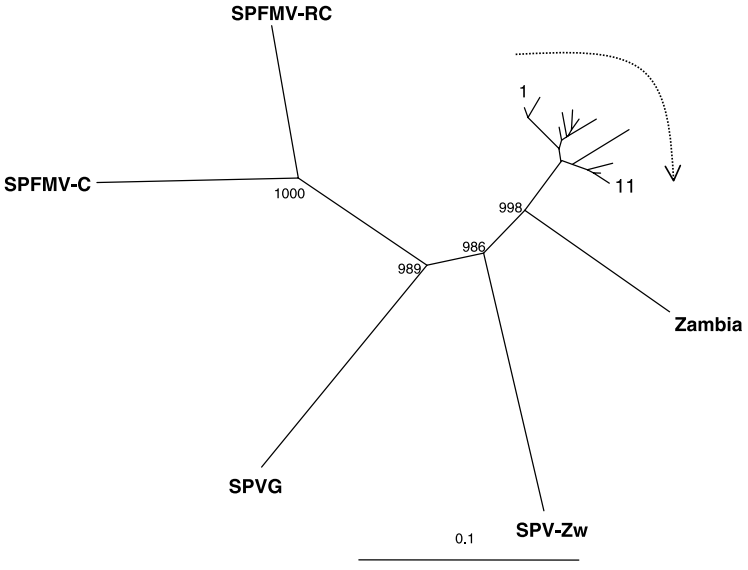

Fig. 3. Unrooted neighbour-joining tree obtained from the alignment of the CP aa sequences using the ClustalX programme. The SPV2 isolates referred to as 1–11 in the phylogenetic tree are MD2, VTSB-Tshilombo, TshilomboIV1-1, SPV2-type, Louro1, 260702/1, XN3, Thomas16A, J51, M521, and PD12, respectively. The accession numbers for the SPFMV-RC and SPFMV-C isolates used are S43450 and S43451, respectively

of the SPV2 isolates formed a compact clade, whereas the isolate from Zambia formed a separate branch clearly distinct from all the other SPV2 isolates and appeared to be phylogenetically intermediate between the other SPV2 isolates and SPV-Zw (Fig. 3). Zambia shared CP aa sequence identities of 87 and 76% with SPV2-type and SPV-Zw, respectively, whereas the CP aa sequences of SPV-Zw were 67 and 71% identical to those of SPFMV (both C and RC strains) and SPVG, respectively.

Discussion

In this study, the host range and nucleotide sequences of the CP-encoding region and the 3′NTR of selected isolates of SPV2 from China, Portugal, South Africa, and Zambia were determined and analysed. Therefore, this study is not only the first biological and molecular comparison of a geographically diverse range of SPV2 isolates but also the first record of the occurrence of this potyvirus in these four countries. Together with reports from Nigeria [26], the U.S.A. [28] and Australia [29], the present study provides evidence that SPV2 has a wide geographic distribution.

There is not much information on the distribution of SPV2 within the major production areas in Africa, Asia and the Americas. SPV2 was not detected in extensive surveys conducted recently in Kenya [5] and Uganda [3]. The reason why SPV2 was readily detected in South Africa but not in Kenya and Uganda is unknown. We also do not know why it took about 25 years until SPV2 was rediscovered since its first description in 1988. We can only speculate that this might be due to the facts that SPV2 alone causes no or only inconspicuous symptoms in sweet potato, that it often occurs in mixed infections with other viruses, notably SPFMV, and that some SPFMV antisera (cross-)react with SPV2 [our unpublished observation]. For the latter reason, some SPV2 infections may have been mistaken for those by SPFMV.

Host range and symptoms have been used for the identification and differentiation of strains and pathotypes of viruses [27, 32, 33]. Sap inoculation of six selected isolates of SPV2 onto a range of test plants revealed differences in symptoms and host range. Some isolates showed similar biological properties, suggesting that they are closely related; others appeared to be exceptional strains, and these observations seemed to be consistent with their phylogenetic grouping. Isolates PD12 and J51 were not only similar in symptoms and host range but also shared a CP aa sequence identity of 98%. Zambia and Thomas16A caused more severe symptoms on most test plants, particularly in *N. hesperis*, and their CP and 3′NTR sequences were most

divergent from the other SPV2 isolates. Moreover, only isolate Zambia did not infect *C. amaranticolor* despite repeated tests, whereas only isolate Thomas16A infected *D. metel*. Experimental results and circumstantial evidence have indicated that differences in the CP and the 3′NTR of potyvirus isolates may be biologically significant [27]. It is not unlikely that some of the observed differences in the CP aa and 3′NTR nt sequences might be responsible for the symptom differences observed among the isolates. However, the extent to which biological properties are regulated by variations in the CP and 3′NTR sequences is unclear and remains to be demonstrated for SPV2.

The geographical origin of the SPV2 isolates was partially correlated with their phylogenetic clustering. The CP and 3′NTR sequence of the type isolate, whose geographical origin is unknown (Taiwan or Nigeria), is very similar (ca. 97%) to that of the Chinese isolate XN3 and the three Portuguese isolates. On the other hand, isolate Thomas16A, the most divergent SPV2 isolate from South Africa, was phylogenetically more closely related to the isolate from Zambia than to other isolates from South Africa. In this context it is particularly noteworthy that there are SPV2 isolates originating from different countries and continents (Australia, China, Portugal, Nigeria [or Taiwan], South Africa, and U.S.A.) but forming a phylogenetically very similar group of isolates. In contrast, isolates originating from South Africa were surprisingly variable and belonged to three distinct groups. Although we cannot rule out the possibility that the higher variability of the South African isolates is the result of the larger number (seven) of isolates analysed from this country, the genetic data on the five Australian SPV2 isolates studied by Tairo et al. [29] and the three Portuguese isolates studied here suggest that isolates from one country tend to be genetically homogeneous. Since genomic divergence between organisms is roughly proportional to the evolutionary distance from a common ancestor [32], the close relationship of isolates originating from geographically distant regions and the occurrence of divergent isolates in one geographic location, predominated by a certain homogeneous group of isolates, may suggest recent evolutionary events. As P1 and P3 gene sequences have been used for establishing correlations between the biological and molecular variability of potyvirus isolates [13, 19, 31], more information on SPV2 genes other than the CP gene studied here might provide a more sensitive discrimination of biologically and/or geographically distinct SPV2 isolates.

The CP, 3′NTR, and CP N-terminus sequences are considered species-specific and have been used extensively for the classification of potyviruses and potyvirus strains [12, 27]. Recent studies of Adams et al. [2] suggest that 76–77% nt sequence identity is the optimal species demarcation criterion for the CP of potyviruses. Since the CP nt sequence identities between the SPV2 isolates were in the range of 81 to 99% (Table 3), all isolates studied here (incl. Zambia) can confidently be assigned to one virus species, SPV2. Other studies have reported similar wide variations in molecular properties among potyvirid strains [1, 18, 27]. For instance, isolates of the SPFMV strain C share CP aa sequence identities of about 78% with those of other SPFMV strains [18, 23, 30], whereas the CP aa sequence identity among SPV2 isolates studied here was considerably higher (>86%).

Our study of the genetic and biological diversity among SPV2 isolates provides a basis for proposing the existence of SPV2 strains. Isolates from Zambia and South Africa (Thomas16A; MD2 and VTSB-Tshilombo) represent at least three distinct strains of SPV2, whereas the other 8 isolates studied here, together with those from Australia [29] and the U.S.A. [28], belong to a fourth strain group of closely related isolates. It is unknown how widespread strains similar to Zambia and Thomas16A or other divergent strains are. More work is required to obtain a better understanding of the range of variability in SPV2 and to determine if there are divergent strains that escape detection with the available SPV2 antisera and RT-PCR primers.

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