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Characterization and variability of sweetpotato virus Y, a hitherto unrecognized potyvirus infecting sweetpotato in Southern Africa

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Abstract. The biological and molecular properties of a sweetpotato virus isolate formerly known as Sweetpotato virus 2 were determined. The virus had flexuous filamentous particles, induced cylindrical inclusions in infected cells and was experimentally transmitted by *Myzus persicae*. Sequence analysis of its coat protein and 3' non-translated region revealed that the virus is a distinct potyvirus, for which the name Sweetpotato virus Y (SPVY) is proposed. *Sweetpotato chlorotic stunt crinivirus* (SPCSV) synergized SPVY in *Ipomoea setosa* in mixed infections. Unlike plants that were infected only with SPVY or a Kenyan isolate (KY-38) of SPCSV and largely showed no symptoms, dually infected plants had significantly higher titres and a more uniform distribution of SPVY and showed conspicuous symptoms of SPVY infections. Comparisons of coat protein CP gene sequences of geographically diverse isolates of SPVY revealed amino acid sequence identities ranging from 86 to 100%. The results strongly suggest the existence of biologically and genetically diverse strains of SPVY from Southern Africa.

Introduction

Sweetpotato is an adaptable and versatile crop that has gained increasing importance as human food worldwide, particularly in developing countries. While in Africa the major areas of sweetpotato production are concentrated in the Great Lakes region, sweetpotato is also important in several West African countries and in South Africa. Its presence and adaptation to the tropical areas, where the *per capita* income is generally low, and its nutritional value make this crop an important component in food production and consumption (CIP, 1996; CIP, 1998; Woolfe, 1992). However, the crop is faced with numerous production constraints including insect pests and diseases.

Virus diseases are the second most significant biotic constraint after the sweetpotato weevil (Geddes, 1990; Matin, 1999). Some of the viruses reported to infect sweetpotato such as Sweetpotato feathery mottle potyvirus (SPFMV) (Ryu *et al*., 1998; Kreuze *et al*., 2000), Sweetpotato chlorotic stunt crinivirus (SPCSV) (Gibson *et al*., 1998), Sweetpotato mild mottle ipomovirus (SPMMV) (Hollings *et al*., 1976), Cucumber mosaic cucumovirus (CMV) (Cohen and Loebenstein, 1991), Sweetpotato latent potyvirus (SwPLV) (Liao *et al*., 1979; Colinet *et al*., 1997), Sweetpotato potyvirus G (SPVG) (Colinet *et al*., 1994), and Sweetpotato mild speckling potyvirus (SPMSV) (Alvarez *et al*., 1997) have been characterized to a large extent and appear to cause severe viral diseases (Moyer and Salazar, 1989; Carey *et al*., 1996; Karyeija *et al*., 1998). However, the significance and taxonomic assignment of other virus isolates remain unclear.

In the early 80s, Rossel and Thottappilly (1988) succeeded in transmitting a potyvirus from sweetpotato to test plants. In *Nicotiana benthamiana* it induced mild vein yellowing followed by chlorotic mottle. This was unusual for an isolate of SPFMV at the time. Since the isolate also differed serologically from SPFMV, it was referred to as 'sweetpotato virus 2` (SPV2) (Rossel and Thottappilly, 1988). The SPV2 isolated from sweetpotato plants from Taiwan showed mild virus-like symptoms such as mottle, vein yellowing and /or ringspots but it did not cause any symptoms in some sweetpotato clones under experimental conditions (Rossel and Thottappilly, 1988).

The fact that this virus had remained incompletely described since the 80s, prompted us to further characterize it in order to generate a basis for its classification. The biological and molecular properties determined for SPV2 in this paper suggest that it is a distinct member of the genus Potyvirus.

Materials and Methods

Mixed infection with SPCSV. In 1989, the last author received a SPV2 isolate from G.Thottappilly, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and was maintained in *Nicotiana benthamiana*. The isolate was used to investigate the effect of SPCSV on SPV2 in *I. setosa*. The treatments comprised plants infected with SPV2 alone, plants infected with SPCSV only, plants dually infected with SPV2 and SPCSV, and non-inoculated control plants. Sixteen *I. setosa* plants were mechanically inoculated with SPV2. Seven days later, SPCSV-Ky38 was graft inoculated onto eight SPV2- infected and eight non-inoculated *I. setosa* plants which were about two weeks old. Eight non-inoculated *I. setosa* plants were included as a control. Symptom development was monitored and, at 21 days post inoculation, a composite sample of three symptomatic leaves was taken from each plant and analysed by DAS- and TAS-ELISA for SPV2 and SPCSV, respectively. Results indicated that the plants were infected by the respective viruses used for inoculation. Since symptoms due to SPV2 were not uniformly distributed in SPV2-infected plants, three different leaf stages (bottom, intermediate, and top) were sampled from three randomly selected plants of each treatment and individually analyzed for SPV2 and SPCSV. Growth parameters such as leaf number, fresh shoot weight was determined for all the treatments and subjected to analysis of variance (ANOVA).

RNA extraction, RT- PCR and sequencing. Total RNA was extracted from SPV2-infected leaves of *N. benthamiana* plants using the Nucleospin® Plant kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Synthesis of complementary DNA (cDNA) from the 3' region of the viral genome was done using an oligo(dT) primer and M-MLV reverse transcriptase (GIBCO) according to the manufacturer's instructions. The 3´ end of the SPV2 genome comprising part of the NIb gene, the coat protein gene and the 3´-nontranslated region (NTR) was amplified in a MJ Research (Massachusetts, USA) thermocycler using the potyvirus-specific, degenerate primers POT1 and POT2 of Colinet *et al*. (1994) as well as an oligo(dT) primer and the viral anti-sense primer 5'-TGAGGATCCTGGTGYATHGARAAYGG-3' (CPUP) of Langeveld *et al*. (1991). For determining the correct sequence of the 'WCIENG' region (binding site of the POT1 and CPUP primers), the SPV2 specific primers 5'-CGAACTTGCTCGAGTAGGCAG-3' (viral sense) and 5'-TCCGTCCATCATCACCCA-3' (viral anti-sense) were used. Cloning experiments into pGEM®-T vector (Promega) and transformations into *E. coli* strain DH5á were conducted as described (Sambrook *et al*., 1989). The CP nucleotide sequences of 12 other SPV2 isolates were also determined. DNA sequencing was done by a commercial company (MWG-Biotech, Ebersberg, Germany). Nucleotide and amino acid sequences were analysed and compared with NCBI databases using the basic local

alignment search tool (BLAST) programs (Altshul *et al*., 1995).

Results

SPV2 caused more severe symptoms in mixed infections with SPCSV *I. setosa* plants infected with SPV2 alone developed vein clearing and mosaic symptoms only on few leaves. These were, in general, the only leaves that had ELISA-detectable concentrations of SPV2, whereas the asymptomatic leaves of SPV2-inoculated plants tested negative in ELISA. The SPCSV alone caused nearly imperceptible symptoms in infected *I. setosa* and was reliably detected by ELISA in the intermediate and top leaves but not in the bottom leaves of infected plants (Table 1). However, plants co-infected with SPV2 and SPCSV showed very conspicuous symptoms on all leaves, such as severe veinal chlorosis, mosaic leaf distortions, leaf size reductions, and plant stunting.

In addition, the number of leaves and the fresh shoot weights of dually infected *I. setosa* plants were significantly ($P \leq 0.05$) lower than those of singly infected plants, whose growth parameters were largely indistinguishable from each other and from those of non-inoculated plants (Figure 1). The SPV2 concentrations in plants infected only with SPV2 were often below the detection limit, particularly in the top leaves (Table 1). In dually infected plants, SPV2 attained higher concentrations and appeared to be more uniformly distributed than in singly infected plants. Unlike SPV2, the titre and distribution of SPCSV were only negligibly enhanced in dually infected plants as compared to singly infected plants (Table 1).

Sequence analyses and comparisons. Sequence analysis of the 3'-terminal 2006 nucleotides of the SPV2 RNA (Acc. No. AY232437) showed that this genome fragment contains the coding sequence for the C-terminal half (296 aa) of the NIb protein and the entire CP (332 aa), which is followed by a 3' untranslated region (UTR) of 216 nucleotides (without the poly (A) tail). The coding sequence not only shared similarity with NIb and CP genes of potyviruses but

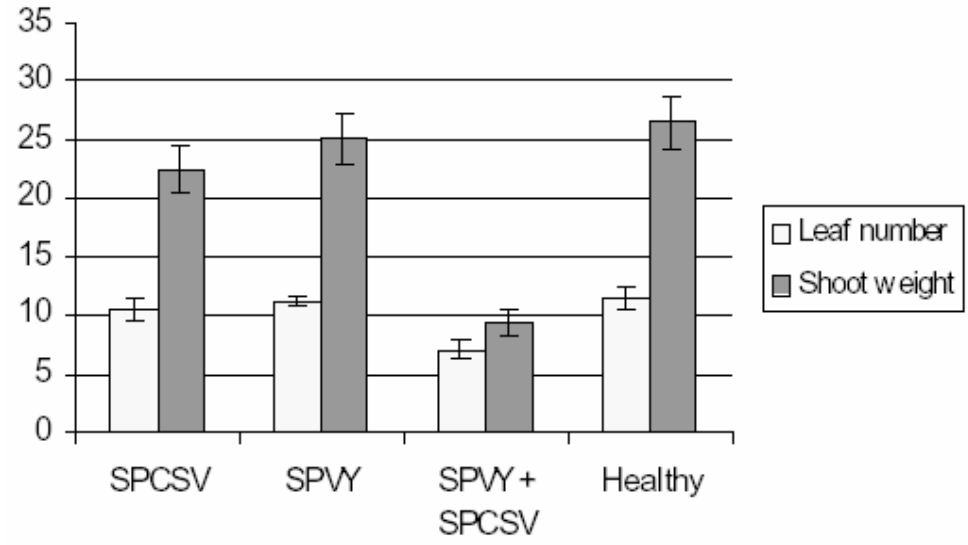

Figure 1: Leaf number and mean fresh shoot weight of plants inoculated with *Sweet potato chlorotic stunt virus* (SPCSV), 'sweet potato virus 2' (SPV2), both viruses (SPCSV + SPVY), and of non-inoculated plants (healthy). Vertical bars represent standard errors of means of 8 plants for each treatment. Treatments were compared by single-factor ANOVA and means separated at Pd"0.05 level.

also contained a characteristic NIa protease cleavage site (VYHQ/S). In close proximity to this cleavage site, the CP amino terminus carries the amino acid triplet DAG that is involved in aphid transmission of poty-viruses (Atreya *et al*., 1995). The non-persistent transmission of SPV2 was confirmed experimentally by *Myzus perscae*.

BLAST search analysis of the SPV2 sequences gave highest scores with potyvirus isolates from sweetpotato. Based on the phylogenetic tree of the CP amino acid sequences (Figure 2A), the closest relatives of SPV2 appear to be SPV-Zw (Chavi *et al*., 1997) and SPVG, which share CP amino acid sequence similarities with SPV2 of 80 % and 77 %, respectively (Table 2). Much lower CP

Table 1: Distribution and titres of SPV2 and SPCSV in *I. setosa* plants infected with SPV2 or SPCSV alone and in dually infected plants.

Leaf stage	DAS-ELISA readings[1] for SPV2 in plants infected with		TAS-ELISA readings for SPCSV in plants infected with	
	SPV2 alone	SPV2 + SPCSV	SPCSV only	SPV2 + SPSV
Bottom	0.618	0.915	0.034	0.154
Intermediate	0.175	0.784	1.103	1.454
Top	0.040	0.767	0.266	0.401

[1]Absorbance readings were measured at A_{405}. Data are means from three randomly selected plants.

Figure 2: Neighbour-joining relationship dendrograms of the coat protein (CP) core amino acid (A) and 3' UTR nucleotide sequences (B) of SPV2 and other sweet potato infecting poty-viruses, with the CP and 3' UTR sequences of PVY^N used as outgroup sequences. Verti-cal branch lengths are arbitrary, horizontal branch lengths are proportional to percent sequence differences, and numbers at each node indicate bootstrap scores higher than 50%. Virus (isolate) designations and accession numbers are SPV2 (AY232437), SPV-Zw (AF016366), SPVG (X76744), SPFMV-RC (S43450), -C (S43451), -S (D86371), -O (D16664), -6 (U96625), SwPLV (X84011), SPMSV (U61228), and PVY^N (AY166867).

Table 2: Percent coat protein amino acid sequence (lower diagonal) and 3'UTR nucleotide sequence identities (upper diagonal) following pairwise alignments of SPV2 and other potyviruses infecting sweet potato.

Virus	SPV2	SPV-Zw	SPVG	SPFMV isolate					SwPLV	SPMSV
				-6	-C	-RC	-S	-O		
SPV2		82.1	82.2	56.3	79.0	78.6	77.8	77.4	55.5	44.0
SPV-Zw	80.0		79.5	55.7	77.9	77.5	75.8	75.3	45.6	43.5
SPVG	77.3	75.0		47.5	75.3	75.3	75.1	74.7	51.8	38.8
SPFMV-6	74.4	71.3	72.3		71.0	70.1	72.1	73.1	44.1	49.5
SPFMV-C	74.7	72.4	72.4	92.7		97.7	99.1	98.6	50.3	45.6
SPFMV-RC	76.4	73.3	74.4	81.2	84.7		98.2	96.4	50.8	45.6
SPFMV-S	76.3	73.3	74.1	82.3	84.4	99.0		97.8	50.3	46.1
SPFMV-O	76.4	72.3	74.5	80.8	83.1	95.6	95.2		51.3	45.2
SwPLV	62.3	61.6	61.6	63.1	62.2	64.1	64.1	62.3		46.1
SPMSV	57.7	57.4	60.4	59.8	60.9	58.7	59.3	60.2	58.8	

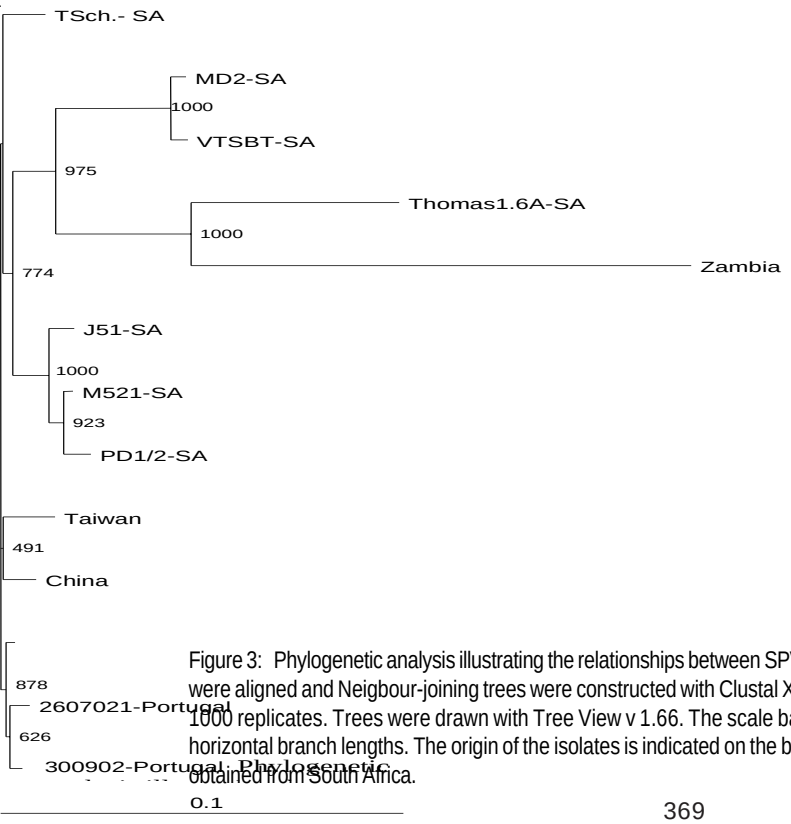

Figure 3: Phylogenetic analysis illustrating the relationships between SPV2 isolates. Coat protein nucleotide sequences were aligned and Neigbour-joining trees were constructed with Clustal X v1.83. Bootstrap values were calculated from 1000 replicates. Trees were drawn with Tree View v 1.66. The scale bar represents a genetic distance of 0.1 for the horizontal branch lengths. The origin of the isolates is indicated on the branches. Isolate names ending with –SA were obtained from South Africa.

amino acid sequence similarities were observed between SPV2 and SPFMV (~75%), SwPLV (69%), and SPMSV (63%) (Table 2). Phylogenetic analysis (Figure 2B) and pairwise alignments (Table 2) of 3' UTR sequences of sweetpotato potyviruses also supported the notion that SPV2 is more closely related to SPV-Zw (82%) and SPVG (82%) than to SPFMV (78%), SwPLV (56%) and SPMSV (44% only). The 3' UTR size (without poly (A) tail) of SPV2 (216 nt) was very similar to that of SPV-Zw (215 nt), SPVG (221 nt), and SPFMV (222 nt), whereas it differed considerably from that of SPMSV (248 nt) and SwPLV (196 nt).

To determine the relationship between geographically diverse isolates of SPV2 the nucleotide (nt) sequences of the CP genes of twelve isolates from different geographical regions were obtained. The comparison of the CP encoding nt sequences gave identities ranging from 81-100%, whereas the CP aa similarities ranged from 86 to 100% (data not shown). Thomas 1.6A and Zambia consistently took a position divergent from all the other isolates. Apart from Thomas 1.6A, all isolates from South Africa were closely related (94 - 100%) to one another and to isolates from China, Portugal and the type isolate from Taiwan (Figure 3).

Discussion

In synergy studies using *I. setosa* as a model host, plants co-infected with SPV2 and SPCSV showed very severe and conspicuous symptoms on all leaves and strikingly enhanced levels in SPV2 accumulation and distribution as compared to singly infected plants (Figure 1; Table 1). Since the symptoms observed on the dually infected plants were typical of potyviruses and the titre and distribution of SPV2 but not those of SPCSV were dramatically enhanced, it is conceivable that the greater damage observed for the dually infected plants was due to SPV2. These observations and conclusions are in agreement with those made for SPFMV, another sweetpotato potyvirus that caused more severe symptoms and accumulated several-fold in sweetpotato and *I. setosa* plants co-infected with SPCSV (Gibson *et al*., 1998; Karyeija *et al*., 2000). It was suggested that the crinivirus suppresses the resistance mechanism of sweetpotato to the potyvirus leading to its accumulation (Karyeija *et al*., 2000). In this type of synergistic interaction, SPCSV-encoded factors might be responsible for the suppression of the host's defense mechanism thereby permitting an enhanced multiplication of potyviruses, such as SPV2 and SPFMV.

In order to further characterize SPV2, an incompletely described potyvirus isolate from sweetpotato in Taiwan, some of its molecular properties were determined. The taxonomic assignment of SPV2 to the genus Potyvirus was supported by the sequence analysis of the 3'-terminal portion of SPV2 RNA. Based on one of the major demarcation criteria for potyvirus species ("CP amino acid identity of < about 80%" Berger *et al*., 2000), SPV2 is clearly different from SPMSV (58%), SwPLV (62%), and SPFMV (~75%) (Table 3). In view of the notion that distinct potyvirus species have 3'-UTR sequence identities of < 80% (Frenkel *et al*., 1989; Shukla *et al*., 1994), it should also be pointed out that the 3'-UTR nucleotide sequence identities between SPV2 and the other potyvirus species SPMSV (38%), SwPLV (49%), and SPFMV (77%) did not exceed 80% (Table 2).

The CP aa sequence similarities among the SPV2 isolates sequenced ranged from 86 to 100%, which is within the suggested thresholds of distinct viruses (Frenkel *et al*., 1992, 1989; Shukla *et al*., 1994). The isolates from Zambia and Thomas 1.6A each represent distinct strains of SPV2 whereas the other 12 isolates belong to a third strain of closely related isolates. It is unknown how widespread strains similar to Zambia and Thomas 1.6A or other divergent strains are. More work needs to be done to get a better understanding of the range of variability in SPV2, and to determine if certain deviant

strains escape serological detection. In conclusion, based on the properties determined in this study, SPV2 is a distinct member of the genus Potyvirus. We propose that it be named Sweetpotato virus Y.

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