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First report of *Sweet potato leaf curl virus* infecting sweet potato in Argentina

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Abstract We report the complete nucleotide sequence of a begomovirus infecting sweet potato in the Northeastern region of Argentina. Sequence comparisons indicated that the isolate under study has the highest nucleotide sequence identity (93.6 %) with *Sweet potato leaf curl virus* Puerto Rico. According to the current taxonomic criteria for begomovirus classification the Argentinean isolate would correspond to a new strain of *Sweet potato leaf curl virus*.

Keywords *Ipomoea batatas* · *Geminivirus* · *Begomovirus* · Sequence

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the most important crops worldwide ranking among the world's seven most important food crops (along with wheat, rice, maize, potato, barley, and cassava). Due to its nutritional qualities (rich in carbohydrates, dietary fiber, beta carotene, vitamin C, and vitamin B6), sweet potato is considered as a crop with great potential not only for human consumption but also for animal feeding and industrial use (Bovell-Benjamin 2007). It is cultivated in over 100 countries but most of the production is concentrated in eastern Asia, with 80 % in China alone. Argentina produced 340,105 metric tones in 2009 (USDA Economics and System 2011), mainly in the Pampean region (Buenos Aires, Córdoba and Santa Fe provinces) and the Northeast (NEA) region with 83 % of the planted surface (43 % and 40 % respectively). The Northwest region (NOA) produces 15 %, and the remaining 2 % of the production corresponds to the Cuyo region (Mendoza and San Juan). One of the major limitations in the sweet potato production is cultivar decline due to the accumulation of viruses on this vegetatively propagated crop (Lozano et al. 2009; Zhang and Ling 2011). The first report on a viral disease in sweet potato in Argentina was published during the 70's, when a symptom named "batata crespa" produced by the potyvirus *Sweet potato vein mosaic virus* (SPVMV) was described affecting Cv Criolla Amarilla (Nome 1973); later *Sweet potato feathery mottle virus* (SPFMV) was detected affecting the same cultivar (Nome et al. 1980). A new cultivar named Morada INTA, tolerant to both virus (SPVMV and SPFMV) was adopted by Argentinean farmers in 1978, but during the 80's, this cultivar was affected by a severe disease called Sweet potato chlorotic dwarf that was produced by a synergistic combination of two aphid-transmitted potyviruses (SPFMV and *Sweet potato mild speckling virus*: SPMSV), with a whitefly-transmitted crinivirus serologically related to *Sweet potato chlorotic stunt virus* (SPCSV) (Di Feo 2000). This viral disease was successfully controlled by using propagation material produced in areas where the disease was not present. In these areas, although the three viruses and their vectors were present, due to environmental conditions, the viral concentrations were too low to produce symptoms or damages. Nevertheless, since 2008/2009 growing season typical begomovirus symptoms appeared in sweet potato crops in the NEA region of Argentina.

Geminivirus (family *Geminiviridae*) are plant viruses with a circular single strand DNA genome encapsidated within twinned isometric particles. Based on their genome organization, host range and insect transmission, geminiviruses are classified into four genera: *Mastrevirus, Curtovirus,*

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Fig. 1 Sweet potato cv Okinawa showing typical *Sweet potato leaf curl virus* symptoms (upward leaf curling and mild vein clearing)

Topocuvirus and *Begomovirus* (Brown et al. 2012). Begomoviruses represent major plant pathogens in tropical, subtropical and, to a more limited extent, temperate regions (Morales and Anderson 2001). Begomoviruses are transmitted by whiteflies (*Bemisia tabaci* Genn.) to dicotyledonous plants. Most of the species in this genus have bipartite genomes consisting of two ssDNA molecules, referred to as DNA-A and DNA-B, but there are some monopartite species, found within the Old World, that have only one genome component similar to DNA-A. The DNA-A virion sense strand encodes the coat protein (CP ORF AV1/V1) that encapsidates the virion-sense ssDNA and may be involved in virus movement in monopartite species, and ORF AV2/V2 which may be also involved in virus movement. This ORF is absent in the New World bipartite viruses. The DNA-A complementary-sense strand encodes the replication – associated protein (Rep ORF AC1/C1) and a replication enhancer protein (REn ORF AC3/C3), responsible for viral replication, a transcriptional activator protein (TrAP ORF AC2/C2) which controls viral gene expression. AC4 protein may counter a host response to Rep expression, while C4 protein (monopartite viruses) is an important symptom determinant involved in cell cycle control. (Hanley-Bowdoin et al. 1999). Genes in DNA-B encode for two proteins (MP and NSP) involved in cell-to-cell movement within the plant, host range and symptom modulation. A region of approximately 200 nucleotides, common to both genomic components, contains *cis*-acting signals required for DNA replication and transcription (reviewed by Rojas et al. 2005). *Ipomoea* infecting begomoviruses are monopartite viruses and phylogenetic analysis showed that these viruses, for which the name of sweepoviruses has been proposed, are

Table 1 Sweepoviruses used in comparative sequence analyses with their corresponding abbreviations, GenBank accession numbers, and percent nucleotide sequence identities with the Argentinean isolate of *Sweet potato leaf curl virus*

Virus	Isolate	Abbreviation	Accession number	Percent identity
Sweet potato leaf curl virus	Brazil	SPLCV-Br	FJ969834	92.1
Sweet potato leaf curl virus	Puerto Rico	SPLCV- Pr	DQ644562	93.6
Sweet potato leaf curl virus	Puerto Rico	SPLCV- Pr Me	DQ644563	93.6
Sweet potato leaf curl virus	USA	SPLCV-USA	Af104036	92.8
Sweet potato leaf curl virus	Korea	SPLCV-K	HM754637	92.5
Sweet potato leaf curl virus	Japan	SPLCV-Jp	Ab433788	92.3
Sweet potato leaf curl virus	China	SPLCV-CN	FN8060776	90.8
Sweet potato leaf curl virus	China F-p3	SPLCV-F-p3	FJ515898	90.2
Sweet potato leaf curl virus	USA MS	SPLCV-MS	HQ333142	91.9
Sweet potato leaf curl virus	Spain	SPLCV-Sp	EU856364	89.6
Sweet potato leaf curl virus	Brazil RS1	SPLCV-RS1	FJ869833	86.3
Sweet potato golden vein associated virus	Brazil	SPGVaV	FJ969830	87.6
Ipomoea yellow vein virus	Italy	IYVV	AJ586885	86.8
Merremia leaf curl virus	Puerto Rico	MLCV	DQ644561	84.9
Ipomoea yellow vein virus	Malaga	IYVV-mal	EU839577	84.3
Sweet potato leaf curl Uganda virus	Uganda	SLCUV	FR751068	81.4
Sweet potato leaf curl South Carolina virus	USA	SPLCSCV	HQ333144	79.1
Sweet potato leaf curl Spain virus	Spain	SPLCESV	EF456741	78.6
Sweet potato leaf curl Georgia virus	USA	SPLCGoV	AF326775	77.6
Sweet potato mosaic associated virus	Brazil	SPMaV	FJ969831	78.7
Sweet potato leaf curl China virus	China	SPLCCNV	DQ512731	46.1
Sweet potato leaf curl Canary virus	Spain	SPLCCaV	EF456745	83.5

Fig. 2 Multiple nucleotide alignment of part of the intergenic region of SPLCV-Ar compared with SPLCV-PR isolate. Text highlighted in gray indicates the iterons, in the box, the TATA box, and underlined, the conserved nanonucleotides sequence found in the loop of the stem loop

grouped in a monophyletic cluster, separated from the main begomovirus branches, the Old and New World groups (Lozano et al. 2009). The objective of this study was to detect, identify and carry out the molecular characterization of Argentinean sweet potato begomoviruses.

Sweet potato cv Okinawa samples showing upward leaf curling and mild vein clearing symptoms (Fig. 1) were collected in Bella Vista, province of Corrientes (NEA), from production fields where 85 % of incidence was observed. Eight plants were transferred to pots and conserved under greenhouse conditions for further analysis.

The presence of sweepoviruses was verified by PCR using primers SPG1 and SPG2 that were designed to bind to conserved regions in open reading frames C2 and C1 and amplify an 912 bp fragment (Li et al. 2004). Total DNA was extracted from infected leaves using a NaOH based method (Wang et al. 1993); basically 50 mg of leaves were grinded with four volumes of 0.5 N NaOH; the supernatant was then centrifuged at 14,000 rpm for 5 min at room temperature and the aqueous phase was diluted (1:100, v/v) with 100 mM Tris-HCl pH 8.0. PCR reactions were prepared in a 25 μl volume, containing 10X buffer, 3.5 mM Mg_2Cl, 0.25 mM dNTP mix, 0.5 μM of primer SPG1, 0.75 μM of primer SPG2, 1 unit of taq DNA polymerase (Promega Corp. Madison, WI, USA) and 2 μl of DNA. The amplification conditions were as follows: 11 cycles of 94 °C for 40 s, (72-n)°C for 30 s (n: cycle number) 72 °C for 90 s; 24 cycles of 94 °C for 40 s, 60 °C for 40 s, 72 °C for 90 s and a final extension of 72 °C for 10 min. PCR products were analyzed by electrophoresis in 1 % agarose gel, then stained with ethidium bromide.

Total DNA, extracted by the CTAB-based method (Doyle and Doyle 1987), was used as template to amplify the putative full-length begomovirus genomes by rolling circle amplification (RCA) with Φ 29 DNA polymerase (Templiphi GE Healthcare) as previously described (Inoue-Nagata et al. 2004). To select an enzyme that could cut at a single site in the genome of begomovirus, generating unit-size molecules, the amplified DNA was digested with seven restriction enzymes (*Cla*I, *Nco*I, *Eco*RV, *Xba*I, *Bam*HI, *Pst*I and *Sac*I). *Eco*RV restriction yielded a DNA fragment of 2,800 Kb, corresponding to a putative monomeric genome component. This fragment was cloned into *Eco*RV digested pBluescript SK +, transformed into *Escherichia coli* DH5α, and sequenced at the Unidad de Genómica, Instituto de Biotecnología-INTA (Argentina).

The nucleotide and deduced amino acid sequences were compared with those of other sweepoviruses available in the GenBank (www.ncbi.nlm.nih.gov). The accession number and assigned abbreviations of these sweepoviruses are listed in Table 1. Database searches were carried out using the Blast*n* algorithm (Altschul et al. 1990) Multiple sequence alignments of nucleotide and deduced amino acid sequences were performed with Clustal V (Higgins et al. 1992). Evolutionary analyses were conducted in MEGA 5 (Tamura et

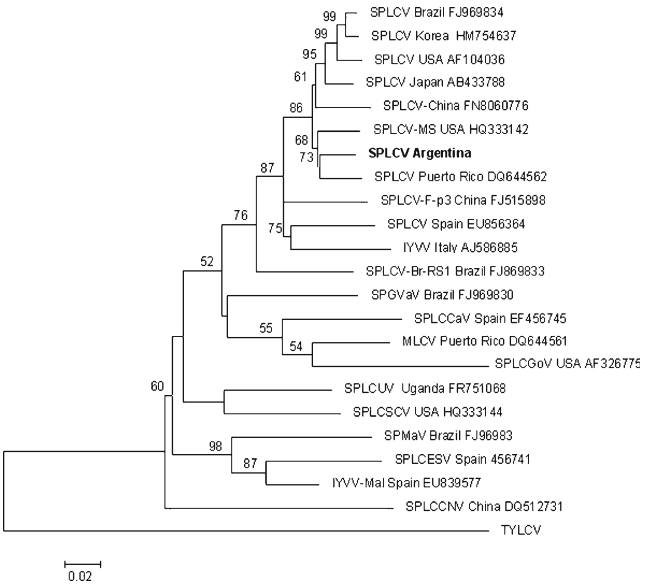

Fig. 3 Phylogenetic tree based on the complete DNA-A nucleotide sequences of *Sweet potato leaf curl virus* Argentinean isolate and other selected sweepovirus. The tree was constructed using the Neighbour joining method with MEGA 5, with *Tomato yellow leaf curl virus* (TYLCV acc no X76319) as an outgroup member. Countries of origin and accession number for each virus isolate are indicated. The used acronyms for the viruses are as follows: SPLCV (*Sweet potato leaf curl virus*), IYVV (*Ipomoea yellow vein virus*), MLCV (Merremia leaf curl virus), SPGVaV (Sweet potato golden vein associated virus), SPLCCaV (*Sweet potato leaf curl Canary virus*), SPLCGoV (*Sweet potato leaf curl Georgia virus*), SPLCCNV (*Sweet potato leaf curl China virus*), SPLCESV (*Sweet potato leaf curl Spain virus*), SPLCSCV (Sweet potato leaf curl South Carolina virus), SPLCUV (Sweet potato leaf curl Uganda virus), and SPMaV (Sweet potato mosaic associated virus)

al. 2011) using the Neighbor-Joining method and the Kimura 2-parameter method (Kimura 1980). The bootstrap consensus tree was inferred from 2000 replicates. All positions containing gaps and missing data were eliminated.

PCR products of the expected size (912 pb) were obtained for eight samples of symptomatic sweet potato plants, while the symptomatic sample was negative in the PCR test.

The complete DNA sequence of the molecule isolated and cloned after *Eco*RV digestion was determined to be 2,828 nt in length (accession number JQ349087). The DNA sequence organization was typical of a monopartite begomovirus. It contained an intergenic region with a putative stem-loop structure sequence and the conserved nanonucleotide sequence (TAATATTAC) in the loop. The sweepoviruses possess four imperfect copies of the iterative elements, three direct (I, II and III) and one in the inverted direction (IV) (Argüello-Astorga et al. 1994) These four elements were identified for the Argentinean isolate (Fig. 2), the iteron related domain in the N- terminal region of the replication protein (Rep IRD) (Argüello-Astorga and Ruiz-Medrano 2001), was also identified and showed to be as follows: MAPPKRFKIQ. Two ORF were found in the positive or virion sense strand, encoding the coat protein (CP) (254 aa) and V2 (123 aa) and four ORFs in the complementary sense strand, corresponding to Rep with 364 deduced aa, REn (144 aa), TrAP (148 aa), and C4 (85 aa).

Sequence comparisons indicated that the isolate under study has the highest nucleotide sequence identity (93.3 %) with *Sweet potato leaf curl virus* Puerto Rico isolate (SPLCV-PR, accession number DQ644562). Thus, in accordance with the current taxonomic criteria for begomovirus classification (Fauquet et al. 2008), the Argentinean isolate would correspond to a new strain of *Sweet potato leaf curl virus* henceforth named SPLCV-Ar

The phylogenetic relationship of the DNA-A sequence of SPLCV-Ar and other selected sweepoviruses (Fig. 3) showed that SPLCV- Ar clustered together with SPLCV-PR in a monophyletic branch with a 73 % bootstrap confidence value, confirming that this Argentinean isolate is closely related with the Puerto Rico isolate.

To our knowledge, this is the first report of SPLCV in Argentina.

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