Arch Virol (2011) 156:1291–1294
DOI 10.1007/s00705-011-1016-x

ANNOTATED SEQUENCE RECORD

A novel monopartite begomovirus infecting sweet potato in Brazil

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Received: 5 March 2011 / Accepted: 30 April 2011 / Published online: 19 May 2011

Abstract The complete genome sequences of two monopartite begomovirus isolates (genus *Begomovirus*, family *Geminiviridae*) present in a single sweet potato (*Ipomoea batatas*) plant collected in São Paulo, Brazil, are presented. Based on the current taxonomic criteria for the genus *Begomovirus*, one of the isolates was shown to represent a novel species, tentatively named Sweet potato leaf curl Sao Paulo virus (SPLCSPV). The other isolate represented a new strain of sweet potato leaf curl virus, named sweet potato leaf curl virus-Sao Paulo (SPLCV-SP). The full genome sequence of the SPLCSPV isolate shared the highest nucleotide identity (87.6%) with isolates of sweet potato leaf curl Spain virus (SPLCESV). Phylogenetic and recombination analyses were used to investigate the relationships of these isolates to other monopartite *Ipomoea*-infecting begomoviruses.

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Geminiviruses (family *Geminiviridae*) are an important group of plant viruses with a genome consisting of one or two circular single-stranded DNA molecules of 2.6-3.0 kb packed into twinned quasi-icosahedral particles. Based on their host range, genome organization and insect vector, geminiviruses are currently classified into four genera: *Begomovirus*, *Curtovirus*, *Mastrevirus* and *Topocuvirus* [15]. A number of monopartite begomoviruses infect several species of the genus *Ipomoea* within the family *Convolvulaceae* [9, 13]. Phylogenetic analysis showed that these viruses, for which the name "swepoviruses" has been proposed [2], are grouped in a monophyletic cluster, separated from the main begomovirus branches, the Old and New World groups [9]. Sweet potato (*I. batatas*) plants infected by swepoviruses are frequently symptomless, although yield losses have been reported in some varieties [8]. Because sweet potato is vegetatively propagated, accumulation of viruses can become a major constraint for its production. Swepoviruses have been described infecting sweet potato in many countries, including Peru [3], Spain [9], China [10], United States of America [6–8] and, recently, in a sweet potato germplasm bank from Brazil [13]. Over the last few years, surveys were conducted across Brazil to identify swepoviruses infecting commercial and backyard sweet potato crops. As a result, we report herein the full genome sequence of a novel monopartite begomovirus infecting sweet potato in the State of São Paulo, Brazil.

Leaf sample #SP71 from a sweet potato plant (var. Londrina) showing chlorosis and vein necrosis symptoms was collected in a commercial field at Álvares Machado, State of São Paulo (22°00'S, 51°27'W) in April 2009. Total DNA was extracted from this sample using a CTAB-based method [1], and then putative full-length begomovirus genome components were amplified by rolling-circle amplification (RCA) with ϕ29 DNA polymerase

(TempliPhi, GE Healthcare) essentially as described previously [5]. The resulting concatamers were digested with ten different restriction enzymes (*Bam*HI, *Cla*I, *Eco*RI, *Eco*RV, *Hind*III, *Kpn*I, *Pst*I, *Sac*I, *Spe*I, and *Xba*I). *Bam*HI and *Sac*I restriction yielded DNA fragments of ca. 2.8 kbp, putatively corresponding to monomeric genome components. These fragments were cloned into *Bam*HI- and *Sac*I-digested pBluescript SK^+ (Stratagene), respectively, introduced into *E. coli* DH5α by transformation and fully sequenced by Macrogen Inc. (Seoul, South Korea).

The DNA molecules isolated from sample #SP71 and cloned after digestion with *Bam*HI (GenBank accession number HQ393477) and *Sac*I (HQ393473) were 2782 and 2837 nucleotides in length, respectively. The sequences contained features typical of monopartite begomoviruses, with two open reading frames (ORFs) in the viral sense strand and four ORFs in the complementary sense strand, separated by an intergenic region containing the nonanucleotide sequence (TAATATT↓AC) conserved in the family *Geminiviridae* with a few exceptions, such as beet curly top Iran virus and Eragrosti curvula streak virus [17, 18]. Within the intergenic region, the repeated elements (iterons) located surrounding the TATA box of the replication-associated protein (Rep) ORF presented the consensus sequence core GGWGD and four iterative elements, three direct (AA ATGGTGGA, AATTGGTGGA and GGTGGA) and one inverted (TCCACCAAAT), identical to those reported for sweet potato leaf curl Lanzarote virus [9].

Identities of begomovirus nucleotide sequences obtained in this work with those of representative swepoviruses were calculated after alignment with CLUSTAL V [4] (included in MegAlign DNASTAR Inc., Madison, WI, USA) (Table 1). The sequence of clone SP71-*Bam*HI (GenBank HQ393477) showed the highest level of nucleotide sequence identity (87.6%) to that of sweet potato leaf curl Spain virus-[ES:CI:BG5:02] (GenBank EF456743), followed (86.5%) by that of sweet potato mosaic-associated virus-[BR:BSB1] (FJ969831), both of which were recently proposed to belong to novel begomovirus species in Spain and Brazil, respectively [9, 13]. The sequence of clone SP71-*Sac*I (GenBank HQ393473) was most closely related (92.2%) to that of sweet potato leaf curl virus-RS2[BR:Ros1] (FJ969837) [13]. Thus, in accordance with the current taxonomic criteria for begomovirus classification [15], the sequence from clone SP71-*Bam*HI likely corresponds to that of an isolate of a novel begomovirus species, for which we propose the name Sweet potato leaf curl Sao Paulo virus (SPLCSPV). The sequence from clone SP71-*Sac*I would correspond to that of an isolate of a new strain of sweet potato leaf curl virus. These isolates were designated sweet potato leaf curl Sao Paulo virus-[Brazil:Alvares Machado:2009] (SPLCSPV-[BR:AlvM:09]) and sweet potato leaf curl virus-Sao Paulo [Brazil:Alvares Machado:2009] (SPLCV-SP[BR:AlvM:09]), respectively.

A phylogenetic tree was constructed using Mega4 by the neighbour-joining method [16] after alignment of the most closely related swepovirus genomes (Fig. 1). The SPLCSPV-[BR:AlvM:09] sequence clustered with sweet potato mosaic-associated virus-[Brazil:Brasilia 1], while SPLCV-SP[BR:AlvM:09] was closer to sweet potato leaf curl virus-Rio Grande do Sul 2[Brazil:Rosario 1], both of which were obtained from a sweet potato germplasm bank in Brazil [13].

Table 1 Pairwise sequence identity percentages of the complete genome sequences of SP71-*Bam*HI and SP71-*Sac*I swepovirus isolates reported here and those of representative *Ipomoea*-infecting begomoviruses

Virus	SP71*Bam*HI	SP71*Sac*I
Sweet potato leaf curl Sao Paulo virus-[Brazil:Alvares Machado:2009] (SP71-*Bam*HI)	100	84.2
Sweet potato leaf curl virus-Sao Paulo [Brazil:Alvares Machado:2009] (SP71-*Sac*I)	84.2	100
Ipomoea yellow vein virus-[Spain:Málaga:IG5:2006]	80.8	77.5
Sweet potato golden vein-associated virus-Pará [Brazil:Belém 1]	74.1	81.2
Sweet potato golden vein-associated virus-Paraíba 1 [BR:Sou1]	80.6	88.1
Sweet potato leaf curl Canary virus-[Spain:Canary Islands:BG21:2002]	75.4	85.2
Sweet potato leaf curl Georgia virus-[United States:Georgia 16]	77.6	77.1
Sweet potato leaf curl Lanzarote virus-[Spain:Málaga:BG30:2006]	79.2	89.6
Sweet potato leaf curl Spain virus-[Spain:Canary Islands: BG5:2002]	**87.6**	79.2
Sweet potato leaf curl virus-[United States:Louisiana:1994]	75.3	89.6
Sweet potato leaf curl China virus-[China:2005]	75.2	74.5
Sweet potato leaf curl virus-Rio Grande do Sul 2[Brazil:Rosario1]	75.2	**92.2**
Sweet potato mosaic-associated virus-[Brazil:Brasília1]	86.5	77.5

Acronyms and GenBank accession numbers are provided in Fig. 1. The highest percentages of nucleotide identity for SP71-*Bam*HI and SP71-*Sac*I isolates are underlined

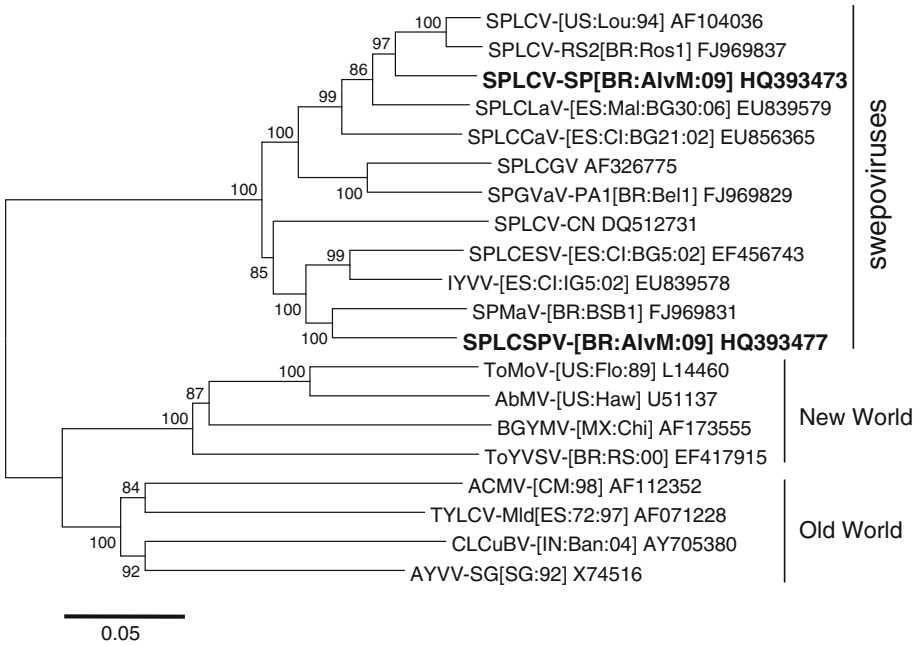

Fig. 1 Phylogenetic tree indicating the relationship between the complete genomes of SPLCSPV-[BR:AlvM:09] and SPLCV-SP[BR:AlvM:09] reported here (in bold) and those of representative *Ipomoea*-infecting begomoviruses. The tree was constructed by the neighbour-joining method with 1,000 bootstrap replicates using MEGA4.1 [16]. The virus used for alignment were as follows: sweet potato leaf curl virus-[United States of America:Louisiana:1994] (SPLCV-[US:Lou:94]), sweet potato leaf curl virus-Rio Grande do Sul 2 [Brazil:Rosario1] (SPLCV-RS2[BR:Ros1]), sweet potato leaf curl Lanzarote virus-[Spain:Málaga:BG30:2006] (SPLCLaV-[ES:Mal:BG30:06]), sweet potato leaf curl Canary virus-[Spain:Canary Islands:BG21:2002] (SPLCCaV-[ES:CI:BG21:02]), sweet potato leaf curl Georgia virus-[United States:Georgia 16] (SPLCGV-[US:Geo16]), sweet potato golden vein-associated virus-Pará [Brazil:Belém 1] (SPGVaV-PA[BR:Bel1]), sweet potato leaf curl China virus-[China:2005] (SPLCCNV-CN:05), sweet potato leaf curl Spain virus-[Spain:Canary Islands: BG5:2002] (SPLCESV-[ES:CI:BG5:02], Ipomoea yellow vein virus-[Spain:Málaga:IG5:2006] (IYVV-[ES:Mal:IG5:06]), sweet potato mosaic-associated virus-[Brazil:Brasília1] (SPMaV-[BR:BSB1]). DNA-A of representative members of the New (tomato mottle virus-[United States:Florida:1989] (ToMoV-[US:Flo:89]), Abutilon mosaic virus-[United States:Hawaii] (AbMV-[US:Hav]), bean golden yellow mosaic virus-[Mexico:Chiapas] (BGYMV-[MX:Chi]) and Old World (African cassava mosaic virus-[Cameroon:1998] (ACMV-[CM:98]), tomato yellow leaf curl virus-Mild[Spain:72:1997] ([TYLCV-Mld[ES:72:97]), cotton leaf curl Bangalore virus-[India:Bangalore:2004] (CLCuBV-[IN:Ban:04]), Ageratum yellow vein virus-Singapore[Singapore:1992] (AYVV-SG[SG:92])) begomoviruses are included. GeneBank accession numbers are shown in the tree. The scale bar indicates the number of substitution per site

The presence of isolates belonging to two different begomovirus species in a single field-grown sweet potato plant, as reported here, is an additional example of a mixed infection, which was previously shown to be frequent in this plant host [9]. This phenomenon is extremely important for virus evolution because mixed infections are the prerequisite for the occurrence of natural recombination events, which may contribute to the appearance of new begomoviruses [12, 14]. We searched for evidence of recombination (using RDP3 with default settings [11]) between the isolates described here and other swepoviruses. Our analyses showed that SPLCSPV probably contributed with genetic material (DNA fragments of $\sim$727 nt [P-value $= 3.0\text{x}10^{-40}$] and $\sim$897 nt [P-value $= 3.0\text{x}10^{-40}$], respectively) to the emergence of two viruses, SPLCV-SP (described here) and sweet potato golden vein-associated virus-PB1 [13].

The only previous study on swepoviruses from Brazil [13] was carried out on a collection of sweet potato germplasm maintained *ex vitro* at Embrapa Vegetables, Brasilia. The results presented here, as part of a survey carried out on commercial crops in Brazil, support the presence of begomoviruses infecting commercial sweet potato crops and point to a complex ecological situation with a great diversity of swepoviruses present, and this diversity might increase as further studies are carried out.

Acknowledgments The authors thank Dr. João Bosco Carvalho Silva and Dr. Amaríllis Rós Golla for assistance on sample collection, and Lucio Flávio Barbosa and Oneilson Medeiros de Aquino for technical assistance. This study was funded by CNPq project 482005/2007-6. L.C.A. is sponsored by CNPq (561405/2008-5) and Capes/Wageningen (014/07), and R.O.R. and A.K.I.N. are CNPq fellows. The work in Spain was supported by grant GEN2006-27770-C2-2 (Ministerio de Educación y Ciencia, Spain, co-financed by FEDER) in the frame of the ERA-NET Plant Genomics project ERA-PG 040B RCA-Genomics of the 6th EU Framework Programme for Research and Technological Development (FP6).

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