Virus Genes
DOI 10.1007/s11262-012-0784-z

Complete nucleotide sequence of an Argentinean isolate of *sweet potato virus G*

P. E. Rodríguez Pardina · N. Bejerman · A. V. Luque · L. Di Feo

Received: 22 June 2012 / Accepted: 4 July 2012
© Springer Science+Business Media, LLC 2012

Abstract *Sweet potato virus G* belongs to the largest plant virus genus *Potyvirus*. This virus was detected for the first time in Argentina and then sequenced using the method of next-generation pyrosequencing. The complete genome was found to be 10,798 nucleotides excluding the poly-A tail with a predicted genome organization typical for a member of the genus *Potyvirus*. This is the first report of the complete genomic sequence of a SPVG isolated from South America.

Keywords Sweet potato · Potyvirus · Molecular characterization · South America

Sweet potato (*Ipomoea batatas* Lam.) is the third most important root crop globally after potato and cassava. It is grown in all subtropical and tropical regions of the world [1]. Although viruses are the most serious pathogens limiting sweet potato production worldwide [2], virus diseases were formerly the most poorly understood of all diseases affecting the crop. However, during the last decades, several viruses of sweet potato have been characterized and there are now more than 20 viruses known to infect sweet potato worldwide, but only 15 of them are currently recognized by the International Committee on Taxonomy of Viruses (ICTV) [3]. In Argentina, a viral disease known as "batata crespa", caused by sweet potato vein mosaic virus (SPVMV), had devastated cv. Criolla Amarilla by 1970 [4]. Later, *sweet potato feathery mottle virus* (SPFMV) was detected in affected plants of the same cultivar [5]. Because of "batata crespa", a new cultivar (Morada INTA), tolerant to both SPFMV and SPVMV, was adopted by Argentinean farmers in 1978. This cultivar was grown on more than 90 % of the area regularly planted with sweet potato in Argentina. However, since 1984, Morada INTA was affected by a severe disease, termed chlorotic dwarf (CD) caused by a synergistic combination of three viruses (SPFMV, *sweet potato mild speckling virus*: SPMSV, and *sweet potato chlorotic stunt virus*: SPCSV) [6]. This viral disease was successfully controlled by using propagation material produced in areas where the disease was not symptomatically expressed. Nevertheless, during the last years cv. Morada INTA has been replaced, in all sweet potato producing area of Argentina, by a new genotype known as Arapey INIA. Since 2009 growing season a new severe viral disease was observed in this cultivar. Serological and molecular analysis showed that this disease is produced by SPFMV, SPCSV, a begomovirus, and *sweet potato virus G* (SPVG) (unpublished data). SPVG is a member of genus *Potyvirus* that has gained relatively little attention so far. It was originally described in China [7] and it is also known to occur in the United States, Africa, Oceania, and Peru [8–11]. To the best of our knowledge, this is the first report of the natural occurrence of SPVG on sweet potato in Argentina.

The objective of this study was to determine SPVG complete nucleotide sequence by pyrosequencing, and to establish its relationship with other SPVG isolates and SPFMV.

Sweet potato cv. Arapey INIA samples showing vein clearing, mosaic, chlorotic designs, chlorotic rings, and blistering were collected in Jesús María, Córdoba Province. Infected plants were maintained at IPAVE–CIAP–INTA under greenhouse conditions for further analysis.

P. E. Rodríguez Pardina · N. Bejerman · A. V. Luque · L. Di Feo (✉)
Instituto de Patología Vegetal, Camino 60 Cuadras Km 5.5, Córdoba, Argentina
e-mail: ldifeo@correo.inta.gov.ar; ldifeoar@yahoo.com.ar

Decoration using SPFMV specific antiserum showed the presence of both decorated and undecorated potyvirus-like particles in electron microscopy observations. Nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) [12, 13] using specific polyclonal antisera to SPVG and SPFMV, revealed the presence of SPVG in some of the tested samples. The viral particles were partially purified using 50 g of infected leaves of cv. Arapey INIA, according to the previously described protocol [6]. After ultracentrifugation of the 25 % sucrose cushion, the pellets were resuspended in 1.5 ml of 0.05 M borate buffer, pH 8.0, containing 0.001 M EDTA. Total RNA was extracted from 100 µl of virus enriched preparation using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and 200 µl (20 ng/µl), were submitted to INDEAR (Rosario, Santa Fe, Argentina) for its pyrosequencing using a 454 genome sequencer FLX Titanium System (Roche, Brandford, CT, USA).

Contigs were assembled de novo from the dataset using Newbler v 2.6 software (Roche), edited manually and subjected to both BlastN and BlastX analysis. A dataset of 43,309 reads was generated totalling 16,336.590 nucleotides (nt); which represents an average length of reads of 377.21 bp. Blast analysis indicated that 3,506 reads (8.09 %) were highly related to SPVG. De novo assembly yielded a contig that covered SPVG complete genome and mean sequence coverage was 122-fold. Coverage was not even over the genomes, being greater in the 3′ terminus than the 5′ terminus.

Sequence analysis was performed using the Lasergene 8.0.2 software package (DNASTAR, Inc., Madison, WI, USA), whereas phylogenetic inference was made using MEGA version 5 [14]. The tree was constructed using the maximum likelihood algorithm with Hasegawa, Kishino and Yanoplus invariable sites model.

The full genome sequence of SPVG-Arg could be assembled from the pyrosequencing run and has been deposited in GenBank under JQ824374 accession number. The complete genome sequence of SPVG-Arg was 10,798 nt in length, and its organization was similar to those reported for potyviruses [15]. It contained a 5′-non-coding region (NCR) of 111 nt, an open reading frame (ORF) of 10,464 nt encoding a polyprotein of 3,488 amino acids (aa) and a 3′-NCR of 223 nt, respectively. In addition, the recently discovered small ORF PIPO (61 aa), was identified at the 5′ end of the SPVG-Arg sequence. This ORF is conserved throughout the family *Potyviridae* [16], it is located in the P3 cistron and it has a conserved G_2A_6 leading motif (nt 3,804–3,989). Recently, a novel ORF (PISPO), which nests within P1 region and encodes a protein of 224 aa, was detected in potyvirus species infecting sweet potato [17].This ORF was also observed in the SPVG-Arg sequence at nt 1,242–1,916. The nine putative protease cleavage sites observed in SPVG-Arg genome were perfectly consistent with those reported by Li et al. [18] for SPVG-NC and -Kr. The sizes of each protein encoded by SPVG-Arg are similar to those reported by Li et al. [18] for SPVG-NC and -Kr; however, its 5′-NCR is 4-nt shorter; whereas its 3′-NCR is 2-nt longer. The SPVG complete genome reported by Li et al. [18], was published during the preparation of this manuscript.

SPVG genome is the third largest among potyvirus genomes, after SPFMV and *sweet potato virus C* (SPVC) [19], which also infect *Convolvulaceae*. Its P1 is the third largest among potyvirus protein after the viruses mentioned above; whereas its CP is the largest of all known species of the genus *Potyvirus.* The large sizes of both P1 and CP result from additional amino acids at the N-terminal regions; which have been mentioned as highly variable regions among potyviruses [20, 21].

Almost all functional motifs that are highly conserved among members of the genus *Potyvirus* [15] were identified in SPVG-Arg. However, the KITC motif located in HC-Pro, involved in aphid transmission [22], was not found in SPVG genome. In this virus, this box is RTTC, which is similar to that of SPFMV-S [23]. Mutation in KITC motif had been associated with loss of aphid transmission [24]; however, SPFMV-S was highly transmitted by aphids [25].

Pairwise comparison of the complete genomic sequence of the SPVG-Arg showed 98.4 % sequence identity with SPVG-NC, whereas SPVG-Arg shares nt sequence

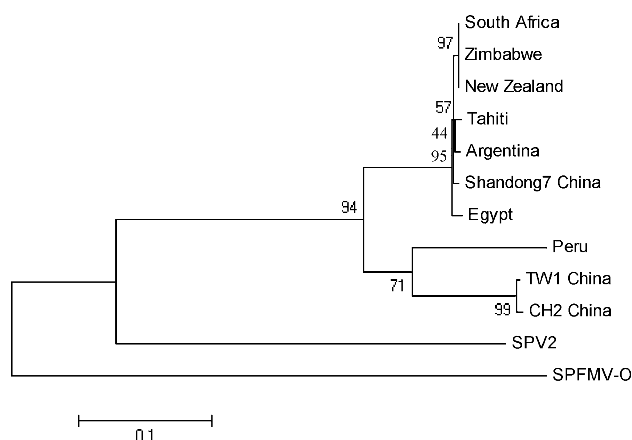

Fig. 1 Rooted tree showing the phylogenetic relationship between SPVG isolated in Argentina with nine other SPVG isolates collected worldwide. The alignment was produced using nucleotide sequences of the complete capsid protein. The horizontal branch-lengths are proportional to the genetic distance and numbers shown at branch points indicate bootstrap values from 1,000 replicates. Sequences of SPVG isolates for comparisons were obtained from the NCBI database: Egypt (AJ515380); Shandong7 China (HQ844192); Tahiti (EU220757); New Zealand (EF514221); South Africa (EU220756); Zimbabwe (EU220751); Peru (EU218528); TW1 China (HQ171932); and CH2 China (Z83314). *sweet potato virus 2* (SPV2, AM050885) and *sweet potato feathery mottle virus*-ordinary strain (SPFMV-O, AB465608) sequences were used as outgroup

identities ranging from 63.1 to 63.9 % with different strains of SPFMV. Therefore, these values show that Arg, and NC are distinct isolates of a same virus species, SPVG, which is a closely related but a distinct species in the genus *Potyvirus* [26]. Nucleotide sequence identities of individual coding regions of SPVG-Arg with SPVG-NC ranged from 98.4 % (P1 and NIa–VPg) to 99.2 % (NIb), consequently, there is a limited genetic diversity among SPVG. A phylogenetic analysis was conducted using the capsid protein nucleotide sequence of several SPVG isolates and *sweet potato virus 2* (SPV2) and SPFMV-O as outgroup. The phylogenetic tree placed the Argentinean isolate in a cluster together with isolates from Africa, Oceania, and China (Shandong7). However, interestingly, our isolate did not cluster together with the other South America isolate (Peru) (Fig. 1).

To our knowledge, the complete nucleotide sequence of SPVG we are reporting is the first one isolated from South America, center of origin, and distribution of sweet potato. The determination of complete genome of SPVG opens the possibility of studying the variability and evolution of this virus in more detail around the world.

References

1. Faostat, FAO statistical databases (2008), http://faostat.fao.org/site/291/default.aspx. Accessed Apr 2012
2. G. Loebenstein, G. Thottappilly, S. Fuentes, J. Cohen, in *The Sweet Potato*, ed. by G. Loebenstein, G. Thottappilly (Springer, New York, 2009), pp. 105–134
3. J. Kreuze, S. Fuentes, in *Encyclopedia of Virology*, 3rd edn, ed. by. B.W.J. Mahi, M.H.V. van Regenmortel (Elsevier Ltd, Kidlington, 2008), pp. 659–669
4. S.F. Nome, Phytopathol. Z **77**, 44–54 (1973)
5. S.F. Nome, L.M. Giorda, A. Vázquez, Rev. Invest. Agropec **15**, 625–634 (1980)
6. L. Di Feo, S.F. Nome, E. Biderbost, S. Fuentes, L.F. Salazar, Plant Dis. **84**, 35–39 (2000)
7. D. Colinet, J. Kummert, P. Lepoivre, J. Semall, Phytopathology **84**, 65–69 (1994)
8. J.A. Ishak, J.F. Kreuze, A. Johansen, S.B. Mukasa, F. Tairo, F.M. Abo El-Abbas, J.P.T. Valkonen, Arch. Virol. **148**, 2449–2460 (2003)
9. M. Rännäli, V. Czekaj, R.A.C. Jones, J.D. Fletcher, R.I.L. Davis, L. Mu, G.I. Dwyer, B.A. Coutts, J.P.T. Valkonen, Plant Dis. **93**, 933–939 (2009)
10. E.R. Souto, J. Sim, J. Chen, R.A. Valverde, C.A. Clark, Plant Dis. **87**, 1226–1232 (2003)
11. M. Untiveros, S. Fuentes, J. Kreuze, Arch. Virol. **153**, 473–483 (2008)
12. C. Lizarraga, E.N. Fernández-Northcote, Plant Dis. **73**, 11–14 (1989)
13. J.G. Parent, F. Berlanger, S. Desjardins, J.D. Brisson, Phytoprotection **66**, 53–57 (1985)
14. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, Mol. Biol. Evol. **28**, 2731–2739 (2011)
15. D.D. Shukla, C.W. Ward, A.A. Brunt, *The Potyviridae* (CAB International, Wallingford, 1994)
16. B.Y. Chung, W.A. Miller, J.F. Atkins, A.E. Firth, Proc. Natl. Acad. Sci. USA **105**, 5897–5902 (2008)
17. C.A. Clark, J.A. Davis, J. Abad, W.J. Cuellar, S. Fuentes, J. Kreuze, R.W. Gibson, S.B. Mukasa, A.K. Tugume, F. Tairo, J.P.T. Valkonen, Plant Dis. **96**, 168–185 (2012)
18. F. Li, D. Xu, J. Abad, R. Li, Virus Genes (2012). doi: 10.1007/s11262-012-0749-2
19. M. Untiveros, D. Quispe, J. Kreuze, Arch. Virol. **155**, 2059–2063 (2010)
20. M.J. Adams, J.F. Antoniw, C.M. Fauquet, Arch. Virol. **150**, 459–479 (2005)
21. M.E. Aleman Verdaguer, C. Goudou Urbino, J. Dubern, R.N. Beachy, C. Fauquet, J. Gen. Virol. **78**, 1253–1264 (1997)
22. C.D. Atreya, P.L. Atreya, D.W. Thornbury, T.P. Pirone, Virology **191**, 106–111 (1992)
23. J. Sakai, M. Mori, T. Morishita, M. Tanaka, K. Hanada, T. Usugi, M. Nishiguchi, Arch. Virol. **142**, 1553–1562 (1997)
24. S. Blanc, E.D. Ammar, S. Garcia-Lampasona, V.V. Dolja, C. Llave, J. Baker, T.P. Pirone, J. Gen. Virol. **79**, 3119–3122 (1998)
25. T. Usugi, M. Nakano, M. Onuki, T. Maoka, T. Hayashi, Ann. Phytopathol. Soc. Jpn. **60**, 545–554 (1994)
26. M.J. Adams, F.M. Zerbini, R. French, F. Rabenstein, D.C. Stenger, J.P.T. Valkonen, in *Virus Taxonomy: 9th Report of the International Committee on the Taxonomy of Viruses*, ed. by A.M.Q. King, M.J. Adams, E.B. Carstens (Elsevier Academic Press, San Diego, 2011), pp. 1069–1089