Acta virologica **59**: 185 – 188, 2015 doi:10.4149/av_2015_02_185

Detection of sweet potato virus C, sweet potato virus 2 and sweet potato feathery mottle virus in Portugal

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Received September 10, 2014; accepted May 12, 2015

Summary. – Field sweet potato plants showing virus-like symptoms, as stunting, leaf distortion, mosaic and chlorosis, were collected in southwest Portugal and tested for the presence of four potyviruses, sweet potato virus C (SPVC), sweet potato virus 2 (SPV2), sweet potato feathery mottle virus (SPFMV), sweet potato virus G (SPVG), and the crinivirus sweet potato chlorotic stunt virus (SPCSV). DsRNA fractions were extracted from symptomatic leaves and used as templates in single and multiplex RT-PCR assays using previously described specific primers for each analyzed virus. The amplified reaction products for SPVC, SPV2 and SPFMV were of expected size, and direct sequencing of PCR products revealed that they correspond to the coat protein gene (CP) and showed 98%, 99% and 99% identity, respectively, to those viruses. Comparison of the CP genomic and amino acid sequences of the Portuguese viral isolates recovered here with those of ten other sequences of isolates obtained in different countries retrieved from the GenBank showed very few differences. The application of the RT-PCR assays revealed for the first time the presence of SPVC and SPFMV in the sweet potato crop in Portugal, the absence of SPVG and SPCSV in tested plants, as well as the occurrence of triple virus infections under field conditions.

Keywords: sweet potato; viruses; Portugal

Sweet potato (*Ipomoea batatas* Lam.), a member of the *Convolvulaceae* family, is an important crop for food security and ranks among the 10 most important food crops worldwide (Clark *et al*., 2012). It is produced by vegetative propagation, which contributes to the rapid spread of pathogens, particularly viruses (Valverde *et al*., 2007). The host range of sweet potato feathery mottle virus (SPFMV), sweet potato virus C (SPVC), sweet potato virus G (SPVG), sweet potato virus 2 (SPV2) and sweet potato chlorotic stunt virus (SPCSV) is limited, with few exceptions, to plants in the *Convolvulaceae* family (Loebenstein *et al*., 2003). SPFMV, SPVG, SPV2 and SPVC, previously considered a strain of SPFMV (Untiveros *et al*., 2010), belong to the genus *Potyvirus*, and SPCSV to the genus *Crinivirus*. They form ca. 850 nm long flexuous particles, which embody single-stranded positive-sense RNA genome. Potyviruses are transmitted in a non-persistent manner by aphids, and the crinivirus SPCSV in a semi-persistent manner by whiteflies (King *et al*., 2012).

SPVG, SPVC, SPV2 and SPFMV are disseminated in several countries (Valverde *et al*., 2007; Li *et al*., 2012), however, to our knowledge, only SPV2 was detected in sweet potato plants growing in Portugal (Ateka *et al*., 2007). SPCSV has been detected in Africa, Israel, Indonesia, Spain, United States and in south and central America (Tairo *et al*., 2005; Valverde *et al*., 2007). SPCSV acts synergistically with several

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Abbreviations: CP = coat protein; M-MLV = moloney murine leukemia virus; NJ = neighbor-joining; SPCSV = sweet potato chlorotic stunt virus; SPFMV = sweet potato feathery mottle virus; SPV2 = sweet potato virus 2; SPVD = sweet potato virus disease; SPVC = sweet potato virus C; SPVG = sweet potato virus G; U = units

unrelated viruses that also affect sweet potato, enhancing replication of SPV2, SPVG, SPFMV, and SPVC (Kokkinos and Clark, 2006). Among these viruses, the synergistic interaction with SPFMV results in the most devastating viral disease in sweet potato, sweet potato virus disease (SPVD) (Gibson *et al*., 1998).

The objectives of this study were to assess the presence of 5 major sweet potato viruses, SPVG, SPV2, SPFMV, SPVC and SPCSV, in a sweet potato field through the application of diagnostic RT-PCR tests.

In 2014, leaves from 80 symptomatic plants from a sweet potato field located in the region of Estremadura, Portugal were collected and used for dsRNA extraction essentially as described by Valverde *et al*. (1990), denatured for 5 min at 100°C, placed on ice for 15 min and used for RT-PCR. For cDNA synthesis, 1 µg of denatured dsRNA was used in a 20 µl reaction with 200 U of M-MLV reverse transcriptase (Invitrogen) in the presence of random hexamers (Promega) and 1x first strand buffer (Invitrogen) in accordance with the manufacturer's instructions. For multiplex PCR, virus-specific forward primers for SPVG (5'-GTATGAAGACTCTCTGACAAATTTTG-3'), SPVC (5'-GTGAGAAAYCTATGCGCTCTGTT-3'), SPFMV (5'-GGATTAYGGTGTTGACGACACA-3'), SPV2 (5'-CGTACATTGAAAAGAGAAACAGGATA-3') and one common reverse primer (5'-TCGGGACTGAARGAYAC GAATTTAA-3') (Li *et al*., 2012) were used. For single PCR, a SPCSV-specific forward primer (5'-CGTCTAGATT GTTAGAAA-3') and a SPCSV-specific reverse primer (5'-TATATGAAAATATAGTTC-3') were used (Alicai *et al*., 1999). For all reactions, one µl of cDNA was used in a 50 µl reaction with 2.5 U of FideliTaqDNA Polymerase (USB corporation) performed in a reaction mix containing 10 mmol/l Tris HCl (pH 8.6), 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs and 0.3 µ mol/l of each primer. Amplifications were carried out in a Thermal Cycler (BioRad) following initial denaturation at 94°C for 2 min, 35 cycles at 94°C for 30 sec, 55°C for 30 sec and 68°C for 1 min and 30 sec, and a final extension step at 68°C for 5 min. The use of above primers in RT-PCR assays originates a fragment of 1200 bp for SPCSV, 1191 bp for SPVG, 836 bp for SPVC, 589 bp for SPFMV and 369 bp for SPV2.

Fig. 1
Foliar virus-like symptoms observed on field-grown sweet potato plants that tested positive for SPVC, SPFMV and SPV2

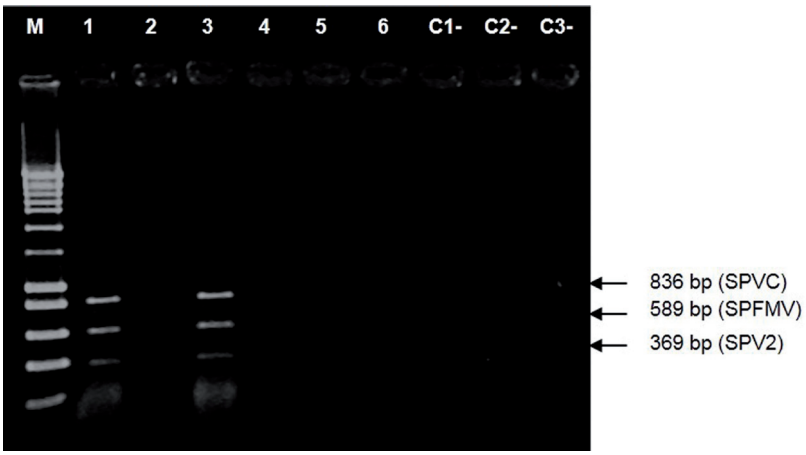

Fig. 2
Products of multiplex RT-PCR applied to dsRNA fraction extracted from sweet potato leaves using primers specific for SPVG, SPVC, SPFMV and SPV2, separated on a 1% agarose gel
Lane M: NZYDNA Ladder III (NZY); Lane 1-6: field plant samples; Lanes C1- and C2-: uninfected plant controls; Lane C3-: water control. Size of generated amplicons is indicated on the right. Plants 1 and 3 are shown to be triple-infected.

Additional virus isolate sequences of SPFMV from Argentina (Acc. Nos. KF386014.1 and KF386013.1) and Japan (Accession numbers NC001841.1, AB439206.1 and AB465608.1); of SPVC from Argentina (Acc. No. KF386015.1), Israel (Acc. No. JX489166.1) and Peru (Acc. Nos. NC014742.1 and GU207957.1); and of SPV2 from USA (Acc. No. NC017970.1), were retrieved from GenBank.

Multiple sequence alignment was performed with BioEdit 7.1.3.0 (Hall, 1999) and CLUSTAL W in MEGA 5.2 software (Tamura *et al*., 2011). The best fit nucleotide substitution model for these data was the Kimura 2-parameter model in the MEGA 5.2 software, showing the lowest Bayesian information criterion (BIC) score. This model was used to estimate nucleotide distance and phylogenetic relationships, which were inferred using neighbor-joining (NJ) method. To validate phylogenetic tree analysis from the NJ method, trees were produced using Minimum Evolution, Maximum Parsimony and Maximum Likelihood methods in the MEGA 5.2 software. Bootstrap analyses with 1000 replicates were performed to evaluate the significance of the inner branches.

A high incidence, ca. 30%, of randomly distributed plants exhibiting virus-like symptoms as leaf distortion, mosaic, chlorosis and purple borders was observed (Fig. 1).

RT-PCR using dsRNA extracted from symptomatic plants as templates showed the presence of triple infections of SPVC, SPFMV and SPV2 in 25% of the tested plants (Fig. 2). No single or double infections were observed. This result is particularly interesting because mixed infections, mainly with SPCSV but also with other viruses, have been reported to interact synergistically, resulting in the manifestation of symptoms or in more severe symptoms (Ateka *et al*., 2004; Valverde *et al*., 2007).

Two amplicons of each 836 bp, 589 bp and 369 bp were purified and directly sequenced. Comparison of the 836 bp consensus sequence revealed 98% identity with the reported sequence of SPVC (GenBank Acc. No. GU207957.1). The 589 bp consensus sequence revealed 99% identity with two reported ones of SPFMV (GenBank Acc. Nos. AB439206.1 and AB465608.1) and the 369 bp consensus sequence revealed 99% identity with the reported sequence of SPV2 (GenBank Acc. No. NC017970.1). This confirms the occurrence of SPFMV and SPVC, which had not been recorded affecting sweet potato in Portugal, and of SPV2.

The distances between isolates show that the SPFMV Portuguese isolate has the lowest value distance (0.007) with the Argentinean isolate KC386014.1 and the highest value distance (0.036) with the Japanese isolate NC001841.1. The SPVC Portuguese isolate shows the lowest distance (0.021) with the two isolates from Peru and the highest distance (0.039) with the Argentinean isolate KF386015.1.

The phylogenetic tree constructed from the sequence alignments revealed segregation of isolates under study into 3 main clusters (Fig. 3). Isolates were grouped according to the respective virus species SPFMV, SPVC or SPV2.

The analyzed viruses are systemically distributed in plants and are easily transmitted through cuttings during vegetative propagation, which contributes to a rapid dissemination of these viruses and may explain the few differences found between Portuguese and foreign isolates.

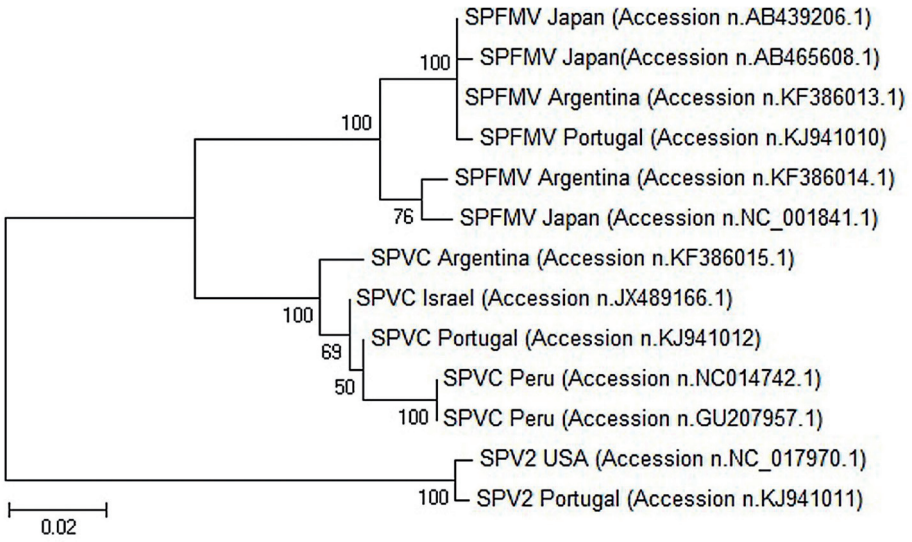

Fig. 3
Phylogenetic tree analysis of SPFMV, SPVC and SPV2 isolates based on 13 partial CP nt sequences (from nt 10217 to nt 10585)
Numbers above the lines indicate bootstrap scores out of 1000 replicates.

Although the most significant sweet potato losses are associated with the severe symptoms of SPVD, sweet potato viruses in general are thought to contribute to the decline in yield and quality of cultivars (Clark *et al*., 2012). Not much is known concerning the effects of specific viruses or the symptomatology the SPFMV-SPVC-SPV2 triple infections cause in the field plants. SPFMV alone causes mostly no symptoms but has already been shown to cause mild virus-like symptoms (Ateka *et al*., 2004) and, when in double infections with SPVG, cause a decrease in yield of 14% (Clark and Hoy, 2006).

Symptoms observed in the field under study may be due to SPFMV, SPVC or SPV2 alone or due to synergistic effects of the combination of two or three of these viruses, or combination with other, here unidentified, viruses. The hypothesis that other yet unidentified viruses may be associated with these viruses has been suggested after observing that the replication of these viruses is enhanced in the field when compared to laboratory-infected plants (Valverde *et al*., 2007). Further studies are needed to understand the relations and interactions that exist between sweet potato viruses. The understanding of their epidemiology will help in the design of efficient control methods.

Acknowledgements. Carla Marisa R. Varanda is recipient of a Postdoctoral fellowship from Fundação para a Ciência e a Tecnologia (FCT), SFRH/BPD/76194/2011, financed by QREN-POPH-Typology 4.1, co-financed by MES national funding and the European Social Fund. Mônica D. M. Oliveira is recipient of a Postdoctoral fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), CsF/2581/31-2. This work was funded by National Funds through FCT - Foundation for Science and Technology under the Strategic Project PEst-OE/AGR/UI0115/2014 and the Operational Programme for Competitiveness Factors - COMPETE and through the Programa Operacional Regional do Alentejo (InAlentejo) Operation ALENT-07-0262-FEDER-001871/ Laboratório de Biotecnologia Aplicada e Tecnologias Agro-Ambientais.

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