Diseases Caused by Viruses

First Report of Sweet Potato Chlorotic Stunt Virus Infecting Sweet Potatoes in Hungary

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Sweet potato chlorotic stunt virus (SPCSV), a crinivirus in the family *Closteroviridae*, is a quarantine pest in Europe and one of the most economically important viruses of sweet potato (*Ipomoea batatas* [L.] Lam) crops globally. It forms synergies with other viruses in sweet potato, leading to yield loss of 30 to 100% (Qin et al. 2014). In summer 2020, 62 symptomatic and 38 symptomless sweet potato vines were randomly collected in farmers' fields in the south (Ásotthalom, Szeged) and central (Galgahévíz) parts of Hungary and transplanted in an insect-proof greenhouse. Six of the plants expressed SPCSV-like symptoms, including stunting, vein clearing, and leaf purpling. To check for common viruses of sweet potato, total RNA and DNA were extracted from leaves of each of the 100 plants using Trizolate reagent (UD-GenoMed, Debrecen, Hungary) and a ZenoGene kit (Zenon Bio, Szeged, Hungary), respectively. Primer pair Ch2N was designed using Primer3 (v. 0.4.0) to amplify a 194-bp fragment of SPCSV RNA1. Presence of the RNA viruses was checked by qPCR using a qPCRBIO SyGreen 1-step qPCR kit (PCR Biosystems, London, U.K.), and DNA viruses were checked by PCR using DreamTaq DNA Polymerase (Thermo Scientific, Vilnius, Lithuania), followed by 1% agarose gel electrophoresis. Four samples (labeled A5.1, A6.1, A6V9-1, and A6V9-2) out of the 100 tested positive for SPCSV. Two of them (A6V9-1 and A6V9-2) were coinfecting with SPCSV, a badnavirus sweet potato pakakuy virus (SPPV), and a potyvirus sweet potato virus 2 (SPV2), whereas the other two (A5.1 and A6.1) lacked SPV2. Plants infected with SPCSV, SPV2, and SPPV displayed more severe symptoms. To confirm the results, cDNA synthesized from the four SPCSV-positive

samples using a RevertAid first strand cDNA synthesis kit (Thermo Scientific, Vilnius, Lithuania) underwent PCR (94°C for 4 min, 94°C for 1 min, 53°C for 30 s, 72°C for 70 s, and 72°C for 10 min for a total of 30 cycles) using primers CL43U and CL43L for the viral heat shock protein 70 gene (Maliogka et al. 2020). An expected band size of 486 bp was obtained in all cases. The amplicon from sample A6.1 was sequenced and found to be identical to SPCSV Guatemalan isolate GT:B3:08 (accession JF699628). RNA1 and RNA2 complete sequences from sample A6.1 were obtained via PCR amplifications of cDNA using primers designed (from accession KC888966 for RNA1 and accession KC888963 for RNA2) to amplify overlapping fragments of a West African strain of SPCSV. QIAquick gel extraction kit (Qiagen, Hilden, Germany) was used to purify the PCR fragments, which were then cloned into pGEM-T Easy Vector (Promega, Madison, WI) and sequenced using Sanger sequencing technique (Biomi, Gödöllő, Hungary). BLASTn search revealed that RNA1 of our isolate Hun_01 (accession MW892835) had 99.63% sequence identity to SPCSV isolate su-17-10 (accession MK802073), whereas RNA2 of Hun_01 (accession MW892836) was 99.68% similar to SPCSV isolate min-17-1 (accession MK802078) and isolate 24-1 (accession MK802080). Phylogenetic analysis using MegAlign (v. 7.1.0, 44.1) showed a close relationship between our isolate and those isolated in China, suggesting that they may have a common origin. Severe stunting and leaf yellowing symptoms developed in *Ipomoea setosa* indicator plants grafted with SPCSV-infected sweet potato scions. qPCR test for the virus confirmed its presence in the *I. setosa* leaves. To the best of our knowledge, this is the first report on the occurrence of SPCSV in Hungary and the third in Europe (EPPO 2021; Valverde et al. 2004).

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