Disease Note

Diseases Caused by Viruses

First Report of Southern Tomato Virus from Tomato (*Solanum lycopersicum*) in Greece

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Southern tomato virus is a member of the genus *Amalgavirus* in the family *Amalgaviridae*. Members of this family are characterized by double-stranded RNA genomes of about 3.4 kbp (Sabanadzovic et al. 2009). Southern tomato virus (STV) was first detected in tomato (*Solanum lycopersicum* L.) plants in the United States and Mexico and since has been reported from many other countries. Although STV has been reported to cause asymptomatic infection, in some cultivars symptoms include interveinal necrosis, stunting, fruit discoloration, and reduced fruit size (Harju et al. 2021; Sabanadzovic et al. 2009). Transmission of STV occurs vertically through infected seeds (Sabanadzovic et al. 2009), and to date no virions have been identified in infected plants. In 2019, tomato seedlings from a breeding collection in Crete (Greece) were assessed for the presence of viruses using high-throughput sequencing (HTS). A total of 40 plant lines were grown, with plants pooled into two groups for HTS (pool 1 with 22 samples and pool 2 with 18 samples). RNA was extracted from leaf tissue using TRIzol (Katsarou et al. 2022), and 2 µg of RNA from each sample was pooled for HTS (Macrogen, the Netherlands). Assembly of raw reads was carried out using metaSPAdes (Nurk et al. 2017). BLASTn analysis against RVDBv20.0 (Goodacre et al. 2018) of the contigs from pool 1 did not result in any sequences matching plant viruses. In contrast, BLASTn of the assembled contigs from pool 2 (NCBI SRA: BioProject PRJNA818693) identified three small contigs of 336, 307, and 230 nt, respectively, with 98 to 100% nucleotide sequence identity to STV. Mapping to the STV reference genome sequence (GenBank accession no. NC_011591) using Geneious R7 confirmed 54 reads that mapped to the STV genome. To confirm the presence of STV in the tomato plants from pool 2, reverse transcription PCR was carried out using the RNA extracts previously prepared for HTS. Two sets of PCR primers were designed, STV_F (5′-TATATTGGAGGAGGAGGCGGT-3′) and STV_R (5′-ATATTCCTTCACCCTGCGCC-3′), which were predicted to amplify a 658-nt region of the RdRP gene, and a second set of nested primers, STVnF (5′-TGGAGATGAGGTGCTCGAAGA-3′) and STVnR (5′-TGGCTATGATGTATCTGTGCTTGA-3′), which amplify 458 nt within the first-round target region. Complementary DNA was synthesized using M-MuLV reverse transcriptase (Minotech, Greece), and two rounds of PCR were subsequently carried out using Taq DNA polymerase (EnzyQuest, Greece) as per the manufacturer's recommendations. Analysis of the PCR products confirmed the presence of amplicons in three samples. The second-round PCR amplicons from the three samples were excised, gel-purified, and Sanger-sequenced (Genewiz, Germany). The trimmed reads (351 nt) were identical to each other, and BLASTn analysis confirmed their identity as STV, with the Crete sequences identical to three isolates from Germany (MK948545) and Switzerland (MF422617 and MF422618). This is the first report of STV in Greece. STV is seed transmitted but often causes no apparent symptoms in infected plants. This is the likely explanation for infected plants to be present in the breeding collection assessed in this study. Knowledge that these plants are not coinfected with other viruses may assist further work to identify symptoms associated with STV infection, as mixed infections are common in previous reports, as well as further investigate plant-host interactions between STV and tomato. Further work is now required to assess the field occurrence, yield effects, or other impacts of STV infections in tomato crops in Crete.

References:

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