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NEW DISEASE REPORT

First report of *Tomato brown rugose fruit virus* in tomato in Slovenia

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In July 2021, during an official survey for *Tomato brown rugose fruit virus* (ToBRFV), a sample composed of leaves and fruit was taken from three tomato (*Solanum lycopersicum* cv. Factor F1) plants growing in a greenhouse producing fresh tomatoes in central Slovenia. The sampled plants were slightly dwarfed and showed deformations such as leaf curling, narrowing and small leaves (Figure 1), while no virus symptoms were observed on the fruit.

Total RNA was extracted from a pool of young leaflets collected from the plant apex and sepals using an RNeasy Plant Mini Kit (Qiagen) and tested by ToBRFV-specific real-time RT-PCRs as described in EPPO PM7/146 (European and Mediterranean Plant Protection Organization, 2021). A duplex test using the methods of International Seed Federation (2020) and Menzel & Winter (2019) gave threshold cycle (Cq) values ranging from 31 to 32. RNA extraction and real-time RT-PCRs were repeated separately on sepals, leaves, and petioles, and again late amplification curves were obtained for all three matrices. No amplification curves were obtained for the negative extraction and amplification controls. A positive result was obtained also by conventional RT-PCR using ToBRFV-specific primers (Panno et al., 2019) and by nested RT-PCR with generic tobamovirus primers

FIGURE 1 Leaf deformation, narrowing and curling

(Dovas et al., 2004). Both RT-PCR products were Sanger sequenced at GATC (Eurofins Genomics, Austria), and BLAST analysis of the 354 bp (GenBank Accession No. ON045074) and 417 bp (ON045075)

consensus sequences obtained with generic tobamovirus primers and ToBRFV-specific primers respectively, showed 100% nucleotide identity with the corresponding genomic regions of different ToBRFV isolates including those from Israel (KX619418) and Jordan (KT383474). However, high throughput sequencing (HTS) of total RNA from the same pooled samples, performed on the Illumina platform (NovaSeq 6000 sequencer, Novogen), was not able to detect any plant virus. In addition, *Nicotiana glutinosa*, *N. tabacum* 'Xanthi', and *S. lycopersicum* cv. Moneymaker were mechanically inoculated with sap of symptomatic leaves and sepals, but no symptoms were produced, and the plants tested negative using ToBRFV specific real-time RT-PCRs six weeks after inoculation. The inability to detect ToBRFV with HTS and to transfer the virus to test plants was probably due to low concentration of ToBRFV in the original sample.

Other tomato plants grown in the vicinity of infected plants showed no ToBRFV symptoms. Additional testing performed in August 2021 found that ToBRFV had not spread to tomato and pepper plants grown in the surrounding greenhouses. Eradication measures have been taken on the infected place of production (European and Mediterranean Plant Protection Organization Global Database, 2021). A prohibition of growing host plants in the infected area for one growing season will be in place until the eradication is confirmed by survey.

To our knowledge this is the first report of ToBRFV in Slovenia. Further studies are needed to monitor the presence of this virus in tomato and other solanaceous crops in Slovenia and, if necessary, to take timely eradication measures to prevent further spread of the virus.

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