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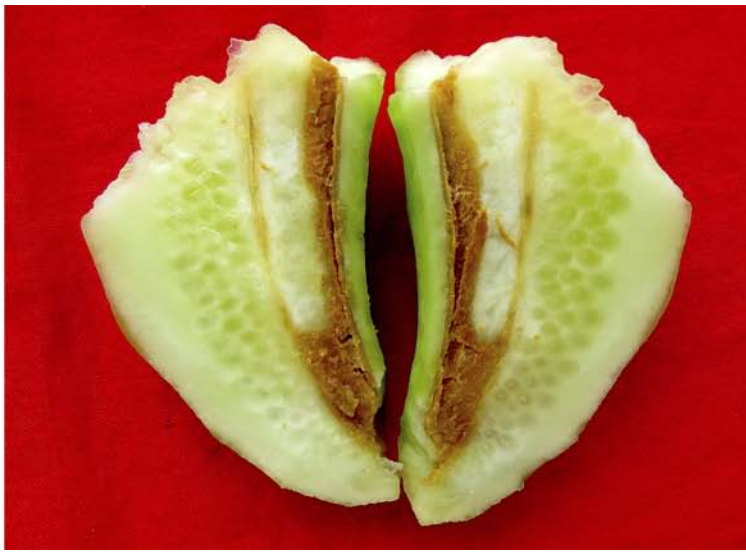

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First Report of Tomato Brown Rugose Fruit Virus on Tomato Crops in Italy

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In October 2018, virus-like symptoms were observed in four different greenhouses of tomato (*Solanum lycopersicum*) in Ragusa province (Sicily, Italy). Symptoms consisted in mosaic, deformation, and necrosis on young leaves, and discoloration and deformations on young fruits. In total 40 symptomatic samples were collected (10 for each greenhouse). Samples were tested by reverse transcription polymerase chain reaction (RT-PCR) using specific primers for different viruses that incite similar symptoms on tomato plants: *Groundnut ringspot virus* (Camelo-García et al. 2014), *Parietaria mottle virus* (Galipienso et al. 2015), *Pepino mosaic virus* (Panno et al. 2012), *Tobacco etch virus* (Zhang et al. 2012), *Tomato brown rugose fruit virus* (Salem et al. 2015), *Tomato chlorotic spot virus* (Webster et al. 2013), *Tomato mosaic virus* (Panno et al. 2012), *Tomato mottle mosaic virus* (Sui et al. 2017), *Tomato necrotic spot virus* (Bratsch et al. 2018), *Tomato necrotic streak virus* (Badillo-Vargas et al. 2016), *Tomato torrado virus* (Panno et al. 2012), and *Tomato yellow leaf curl virus* (Davino et al. 2008). Thirty-seven out of the 40 samples analyzed yielded fragments of the expected size only for tomato brown rugose fruit virus (ToBRFV). This screening identified ToBRFV as a putative causal agent of this disease. To confirm the presence of this virus, two new primers named ToBRFV-F-5722, 5'-CACAATCGCAACTCCATCGC-3' (coordinates: 5,722 to 5,742 nt referred to GenBank no. KT383474), and ToBRFV-R-6179, 5'-CAGAGGACCATTGTAAACCGG-3' (coordinates: 6,179 to 6,200 nt referred to GenBank no. KT383474), based on the sequence

of the coat protein gene, were designed. RT-PCR, in one-step format, was performed in 25 µl (final volume) containing 2 µl of total RNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 3 mM $MgCl_2$, 0.4 mM dNTPs, 1 mM of primers, 4U of RNaseOut, 20 U of superscript II reverse transcription-RNaseH, and 2U of Taq DNA polymerase (Thermo Fisher, U.S.A.). RT-PCR was carried out according to the following conditions: 42°C for 45 min; 95°C for 5 min; 40 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C; and a final elongation of 10 min at 72°C. The RT-PCR yielded the expected amplicons of 458 bp, confirming the previous results. The amplification products were purified using the UltraClean PCR Clean-Up kit (Mo-Bio, U.S.A.), and the nucleotide sequences were determined in both directions using an ABI PRISM 3100 DNA sequence analyzer (Applied Biosystems, U.S.A.). The sequences obtained from the 37 samples showed 99% identity. BLAST analysis showed an identity >99% with ToBRFV isolates Tom1-Jo (accession no. KT383474) and ToBRFV-IL (accession no. KX619418). Only one sequence was deposited in GenBank (accession no. MK313803). Sap extracts of four samples retrieved from the four different greenhouses were mechanically inoculated into tomato cultivar Marmande (three plants per isolate). Plants were grown in sterilized soil in an insect-proof glasshouse, with a photoperiod of 14 h light at 28/20°C day/night. Symptoms were recorded weekly, with all plants showing the symptoms described for ToBRFV at 30 days postinoculation. *Tomato brown rugose fruit virus* is a single-stranded positive RNA virus, belonging to the genus *Tobamovirus*, family *Virgaviridae* (**Salem et al. 2015**). Sicily is an important region for horticulture in Southern Europe. This virus represents a serious problem for tomato crops in Sicily and in all regions where tomato is grown, owing to its ability to be transmitted by plant-to-plant contact, by manipulations, and particularly by seeds. To our knowledge, this is the first report of ToBRFV in Italy and in Southern Europe.

The author(s) declare no conflict of interest.

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