Diseases Caused by Viruses

First Report of Tomato Brown Rugose Fruit Virus on Greenhouse Tomato in Syria

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Tomato (*Solanum lycopersicum* L.) is an important vegetable crop worldwide. In spring and autumn 2017, virus-like symptoms were observed on greenhouse-grown tomato plants in the east of Akkar plain (south of coastal region, Tartous governorate, Syria). These symptoms were mild to severe mosaic on the apical leaves, brown necrosis on sepals, receptacle and flower's cluster carrier, and severe brown rugose and discoloration on fruit. During the next growing seasons, disease symptoms were observed in other sites of the Syrian coastal region. Tomato brown rugose fruit virus (ToBRFV) was suspected as a main causal agent of the disease, especially since its first report in Jordan (Salem et al. 2016), Palestine (Alkowni et al. 2019), Turkey (Fidan et al. 2019), Germany (Menzel et al. 2019), the U.S. (Camacho-Beltrán et al. 2019), and recently in Spain (Alfaro-Fernández et al. 2021). In November and December 2020, 71 leaf samples from symptomatic plants (59 from Tartous and 12 from Lattakia governorates) and seven from asymptomatic ones (five from Tartous and two from Lattakia) were collected and tested for the presence of ToBRFV by DAS-ELISA, using ToBRFV commercial kit (LOEWE Biochemia, Germany) following the manufacturer's instructions. Results showed 43 symptomatic samples reacted positively (38 in Tartous and five in Lattakia) and none of the asymptomatic ones. On the other hand, sap mechanical inoculation of 10 tomato cv. Mandaloun F1 (Enza Zaden, the Netherlands) plants using a positive tomato isolate gave systemic mosaic symptoms in all plants identical to those observed in the field, after 13 days of inoculation, and necrotic local lesions on 10 plants of *Nicotiana tabacum* after 5 days, indicating the presence of a tobamovirus. ToBRFV infection was confirmed in all mechanically inoculated plants by DAS-ELISA. Further tests were necessary to investigate ToBRFV presence, because of its serological relationships with

other tobamoviruses. Six representative symptomatic samples (ELISA-positive) and two asymptomatic (ELISA-negative) samples were subjected to total RNA extraction using the SV-Total RNA Extraction kit (Promega, U.S.A.) following the manufacturer's instructions. The samples were tested by two-step RT-PCR using species-specific primers and protocols for most common tomato-infecting viruses, including tomato chlorosis virus and tomato infectious chlorosis virus (Dovas et al. 2002), pepino mosaic virus (PepMV) and tomato torrado virus (Wieczorek et al. 2013), alfalfa mosaic virus (Parrella et al. 2000), tomato spotted wilt virus (Salem et al. 2012), and a pair of primers: ToBRFV-F2 (5'-CATATCTCTCGACACCAGTAAAAGGACCCG-3') and ToBRFV-R2 (5'-TCCGAGTATAGGAAGACTCTGGTTGGTC-3') targeting a region of the RNA-dependent RNA polymerase (RdRp) of the ToBRFV genome (KT383474; Salem et al. 2016). First-strand cDNA synthesis was carried out using Moloney murine leukemia virus reverse transcription (M-MLV RT; Promega) and random primer according to the manufacturer's protocol, then followed by PCR with the seven species-specific primers. Only ToBRFV was detected among all tested viruses in symptomatic samples, and none of the tested viruses was detected in the asymptomatic plants. To confirm the presence of ToBRFV, two selected RdRp-specific PCR amplicons (872 bp) were purified and ligated into pGEM T-Easy Vector (Promega), and three clones were sequenced (GenBank MZ447794 to 96). BLASTn analysis showed that the nucleotide sequences are 99.77 to 100% identical and shared around 99% identity to RdRp of ToBRFV isolate (MT118666) from Turkey available in GenBank. Accordingly, the presence of ToBRFV was confirmed by bioassays on indicator plants, DAS-ELISA, RT-PCR, and further sequencing. To our knowledge, this is the first report of ToBRFV infecting tomato in Syria, and this requires special emphasis for further investigations because of the virus severity, easy transmission ability, and absence of commercial resistance varieties.

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