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NEW DISEASE REPORT

First report of *Tomato fruit blotch fruit virus* infecting tomato in Greece

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In autumn 2022, a tomato sample (*Solanum lycopersicum*, cv. Ekstasis F1 hybrid) from Aspropyrgos, Attiki, Greece, infected with *Tomato brown rugose fruit virus* (ToBRFV) was analysed with RNA-Seq. Total RNA was isolated from leaves with an RNeasy Plant Mini Kit (Qiagen, Germany) and was subjected to RNA-Seq in an Illumina Novaseq 6000 platform. The BLASTn annotation of the contigs obtained from the analysis of the 12 M, single-end, 100 bp reads with Geneious (v. 11.1.5), revealed the presence of ToBRFV, *Southern tomato virus* (genus *Amalgavirus*), a virus already described in Greece, and *Tomato fruit blotch virus* (ToFBV). ToFBV has been recently reported in Italy, Australia, Brazil, Spain, Portugal, Slovenia and Tunisia (Kitajima et al., 2023) and is associated with fruit blotch disease, although Koch's postulates have not yet been fulfilled, and the virus is not mechanically or seed transmitted. ToFBV is a member of the genus *Blunervirus*, and its genome consists of four single stranded, positive sense RNAs. The RNA 1 (5764 bp; GenBank Accession No. OQ473416) and RNA 2 (3618 bp; OQ473417) sequences of the Greek isolate showed the highest identity with those of the Tunisian isolate (99.7 and 99.6% nucleotide identity with MZ401001.1 and MZ401002.1, respectively). The RNA 3 (2829 bp; OQ473418) and RNA 4 (1905 bp; OQ473419) segments showed 99.4 and 99.7% nucleotide identity with the RNA 3 (NC_078394.1) and RNA 4 (NC_078393.1) of the Italian isolate, respectively. Finally, RT-PCR was used for the amplification of a 500 bp region of RNA 1 (Nakasu et al., 2022). The PCR product was Sanger sequenced and the resulting sequence was identical to that obtained from the RNA-Seq analysis.

To further associate the virus with the fruit blotch symptoms, 11 tomato fruit samples (cv. Esperia F1 hybrid) from a greenhouse in Ierapetra (Crete, Greece) and a sample from field in Prokopi (Evia, Greece), collected in February and August 2023 respectively, and exhibiting fruit blotches (Figure 1), were tested for the presence of ToFBV. Sap inoculation on indicator plants (*Chenopodium quinoa*, *Nicotiana benthamiana* and *N. tabacum* cvs. Xanthi-nc and Turkish) did not induce any symptoms, suggesting the presence of a non-mechanically transmitted virus. Total RNA was extracted from all samples and analysed with RT-PCR for the presence of *Tomato chlorosis virus* (ToCV, Louro et al., 2000), *Tomato infectious chlorosis virus* (TICV, Vaira et al., 2002) and ToFBV. All samples tested positive for ToFBV and negative for ToCV and TICV. Moreover, in both cases plants were infested by tomato russet mite (*Aculops lycopersici*) which is the putative vector of the virus. To the best of our knowledge this is the first report of ToFBV infecting tomato in Greece. The fact that the virus was identified in three distinct regions of Greece, its association with fruit blotch disease together with the probability of mite transmission suggests that this emerging virus poses a risk for tomato cultivation.

FIGURE 1 Blotch symptoms observed in *Tomato fruit blotch virus*-infected tomato fruits collected from a greenhouse in Ierapetra (Crete, Greece).

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