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NEW DISEASE REPORT

First report of *Tobacco necrosis virus D* infecting tomato in Greece

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In winter 2023 fruit blotch symptoms (Beris et al., 2023) were observed in tomato samples (*Solanum lycopersicum* cv. Esperia F1 hybrid) from a greenhouse in Ierapetra (Crete, Greece). To identify the causal agent of the disease, two tomato samples were individually analysed with RNA-Seq. Total RNA was extracted with an RNeasy Plant Mini Kit (Qiagen, Germany) from leaf and fruit tissues and after ribodepletion, it was analysed using an Illumina Novaseq 6000 platform. The analysis of the obtained 30 M, paired-end, 100 bp reads with Geneious (v. 11.1.5) and the subsequent annotation of the resulting contigs with BLASTn revealed the presence of *Tomato fruit blotch virus* (ToFBV, genus *Blunervirus*) associated with fruit blotch disease (Beris et al., 2023), *Olive latent virus 1* (OLV-1, genus *Alphanecrovirus*) and *Tobacco necrosis virus D* (TNV-D) in both samples. The nearly complete genome of the Greek TNV-D isolate (GenBank Accession No. PP580073) consisted of 3757 nucleotides and had 98.4% nucleotide identity with a Dutch isolate from cucumber (ON013905). For the amplification of a 334 bp fragment of the RNA genome, an RT-PCR assay was developed using the TNVD-1F (5′-CTCCTCCAGTTTTCTGGGGCATG-3′) / TNVD-1R (5′-CTGCGACAATGATAATAACAATGATCC-3′) primer pair. The resulting PCR product was Sanger sequenced and the sequence was identical to that obtained from the RNA-Seq.

Plant tissue derived from the initial tomato sample was used for the isolation of TNV-D through sap inoculation in indicator plants, namely *Nicotiana tabacum* cv. Xanthi NC, *Solanum lycopersicum* cv. Ace 55 VF, *Cucumis sativus* cv Lungo Verde Degli Ortolani and *Cucurbita pepo* cv Géode HF1. At five days post inoculation all plants showed local necrotic lesions (Figure 1) and TNV-D presence was further confirmed with RT-PCR using the primer pair described above. Finally, 11 tomato samples from the same greenhouse were analysed with RT-PCR for the presence of TNV-D. In two plants TNV-D was found in mixed infection with ToFBV and OLV-1, in two TNV-D was present along with ToFBV and only one plant was solely infected with TNV-D.

To the best of our knowledge this is the first report of TNV-D in Greece and the first nearly complete annotated genome derived from tomato. TNV-D belongs to the *Betanecrovirus* genus (family *Tombusviridae*) and was originally described from tobacco (Smith & Bald, 1935). The virus is thought to be spread worldwide, infecting more than 37 botanical families and in nature it is transmitted by the fungus *Olpidium brassicae*. TNV-D infection results in local necrotic lesions and systemic infection is observed only in a limited number of plant species (Díaz-Cruz et al., 2017). The virus is not a direct threat to tomato cultivation, however its ability to recombine with OLV-1 and to give rise to new viral species (Borodynko et al., 2010), support the need for further investigation of TNV-D spread, epidemiology and impact on tomato cultivation.

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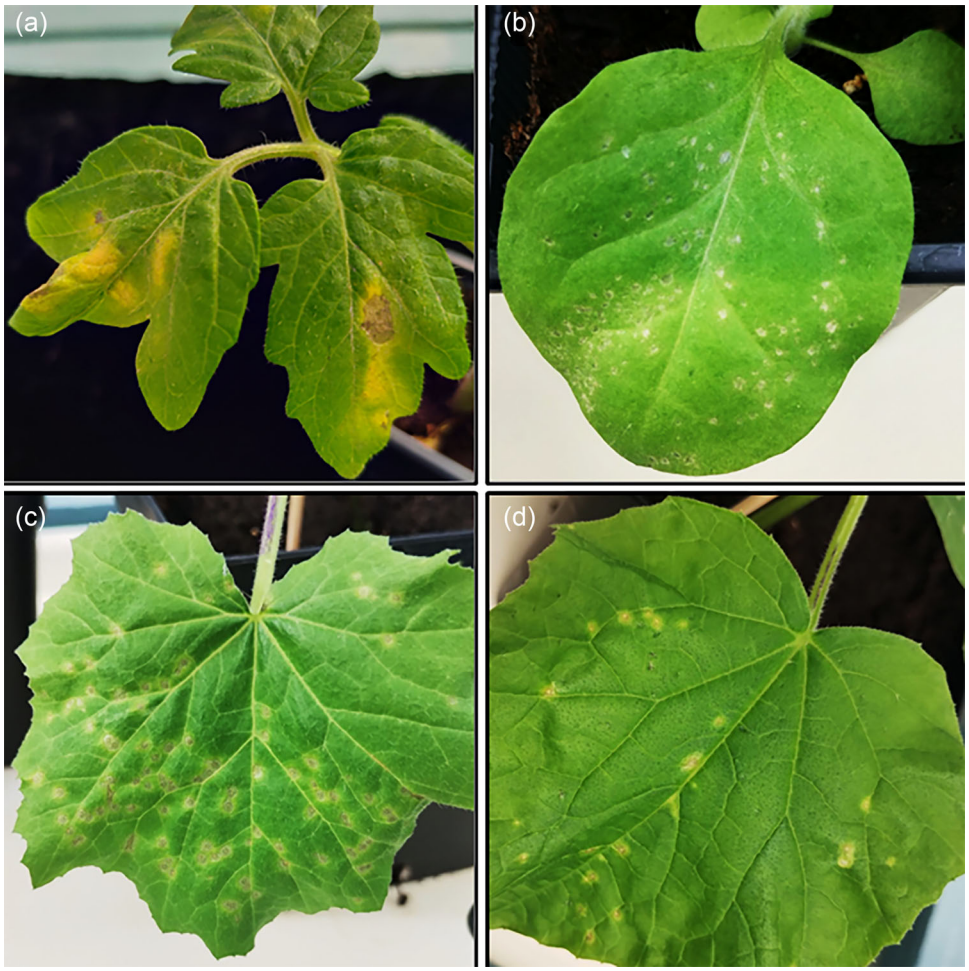


FIGURE 1 Local necrotic lesions caused by *Tobacco necrosis virus D* on plants of (a) *Solanum lycopersicum* cv. Ace 55 VF, (b) *Nicotiana tabacum* cv. Xanthi NC (c) *Cucurbita pepo* cv Géode HF1 and (d) *Cucumis sativus* cv Lungo Verde Degli Ortolani.

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