Diseases Caused by Viruses

First Report of Eggplant Mottled Crinkle Virus Infecting Eggplant in Greece

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During winter 2020–2021, a severe virus-like disease outbreak was observed in eggplant (*Solanum melongena* L.) hybrids ‘Monarca’ (F1) and ‘Angela’ (F1) growing under protected conditions in Heraklion, Crete, Greece. In three greenhouses, the percentage of infected plants reached 100%, leading to crop abandonment. Symptoms included leaf mottling and yellowing accompanied by plant stunting and apical necrosis. Extensive fruit damage was due to severe malformation and necrotic lesions on the calyx, peduncle, and endocarp. To identify the causal agent, total RNA was extracted from a symptomatic eggplant fruit with PureLink RNA Mini Kit (ThermoFisher Scientific, U.S.A.), which was subjected to high-throughput sequencing (HTS) analysis (Illumina, U.S.A.). The de novo assembly of the obtained 25 million 75-bp single-end reads with Geneious Prime (Biomatters, New Zealand) and the annotation of the resulting contigs with BLASTn revealed the presence of only eggplant mottled crinkle virus (EMCV) in the sample. The assembled sequence of EMCV isolate from Greece (EMCV-Gr, GenBank no. MW716271) was 4,764 bp in length, covering the full genome of the virus and showing 96.3% nucleotide (nt) identity with an isolate identified from calla lilies (*Zantedeschia* sp.) in Taiwan (AM711119). Five symptomatic and seven asymptomatic Monarca (F1) eggplants, as well as two symptomatic Angela (F1) eggplants, were

tested by RT-PCR that targeted the capsid protein gene of the virus (Dombrovsky et al. 2009). PCR products of 1,184 bp were obtained from the seven symptomatic samples, and their Sanger sequencing revealed 100% nt identity with the respective HTS-derived EMCV sequence. No product was obtained from the analysis of the asymptomatic samples. Mechanical sap transmission of the HTS-analyzed eggplant sample resulted in necrotic local lesions on *Nicotiana rustica* and *Chenopodium quinoa*, necrotic local lesions plus systemic necrosis on *N. tabacum* cv. Xanthi-nc, cv. Samsun, and *N. glutinosa*, systemic collapse of *N. benthamiana*, and leaf mottling plus stunting of pepper cv. Yolo Wonder plants. Although no symptoms were observed on tomato plants cv. Ace 55, systemic EMCV infection was detected by RT-PCR. To establish the relationship between the disease and EMCV, infected tissue from *N. benthamiana* plants was used for the mechanical inoculation of virus-tested negative eggplant seedlings cv. Black Beauty. Necrotic spots, shoot necrosis, leaf mottling, and mosaic symptoms were observed on the test plants 10 days postinoculation, and the presence of the virus was confirmed by RT-PCR as described. To the best of our knowledge this is the first report of EMCV infecting eggplant in Greece. *Eggplant mottled crinkle virus* belongs to the genus *Tombusvirus*; the virus was originally described in eggplant in Lebanon (Makkouk et al. 1981), and it is mainly present outside the European Union (EU) territory, including India, Japan, Taiwan, Iran, and Israel (Dombrovsky et al. 2009 and references therein). A latent EMCV infection was detected in pear in Italy (Russo et al. 2002), and the virus is considered by the European Food Safety Authority as an exotic virus of the genera *Cydonia*, *Malus*, and *Pyrus* that meets all the criteria to qualify as an EU quarantine pest (Bragard et al. 2019). The severity of the disease observed in Crete leading to the destruction of eggplant greenhouse cultivations constitutes EMCV as an emerging threat to eggplant and other solanaceous crops for Greece and Europe.

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