TOMATO LEAF CURL NEW DELHI VIRUS FOUND ASSOCIATED WITH EGGPLANT YELLOWING DISEASE IN ITALY

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Tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus (genus *Begomovirus*, family *Geminiviridae*), was originally described in tomato in India in 1995 (Padidam et al., 1995). The virus apparently remained confined to the Asian continent for about twenty years, and has only recently been discovered in Europe. In Italy, ToLCNDV was detected for the first time in 2015 in zucchini squash in Sicily (Panno et al., 2016), and later on in other regions of continental Italy (Panno et al., 2018). Recently, epiphytotics of ToLCNDV were reported in central and south Italy, associated with yellowing and leaf curling symptoms in pepper crops (Luigi et al., 2019). In 2016, a large-scale survey was conducted to assess the distribution and the genetic diversity of the viral isolates spreading in Italy. Samples collected at that time included cucurbits and solanaceous plants, including five leaf samples from five distinct eggplant (*Solanum melongena* L.) plants cv. Violetta di Napoli that showed yellowing and light curling of the apical leaves and noticed in a cultivation located in Campania region (Castel Volturno municipality). Few *Bemisia tabaci* individuals were noticed associated to the cultivation. All five leaf samples tested positive for ToLCNDV with ImmunoStrip® (Agdia Inc., Elkhart, IN). These results were confirmed by PCR using ToLCNDV specific primers TLCNDVCP1/TLCNDVCP2 (Parrella et al., 2017), on the total DNA extracted from each sample using E.Z.N.A.® Plant DNA kit (Omega Bio-tek, Norcross, GA). Amplicons of the expected size (~1.0 kb) were obtained only from the five symptomatic plants, and the nucleotide sequences of these isolates were identical. One representative sample (Som-166/16) was selected for full-length amplification of the genome (DNA-A and DNA-B-like sequences) using the rolling circle amplification method with an Illustra TempliPhi amplification kit (GE Healthcare, Piscataway, NJ), in accordance with the manufacturer's instructions. RCA products were digested with different

restriction endonucleases to obtain a 2.8 kb linear DNA fragment. Among the different enzymes tested, *Bam*HI resulted in a maximum DNA fragment length of 2.8 kb, which was cloned into a *Bam*HI-linearized pUC19 plasmid. The ligated products were transformed into a competent DH5α strain of *Escherichia coli*, and the positive clones were sequenced in both orientations at Microsynth Seqlab (Göttingen, Germany). The obtained full-length DNA-A (2738 nt; GenBank Acc. No. MN782303) and DNA-B (2686 nt; GenBank Acc. No. MN782304) sequences showed the highest percentage of nucleotide identity with the ToLCNDV Italian isolates Caa-164/16 (GenBank Acc. No. MK732932) for the DNA-A (99.82%) and Cum-45/16 (GenBank Acc. No. MF688671) for the DNA-B (99.48%). Eggplant leaf yellowing caused by a ToLCNDV variant has been previously described in India (Acc. Nos. HQ264185 for DNA-A and HQ264186 for DNA-B) (Pratap et al., 2011), but the Italian and the Indian isolates exhibited only 90.20% and 79.00% nucleotide identity with respect to the nucleotide sequences of DNA-A and DNA-B. The Som-166/16 isolate was very similar at the molecular level to previously reported isolates from Mediterranean countries and belonged to the European strain ToLCNDV-ES, since the percentage of nucleotide identity was of 98.83% for DNA-A with the ToLCNDV-ES strain A-MU.13.ME/4.3 (GenBank Acc. No. MH577751) and of 97.88% for DNA-B with the ToLCNDV-ES strain B-AL.15.ZU/2.1 (GenBank Acc. No. MH577658) (Moriones et al., 2017; Panno et al., 2018). The ToLCNDV-ES strain evolved from ToLCNDV isolates of Asian origin and adapted to infect cucurbits (Moriones et al., 2017). Nevertheless, this report presents further evidence, in addition to a previous report (Luigi et al., 2019), that ToLCNDV-ES isolates can potentially pose a threat not only to cucurbits but also to solanaceous crops as well since this is the first finding of ToLCNDV in eggplant in Italy. Control measures against ToLCNDV are limited and mainly based on vector control, cultivation in protected environments and the prompt elimination of infected materials.

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