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First Report of Tomato Leaf Curl New Delhi Virus Causing Yellow Leaf Curl of Pepper in Europe

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Tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus (family *Geminiviridae*) with two circular ssDNA genome components (DNA-A and DNA-B), is transmitted in a circulative nonpropagative manner by the whitefly *Bemisia tabaci* (Gennadius). Although it was first reported in Asia on tomato and other solanaceous crops such as eggplant, potato, and chilli pepper in the Mediterranean basin, this virus was mainly detected on cucurbits and only sporadically on tomato and on two wild solanaceous species, *Datura stramonium* L. and *Solanum nigrum* L. (**Juárez et al. 2019**). In 2018, separate surveys were carried out in protected cultivations of sweet pepper (*Capsicum annuum* L.) in two Italian regions: Lazio and Campania. The greenhouses were in areas with high density of *B. tabaci* and where ToLCNDV outbreaks occurred on cucurbits since 2016 (**Panno et al. 2019**). Some plants showing symptoms of yellowing and leaf curling were found in both regions, whereas fruit symptoms were neither observed nor reported by farmers. This disease syndrome, known

as yellow leaf curl disease (YLCD), can be caused in pepper by several begomoviruses, as reported recently in a review listing the viruses causing YLCD in peppers in Thailand (**Chiemsombat et al. 2018**). Symptomatic leaves were collected during late summer 2018 from different pepper plants as well as from the neighboring zucchini cultivations, showing the typical symptomatology induced by ToLCNDV. Total DNA was extracted (DNeasy Plant Mini kit, Qiagen, Germany), and the presence of ToLCNDV was ascertained by PCR with the specific primers ToLCNDV-CP1 and ToLCNDV-CP2 (**Panno et al. 2019**; **Parrella et al. 2018**). ToLCNDV infection was further ascertained in three symptomatic leaf samples from Campania by using specific ToLCNDV ImmunoStrips (Agdia, Elkhart, IN). Successively, one symptomatic pepper sample from each greenhouse was selected and amplified by rolling circle amplification technique (RCA; **Inoue-Nagata et al. 2004**). The amplicons were cloned, and the DNA-A and DNA-B were full-length sequenced. The sequences were deposited in GenBank NCBI database (MK732932 DNA-A and MK732933 DNA-B, pepper sample from Campania; MK756106 DNA-A and MK756107 DNA-B, pepper sample from Lazio). The RCA analysis was performed also on a ToLCNDV-infected zucchini sample collected in the same area in Lazio region (MK756108 DNA-A and MK756109 DNA-B). The analysis of the ToLCNDV sequences showed a low level of genetic variability between the two pepper isolates from Lazio and Campania regions (rate of substitutions: 0.016 for DNA-A and 0.023 for DNA-B). A high genetic similarity was recorded between the zucchini isolate and both the pepper isolates from Campania (0.019 for DNA-A and 0.023 for DNA-B) and Lazio (0.003 for both DNA-A and B). The three characterized isolates showed a high sequence homology also with both the DNA-A (MH577751 from a melon isolate) and DNA-B (MH577673 from a zucchini isolate) of the ToLCNDV-ES genotype (**Fortes et al. 2016**), which differed in 15 and 13 nucleotide substitutions from pepper sample from Lazio, 29 and 51 substitutions from Campania sample, and 10 and 5 substitutions from zucchini sample. High homology was also identified compared with the other Spanish isolates collected since the first appearance of the virus (2014) and to the Tunisian (2015) and Moroccan (2018) isolates, confirming the hypothesis that the Mediterranean population of ToLCNDV is highly conserved (**Juárez et al. 2019**). To our knowledge, this is the first report of ToLCNDV infection on pepper in Europe and indicates that sweet pepper could also act as a reservoir of the virus for further spread to other solanaceous plants and cucurbits.

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