First report of tomato leaf curl New Delhi virus in *Lagenaria siceraria* var. *longissima* in Italy

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**Summary.** During 2022, a new disease of bottle gourd, causing leaf mosaic and yellowing symptoms, was observed in a private garden in the Campania region, Southern Italy. Incidence of disease was high (up to 80% of plants with symptoms). Polymerase chain reaction (PCR) with coat protein specific primers to tomato leaf curl New Delhi virus (ToLCNDV) indicated association of a begomovirus with the disease. The sequence comparison and phylogenetic analysis of the complete DNA genome further revealed the virus as within ToLCNDV-ES strain. Nevertheless, phylogenetic relationships showed two distinctive subgroups among ToLCNDV-ES isolates, with subgroup I composed only of ToLCNDV-ES isolates identified in the Campania region, including the isolate found in bottle gourd. The possible evolutionary forces that determined evolution of the two subgroups within the ToLCNDV-ES strain, including the role of the vector and cultural practices, are briefly analyzed and discussed.

**Keywords.** ToLCNDV-ES, ToLCNDV-In, bottle gourd, begomoviruses, subgroups division.
samples were analyzed by double antibody sandwich ELISA using commercial kits (Bioreba AG) for cucumber mosaic virus (CMV), and by indirect plate trapped antigen ELISA for potyviruses (potygroup test). No positive reactions were observed for all the samples analyzed by ELISA. Loop-mediated isothermal amplification (LAMP)-based kit (Enbiotech), including positive and negative controls specific for tomato leaf curl New Delhi virus (ToLCNDV, genus Begomovirus, family Geminiviridae), was then used to check samples for ToLCNDV infections. Results showed that all samples tested positive for this virus.

DNA was extracted from all symptomatic leaf samples, and from leaves of a healthy and a ToLCNDV-infected zucchini plant, and was used in PCR reactions with primer pair TLCNDVCP1 (5'-CTCCAAGAGATTGAGAAGTCC-3') and TLCNDVCP2 (5'-TCTG-GACGGGCTTACGCCCT-3'), designed to amplify a 1.0 kb fragment encompassing the AV1 (coat protein) gene of ToLCNDV. The expected amplicon was obtained only from symptomatic plants, indicating the presence of ToLCNDV in the bottle gourd samples (Figure 2). The identity of the virus was further confirmed by sequencing 3 out of 10 amplicons. The three sequences showed 100% nucleotide sequence similarity among them, and the greatest level of similarity (99.8%) with the Italian isolates Som-166/16, Caa-164/16 and Cum-45/16 (GenBank nos. MN782303, MK732932 and MF688670, respectively), whereas the DNA-B sequence (2,686 nt, GenBank no. OP588912) showed greatest nucleotide similarity (99.5%) with the DNA-B of the Italian isolate Cum-45/16 (GenBank no. MF688671). Phylogenetic and molecular evolutionary analyses were performed with MEGA version 11, using both Maximum Likelihood and Neighbor-Joining methods and Tamura-Nei model (Tamura et al., 2021).

Phylogenetic reconstructions placed all the ToLCNDV isolates from the Campania region in a distinct subgroup within the ToLCNDV-ES phylogenetic group (Figure 3).

ToLCNDV European isolates belong to the ToLCNDV-ES strain, which are particularly adapted to cucurbits and poorly infectious in other hosts, including tomato (Fortes et al., 2016). In addition, a pumpkin ToLCNDV-ES isolate, belonging to the subgroup I of Italian ToLCNDV isolates and identified in continental Italy (Campania region), has been shown to infect tomatoes only when coinfected with TYLCV, which may complement functions (e.g. virus movement within hosts) that are blocked in the ToLCNDV-ES-tomato interaction (Vo et al., 2022).
The results of the present study highlight the poor adaptation of ToLCNDV-ES isolates to tomato, at least those belonging to subgroup I. In this apparent specialization of ToLCNDV-ES isolates to infect cucurbits, the MED-Q2 genotype of B. tabaci may have played a role. The MED-Q2 strain is widespread in the Campania region, especially on intensive cultivation of zucchini that overlaps from spring to autumn-winter. The MED-Q2 populations characterized in this region are also extremely invasive, characterized by abundant populations and high numbers of annual generations, due to an unbalanced sex ratio in favour of females, apparently correlated with an almost fixed infection of the Rickettsia sp. as secondary endosymbionts (Parrella et al., 2018).

The combination of these factors may have contributed to the emergence, selection and spread of ToLCNDV isolates that are highly adapted to cucurbits. In addition, from a phylogenetic point of view, within the ToLCNDV-ES major clade and both considering the coat protein (Panno et al., 2019) or the whole genome (the present study), the ToLCNDV-ES isolates identified in Campania group together, forming a distinct subgroup, both for the DNA A and DNA B sequences (Figure 3). Therefore, the phylogenetic relationships among ToLCNDV-ES isolates correlated with their different biological features.

This report represents the first finding of ToLCNDV in bottle gourd in Italy, confirming the widespread occurrence of this virus in different cucurbit crops in Italy. Although Lagenaria siceraria has been already reported as a host of ToLCNDV in Thailand (by Ito et al., 2008) and India (by Sohrab et al., 2010), both publications described symptoms of stunting and very small, yellow or chlorotic, slightly curled leaves, that are different from the symptoms we observed on bottle gourd in Italy and reported here (Figure 1). Genetic differences both of the virus strains, spreading in the two continents (Fortes et al., 2016), and of the bottle gourd varieties cultivated in Europe and Asia (Levi et al., 2009) could explain these differences.

Based on the evidence collected in the field during several years of monitoring in the Campania region, relating to the constant and abundant presence of the MEDQ2 variant of B. tabaci and to the absence of ToLCNDV infection in tomato, further research on the possible contribution in ToLCNDV evolution by this B. tabaci genotype would be appropriate.

**LITERATURE CITED**


