Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

New or Unusual Disease Reports

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

Elisa TROIANO, Giuseppe PARRELLA*

Institute for Sustainable Plant Protection of the National Research Council (IPSP-CNR), Piazzale Enrico Fermi 1 – 80055 Portici (NA), Italy
*Corresponding author. E-mail: giuseppe.parrella@ipsp.cnr.it

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.

OPEN ACCESS

Citation: E. Troiano, G. Parrella (2023) First report of tomato leaf curl New Delhi virus in *Lagenaria siceraria* var. *longissima* in Italy. *Phytopathologia Mediterranea* 62(1): 25-28. doi: 10.36253/phyto-14147

Accepted: February 9, 2023

Published: April 6, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Arnaud G Blouin, Institut des sciences en production végétale IPV, DEFR, Agroscope, Nyon, Switzerland.

ORCID:
ET: 0000-0001-7755-4915
GP: 0000-0002-0412-4014

During field monitoring for cucurbit viruses carried out in 2022 in the Campania region of Southern Italy, an unusual disease was noted on some bottle gourd plants (*Lagenaria siceraria* (Molina) Standl.) of the *longissima* variety, growing in a private garden. Symptoms consisted of stunting, reduced leaf area and severe bright-yellow mosaic of the younger leaves and yellowing of the oldest leaves (Figure 1). The flowers were also affected, with deformations and anomalies such as blistering, fraying and reduced size. Fruit development was stunted, many turned necrotic and dropped a few days from fruit set. In many plants production was completely compromised. Incidence of the disease was high with 80% of the plants affected (n = 23). The insect vector *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) was found associated to the bottle gourd plants inspected, and the genotype of ten specimens of the vector was identified as previously described (Parrella *et al*., 2012; Parrella *et al*., 2014). Only the Q2 variants of the Mediterranean (MED) species were found on the plants.

To identify the putative virus(es) associated with the syndrome observed, ten symptomatic plants were chosen, and one leaf per plant was sampled. The

Figure 1. Symptoms associated to tomato leaf curl New Delhi virus (ToLCNDV) infections in bottle gourd (*Lagenaria siceraria* var. *longissima*): severe bright-yellow mosaic of young leaves and yellowing of oldest leaves.

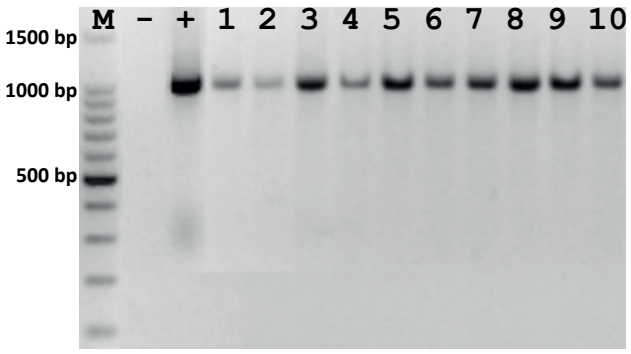

Figure 2. PCR detection of ToLCNDV in symptomatic bottle gourd (*Lagenaria siceraria* var. *longissima*) plants. Lanes M, 100 bp DNA ladder; -, negative control (healthy bottle gourd plant); +, positive control (ToLCNDV infected zucchini plant); 1-10, symptomatic bottle gourd plants.

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'-TCTGGACGGGCTTACGCCCT-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 ToLCNDV pepper isolate Caa-164/16 (GenBank no. MK732932), identified in 2019 in the Campania region (Luigi *et al*., 2019).

The full-length genome sequence (DNA-A and DNA-B) was determined from two ToLCNDV-infected bottle gourd plants. DNA from these plants was used for rolling-circle amplification using ϕ29 DNA polymerase (TempliPhi kit, GE Healthcare) and digested with a set of restriction endonucleases (Haible *et al*., 2006). The two samples yielded amplification products with identical restriction patterns. One sample was selected to clone the putative DNA-A and DNA-B begomovirus genome components using single *Bam*HI or *Nco*I sites. Inserts of two clones, one corresponding to DNA-A and the other to DNA-B, were completely sequenced.

The DNA-A sequence (2738 nt, GenBank no. OP588911) showed the greatest nucleotide similarity (99.4%) with the DNA-A of 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).

Figure 3. Phylogenetic analyses based on the complete nucleotide sequences of ToLCNDV DNA-A (A) and B (B) components of different virus isolates from the Mediterranean area (ToLCNDV-ES) and Asian continent (ToLCNDV-In). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018), using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993), with 1000 bootstrap replicates. The percentage of trees in which the associated taxa clustered together is shown next to the branches for values >75%. In both trees, the ToLCNDV isolate from bottle gourd (*Lagenaria siceraria* var. *longissima*) Las-1 groups together with the other three isolates identified in the same Italian region (Campania), thus forming a distinct subgroup, the subgroup I (red box), within the ToLCNDV-ES major clade. The green box groups the ToLCNDV isolates belonging to subgroup II (isolates from Spain and Lazio, central Italy) within the ToLCNDV-ES major clade.

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 *Rikkettsia* 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

Bertin S., Luigi M., Parrella G., Davino S., Tomassoli L., 2018. Survey of the distribution of *Bemisia tabaci*

(*Hemiptera*: *Aleyrodidae*) in Lazio region (Central Italy): a threat for the northward expansion of tomato leaf curl New Delhi virus (*Begomovirus*: *Geminiviridae*). *Phytoparasitica* 46: 171–182.

Fortes I.M., Sánchez-Campos S., Fiallo-Olivé E., Díaz-Pendón J.A., Navas-Castillo J., Moriones E., 2016. A novel strain of tomato leaf curl New Delhi virus has spread to the Mediterranean Basin. *Viruses* 8: 307.

Haible D., Kober S., Jeske H., 2006. Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *Journal of Virological Methods* 135: 9–16.

Ito T., Sharma P., Kittipakorn K., Ikegami M., 2008. Complete nucleotide sequence of a new isolate of tomato leaf curl New Delhi virus infecting cucumber, bottle gourd and muskmelon in Thailand. *Archives of Virology* 153: 611–613.

Kumar S., Stecher G., Li M., Knyaz C., Tamura K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549.

Levi A., Thies J., Ling K.-S., Simmons A.M., Kousik C., Hassell R., 2009. Genetic diversity among *Lagenaria siceraria* accessions containing resistance to root-knot nematodes, whiteflies, ZYMV or powdery mildew. *Plant Genetic Resources*, 7: 216–226.

Luigi M., Bertin S., Manglli A., Troiano E., Davino S., Tomassoli L., Parrella G., 2019. First report of tomato leaf curl New Delhi virus causing yellow leaf curl of pepper in Europe. *Plant Disease* 103: 2970.

Panno S., Caruso A.G., Troiano E., Luigi M., Manglli A., Vatrano T., … Davino S., 2019. Emergence of tomato leaf curl New Delhi virus in Italy: estimation of incidence and genetic diversity. *Plant Pathology* 68: 601–608.

Parrella G., Scassillo L., Giorgini M., 2012. Evidence for a new genetic variant in the *Bemisia tabaci* species complex and the prevalence of the biotype Q in southern Italy. *Journal of Pest Science* 85: 227–238.

Parrella G., Nappo A.G., Manco E., Greco B., Giorgini M., 2014. Invasion of the Q2 mitochondrial variant of Mediterranean *Bemisia tabaci* in southern Italy: possible role of bacterial endosymbionts. *Pest Management Science* 70: 1514–1523.

Parrella G., Troiano E., Formisano G., Accotto G.P., Giorgini M., 2018. First report of tomato leaf curl New Delhi virus associated with severe mosaic of pumpkin in Italy. *Plant Disease* 102: 459.

Sohrab S.S., Mandal B., Ali A., Varma A., 2010. Chlorotic curly stunt: a severe begomovirus disease of bottle gourd in northern India. *Indian Journal of Virology* 21: 56–63.

Tamura K., Nei M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10: 512–526

Tamura K., Stecher G., Kumar S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38: 3022–3027.

Vo T.T.B., Troiano E., Lal A., Hoang P.T., Kil E.-J., Lee S., Parrella G., 2022. ToLCNDV-ES infection in tomato is enhanced by TYLCV: Evidence from field survey and agroinoculation. *Frontiers in Microbiology* 13: 954460.