New Disease Reports

First report of *Tomato leaf curl New Delhi virus* affecting zucchini squash in an important horticultural area of southern Italy

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Received: *06 Dec 2105.* ***Published:*** *01 Feb 2016.* ***Keywords:*** *begomovirus, Cucurbitaceae, emerging pathogen, ToLCNDV*

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (family *Geminiviridae*) which infects species in the families Cucurbitaceae and Solanaceae (Padidam *et al*., 1995; Mizutani *et al*., 2011). Begomoviruses are transmitted by the whitefly *Bemisia tabaci* in a persistent manner (Rosen *et al*., 2015). In October 2015, severe symptoms not previously reported by growers in the horticultural area of the Province of Trapani (Sicily, Italy) were observed on zucchini squash (*Cucurbita pepo*) in open fields. The symptoms included yellow mosaic, severe leaf curling, swelling of veins of young leaves, shortening of internodes, roughness of the skin of fruit and reduced fruit size; the symptoms were reminiscent of those caused by begomoviruses. Total DNA was extracted from young leaves of 22 plants by phenol/chloroform extraction and ethanol precipitation. PCR was performed with the A1F/A1R primer pair (Mizutani *et al*., 2011) for the DNA-A component and the pair described by Ruiz *et al*. (2015) for the DNA-B component to amplify a ~1200-bp fragment of DNA-A and a ~890 bp fragment of DNA-B, respectively. All 10 samples were positive by PCR with both primer pairs. No amplification products were obtained using primers specific for the monopartite begomoviruses *Tomato yellow leaf curl virus* and *Tomato yellow leaf curl Sardinia virus* (Davino *et al.*, 2008). DAS-ELISA analysis for *Cucumber mosaic virus*, *Papaya ring spot virus* and *Zucchini yellow mosaic virus* (Loewe Phytodiagnostica, Germany) yielded negative results.

The amplification products of DNA-A and DNA-B components from three plants were purified using the UltraClean® PCR Clean-Up kit (Mo-Bio, USA) and the nucleotide sequences were determined in both orientations using an ABI 3130XL Genetic Analyzer (Life Technologies, USA). The sequences obtained from the three samples showed 99% identity. A Basic Local Alignment Search Tool (BLAST) analysis showed an identity >99% with sequences of DNA-A and DNA-B of *Tomato leaf curl New Delhi virus* (ToLCNDV) isolates recently reported in Spain on zucchini (GenBank Accession Nos. KF749224 and KF749227) and tomato (KM977733 and KM977734). The DNA-A and DNA-B sequences of one sample were deposited in GenBank (KU145141 and KU145142).

Sicily is an important region for horticulture in the Mediterranean basin and Europe in general. With the possible opening of a free trade area in countries bordering the Mediterranean basin, Sicily may become a transit centre for flow of plant materials, potentially carrying new pathogens, between Europe and North Africa. To our knowledge, this is the first report of ToLCNDV in Italy, as well as the first identification of a bipartite begomovirus in Italian crops.

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Figure 1

To cite this report: Panno S, Iacono G, Davino M, Marchione S, Zappardo V, Bella P, Tomassoli L, Accotto GP, Davino S, 2016. First report of *Tomato leaf curl New Delhi virus* affecting zucchini squash in an important horticultural area of southern Italy. *New Disease Reports* **33**, 6. http://dx.doi.org/10.5197/j.2044-0588.2016.033.006

 This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.