



First report of *Tomato leaf curl New Delhi virus* infecting cucurbits in Tunisia

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Due to the favourable climate of the southern Mediterranean countries including Tunisia, cucurbits are grown year-round in open fields, and unheated and geothermally heated plastic tunnels. In January 2015, a serious disease affecting many plants, and that may generally impede continued growth of crops until the end of the season, was observed on cucurbits (melon, cucumber and zucchini) cultivated in geothermally heated plastic tunnels in the Kébili region (southeastern Tunisia). The symptoms consisted of severe yellowing and mosaic in young leaves and included curling, vein swelling and short internodes (Fig. 1), together with fruit skin roughness and longitudinal cracking (Fig. 2). A virus disease was suspected based on (i) the importance of the affected plants, (ii) the recent detection of *Tomato yellow leaf curl virus* (TYLCV) on watermelon in Tunisia (Mnari-Hattab *et al.*, 2014a) and (iii) the outbreak of *Tomato leaf curl New Delhi virus* (ToLCNDV) on zucchini in Spain (Juárez *et al.*, 2014). Consequently, the involvement of a *Begomovirus* (family *Geminiviridae*) not previously reported in Tunisia was suggested.

In order to establish the nature of the suspected virus infection, a total of nine samples (i.e. leaves, and fruit skins) collected from plants exhibiting severe symptoms were tested for potential begomovirus infection. To investigate this, DNA was purified from the tissue and PCR amplification was performed using degenerate primers designed to amplify the sequence encoding the begomovirus coat protein (CP) gene (Wyatt & Brown, 1996; Mnari-Hattab *et al.*, 2014b). A product of ~560 bp was obtained from all the tested symptom-bearing samples and absent from samples collected from symptomless plants. Multiplex PCR (Davino *et al.*, 2008) was used to attempt to identify the begomovirus species responsible for the infection. However, neither a 570 bp amplicon for TYLCV nor a 800 bp amplicon for *Tomato yellow leaf curl Sardinia virus* (TYLCSV) were generated with DNA extracted from the infected plants investigated here.

Three CP amplicons from the degenerate priming study were cloned and sequenced and shared 99% identity with one another. The nucleotide sequences were deposited in GenBank (Accession Nos. KP979713-KP979715). BLAST analysis showed that the three sequences shared highest nucleotide identity (97.6 to 99.2%) with partial CP gene

sequences from isolates of ToLCNDV infecting tomato and zucchini in Spain (KM977733 and KF749225) but were also related to the corresponding sequence of an Indian isolate of ToLCNDV (KC846817) where homology scores between 96.9 and 97.3% were found. ToLCNDV, a bipartite begomovirus first reported in India and neighbouring countries infecting solanaceous and cucurbitaceous crops and more recently reported in Spain on zucchini and tomato, is described to the best of our knowledge for the first time in cucurbit crops in Tunisia. Based on our findings, further targeted surveys must be undertaken to advance our knowledge of this emerging disease.

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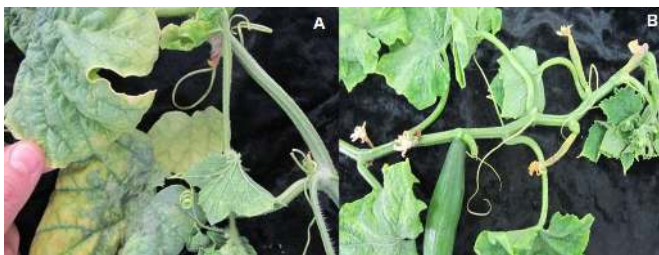


Figure 1

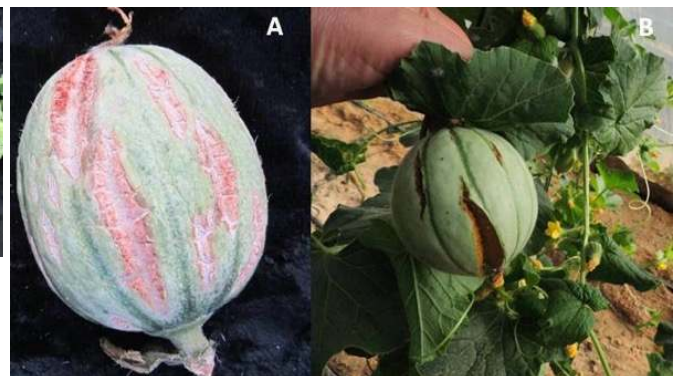


Figure 2

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