First report of Tomato leaf curl New Delhi virus infecting courgette in France

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NEW DISEASE REPORT

First report of *Tomato leaf curl New Delhi virus* infecting courgette in France

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Viruses constitute important problems for cucurbit production. In France, aphid-transmitted viruses remain the most important (Desbiez *et al.*, 2020), but whitefly-transmitted viruses, particularly begomoviruses, now emerging in the Mediterranean Basin (Lecoq & Desbiez, 2012) constitute major threats.

A few plants showing leaf crumpling, yellowing and downward curling were observed in a courgette (*Cucurbita pepo*) plot in Bouches-du-Rhône department (southeastern France) in September 2020. The symptoms were similar to those of *Tomato leaf curl New Delhi virus* (ToLCNDV), an emerging bipartite begomovirus affecting cucurbits in the Mediterranean Basin (Fortes *et al.*, 2016). After DNA extraction, PCR was performed on a symptomatic sample (CD20001) with specific primers TLCNDV-CP-5 and TLCNDV-CP-3, and ToLCNDV-B-900-5 and ToLCNDV-B-1940-3 (Romay *et al.*, 2019) targeting ToLCNDV DNA-A and DNA-B respectively. The amplified fragments (760 bp and 1072 bp) displayed 99.1% and 99.6% identity respectively to ToLCNDV isolate MU.12.ZU/1/2 (MH577761) from Spain. To complete the sequence of DNA-A, PCR amplification was performed with primers TLCNDV-fCP-F (5′-GTGACGGGAGGAACRTATGC-3′) and TLCNDV-dCP-R (5′-CTAACACACATGACTTTGCC-3′) before sequencing. For DNA-B, rolling circle amplification (RCA) was performed on the extracted DNA with the TempliPhi kit (GE Healthcare). A 2.7 Kb fragment was obtained after digestion with *Bam*HI and cloned in linearised pBlueScript(KS)+ before sequencing. Two clones were sequenced and were identical. The complete sequences of DNA-A and DNA-B (GenBank Accession Nos. MW310624 and MW310625. respectively) were 98–99% identical to isolates from the "Mediterranean" ToLCNDV clade (Fortes *et al.*, 2016).

FIGURE 1 Foliar symptoms in a zucchini plot in which *Tomato leaf curl New Delhi virus* was detected in September 2021 in Bouches-du-Rhône, southeastern France

Five other symptomatic courgette plants were collected in the same plot and 12 plants in two other plots in Bouches-du-Rhône, at a distance of 10 km (8 plants) and 6 km (4 plants) respectively from the first plot (Figure 1). Four courgette samples with similar symptoms were also collected in Gard department, 30 km away from the first plot. The samples were tested by PCR with primers Beg-CP-F and Beg-580-R (Saison *et al.*, 2015). A 580 bp product was obtained for 17 of the 21 tested samples (3/5 in the first plot, 8/8 and 4/4 respectively in the two

other plots in Bouches-du-Rhône and 2/4 in Gard) and determined as ToLCNDV by sequencing, confirming the presence of the virus in all four areas. The sequences displayed 99.3-100% identity with isolate CD20001.

FIGURE 2 Foliar symptoms in a zucchini plot in which *Tomato leaf curl New Delhi virus* was detected in September 2021 in Bouches-du-Rhône, southeastern France

FIGURE 3 Fruit symptoms in a zucchini plot in which *Tomato leaf curl New Delhi virus* was detected in September 2021 in Bouches-du-Rhône, southeastern France

This is the first report of ToLCNDV in France. Since ToLCNDV is highly damaging in cucurbits and is a quarantine pest in Europe, it is important to prevent its emergence in France, particularly in the southeast which constitutes France's most important cucurbit production area. The presence of ToLCNDV in four locations within a 30 km range suggests that it has begun to spread efficiently or that multiple introductions have occurred. It is now important to prevent virus overwintering in weeds (Juárez *et al*., 2019) and be careful to detect and eradicate potential infections in the next growing season.

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