Disease Notes

Diseases Caused by Viruses

First Report of Lettuce Necrotic Leaf Curl Virus Infecting Cultivated Lettuce in France

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Lettuce necrotic leaf curl virus (LNLCV, genus *Torradovirus*, family *Secoviridae*) has a bipartite single-stranded RNA genome and has so far only been reported in the Netherlands in open field lettuce (Verbeek et al. 2014). It was the first torradovirus described from a non-tomato host, and contrary to whitefly-transmitted tomato torradoviruses, aphids are its natural vectors (Verbeek et al. 2017). In October 2019, a symptomatic lettuce (JG3, cv. 'Tregoney') was collected in an open field in southwestern France. Symptoms included stunted and deformed leaves with light necrosis and yellow spotting along minor veins of older leaves. Double-stranded RNAs were purified from JG3 leaves as described (Marais et al. 2018) and a cDNA library prepared and subjected to high-throughput sequencing (HTS) using an Illumina NovaSeq sequencer. Analysis of sequence data (3.51 million trimmed reads, average length 112 nt) identified two nearly fully assembled RNAs integrating respectively 28.9 and 60.9% of the sequencing reads (average coverage of respectively 14,720× and 44,829×) and sharing respectively 85.5 and 83.3% nucleotide (nt) identity with the RNAs 1 and 2 of the LNLCV reference isolate (NC_035214 and NC_035219, respectively). To confirm the presence of LNLCV in the original JG3 plant, an extract from this plant was used to mechanically inoculate indicator *Nicotiana benthamiana*, *Chenopodium quinoa*, and *C. amaranticolor* plants. Only *N. benthamiana* developed symptoms, in the form of smaller and yellowed leaves. All inoculated plants were tested 1 month post-inoculation for the presence of LNLCV. Total RNAs were extracted according to the method of Foissac et al. (2005) and used for RT-PCR tests with primers designed from the alignment between NC_035214 and our RNA1 sequence (LNLCV-S, 5′-ATATTTTCCAAGTTGGAGGCTC-3′; LNLCV-R, 5′-AGTRA-CAAAGGGACTAACTG-3′). LNLCV was detected in three out of four inoculated *N. benthamiana* plants, and only those three plants developed symptoms. Sequencing of the 858-nt amplicons confirmed their identity with the HTS sequence. The full-length RNA1 sequence (7,577 nt) and the near-complete RNA2 (5,286 nt, lacking 3 nt at the 5′ end as compared with NC_035219) could be assembled from the JG3 sequencing data and have been deposited in GenBank (MW172270 and MW172271, respectively). The lettuce JG3 isolate RNA1 shows 86.5% nt identity with the reference isolate, and in the taxonomically informative region between the conserved protease CG and polymerase GDD motif (Thompson et al. 2017) they share 96.8% aa identity. JG3 RNA2 shares 84.8% nt identity with NC_035219, whereas the movement protein and capsid subunits share respectively 92.5 and 98.3% aa identity. The smaller upstream open reading frame (ORF) that slightly overlaps with the large MP-CP1/2/3 ORF is also conserved and shows 94.8% aa identity with the reference isolate. To our knowledge, this represents the first report of a natural infection of LNLCV in cultivated lettuce in France and anywhere outside the Netherlands. Because no other virus was detected in the sequence dataset, LNLCV is most likely responsible for the mild necrosis and leaf deformation symptoms observed on the JG3 plant that appear to be similar to those initially described for LNLCV (Verbeek et al. 2014). Although the pathogenicity of LNLCV in lettuce appears to be firmly established, further studies are needed to establish its distribution and prevalence, to understand why this pathogenic and aphid-transmitted virus does not seem widely reported and whether it has the potential to increase in impact as a potential emerging agent on field lettuce crops.

References:

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