Disease Note

Diseases Caused by Viruses

First Report of Little Cherry Virus 1 (LChV-1) Infecting Plum (*Prunus domestica*) in Bosnia and Herzegovina

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Little cherry virus 1, newly categorized as *Velarivirus nanoavii*, belongs to the genus *Velarivirus* in the family *Closteroviridae*. Over the past 15 years, new data on the virus have been collected, leading to an expansion of its host range, geographical distribution, and further insights into its genetic diversity. In terms of symptoms, little cherry virus 1 (LChV-1) mostly causes latent infections but is also associated with Kwanzan stunt and Shirofugen stunt disease (Matic et al. 2009; Candresse et al. 2013), as well as little cherry disease (Jelkmann and Eastwell 2018). Samples of plum (*Prunus domestica*) cultivar Čačanska ljepotica were randomly collected from an orchard in the northwestern region (Orašac, Una-Sana Canton) of Bosnia and Herzegovina (B&H). Total RNA was extracted, followed by removal of ribosomal RNA. The RNA library was prepared using the TrueSeq Standard Total RNA Library Prep Kit (Illumina). Samples were analyzed by high-throughput siRNA sequencing on the TrueSeq Illumina 500 platform and submitted to GenBank. Data analysis and de novo assembly were performed using SPAdes on the Geneious platform (version 10.1.2) and revealed the presence of plum pox virus strain D (PPV-D) (accession no. MW412433) and LChV-1 (accession no. MW283320) in one sample. A total of 2.5 million reads of 2 × 150 nt were generated. Mapping the Illumina reads to the reference genome of LChV-1, Kyoto-2 (GenBank accession no. MG934545), allowed the recovery of approximately 80% of the viral genome, distributed across various regions of the reference sequence. Nucleotide identity comparisons of the obtained partial sequence that covered ORF1a/1b to ORF3, performed using BioEdit software, showed the highest nucleotide identity with isolate ALM138 (accession no. MZ570904) of 98.3%. To validate the presence of LChV-1, additional asymptomatic plum leaf samples from various regions of B&H were collected over the following two seasons and tested together with seven previously collected samples from the orchard using RT-PCR. The primers reported by Jelkmann et al. (1997), amplifying the CP region, failed to detect the B&H isolates. Similarly, Katsiani et al. (2018) reported difficulties in reliably detecting LChV-1 in highly divergent isolates due to primer mismatches across different phylogroups. Therefore, new primers were developed to amplify a 370-bp fragment within the CPd region of LChV-1 using RT-PCR: LChV-1-13807-13825s (5′-TAGGYAGTTGGTATTTRAA-3′) and LCV1-14178-14160as (5′-AATTTTTCCAAACTTCACA-3′). In silico comparison of these primers with all available LChV-1 sequences from GenBank showed their suitability for the detection of different phylogroups of LChV-1. LChV-1 was detected in 7 out of 44 analyzed samples, all originating from a single orchard in the northwestern region. A 370-bp RT-PCR amplicon from one positive sample was cloned and sequenced (accession no. MW283319), confirming the LChV-1 sequence. To our knowledge, this is the first report of LChV-1 in B&H, as well as the first report of a mixed infection of LChV-1 and PPV-D in plums in general. Considering the wide host range of the virus, this finding represents a phytosanitary concern for B&H and its economically important stone fruit production. Although currently only found in the northwestern region of B&H (Orašac, Una-Sana Canton), this finding signals the need for large-scale surveys among different regions and host species of LChV-1 within B&H for risk assessment.

References:

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