Disease Note

Diseases Caused by Viruses

First Report of Watermelon Crinkle Leaf-Associated Virus 1 (WCLaV-1) and WCLaV-2 in Watermelon in Slovenia

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In July 2024, a pooled leaf sample (D760/24) was collected from several plants of three watermelon cultivars (*Citrullus lanatus* cvs. Crimson Sweet, Asahi Miyako Hybrid F1, and Top Gun) grown in an open field (approximately 0.5 ha) in Dombrava, Slovenia. The plants which were included in the pooled sample showed virus-like symptoms, such as leaf mosaic, wilting, and necrosis. The disease incidence was estimated at 10%. DNA and RNA were extracted following Mehle et al. (2013) and RNeasy Plant Mini Kit (Qiagen, Germany) protocols, respectively. The sample was tested positive by reverse-transcription (RT)-PCR for watermelon crinkle leaf-associated virus 1 (WCLaV-1) and WCLaV-2 (Hernandez et al. 2021) and negative for other viruses (details on viruses tested and primers used are available in supplementary materials). The obtained amplicons of expected sizes of WCLaV-1 and WCLaV-2 movement protein (MP) and RNA-dependent RNA polymerase (RdRp) genes were then subjected to Sanger sequencing (Eurofins Genomics, Germany) and BLAST analysis. The MP (PQ570004 and PQ570006) and the RdRp (PQ570005 and PQ570007) sequences exhibited 100% identity with multiple accessions of WCLaV-1, such as PP792977 and PP792976, and WCLaV-2, such as LC636073 and LC636074. Illumina high-throughput sequencing (HTS, Novogene, Germany, NovaSeq X Plus, PE150) identified WCLaV-1 (PV012703 and PV012704) and WCLaV-2 (PV012705 and PV012706) reads, along with cucumis melo amalgavirus 1 (CmAV1, PV012707) and solanum nigrum ilarvirus 1 reads (insufficient reads to reconstruct genome segments; it may originate from pollen contamination of nearby infected plants in the field [Rivarez et al. 2023]). HTS data were analyzed in CLC Genomics Workbench version 24 (Qiagen, U.S.A.) using the pipeline by Pecman et al. (2022). Consensus genome sequences were reconstructed by iterative read mapping to the most similar reference sequence of the virus obtained from NCBI GenBank. To check for WCLaVs in watermelon seeds sold in Slovenia, we tested five seed samples from Sugar Baby, Crimstar F1, and Crimson Sweet (three lots) by RT-PCR. We also tested four leaf samples from plants grown from these seeds at the 3 to 5 true leaf stage. Both viruses were found in all seed and leaf extracts. However, mechanical inoculations with the sap of two samples (plants grown from the infected seed sample and the sample D760/24) on several commonly used indicator plants including *Chenopodium quinoa*, *Capsicum annuum*, *Nicotiana clevelandii*, *N. glutinosa*, *N. benthamiana*, *N. tabacum* cv. White Burley, *N. rustica*, *Datura stramonium*, *Cucurbita pepo* cv. Bianca di Trieste, and *C. maxima* did not result in their infection. Retrospective analyses of our HTS data of two watermelon and 84 other cucurbits samples from previous years showed WCLaV-1 and WCLaV-2 reads in two pooled samples (containing equal amount of RNA of each sample): one from 2018 and another from 2019. RT-PCR confirmed the presence of WCLaVs only in watermelons. The pool from 2018 was sequenced at GATC (Germany, NovaSeq 6000 S2, PE 150) and from 2019 in-house using Oxford Nanopore Technologies (U.K., MinION Mk1B device, SQK-PCS108, R9 flow cell). All HTS reads are deposited in the NCBI Short Reads Archive (PRJNA1202089). This is the first report of WCLaV-1 and WCLaV-2 in Slovenia and Europe, the two viruses which were included to the Alert List of the European and Mediterranean Plant Protection Organization, due to limited knowledge about their epidemiology (EPPO 2023). Further research is necessary to determine the incidence of these viruses in Europe and elucidate their epidemiology, symptoms association, and their potential impact on the production of watermelons in the region.

References:

EPPO. 2023. EPPO Reporting Service no. 05 - 2023. https://gd.eppo.int/reporting/article-7598
Hernandez, R. N., et al. 2021. Plant Dis. 105:2025.
Mehle, N., et al. 2013. Page 139 in: Methods in Molecular Biology, vol. 938. Humana Press, Totowa, NJ, U.S.A.
Pecman, A., et al. 2022. Front. Microbiol. 13:883921.
Rivarez, M. P. S., et al. 2023. Phytopathology 113:1729.

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