DISEASE NOTES

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First Report of Cucurbit Aphid-Borne Yellows Virus in *Cucurbita pepo* and *Cucurbita maxima* in Slovenia

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In summer of 2017 and 2018, a survey was conducted in the major cucurbit-growing areas in Slovenia to assess the occurrence of viral diseases. Leaf samples of 20 plants of *Cucurbita pepo*, three *Cucurbita maxima*, seven *Cucumis melo*, one *Cucumis sativus*, and one *Citrullus lanatus* showing mosaic, interveinal mottling or yellowing, blisters, and distortions were collected from 11 farms in southwestern Slovenia, seven in northeastern Slovenia, and five in southeastern Slovenia. Total RNA was extracted from leaf tissue using RNeasy plant mini kits (Qiagen, Germantown, MD). Five composite RNA samples were generated by pooling together total RNAs from three to 11 individual samples. Two

of those were sent for Illumina small RNA sequencing to SeqMatic (Fremont, CA), two for ribosomal RNA depleted total RNA sequencing at Eurofins Genomics AT (Vienna, Austria), and one for ribosomal RNA depleted total RNA sequencing at Liege University. The sequencing reads (10 to 20 thousand) were analyzed using CLC Genomics Workbench 11 (Qiagen Bioinformatics, Aarhus, Denmark) with the pipeline for plant virus discovery (**Pecman et al. 2017**) or with Geneious version 10.1.5 (using the plugins Dedupe, SPADES, and blasting the contigs against viral reference database). In four out of five sample pools, sequences of cucurbit aphid-borne yellows virus (CABYV, genus *Polerovirus*) were detected along with sequences of cucumber mosaic virus (CMV, genus *Cucumovirus*), watermelon mosaic virus (WMV, genus *Potyvirus*), and/or zucchini yellow mosaic virus (ZYMV, genus *Potyvirus*). To confirm the presence of CABYV, each of the 26 individual plants, which were part of the pools of CABYV-positive samples, were tested by reverse transcription PCR using the coat protein (CP) gene-specific primers CE9/CE10 (**Juarez et al. 2004**). An amplicon of the expected size (600 bp) was generated from samples of 13 plants of *C. pepo* and from one of the *C. maxima* samples. All CABYV-positive plants were screened by double-antibody sandwich ELISA for CMV, WMV (both Loewe Biochemica, Germany), and ZYMV (Prime Diagnostics, Wageningen University & Research, the Netherlands). Single infection by CABYV was found in one plant of *C. pepo*, whereas other 13 plants were coinfected with CMV, WMV, and/or ZYMV. PCR products of the CP gene from all positive samples were purified, Sanger sequenced in both directions, and seven unique sequences were deposited in GenBank (accession nos. MN145451 to MN145457), and subjected to sequence analysis using the BLASTn algorithm (NCBI *nt* database, 20 June 2019) and MEGA7 software. Sequence comparisons showed 97.3 to 100% nucleotide sequence identity between Slovenian CABYV-positive samples. A maximum likelihood phylogenetic tree obtained based on the alignment of sequences of CABYV isolates from this study and isolates available in the GenBank database revealed that all Slovenian isolates of the virus cluster within the CABYV-Mediterranean group of isolates (**Kwak et al. 2018**). CABYV is widespread throughout the Mediterranean Basin, but it was found for the first time in Slovenia in this study. We confirmed the presence of CABYV in five different farms in southwestern Slovenia (eight samples) and in six different farms in northeastern Slovenia. Leaf yellowing symptoms were expressed on *C. pepo* infected by CABYV only. In some of the mixed infected plants, severe symptoms were observed. Further investigation is needed to clarify the significance of CABYV infection.

The author(s) declare no conflict of interest.

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