Diseases Caused by Viruses

First Report of Watermelon Crinkle Leaf-Associated Virus 1 (WCLaV-1) and WCLaV-2 Infecting Watermelon (*Citrullus lanatus*) in the United States

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Watermelon (*Citrullus lanatus*) and other cucurbits are cultivated globally, and Texas ranks among its top five producers in the United States. In July 2020, plants with virus-like disease symptoms consisting of mild leaf crinkling and yellow mosaic patterns were observed in a 174-ha watermelon field in Burleson Co., Texas; disease incidence was visually estimated at 5%. Total nucleic acids were extracted from leaf tissues of five randomly sampled plants (Dellaporta et al. 1983), and their equimolar amounts were made into a composite sample that was used for cDNA library construction with TruSeq Stranded Total RNA with Ribo-Zero Plant Kit (Illumina). The cDNA library was sequenced on the Illumina NextSeq 500 platform, generating ~37M single-end reads (each 75 nt), which were analyzed according to the method of Al Rwahnih et al. (2018). Of these, 58,200 and 27,500 reads mapped to the genomes of watermelon crinkle leaf-associated virus 1 (WCLaV-1) and WCLaV-2 (Xin et al. 2017), respectively, along with four other virus-specific reads (data not shown). The near-complete RNA1 to RNA3 segments of WCLaV-1 (354-652X) and WCLaV-2 (144-258X) were generated from the mapped reads, and they shared ≥96% nt identities with published RNA segments of both viruses. The results were verified by RT-PCR using newly designed primers WCLaV-1vRP (5'-GGTGAGT-TAGTGTGTCTGAAGG)/WCLaV-1cRP (5'-GAGGTTGCCTGAGGT-GATAAG) to target 881 bp of the RNA1-encoded RNA-dependent RNA polymerase (RdRP), WCLaV-1vMP (5'-GAAGGTTTGCTCCCTT-GAAATG)/WCLaV-1cMP (5'-GACTGTGGCTGAAGAGTCTATG) to target 538 bp of the RNA2-encoded movement protein (MP), and

WCLaV-1vNP (5'-CGAATAGACTCTGGAGGGTAGA)/WCLaV-1cMP (5'-GAAAGCAAGAAAGCTGGCTAAA) to target 786 bp of the RNA3-encoded nucleoprotein (NP). Similarly, the WCLaV-2-specific primers WCLaV-2vRP (5'-GTCTCACATTCTGCACTAACT)/WCLaV-2cRP (5'-ATCGGTCTGGGTTATTGTATC) target 968 bp of the RdRP, WCLaV-2vMP (5'-GACTTCAGAACCTCAACATCCA)/WCLaV-2cMP (5'-CAAGGGAGAGTGCTGACAAA) target 562 bp of the MP, and WCLaV-2vNP (5'-ATTCCCAGTGAGAGCAACAA)/WCLaV-2cMP (5'-GAGGTGGAGGTAGGAAAGAAAAG) target 449 bp of the NP. Fresh cDNA synthesized from the five samples with a PrimeScript First Strand cDNA synthesis kit (Takara Bio) was tested by PCR with all six primer pairs using the PrimeSTAR GXL DNA Polymerase kit (Takara Bio). Three of the five samples were positive for both viruses, and one sample was positive for each virus. The obtained products from four samples were cloned individually into pJET1.2/Blunt vector (Thermo Scientific, U.S.A.), followed by bidirectional Sanger sequencing of the plasmids obtained with the GenElute Five-Minute Plasmid Miniprep kit (Sigma-Aldrich). In pairwise comparisons, the partial RNA1 to RNA3 sequences of WCLaV-1 (GenBank accession nos. MW559074 to MW559082) shared 100% nucleotide (nt)/amino acid (aa) identities with each other and with corresponding sequences of WCLaV-1 isolate KF-1 from China (KY781184 to KY781186). The partial RNA1 to RNA3 sequences of WCLaV-2 (MW559083 to MW559091) shared 97 to 100% nt/96 to 100% aa identities with each other and with corresponding sequences of WCLaV-2 isolate KF-15 from China (KY781187 to KY781189). This is the first report of WCLaV-1 and WCLaV-2 in Texas and the first record of both viruses in the United States and elsewhere outside of China. Both negative-sense, single-stranded RNA viruses represent a novel taxon in the family *Phenuiviridae* (order *Bunyavirales*) (Xin et al. 2017). Although aspects of the biology of both viruses are yet to be elucidated, our results expand their geographical range. The detection primers developed here will be useful for screening cucurbit germplasm to avert their spread.

References:

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