Diseases Caused by Viruses

First Report of Watermelon Crinkle Leaf-Associated Virus 1 Naturally Infecting Watermelon (*Citrullus lanatus*) in Georgia, United States

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Watermelon (*Citrullus lanatus*) is one of the major vegetable crops grown in Georgia during the spring and summer seasons, contributing \$180 million of farmgate value to the state's economy (Center for Agribusiness and Economic Development 2019). During the summer of 2021, watermelon plants with foliar symptoms such as yellow mottling, chlorosis, and wrinkling with thickened, bunchy, and upward curling were observed on commercial fields in Georgia, United States. A disease incidence of 15 to 20% in ~56 ac. in Tift County and 10 to 15% in ~60 ac. in Wilcox County was observed. The symptoms observed were similar to those described for watermelon crinkle leaf-associated viruses (WCLaV-1 and WCLaV-2) from Florida (Hendricks et al. 2021) and Texas (Hernandez et al. 2021). Symptomatic leaves from Tift ($n = 40$) and Wilcox ($n = 20$) Counties were collected, surface sterilized with 0.1% bleach, and used for total nucleic acid extractions using the Mag-MAX 96 Viral RNA isolation kit (ThermoFisher Scientific, Waltham, MA, U.S.A.) following the manufacturer's instructions without DNase treatment. The potential introduction of WCLaV-1 and WCLaV-2 into Georgia was tested by a reverse transcription (RT)-PCR assay using specific primers targeting RNA-dependent-RNA polymerase (RdRp) and movement protein (MP) genes of both viruses (Hernandez et al. 2021). The expected amplicon sizes for RdRp (~900 nt) and MP (~500 nt) genes of WCLaV-1 located on RNA 1 and RNA 2 segments, respectively, were observed in 39 of 40

(97.5%) samples from Tift and 7 of 20 (35%) samples from Wilcox. However, WCLaV-2 was not detected in any of the tested samples. All 60 samples also tested negative for the whitefly-transmitted viruses prevalent in the region, including cucurbit chlorotic yellows virus, cucurbit yellow stunting disorder virus, and cucurbit leaf crumple virus using virus-specific primers (Kavalappara et al. 2021). A subset of the samples analyzed by RT-PCR was also tested by SYBR green-based real-time RT-PCR assay targeting the MP gene of WCLaV-1 using primers WCLaV-1FP (5'TCCACAAGCTTGATGGAGGG3') and WCLaV-1RP (5'TCCCGAGTGAGGAAGCTAGT3'). The virus was detected in samples from both counties, and the results matched those obtained by the conventional RT-PCR assays. The presence of WCLaV-1 was further confirmed by sequencing (Genewiz, South Plainfield, NJ, U.S.A.) coupled with BLASTn analysis of amplicons that resulted from the conventional RT-PCR from three randomly selected samples. The partial RdRp sequences (OL469153 to OL469155) were 99.3 and 99.9% identical to the corresponding sequences of WCLaV-1 isolates from China (KY781184) and Texas (MW559074), respectively. The partial MP sequences (OL469150 to OL469152) were 100% identical to those from China (KY781185) and Texas (MW559077). WCLaV-1 and WCLaV-2 were first discovered in Asia (Xin et al. 2017). Both viruses were subsequently reported from North and South America (Hendricks et al. 2021; Hernandez et al. 2021; Maeda et al. 2021), indicating their geographical expansion. Biological information, including vector relations, is unknown for both viruses and other members of the genus *Coguvirus* (family *Phenuiviridae*), to which they are provisionally assigned (Zhang et al. 2021). Further studies are also required to understand the biology and impact of both viruses on watermelon production and other crops, if any.

References:

- Center for Agribusiness and Economic Development. 2019. Georgia Farm Gate Value Report. <https://caed.uga.edu/publications/georgia-agricultural-statistics.html> (accessed on January 19, 2022).
- Hendricks, K. E. M., et al. 2021. Plant Dis. 106:339.
- Hernandez, R. N., et al. 2021. Plant Dis. 105:2025.
- Kavalappara, S. R., et al. 2021. Plant Dis. 105:1862.
- Maeda, M. H. K., et al. 2021. Plant Dis. 106:773.
- Xin, M., et al. 2017. Front. Microbiol. 8:1514.
- Zhang, S., et al. 2021. Arch. Virol. 166:2829.

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