Diseases Caused by Viruses

First Report of Watermelon Crinkle Leaf-Associated Virus 1 and 2 Infecting Watermelon (*Citrullus lanatus*) Plants in Brazil

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Watermelon (*Citrullus lanatus*) is an important crop in Brazil both for export and domestic consumption. In this study, the cause of a severe leaf curling, distortion, and vein clearing/yellowing disease of watermelon was investigated by high-throughput sequencing (HTS). The RNA extract of virus semipurification preparation (Blawid et al. 2017) from leaf samples of 10 symptomatic plants collected from a commercial field in Juazeiro, Bahia State in May 2019, were pooled. Then, cDNA prepared with TruSeq Stranded Total RNA with Ribo-Zero Plant Kit (Illumina, San Diego, CA, U.S.A.) was analyzed by HTS using Novaseq with 100 paired ends of 3G scale (~31M reads). De novo assembly of total reads was performed using Megahit (Li et al. 2015), and the tBlastX search against the RefSeq virus genomes (NCBI) was done using Geneious 11.1 (Biomatters, Auckland, New Zealand). Reads of groundnut ringspot virus (GRSV) were identified, in addition to those of watermelon crinkle leaf-associated virus 1 (WCLaV-1, LC636070-72) and 2 (WCLaV-2, LC636073-75), which are putative members of the genus *Coguvirus* in the family *Phenuiviridae* (Zhang et al. 2021) and have not been reported in Brazil. Other plant viruses were not found. The means of read coverage over the genes ranged from 1,652 to 2,532 for WCLaV-1 and from 404 to 1,025 for WCLaV-2. These viruses were recently reported infecting watermelon in China (Xin et al. 2017) and the U.S.A.

(Hernandez et al. 2021). The nucleotide identities between consensus sequences of isolates from Brazil and reference sequences of the WCLaV-1 KF-1 isolate from China (KY781184–86) were 99.0% in RdRp, 98.9% in nucleocapsid (NC), and 99.1% in putative movement protein (MP) genes, and between the WCLaV-2 KF-15 isolate from China (MW559083–91) and the sequences from Brazil, 97.2% in RdRp, 96.6% in NC, and 96.9% in putative MP genes. To confirm the presence of these viruses in individual samples, RT-PCR was conducted with specific primers to WCLaV-1 (WCLaV-1vNP and WCLaV-1cMP) and WCLaV-2 (WCLaV-2vNP and WCLaV-2cMP) (Hernandez et al. 2021), targeting the NC protein genes with the expected amplicon sizes of 786 and 449 nt, respectively. In addition, GRSV-specific primers (GRSVS: GTGCATCATCCATTGTAAATCC and GRSVA: CGCCAAAGCATCAT GAAAG), targeting the NC protein gene, with the expected amplicon size of 445 nt, were also used in the test. Twelve samples from watermelon plants with similar symptoms were analyzed by RT-PCR, six from a field in Mossoró, Rio Grande do Norte State collected in 2020, and six from the same field in Juazeiro, Bahia sampled in 2019. All plants from both locations were positive for WCLaV-1 and GRSV in RT-PCR tests, whereas two samples from Juazeiro were positive for WCLaV-2. Six cDNA fragments (two from Mossoró, two from Juazeiro for WCLaV-1, and two from Juazeiro for WCLaV-2) were sequenced (MZ819081–6) and showed very high identities within the species among them (99.8 to 100%). Finally, leaf samples were also collected from watermelon plants with these symptoms in Guadalupe County, Piauí State in 2015. An HTS analysis of this sample was conducted at the University of California at Davis and revealed infection with a divergent strain of GRSV and WCLaV-1 (LC636068-9), but not WCLaV-2. The nucleotide identities between consensus sequences of isolates from Piauí and Juazeiro were 99.9% in RdRp and NC, and 100% in putative MP genes. These results indicate that WCLaV-1 and WCLaV-2 are present in Brazil in association with severe virus-like disease symptoms in watermelon plants.

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