Diseases Caused by Viruses

First Report of Cucurbit Chlorotic Yellows Virus Affecting Watermelon in the United States

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Watermelon (*Citrullus lanatus*) is a high-nutrient crop, high in vitamins and very popular in the United States and globally. The crop was harvested from 101,800 acres with a value of \$560 million in the United States (USDA-NASS 2020). California, Florida, Georgia, and Texas are the four leading watermelon-producing states in the United States. During the fall season of 2020, plants in two North Florida watermelon fields, one in Levy County (~20 acres) and one in Suwannee County (~80 acres) with varieties Talca and Troubadour, respectively, exhibited viral-like symptoms. The fields had 100% disease incidence that led to fruit quality issues and yield losses of 80% and above. Symptoms observed in the watermelon samples included leaf crumpling, yellowing and curling, and vein yellowing similar to that of single/and or mixed infection of Cucurbit leaf crumple virus, genus *Begomovirus*, family *Geminiviridae*, Cucurbit yellow stunting disorder virus, genus *Crinivirus*, family *Clsteroviridae*, and Squash vein yellowing virus, genus *Ipomovirus*, family *Potyviridae*, although the vine decline symptoms often associated with squash vein yellowing virus (SqVYV) infection of watermelon were not observed. All three viruses are vectored by whiteflies and previously described in Florida (Adkins et al. 2009; Akad et al. 2008; Polston et al. 2008). To confirm the presence of these viruses, RNA was isolated from 20 symptomatic samples using the RNeasy Plant Mini Kit (Qiagen, U.S.A.) as per protocol. This was followed by RT-PCR (NEB, U.S.A.) using gene-specific primers described for cucurbit leaf crumple

virus (CuLCrV), cucurbit yellow stunting disorder virus (CYSDV), and SqVYV (Adkins et al. 2009). Amplicons of expected sizes were obtained for all the viruses with the infection of CuLCrV in 17/20, CYSDV in 16/20, and SqVYV in 8/20 samples. In addition, the presence of Cucurbit chlorotic yellows virus, genus *Crinivirus*, family *Clsteroviridae* in mixed infection was confirmed in 4/20 samples (three leaves and one fruit) by RT-PCR with primers specific to the cucurbit chlorotic yellows virus (CCYV) coat protein (CP), heat shock protein 70 homolog (HSP70h), and RNA dependent RNA polymerase (RdRp) designed based on the available CCYV sequences. The RT-PCR amplification was performed using a symptomatic watermelon sample, and the amplicons of RdRp, HSP70h, and CP were directly sequenced by the Sanger method, and the sequences of the amplicons were deposited in GenBank under the following accession numbers: MW527462 (RdRp, 952 bp), MW527461 (HSP70h, 583 bp), and MW527460 (CP, 852 bp). BLASTn analysis demonstrated that the sequences exhibited an identity of 99 to 100% (RdRp and HSP70h, 100%; and CP, 99%) with the corresponding regions of the CCYV isolate Shanghai from China (accession nos. KY400636 and KY400633). The presence of CCYV was further confirmed in the watermelon samples by ELISA (Loewe, Germany) using crude sap extracted from the RT-PCR-positive, symptomatic watermelon samples. CCYV was first identified in Kumamoto, Japan, in 2004 on melon plants (Gyoutoku et al. 2009). CCYV was previously reported on melon from Imperial Valley, California (Wintermantel et al. 2019) and more recently on squash in Tifton, Georgia (Kavalappara et al. 2021) and cantaloupe in Cameron, Texas (Hernandez et al. 2021). To our knowledge, this is the first report of CCYV on field watermelon production in the United States. Continued monitoring of CCYV in the spring and fall watermelon crop, and cucurbit volunteers and weeds, will be critical toward understanding the spread of this virus and its potential risk to watermelon in Florida and other regions of the United States.

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