Plant Health Brief

First Report of Cucurbit Leaf Crumple Virus Infecting Three Cucurbit Crops in South Carolina

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South Carolina watermelon (*Citrullus lanatus*) growers harvested 2,874 ha in 2017, the fifth largest area in the United States. Virus-like symptoms of curled and crumpled leaves, bright yellow chlorosis, and marginal leaf necrosis typical of begomovirus infection were observed on younger leaves of watermelon on two farms in Beaufort County in southeastern South Carolina in August 2017 (Fig. 1). Several watermelon fields displayed these symptoms on 20 to 50% of the plants. Large populations of whiteflies (*Bemisia tabaci*) noted by growers before the symptoms appeared also suggested possible begomovirus infection. Although yield lost to the disease was not measured, one grower reported adverse effects on plant vigor and 15 to 20% reduction in the number of harvestable fruit. Affected plants appeared to produce smaller than normal fruit.

To investigate the causal agent, five samples of watermelon leaves were collected from each of two adjoining farms, where >50% of plants displayed symptoms. Additional leaf samples were collected from plants with crinkled leaves, one from watermelon and four from muskmelon (*Cucumis melo*) at the Coastal Research and Education Center in Charleston County, and one and two from summer squash (*Cucurbita pepo*) on farms in Colleton and Charleston counties, respectively. One additional muskmelon sample came from a fruit with external symptoms of mosaic and mottling. Genus-specific primers for begomoviruses (Rojas et al. 1993) and potyviruses (Gibbs and Mackenzie 1997) and species-specific primers for cucurbit chlorotic yellows virus (forward, 5′-CGCAATCAATAAGGCGGCGACC-3′; reverse, 5′-ACTACAACCTCCCGGTGCCAACT-3′; accession no. KU507602) and cucurbit yellow stunting disorder virus (CYSDV; Polston et al. 2008; accession no. FJ492808) were used in polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR). Genus-specific primers for the begomovirus-A component (PAR1c496 and PALv1978; Rojas et al. 1993) produced amplicons of the expected size (approximately 1,100 bp) from 18 of the 19 samples: all 11 watermelon, all five muskmelon, and one squash from each county. Additional genus-specific primers for the begomovirus-B component (PBL1v2040 and PCR154; Rojas et al. 1993) confirmed the presence of a begomovirus in these 18 samples. Sanger sequencing of amplicons from both A and B components and BLASTn analysis revealed a high nucleotide sequence identity in seven watermelon samples from Beaufort and Charleston counties and all muskmelon samples with cucurbit leaf crumple virus (CuLCrV). BLASTn analysis showed that 1,042 nt of the A component amplicon (GenBank accession no. MH013228) shared 97% nucleotide sequence identity with both Arizona and California isolates of CuLCrV (AF256200 and AF224760, respectively). The 377-nt B component amplicon (GenBank accession no. MH013229) shared 94 to 95% nucleotide sequence identity with the same Arizona and California CuLCrV isolates (AF327559 and AF224761, respectively). No amplicons were obtained with the other primers.

Seven additional watermelon leaf samples collected from farm 2 in Beaufort County were tested with RT-PCR primers specific for CuLCrV, CYSDV, squash vein yellowing virus (SqVYV), or papaya ringspot virus type W (PRSV-W). Total nucleic acids were extracted from a pool of all seven samples using an RNeasy Plant Mini kit (Qiagen, Germantown, MD) followed by RT-PCR with previously described virus-specific primers and conditions capable of generating proper amplicons for begomoviruses and the other three viruses (Turechek et al. 2010). The CuLCrV primers yielded an amplicon of 996 nt (GenBank accession no. MG920141) that shared 96 to 97% nucleotide identity with CuLCrV isolates in

FIGURE 1

Symptoms of stunted, crumpled leaves on watermelon infected with cucurbit leaf crumple virus in South Carolina in August 2017. The field had been cropped to tomato in spring 2017; thus, a shriveled tomato appears in the image. Note that the black polyethylene mulch had been spray painted white for reuse in fall production.

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GenBank. No amplicons were obtained with SqVYV, CYSDV, or PRSV-W primers.

Transmission experiments used watermelon vine cuttings with symptoms of CuLCrV from farm 1 in Beaufort County as the inoculum source. Cuttings in vials of water were placed inside an insect-proof cage in which approximately 1,000 whiteflies (*B. tabaci* Middle East-Asia Minor 1) were allowed to feed for 2 days. Thereafter, six muskmelon seedlings were added to the chamber to allow transmission of CuLCrV. After 4 weeks, all recipient plants exhibited CuLCrV symptoms. A PCR test using primers PBL1v2040 and PCR154 produced the same size amplicons from two recipient plants and the source material.

Although CuLCrV has been identified in California, Arizona, Texas, Georgia, and Florida (Akad et al. 2008; Gadhave et al. 2018), this is the first report of CuLCrV infecting cucurbits in South Carolina. Because CuLCrV could have significant implications for cucurbit crops in the southeastern United States, preventative management of the whitefly vector in fall crops may be necessary in the future.

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