First Report of *Cucurbit chlorotic yellows virus* Infecting Cantaloupe (*Cucumis melo*) in Texas

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Plant Dis. 105:3313, 2021; published online as <https://doi.org/10.1094/PDIS-02-21-0378-PDN>. Accepted for publication 29 March 2021.

Texas is a major producer of cucurbits such as cantaloupe (*Cucumis melo* L.), but outbreaks of virus-like diseases often adversely affect yields. Little is known about the identity of the causal or associated viruses. During studies conducted in fall 2020 to explore the virome of cucurbit fields in Texas, a commercial cantaloupe field (~4.1 ha) in Cameron County was observed with virus-like symptoms of interveinal chlorotic mottle and foliar chlorosis, and disease incidence was estimated at 100%. Virus-like symptoms including mosaic and leaf curl were also observed in six additional fields across five south and central Texas counties of Atascosa, Hidalgo, Fort Bend, Frio, and Wharton. Forty-six plants, which included 32 symptomatic and 14 nonsymptomatic, were sampled from these fields for virus diagnosis, and each sample was subjected to total nucleic acid extraction according to the method of Dellaporta et al. (1983). Initially, equal amounts of nucleic acids from 14 symptomatic plants (two/field) were pooled into one composite sample for preliminary diagnosis by high-throughput sequencing (HTS). The cDNA library obtained from the composite sample with a TruSeq Stranded Total RNA with Ribo-Zero Plant Kit (Illumina) was sequenced on the Illumina NextSeq 500 platform, generating ~26.3 million single-end HTS reads (75 nt each). Analyses of the reads according to the method of Al Rwahnih et al. (2018) revealed several virus-like contigs; among them 23 contigs (206 to 741 nt) shared 98 to 100% nt identities to isolates of cucurbit chlorotic yellows virus (CCYV), genus *Crinivirus*, family *Closteroviridae*. Three pairs of

CCYV-specific primers were designed from the HTS contigs with primers CCYV-v1330 (5'-AGTCCCTTACCCTGAGATGAA)/CCYV-c2369 (5'-CGGAGCATTCGACAACCTGAATA) targeting a ~1-kb fragment of the ORF1a (RNA1), primers CCYV-v4881 (5'-ATAAGGCGGCGACCTAAT C)/CCYV-c5736 (5'-GATCACTTGACCATCTCCTTCT) targeting a ~0.9-kb fragment encompassing the coat protein (CP) cistron of CCYV (RNA2), and primers CCYV-v6362 (5'-CACCTCTTCCAGAACCAAGTTAAA)/CCYV-c7423 (5'-TGGGAACAACCTATTTCTCCTAGC) targeting ~1 kb spanning partial minor coat protein (CPm) and p26 sequences (RNA2). Total nucleic acid extracts of each of the 46 samples from the seven fields were tested by two-step reverse transcription polymerase chain reaction using all three CCYV-specific primer pairs, and they yielded amplicons of the expected sizes from all five symptomatic cantaloupe samples from the Cameron County field and one additional symptomatic butternut squash sample from a field in Hidalgo County. The DNA bands from three randomly chosen cantaloupe samples were cloned and sequenced as previously described (Oke et al. 2020). In pairwise comparisons, the obtained 1,040-nt ORF1a (MW584332 to 334), 753-nt complete CP (MW584335 to 337), and 1,062-nt CPm/p26 (MW584338 to 340) gene specific sequences from the three samples shared 100% nt identity with each other and 99 to 100% nt identities with corresponding RNA1 (AB523788) and RNA2 (AB523788) sequences of the exemplar isolate of CCYV. This is the first report of CCYV in Texas, thus expanding the current geographical range of the virus in the United States, which includes California (Wintermantel et al. 2019) and Georgia (Kavalappara et al. 2021). The abundance of whiteflies of the *Bemisia tabaci* species complex in south Texas and other major U.S. cucurbit production areas presents additional challenges to virus disease management.

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- The author(s) declare no conflict of interest.

Keywords: cucurbits, viruses, RNA viruses, *Crinivirus*, *Closteroviridae*, high-throughput sequencing, whitefly, *Bemisia tabaci*, epidemiology

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