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NEW DISEASE REPORT

First report of Watermelon crinkle leaf-associated virus 1 (WCLaV-1) on Cucurbita pepo in the United States

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Zucchini (Cucurbita pepo) is widely grown in Florida and contributes to the US$35 million value production of all types of squash in the state (United States Department of Agriculture/ National Agricultural Statistics Service, 2021). In August 2022, zucchini (cv. Renegade) in a commercial field in North Florida, USA, showed pronounced chlorosis and mild crinkle on leaves in scattered areas (Fig. 1). The disease incidence was approximately 35%.

One diseased leaf from each of six zucchini plants were collected randomly for diagnosis; three from 21-day-old plants (noted as Z272), and three from 28-day-old plants (noted as Z273). All samples tested negative for *Cucumber mosaic virus*, *Squash mosaic virus* and *Zucchini yellow mosaic virus* using ImmunoStrips (Agdia, USA). Subsequently, total plant RNA was extracted using a Quick-RNA MiniPrep kit (Zymo Research, USA). A conventional one-step RT-PCR (New England Biolabs, USA) was used to test the samples using primer pairs for *Cucurbit chlorotic yellows virus* (CCYV; Jailani et al., 2022), *Watermelon crinkle leaf-associated virus 1* and *2* (WCLaV-1 and WCLaV-2; genus *Coguvirus*) (Hernandez et al., 2021) and *Watermelon mosaic virus 2* (WMV-2; 5′-AAAGCTCCATACATAGCTGAGAC-3′/ 5′-CGGCCTTCATTTGTGCTATTG-3′). The samples were negative for CCYV, WMV-2 and WCLaV-2, but positive for WCLaV-1 using specific primers for the RNA-dependent RNA polymerase (RdRP) region on RNA-1 (Table 1). To confirm the detection of WCLaV-1, specific RNA-2 primers, targeting the coding regions of movement protein (MP) and nucleocapsid protein (NP), respectively, were used in an RT-PCR assay (Table 1, Hernandez et al., 2021). The sequences of the RdRP amplicon (881 bp) (GenBank Accession No. OP484885) had 99% nucleotide identity with WCLaV-1 isolates from China (KY781184) and the USA (OL469153 and OL469154). The sequences of the MP amplicon (538 bp) (OP484884) shared 99% nt identity with Australian WCLaV-1 isolate S6 (ON934517) and American isolates "Turner" and



FIGURE 1 Zucchini leaf showing yellowing and leaf crinkling symptoms caused by infection with *Watermelon crinkle leaf-associated virus 1*

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TABLE 1 Primers used in RT-PCR for the detection of *Watermelon crinkle leaf-associated virus 1* in zucchini (Hernandez et al., 2021)

Name	Primer sequences (5′-3′)	Gene, Genome	Location	Size (bp)
WCLaV-1vRP	GGTGAGTTAGTGTGTCTGAAGG	RdRp, RNA1	3856	881
WCLaV-1cRP	GAGGTTGCCTGAGGTGATAAG		4736	
WCLaV-1vMP	GAAGGTTTGCTCCCTTGAAATG	MP, RNA 2	702	538
WCLaV-1cMP	GACTGTGGCTGAAGAGTCTATG		1245	
WCLaV-1vNP	CGAATAGACTCTGGAGGGTAGA	NP, RNA 2	341	786
WCLaV-1cNP	GAAAGCAAGAAAGCTGGCTAAA		1126	

"Wilcox-02" (OM751929 and OM751928). The NP amplicon sequences (786 bp) (OP484883) shared 99% nt identity with Chinese isolate KF-1 (KY781186) and American isolate Wig-WM-D10 (MW559081). Both the samples (Z272 and Z273) had similar results, therefore only sequences from sample Z272 were submitted to GenBank. The results were further confirmed by SYBR®-based real-time RT-PCR using the WCLaV-1 MP gene-specific primer pairs (WCLaV-1FP/WCLaV-1RP) (Adeleke et al., 2022).

WCLaV-1 and WCLaV-2 were first reported in watermelon in China (Xin et al., 2017), followed by America (Hernandez et al., 2021), Brazil (Maeda et al., 2022) and Australia (Mulholland et al., 2023). To our knowledge, this is the first report of WCLaV-1 on zucchini in America and globally. The mode of transmission of the virus in currently unknown. Studies to understand the potential of vector and seed transmission should be conducted to aid the development of best management practices. In the meantime, growers will have to rely on keeping pests of zucchini crop under control, practicing tillage of plant residues, and removing weeds routinely as general good practices.

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