DISEASE NOTE

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First Report of the Hibiscus Strain of Citrus Leprosis Virus C2 Infecting Passionfruit (*Passiflora edulis*)

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Published Online: 3 Aug 2022 | https://doi.org/10.1094/PDIS-10-21-2314-PDN

In Hawaii, passionfruit (*Passiflora edulis*; Passifloraceae) is grown primarily in residential properties and community gardens (CG). In 2019, passionfruit plants displaying chlorotic spots on young leaves and green spots in senescing leaves

were observed at two CG in Honolulu. Symptoms resembled those of passionfruit green spot virus (PfGSV) infection in *Passiflora* spp. (**Ramos-González et al. 2020**) and of the hibiscus strain of citrus leprosis virus C2 (CiLV-C2H) infection in hibiscus in Hawaii (**Melzer et al. 2013**). Both viruses belong to the genus *Cilevirus*, family *Kitaviridae*. Total RNA was extracted from two sample pools comprised of 40 symptomatic leaves collected from both CG following a CTAB-based procedure (**Li et al. 2008**). To identify the virus associated with the *P. edulis* infection, reverse transcription (RT)-PCR was performed using CiLV-C2 (**Olmedo-Velarde et al. 2021**) and PfGSV specific primers (**Ramos-González et al. 2020**). The RT-PCR assay amplified the CiLV-C2 amplicon but failed to produce the PfGSV amplicon from infected leaves. Amplicon sequencing followed by a BLASTn search showed the nucleotide sequence had >99% identity with the CiLV-C2H-RNA1 (KC626783). A ribo-depleted RNA library created using the TruSeq Stranded Total RNA Library Prep kit (Illumina) underwent high throughput sequencing (HTS) on a NextSeq550 Illumina platform (2 × 75 cycles). The 6.5 million raw reads obtained were trimmed, filtered, and de novo assembled using Metaviral SPAdes v. 3.15.02 (**Antipov et al. 2020**). The resulting contigs were searched against an in-house database generated from GenBank virus and viroid sequences using BLASTn. This identified 12 and 3 contigs corresponding to CiLV-C2H and watermelon mosaic virus, respectively, with the latter being previously reported in passionfruit (**Watanabe et al. 2016**). RNA1 contigs covered 80.17% of the CiLV-C2H genome, whereas RNA2 contigs covered 94.5% with an average coverage depth of 31.660 and 57.121, respectively. To obtain the near complete genome of CiLV-C2H, gaps from the assembled HTS data were filled by overlapping RT-PCR followed by Sanger sequencing. RNA1 (8,536 nt, acc. no. MW413437) and RNA2 (4,878 nt, MW413438) genome sequences shared 99.2% and 97.0% identity with CiLV-C2H-RNA1 (KC626783) and -RNA2 (KC626784). To further confirm the presence of CiLV-C2H in symptomatic *P. edulis* plants, 40 symptomatic leaf samples were individually tested by RT-PCR, and 30 samples were positive. *Brevipalpus* mites collected from CiLV-C2H-positive *P. edulis* leaves were transferred to common bean (*Phaseolus vulgaris*) seedlings. At 15 to 30 days posttransfer, RNA extracted from lesions observed in recipient plants tested positive for CiLV-C2H by RT-PCR. Total RNA from individual *Brevipalpus* mites was isolated, and cDNA was prepared to tentatively identify the

mite species involved in CiLV-C2H transmission in passionfruit (Druciarek et al. 2019; Olmedo-Velarde et al. 2021). CiLV-C2H was detected in individual mites, and the 28S ribosomal mite RNA sequence (MZ478051) shared 99 to 100% nucleotide identity with *B. yothersi* (MK293678 and MT812697), a vector of CiLV-C2 (Roy et al. 2013). CiLV-C2 currently has a host range limited to the families Malvaceae, Araceae, and Rutaceae (Roy et al. 2015). CiLV-C2H infects hibiscus alone and citrus in mixed infection with CiLV-C2 (Roy et al. 2018), which is responsible for causing citrus leprosis disease. Detection of CiLV-C2H in passionfruit expands the number of host families of CiLV-C2H.

Funding: This project was funded, in part, by USDA-NIFA Hatch Project HAW09050-H managed by the College of Tropical Agriculture and Human Resources at the University of Hawaii at Manoa.

The author(s) declare no conflict of interest.

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