Diseases Caused by Viruses

First Report of Clover Yellow Vein Virus in *Crotalaria micans* in Hawaii

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Rattlepods (*Crotalaria* spp.) are nitrogen-fixing legumes that were introduced to Hawaii, where they have been used as windbreaks (Shigeura and McCall 1982), stink bug trap crops in macadamia nut orchards (Jones et al. 2001), and green manure/cover crops (Wang et al. 2002). Many rattlepod species have since become naturalized on several of the Hawaiian Islands. In September 2018, conspicuous virus-like symptoms of chlorotic mosaic and yellowing were observed in a roadside patch of *Crotalaria micans* on the Big Island (Island of Hawaii), where foliar samples from five individual plants were collected and subjected to molecular and biological characterization. All five samples were found to be bean common mosaic virus (BCMV)-positive using an ELISA test (Feng et al. 2014); all five were mechanically inoculated to *Nicotiana benthamiana* and 2 weeks after inoculation developed clear virus-like symptoms and were confirmed to be BCMV-positive using the ELISA test. To examine samples for possible mixed infections, two original *C. micans* and one *N. benthamiana* samples were collected, total RNA was extracted, depleted of ribosomal RNA, and submitted to the high-throughput sequence (HTS) analysis on a MiSeq platform, generating 3,533,626, 4,034,693, and 9,604,399 300-bp paired-end reads, respectively. The paired-end reads were adapter- and quality-trimmed, and following assembly the resulting contigs over 1 kb were screened with BLASTn and BLASTx programs against a custom-made viral sequence database. Three potyviruses were identified among the virus-specific contigs recovered from *C. micans*: three distinct isolates of BCMV, bean yellow mosaic virus (BYMV), and clover yellow vein virus (CIYVV). A nearly

complete genome was assembled for the CIYVV (9,520 nt with a coverage of 1,867 reads, deposited in GenBank under the accession no. MT631721) that displayed 95% identity to the Korean CIYVV isolate (KF975894) from soybean (Shin et al. 2014). All three viruses (BCMV, BYMV, and CIYVV) were detected in the original samples by RT-PCR with specific primers designed based on sequences of the newly identified contigs: BC_F1 (5'-TC GCTGCATCGTTTAGGGAG-3') and BC_R1 (5'-TTGGTGGCTGCAACTG TTCT-3') for BCMV (474 bp), BY_F (5'-TGCGATGCTGACGGATCTC-3') and BY_R (5'-CCTTTGAAGGGTTGTCATCAATCT-3') for BYMV (898 bp), and CY_F1 (5'-ACCGAAGGATATTGGGCACA-3') and CY_R1 (5'-CATGGTTTCCTTTCATTTGGTCCC-3') for CIYVV (754 bp). These RT-PCR products were sequenced directly, and corresponding partial sequences were deposited under the accession numbers MT631723 (BCMV), MT631722 (BYMV), and MT631724 (CIYVV). The RT-PCR derived partial sequence of CIYVV was found 100% identical to the HTS-generated nearly whole genome of the virus. The presence of two of the HTS-uncovered viruses in the *N. benthamiana* samples was confirmed by ELISA tests for BYMV (Agdia, Elkhart, IN) and for BCMV (Feng et al. 2014). BCMV and CIYVV were biologically separated from this mixed infection in *N. benthamiana* through a single-lesion selection on *Chenopodium quinoa* with subsequent back inoculations to *N. benthamiana*. These BCMV and CIYVV isolates produced distinct symptoms in *N. benthamiana*: vein clearing for CIYVV and mild mosaic and leaf deformation for BCMV. BYMV was previously reported in Hawaii from vanilla (Wang et al. 2017) and nasturtium (Wang et al. 2019), and BCMV was reported in Hawaii from flowering ginger (Larrea-Sarmiento et al. 2020) and from lima bean (Green et al. 2017). To the best of our knowledge, this is the first report of CIYVV found in Hawaii, and the first report of BYMV, BCMV, and CIYVV infecting *Crotalaria* spp.

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