Diseases Caused by Viruses

First Report of *Bougainvillea spectabilis* chlorotic vein-banding virus Infecting *Bougainvillea* Species in Hainan, China

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Bougainvillea, a species including *B. glabra*, *B. spectabilis*, and *B. peruviana*, is a perennial flowering plant widely grown in the world that is the symbol flower of Hainan Province in China. In August 2019, bougainvillea plants with leaves exhibiting virus-like symptoms including foliar chlorosis, vein-banding, leaf distortion, and wrinkling were observed in Danzhou, Haikou, and Baoting cities in Hainan Province with disease incidence exceeding 30% ($n = 100$). Symptomatic leaves of *B. glabra* from two different plants from Danzhou were collected and divided into two groups based on their symptoms: one group included leaves with leaf distortion and wrinkling, and the other leaves exhibiting chlorosis and vein-banding. Crude extracts of leaf samples from the two groups and an asymptomatic control were prepared by grinding 1 g of leaf tissue in 9 ml of sterilized pure water with a mortar and pestle. They were first examined by transmission electron microscopy after negative staining by phosphotungstic acid. Bacilliform virus particles of 160 to 185 nm long and 29 to 38 nm wide were observed in crude extracts of symptomatic leaf tissues, whereas no virus particles were observed in the asymptomatic control. The virus present in these samples was identified using

high-throughput sequencing (HTS). Leaf samples were sent to the Shanghai Sangon Biotech Co. for total RNA preparation. Small RNA libraries were subsequently built and sequenced using an Illumina HiSeq 2500 platform. A total of 9,488,366 and 7,811,515 reads were obtained for the two groups, respectively. After removing adaptors, the reads were de novo assembled using VELVET with a k-mer of 17, which generated 1,210 and 1,258 contigs (>50 nucleotides [nt]), respectively. Two longer contigs with a size of 8,715 and 8,697 nt were, respectively, obtained from these two sample groups. BLAST analysis revealed the first assembled contig (7,927 out of 8,715 bp) shared 98.9 and 98.6% sequence homology with the complete genome of *bougainvillea spectabilis* chlorotic vein-banding virus (BsCVBV) isolates from Malaysia (MK816926) and Taiwan (EU034539), respectively. The other assembled contig (7,922 out of 8,697 bp) shared 99.0 and 98.6% identity with the complete genome of BsCVBV isolates from Malaysia and Taiwan, respectively. To further confirm the occurrence of this virus in source samples, viral DNA of the two samples used for HTS was extracted, and polymerase chain reaction (PCR) was performed using the primers BadnaF (5'-AAAGACCATGAGAAGCATC-3') and BadnaR (5'-ATAAGCAT CAGGGGGTG-3') (Alexandre et al. 2016). Amplicons of the expected size of 400 bp were generated by PCR and sequenced. The two amplicons shared 100% nucleotide identity, and one sequence was deposited in GenBank (accession no. MN764362). BLASTn analysis showed that the amplified fragment was 99.8% identical to BsCVBV isolate from Taiwan (DQ347841). Furthermore, 14 leaf samples, from eight symptomatic (six from *B. glabra* and two from *B. spectabilis*) and six asymptomatic plants (five from *B. glabra* and one from *B. spectabilis*) were used for the molecular detection of the virus by PCR. Amplicons of the expected size (400 bp) were generated for the eight symptomatic plants, whereas no PCR product was obtained using leaves of six asymptomatic plants. Bougainvillea infections by BsCVBV have been reported from Brazil, India, Malaysia, and Taiwan (Alexandre et al. 2016; Baranwal et al. 2010; Tsai et al. 2008; Yusop et al. 2019). To our knowledge, this is the first report of BsCVBV infection of *Bougainvillea* in Hainan, China. This study lays the groundwork for the detection of the virus, and further work is necessary to determine the distribution of the virus and its impact on *Bougainvillea* cultivation in Hainan.

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