Diseases Caused by Viruses

Mulberry (*Morus alba*) Is a New Natural Host of Citrus Leaf Blotch Virus in China

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Mulberries (*Morus* spp., family Moraceae) are economically important deciduous woody plants. Their leaves are food for silkworms, and both the fruits and leaves have nutritional and medicinal values (Qin et al. 2012). The plants are widely distributed globally and have been cultivated in China for more than 5,000 years (Xie et al. 2014). In April 2019, virus-like symptoms of chlorotic leaf spots and occasionally witches' broom were observed in trees of white mulberry (*M. alba*) in Shapingba district of Chongqing province. To investigate if any potential viral agent is associated with the symptoms, total RNA was extracted from leaves of one symptomatic tree using an RNAPrep Pure Plant Plus Kit (Tiangen, China). Ribosomal RNA was depleted using a TruSeq RNA Sample Prep Kit (Illumina, U.S.A.), and the depleted RNA was used for construction of a cDNA library for sequencing using an Illumina HiSeq X-ten platform with paired-end reads length of 150 bp. Adaptors, low-quality reads, and mulberry genome-derived reads (He et al. 2013) were removed from a total of 25,433,798 reads using CLC Genomics Workbench 11 (Qiagen, U.S.A.), and the clean reads of 936,562 were

subjected to de novo assembly that generated 4,278 contigs (200 to 3,862 bp). These sequences were annotated by BLASTx searches to local Viruses_NR and viroid datasets downloaded from GenBank. Finally, except for three contigs (3,862, 1,950, and 1,179 nt) with 81.4 to 90% nucleotide sequence identities to *Citrus leaf blotch virus* (CLBV, genus *Citrivirus*, family *Betaflexiviridae*), no other contigs were identified as viral-related. Total clean reads of 113,185 were mapped to the viral contigs with average coverage depth of 1,915, suggesting the presence of CLBV in the symptomatic tree. To recover the complete genome of CLBV, overlapping fragments were amplified by RT-PCR using virus-specific primer pairs. The 5' and 3' termini were determined by rapid amplification of cDNA ends (RACE kit, Invitrogen, U.S.A.). Five clones per amplicon were sequenced in two directions (Cao et al. 2018). The complete genome of the mulberry strain of CLBV (CLBV-ML, GenBank accession no. MT767171) is 8,776 nt in length, excluding the poly(A) tail. CLBV-ML is similar to extant CLBV isolates in genome structure. BLASTn analysis showed that CLBV-ML had the highest nucleotide sequence identities of 79.65 to 81.56% with *Actinidia* isolates (Liu et al. 2019) of CLBV at the whole genome level. Phylogenetic analysis also placed it with the *Actinidia* isolates, indicating they are closely related. Thus, CLBV-ML is a highly divergent strain of CLBV. To study the occurrence of CLBV-ML, a total of 62 mulberry samples (42 with similar symptoms and 20 without symptoms) were randomly collected from Shapingba and tested by conventional RT-PCR using an isolate-specific primer pair (CLBV-F7182: ACCAATGACAATGCCACA; CLBV-R7857: TTATGAAACTCTTCCCACTT) designed in the CP gene to amplify a 676-bp fragment. The virus was detected in 37 symptomatic trees (88%) and 2 (10%) asymptomatic trees, suggesting the association of CLBV-ML with the symptoms. To the best of our knowledge, this is the first report of CLBV infection in mulberry, which expands the host range of CLBV.

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