Diseases Caused by Viruses

First Report of Peach Leaf Pitting-Associated Virus, Plum Bark Necrosis Stem Pitting-Associated Virus, and Mume Virus A from Mei (*Prunus mume*) in China

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Mei (*Prunus mume* Sieb. et Zucc.), widely distributed in East Asian countries for both fruiting and flowering purposes, is susceptible to viral infections (Marais et al. 2018). Infection by plum bark necrosis stem pitting-associated virus (PBNSPaV) or little cherry virus 2 possibly caused overall yield loss in mei in Japan due to incomplete flower development, low fruit bearing rate, and interveinal chlorosis (Numaguchi et al. 2019). Virus-like disease showing mosaic, interveinal chlorosis, vein clearing, or necrotic spot on leaf was observed in mei trees in Beijing, Wuhan, Wuxi, and Nanjing in spring and early summer from 2017 to 2018. Symptomatic leaves collected from the four regions were pooled as two samples for RNA-sequencing analysis. After ribosomal RNA depletion, total RNA extracted by TRNzol reagent (Tiangen, China) was subjected to library construction using a NEBNext Ultra RNA Library Prep Kit (New England Biolabs, U.S.A.) and sequenced on an Illumina HiSeq 4000 (Novogene, China). Sequencing data were filtered, screened, and assembled as described previously (Zhou et al. 2020) to generate contigs, followed by BLAST-x/n search in viral genomes in GenBank. We identified >300 contigs (208 to 10,756 nt) homologous to Asian prunus virus 1 and Asian prunus virus 2 (APV1 and 2), mume virus A (MuVA), PBNSPaV, and peach leaf pitting-associated virus (PLPaV), with 71 to 100% nucleotide sequence identity values. APV1 and 2 have been reported in mei in China (Wang et al. 2018). Here, we focused on the other three viruses. Contigs

homologous to these three viruses were further assembled into three scaffolds of 14,224, 1,107, and 753 nt for PBNSPaV, MuVA, and PLPaV, respectively. The scaffold of PBNSPaV (MW217574) nearly covered the whole genome of the isolate VIC3 from Australia (LC523039.1) (Kinoti et al. 2020) with 92.30% sequence identity; the scaffold of MuVA (MW217572) covered 14.50% of the genome of the isolate pm14 from Japan (NC_040568.1) (Marais et al. 2018) with 98.47% sequence identity; and the scaffold of PLPaV (MW217573) covered 15.26% of the genome of the isolate XJ-6 from peach (KY867750.1) (He et al. 2017) with 85.23% sequence identity. Presence of the three viruses was verified by RT-PCR detection using designed specific primers for PBNSPaV (forward: 5'-CAACAAACTCCACAGCGG-3' [positions 4,014 to 4,033, NC_009992.1] / reverse: 5'-GCCAAAAGAAGTGCTGGTGG-3' [positions 4,659 to 4,640, NC_009992.1]), MuVA (forward: 5'-AAGAGAATTACTTCAATGCCCTC-3' [positions 171 to 194, NC_040568.1] / reverse: 5'-GATATCCAAGATACGATTAGCCAG-3' [positions 533 to 510, NC_040568.1]), and PLPaV (forward: 5'-GCTATATCTCAACA CTGCAAGAA-3' [positions 5,798 to 5,821, KY867750.1] / reverse: 5'-GAG TGATACATAGTCCACAGAGAT-3' [positions 6,045 to 6,022, KY867750.1]). The amplified 626-, 350-, and 251-bp fragments of PBNSPaV, MuVA, and PLPaV had 91.47, 98.07, and 81.89% sequence identity to their respective reference sequences. This is the first report of PBNSPaV and MuVA infecting mei in China, and more importantly, the first report of a new host for PLPaV. In addition, 30 collected leaf samples from Nanjing and Wuhan were analyzed by RT-PCR, and 15, six, and five samples tested positive to PLPaV, PBNSPaV, and MuVA, respectively. Although it is difficult to link a particular virus with the observed symptoms due to mixed infections, the symptoms were significantly associated with viral infection because almost all symptomatic leaf samples were positive for one or more viruses. Further studies would be required to determine the distribution and impact of these viruses on mei trees and other stone fruit species and to understand the possibility that mei trees may play a role in PLPaV epidemiology.

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