First Report of Cherry Virus F Infecting Japanese Plum in Korea

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Cherry virus F (CVF) is a tentative member of the genus *Fabavirus* in the family *Secoviridae*, consisting of two RNA segments (Koloniuk et al. 2018). To date, CVF has been documented in only sweet cherry (*Prunus avium*) in the Czech Republic (Koloniuk et al. 2018), Canada, and Greece. In May 2014, we collected leaf samples from four symptomatic (leaf spots and dappled fruits) and two asymptomatic Japanese plum cultivars (Sun and Gadam) grown in an orchard in Hoengseong, South Korea, to identify viruses and viroids infecting plum trees. Total RNA from individual plum trees was extracted using two commercial kits: Fruit-mate for RNA Purification Kit (Takara, Shiga, Japan) and RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). We generated six mRNA libraries from the six different plum cultivars for RNA sequencing using the TruSeq RNA Library Preparation Kit v2 (Illumina, San Diego, CA) as described previously (Jo et al. 2017). The mRNA libraries were paired-end (2 × 100 bp) sequenced with a HiSeq 2000 system (Macrogen, Seoul, Korea). The raw sequence reads were de novo assembled by Trinity program v. 2.8.6, with default parameters (Haas et al. 2013). The assembled contigs were subjected to BLASTX search against the nonredundant protein database in NCBI. Of

the two asymptomatic cultivars, the transcriptome of asymptomatic plum cultivar Gadam contained five contigs specific to CVF. Two and three contigs were specific to CVF RNA1 (2,571 reads, coverage 42.15%) and RNA2 (2,025 reads, coverage 53.04%), respectively. The size of these five contigs ranged from 241 to 5,986 bp. Contigs of 5,986 and 3,867 bp in length, referred to as CVF isolate Gadam RNA1 (GenBank MN896996) and RNA2 (GenBank MN896995), respectively, were subjected to BLASTP search against NCBI's nonredundant protein database. The results showed that the polyprotein sequences of RNA1 and RNA2 shared 95.3 and 93.11% amino acid identities with isolates SwC-H_1a from the Czech Republic (GenBank AWP36326) and Stac-3B_c8 from Canada (AZZ10055), respectively. To confirm the infection of CVF in cultivar Gadam, RT-PCR was conducted using CVF RNA1-specific primers designed based on the CVF reference genome sequences (MH998210 and MH998216), including 5'-CCACCAAATAGGCAGAGGTCAC-3' (position 3,190 to 3,212) and 5'-CACAATCACCATCAATGGTCTCTGC-3' (position 3,742 to 3,766), and CVF RNA2-specific primers, including 5'-CTGCTTTATGATGCTAGACATCAAGATG-3' (position 1,015 to 1,042) and 5'-ACAATAGGCATGCTCATCTCAACCTC-3' (position 1,594 to 1,619). We amplified 577-bp RNA1-specific and 605-bp RNA2-specific amplicons that were cloned and then performed Sanger sequencing. Sequencing of the cloned amplicons for isolate Gadam RNA1 (GenBank MN896993) and RNA2 (GenBank MN896994) revealed values of 99.48 and 99.17% nucleotide identity to that of RNA1 and RNA2 determined by high-throughput sequencing, respectively. Additionally, we tested five plants for each of the six plum cultivars grown in the same orchard. The detection of CVF was carried out through PCR using the primers and protocol described above. Of the 30 trees, CVF was detected in three trees of cultivar Gadam by both primer pairs. To our knowledge, this is the first report of CVF infecting Japanese plum and the first report of the virus in Korea. However, its prevalence in other *Prunus* species, including apricot, European plum, and peach, should be further elucidated.

References:

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