Diseases Caused by Viruses

First Report of Apple Rubbery Wood Virus 2 Infection of Apples in China

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Apple (*Malus*) is one of the most widely grown fruit trees worldwide, and viral diseases can severely inhibit its growth and development. Apple rubbery wood virus 2 (ARWV-2, family *Phenuiviridae*) is a negative-sense single-stranded RNA virus whose genome comprises three RNA segments (large [L], medium [M], and small [S]) (Rott et al. 2018). This virus is associated with apple rubbery wood disease (Rott et al. 2018) and has previously been found in pear (*Pyrus* spp.) in China (Wang et al. 2019). In autumn 2019, six trees (one each of cvs. Honglu, Hongzhengzhu, Jinxiuhaitang, Liquanduanfu, Huahong-1, and Huahong-2) showing mosaic disease-like symptoms in the leaves and two trees (one each of cvs. Qingming-1 and Qingming-2) showing rusty skin symptoms (i.e., many irregular rust spots on the peel's surface) in the fruits were found in Xingcheng, Liaoning province, China. Shoots of the diseased plants were collected, and total RNA was extracted from the phloem of the samples as described by Hu et al. (2015). RT-PCR was used to detect various viruses including apple chlorotic leaf spot virus (ACLSV), apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), apple necrotic mosaic virus (ApNMV), and ARWV-2 as well as apple scar skin viroid (ASSVd) using their respective primers. ACLSV, ASPV, and ASGV were detected in all samples. ApNMV was detected in the six trees with leaf mosaic symptoms, and ASSVd was detected in the two trees with apple rusty skin symptoms. Moreover, five trees (cvs. Honglu, Hongzhengzhu, Jinxiuhaitang, Qingming-1, and Qingming-2) tested positive for ARWV-2 in the RT-PCR assay. The

PCR products of ARWV-2 from Honglu and Qingming-2 were cloned into the pMD18-T vector (Takara, China), and one clone of each of the samples was sequenced. BLASTn analyses showed that they shared 98.2 to 99.2% nt identity with ARWV-2 sequences (MT901298 to MT901299) deposited in GenBank. A small RNA library was prepared for high-throughput sequencing (HTS) with the Solexa-Illumina platform using phloem tissue collected from a Qingming-2 tree in which apples with rusty skin symptoms were observed. A total of 3,7746,671 reads were obtained from the library. De novo assembly of the reads yielded 1,378 viral sequence contigs. Of those, 20 contigs with lengths ranging from 82 to 387 nt were mapped to the reference genome of ARWV-2 (MT733339 to MT733344, MT901300 to MT901313). In addition, contigs of ACLSV, ASPV, ASGV, ApNMV, and ASSVd were detected. To further confirm the HTS results, partial length fragments of segments L (717 bp), M (645 bp), and S (657 bp) of the ARWV-2 genome were amplified from Qingming-2 using primers and sequenced. The resulting sequences, which were deposited in GenBank as MT364372 to MT364374, showed 97.2, 97.8, and 98.0% nt identity, respectively, with the corresponding segments of ARWV-2 isolate R7 (MF062144 to MF062146). To understand the infection status of apple trees in China with regard to ARWV-2, 116 apple shoot samples were randomly collected from commercial orchards in Liaoning, Shanxi, and Shandong provinces and subjected to RT-PCR to detect ARWV-2, ACLSV, ASGV, ASPV, ASSVd, and ApNMV. In total, 49 (42.2%) of the 116 samples tested positive for ARWV-2, suggesting that this virus is widespread in apple trees in China. The mixed-infection rates of ARWV-2/ApNMV and ARWV-2/ASSVd were 18.1% (21/116) and 3.4% (4/116), respectively. Among the 46 ARWV-2-positive samples, seven had mosaic disease-like symptoms in the leaves and three had rusty skin symptoms in the fruits. To our knowledge, this is the first report of ARWV-2 infection in apples showing rusty skin symptoms, as well as the first report of ARWV-2 infection in domestic apples in China. Further research is needed to understand the distribution of ARWV-2 in apple orchards throughout China, to confirm the relationship of ARWV-2 with different symptoms, and to evaluate how ARWV-2 affects the performance and quality of apple.

References:

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