First Report of Apple Rubbery Wood Virus 1 in Apple in China

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More than 30 viral and subviral pathogens infect apple (*Malus domestica*, an important fruit crop in China) trees and rootstocks, posing a threat to its production. With advances in diagnostic technologies, new viruses including apple rubbery wood virus 1 (ARWV-1), apple rubbery wood virus 2 (ARWV-2), apple luteovirus 1 (ALV), and citrus virus A (CiVA) have been detected (Beatriz et al. 2018; Hu et al. 2021; Rott et al. 2018). ARWV-1 (family *Phenuiviridae*) is a negative-sense single-stranded RNA virus with three RNA segments (large [L], medium [M], and small [S]). It causes apple rubbery wood disease (Rott et al. 2018) and is found in apple rootstocks, causing leaf yellowing and mottle symptoms in Korea (Lim et al. 2019). To determine virus prevalence in apple trees in China, 200 apple leaf and shoot samples were collected from orchards in Hebei ($n = 26$), Liaoning (40), Shandong (100), Yunnan (25), Shanxi (4), and Inner Mongolia (5) in 2020. Total RNA was extracted from the shoot phloem or leaf (Hu et al. 2015) and subjected to reverse transcription (RT)-PCR to detect apple chlorotic leaf spot virus (ACLSV), apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), apple necrotic mosaic virus (ApNMV), apple scar skin viroid (ASSVd), ARWV-2, ARWV-1, ALV, and CiVA, using primers specific to respective viruses. The prevalence of ACLSV, ASPV, ASGV, ApNMV, ASSVd, ARWV-2, ARWV-1, ALV, and CiVA was found to be 75.5, 85.5, 86.0, 43.0, 4.0, 48.5, 10.5, 0, and 0%, respectively. Among the 21 positive

samples for ARWV-1, three, five and 13 samples were from Hebei, Liaoning, and Shandong, respectively. Five ARWV-1-positive samples (cultivars Xinhongjiangjun, Xiangfu-1, Xiangfu-2, and Tianhong) showed leaf mosaic symptoms. To confirm ARWV-1 by RT-PCR, amplicons from Xiangfu-1 and Tianhong were cloned into the pMD18-T vector (Takara, Dalian, China), and three clones of each sample were sequenced. BLASTn analyses demonstrated that the sequences (accession nos. MW507810 to MW507811) shared 96.9 to 98.9% identity with ARWV-1 sequences (MH714536, MF062127, and MF062138) in GenBank. An lncRNA library was prepared for high-throughput sequencing (HTS) with the Illumina HiSeq platform using Xiangfu-1 RNA. A total of 71,613,294 reads were obtained. De novo assembly of the reads revealed 135 viral sequence contigs of ACLSV, ASGV, ASPV, ApNMV, ARWV-1, and ARWV-2. The sequences of contig-100_88981 (302 nt) and contig-100_25701 (834 nt) (accession nos. MW507821 and MW507820) matched those of segment S from ARWV-1, whereas the sequences of contig-100_6542 (1,660 nt) and contig-100_27 (7,364 nt) (accession nos. MW507819 and MW507818) matched those of segments M and L, respectively. To confirm the HTS results, fragments of segments L (744 bp), M (747 bp), and S (554 bp) from Xiangfu-1 and Tianhong were amplified and sequenced. The sequences (accession nos. MW507812 to MW507817) showed 94.8 to 99.9% nucleotide identity with the corresponding segments of ARWV-1. Coinfection of ARWV-1 with ApNMV and/or ARWV-2 was confirmed in 17/21 ARWV-1-positive samples. The prevalence of ARWV-1/ApNMV, ARWV-1/ARWV-2, and ARWV-1/ApNMV/ARWV-2 infections was 61.9, 71.4, and 52.4%, respectively. To our knowledge, this is the first report of ARWV-1 infecting apple trees in China. Further research is needed to determine whether and how ARWV-1 affects apple yield and quality.

References:

- Beatriz, et al. 2018. Front. Microbiol. 2:203.
- Hu, G. J. et al. 2015. Australas. Plant Pathol. 44:167.
- Hu, G. J., et al. 2021. Plant Dis. 105:519.
- Lim, S., et al. 2019. Plant Dis. 103:591.
- Rott, M. E., et al. 2018. Plant Dis. 102:1254.

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