Diseases Caused by Viruses

First Report of *Apple rubodvirus 2* Infecting Pear (*Pyrus communis*) in South Africa

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Apple rubbery wood virus 2 (ARWV-2; Rott et al. 2018) belongs to the species *Apple rubodvirus 2*, a member of the genus *Rubodvirus* (family *Phenuiviridae*; Kuhn et al. 2020). ARWV-2 was first identified in apples and is associated with apple rubbery wood disease (ARWD), which is characterized by unusual flexibility of stems and branches, reduced growth, shortened internodes, and increased cold sensitivity (Jakovljevic et al. 2017; Rott et al. 2018). ARWD was first reported in 1935 in England on apple and has since been found on quince and pear (Jakovljevic et al. 2017; Rott et al. 2018). In January 2021, leaves were collected from a pear tree (*Pyrus communis* cv. 'Forelle', F514) in a commercial orchard near Villiersdorp, South Africa. The tree displayed no foliar or tree branch symptoms except for malformed fruits potentially due to insect feeding damage or pear stony pit disease previously associated with infection of apple stem pitting virus (ASPV) (Paunovic et al. 1999). Leaf petioles (1 g) were used for total RNA extraction, using a modified CTAB extraction protocol (Ruiz-García et al. 2019). A sequencing library was constructed (Illumina TruSeq Stranded Total RNA with plant Ribo-Zero) and sequenced on an Illumina HiSeqX instrument (Macrogen, South Korea). A total of 30,709,182 paired-end reads (100 nt) were obtained and trimmed for quality with Trimmomatic (SLIDINGWINDOW:3:20, MINLEN:20) (Bolger et al. 2014). De novo assembly, using default parameters of CLC Genomics Workbench 11.0.1 (Qiagen), resulted in 97,294 contigs. BLASTn analysis identified 17 viral

contigs, with 14 contigs having high nucleotide identity to ASPV and three to ARWV-2. The latter contigs included all three segments of ARWV-2. The L contig was 7,371 nt, M was 1,289 nt, and S was 1,463 nt in length, generated with 7,341, 626, and 9,161 reads for segments L, M, and S, respectively. Segment S had the highest read coverage (524.87×), followed by segments L (88.07×) and M (36.60×). The ARWV-2 GenBank accessions with the highest percentage identity to the contigs were MF062128.1 from the United States of America (98.2% to segment L), MN163134.1 from China (97.5% to segment M), and NC_055535.1 from Germany (93.5% to segment S). The contigs spanned 100, 80.92, and 100% of these accessions of segments L, M, and S, respectively, and were deposited in GenBank as accessions MZ593725 to MZ593727. Reverse transcription polymerase chain reaction was used to validate the presence of ARWV-2 in sample F514, using primers directed at segments L (con708_178F/con708_666R), M (ARWV-2S1_38F/ARWV-2S1_682R), and S (ARWV-2M567F/ARWV-2M1342R) (Rott et al. 2018). Amplicon sequences (510 bp [L], 645 bp [M], and 799 bp [S]) were confirmed with bidirectional Sanger sequencing. Fifty-nine additional pear samples were surveyed in 2021 for ARWV-2 using the M segment assay mentioned above. The survey included the Koue Bokkeveld and Elgin areas, and cultivars 'Bosc' (22 samples), 'Abate' (10 samples), 'Rosemarie' (3 samples), 'Forelle' (9 samples), 'Packham's Triumph' (12 samples), and 'Early Bon Chretien' (3 samples). A total of 27 samples (11 samples from the Koue Bokkeveld region and 16 samples from the Elgin region) tested positive for ARWV-2, demonstrating the common presence of this virus in pears in South Africa. This is the first report of ARWV-2 infecting pear in South Africa. Although no association with disease symptoms were observed, this study expands the data on the incidence and distribution of this virus in South Africa.

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