First Report of Plum Bark Necrosis Stem Pitting-Associated Virus in Japanese Plums in South Africa

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Plum bark necrosis stem pitting disease was first reported in 1996 on Japanese plum (*Prunus salicina*) cultivar Black Beaut when graft-inoculated trees developed disease symptoms (Uyemoto and Teviotdale 1996). The potential causal agent was identified to be *Plum bark necrosis stem pitting-associated virus*, a member of the genus *Ampelovirus* in the family *Closteroviridae* (Marini et al. 2002; Martelli et al. 2012). Symptoms associated with plum bark necrosis stem pitting-associated virus (PBNSPaV) are host dependent and include bark necrosis and stem pitting (Boscia et al. 2011); however, latent infections have also been observed in sweet cherry and apricot (García-Ibarra et al. 2010; Ruiz-García et al. 2020). In the summer of 2018, a representative sample was collected from a *P. salicina* cv. Angeleno tree (LH4) in the Western Cape, South Africa, and total RNA was extracted from 1 g of leaf petioles using a modified CTAB extraction protocol (Ruiz-García et al. 2019). Ribo-depleted RNA was prepared (Ribo-Zero Plant kit) and a sequencing library constructed (Illumina TruSeq Stranded Total RNA). The RNA library was paired-end (2 × 100 bp) sequenced on an Illumina NovaSeq instrument (Macrogen, South Korea). Quality-trimmed data (26.4 million read pairs) were used in a de novo assembly using SPAdes version 3.14 (Nurk et al. 2013) (coverage cutoff = 20). BLASTn analysis found 34 PBNSPaV-related contigs from 29,332 assembled. The longest contig (14,209 nucleotides [nt]) had an average read depth of 88.5, indicating the presence of PBNSPaV in sample LH4. This contig had the highest nt sequence identity to isolate PR258-2 (KC590346) (98.95%), spanning 99.83% of the genome. Contigs with the closest nt similarity (~98 and ~92%) to accessions EF546442 and KU240013, respectively, were also identified, signifying the potential presence of three PBNSPaV variants in a single tree. Simultaneous read mapping to the three accessions (KC590346, EF546442, and KU240013) was performed using CLC Genomics Workbench 11.0.1 (Qiagen) (length

fraction = 0.9, similarity fraction = 0.9; reads with more than one match were assigned randomly). A total of 80,405 reads mapped to KC590346, EF546442, and KU240013, representing 43.74, 39.79, and 16.47% of the mapped reads. The consensus sequence obtained after mapping the reads to KC590346 spanned 99.84% of the KC590346 genome. This consensus sequence was submitted to GenBank as a near-complete genome of 14,197 nt (MT271231). The consensus sequences generated from mapping the reads to EF546442 and KU240013 had 97.29 and 95.29% nt identity to the respective references and spanned 99.79 and 76.46% of the genomes, respectively. These two consensus sequences had 96% nt sequence similarity and were 83.12 and 86.49% similar to MT271231. No other virus- or viroid-related contigs were identified in the sample. To validate the presence of PBNSPaV in sample LH4, reverse transcription polymerase chain reaction (RT-PCR) was used to amplify a partial region of P61 using primers PBN-F1 and PBN-R1 (Marais et al. 2014). The 319-bp amplicon was bidirectionally Sanger sequenced (Central Analytical Facility, Stellenbosch University) and additionally confirmed PBNSPaV in sample LH4. Ten additional samples from the same orchard as LH4, 10 samples from a different Angeleno orchard on the same farm, and 19 Angeleno samples from a young orchard on a different farm in the Western Cape were sampled and screened using the PBN-F1/PBN-R1 RT-PCR assay. Thirty-eight of these samples were positive for PBNSPaV, further confirming the presence of the virus in Japanese plum cultivar Angeleno in South Africa. No stem pitting and bark symptoms were observed in any of the trees analyzed. To our knowledge, this is the first report of PBNSPaV infecting Japanese plums in South Africa. This study adds information on the distribution of this pathogen and will contribute to the updating of the South African certification scheme.

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