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First Report on Detection of Three Bunya-Like Viruses in Apples in Brazil

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Apple chlorotic leaf spot virus (ACLSV), apple stem grooving virus (ASGV), and apple stem pitting virus (ASPV) cause significant losses to Brazilian (BR) apple production. Looking beyond these latent viruses, high-throughput sequencing (HTS) of three samples (Vacaria, Brazil) was performed on an Illumina HiSeq X Ten platform (USA), cv. Braeburn (BB), and a BGISEQ-500 platform (China), cvs. Royal Gala (RG) and Mishima (MI). Total nucleic acids (TNA) extracts were enriched for ds-RNA (Valverde et al. 1990) to prepare cDNA


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for HTS libraries using a TruSeq Stranded mRNA kit (Illumina). CLC Genomics Workbench v. 8.5.1 (CLC Bio, Qiagen, USA) was used for quality trimming and de novo contig assembly of the reads. All contigs were annotated by BLASTn and BLASTx against the nr or protein GenBank databases using a cut-off e-value of 10^{-4} . Multiple nearly complete genome contigs corresponding to ACLSV, ASGV, and ASPV were obtained from all three cvs., showing 78 to 95% (ACLSV; MK929793 to 96), 80 to 98.5% (ASGV; MK923757, MK929791 to 92), and 75 to 87% (ASPV; MK923753 to 56) nucleotide (nt) identities to sequences in GenBank; reads mapping to reference genomes are in the supplementary materials. Long contigs were obtained for apple rubbery wood virus 1 (ARWV 1; MI), ARWV 2 (RG), and citrus concave gum-associated virus (CCGaV; RG and MI). The three viruses are related to the order *Bunyavirales*, family *Phenuiviridae*. Total reads mapped to the reference genomes were 1,958 (ARWV 1; MI) and 905 (ARWV 2, RG), corresponding to average genome coverages of 19.3 and 8.6 times, respectively. The two viruses represented 0.004% of total reads for each of the apple samples. For CCGaV, a higher representation in the sequencing reads was observed, with 50,835 mapped reads (0.24% of total reads, average coverage 541.7 times) and 32,099 mapped reads (0.07% of total reads, average coverage 342.1 times), respectively, in RG and MI. Using the nearly complete assembled contigs for the various genomic RNAs, the BR isolates of ARWV 1 (MK936225 to 27) and ARWV 2 (MK936228 to 30) were shown to be closely related to those in GenBank with 95 to 96%, 94 to 96%, and 95.8 to 98.7% nt identities with the L, M, and S segments of ARWV 1 and 96 to 97%, 96.9 to 97.2%, and 97.2 to 97.7% nt identities with the L, Ma, and Sa segments of ARWV 2, respectively. CCGaV,

recently described in citrus, is phylogenetically related to phlebo-like viruses (Navarro et al. 2018) and possibly associated with an apple decline (Wright et al. 2018). Based on the long contigs assembled, the RNAs 1 (MK940540 to 41) and 2 (MK940542 to 43) of the two BR isolates had nt identities of 97 to 99% with the homologous sequences in GenBank. To validate the HTS-based results in apples, RT-PCR assays were performed on TNA of three resampled original source plants using primers based on the HTS contigs. ARWV 1 S (MN652009) and ARWV 2 Sa (MN652010) RT-PCR amplicons from BB showed 98.4 and 99.6% nt identities with sequences obtained by HTS (MK936225 and MK936228, respectively). The CCGaV RNA 2 (MN631048) RT-PCR amplicon from RG showed 94.8% nt identity with the sequence obtained by HTS (MK940542). To determine prevalence of ARWV 1 and 2 and CCGaV, TNA from 12 additional apple cvs. was confirmed by RT-PCR, using specific primers. Amplicons of the expected sizes were detected, respectively, in nine, one, and two of the analyzed plants. To our knowledge, this is the first report of ARWV 1, ARWV 2, and CCGaV infecting apples in Brazil. Apple rubbery wood disease (ARWD), first described almost a century ago, damaged apple orchards in the 1970s to 1990s in Brazil. Recently, Rott et al. (2018) reported ARWV 1 and ARWV 2 in ARWD-affected apple plants in Germany. These results confirmed the findings of Wright et al. (2018) and highlight the presence of ARWV 1 and 2 and CCGaV in BR apple materials, suggesting the need to consider them in certification programs. Additional studies on the prevalence and possible effects of these viruses on apples in this region are necessary.

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