First report on occurrence of apple hammerhead viroid in apples in Brazil

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Latent viruses have historically caused heavy losses to apple production in Brazil. Other uncharacterized agents of virus or virus-like nature and viroids also cause damages to the apple industry. Eight viroids are known to infect apples. Apple hammerhead viroid (AHVd), first reported in China (Zhang et al., Plant Disease, 10.1371/journal.ppat.1004553, 2014), was detected in plants showing shoot decline/dieback, trunk splitting, mosaic and/or ringspots, and is now considered to be spread worldwide. AHVd was recently proposed as a new member of the genus *Pelamoviroid*, family *Avsunviroidae* (Serra et al., Virus Research, 249, 8, 2018). To acquire a more comprehensive picture on viruses and viroids present in Brazilian apple orchards, high-throughput sequencing (HTS) of leaf extracts of cv. Royal Gala (Vacaria, RS, Brazil) was performed on the BGI platform (China). Complementary DNA of total nucleic acid extracts enriched for double-stranded RNA (Valverde et al., Plant Disease 74, 255, 1990) was used to prepare sequencing libraries for HTS using TruSeq Stranded mRNA kit (llumina, USA). CLC Genomics Workbench v. 8.5.1 (CLC Bio, Qiagen, Germany) was used for quality trimming and *de novo* contig assembly from reads. Contigs were annotated by BLASTn and BLASTx against the GenBank database using 10^{-4} as cut-off e-value. Additionally to several full-length sequences of latent viruses and three other viruses reported elsewhere (Nickel et al., Plant Disease, 104, 11, 2020), a 436 nucleotides (nt) contig was detected showing 84.02 to 97.48% nt sequence identity with AHVd GenBank sequences. This full-length sequence was deposited at NCBI GenBank under accession number MK947213; phylogenetically the Brazilian isolate is closest to Canadian and Korean AHVd isolates. Presence of AHVd in the original cv. Royal Gala

HTS-samples was confirmed by conventional RT-PCR assays using the OneStep RT-PCR kit (Qiagen, USA) and primers AHVd1-F1 5´ CCCCTCCGGTCTTGTCCAAC 3´ and AHVd1-R1 GAGTCCTTTTAGGACGAAACTTA whose sequence was derived from that of the original contig. RT-PCR fragments of the expected size were also detected in cvs. Fuji, Belgolden, Mishima, Braeburn and two hybrid apple plants out of twelve different cvs. analysed to estimate the prevalence of AHVd in commercial apple orchards. The Brazilian isolate of AHVd showed high nucleotide variability. Additional studies are required for a more comprehensive view on prevalence and possible damaging effects of AHVd to apples.

Palavras-chave: *Malus domestica,* HTS, *Pelamoviroid*, *Avsunviroidae.*
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