First Report of Grapevine Virus H in Grapevine in Greece

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Grapevine virus H (GVH) is a member of the genus *Vitivirus* in the family *Betaflexiviridae* (subfamily *Trivirinae*, order *Tymovirales*) that infects grapevine (Candresse et al. 2018). GVH was first identified in a symptomless grapevine of an unknown cultivar from Portugal in 2018 (Candresse et al. 2018), and since then the virus has been reported only from California (Diaz-Lara et al. 2019). Several vitiviruses have been detected in Greek vineyards (Avgelis and Rumbos 2000; Dovas and Katis 2003a, b; Lotos et al. 2020; Panailidou et al. 2019), but no information was available on the presence of GVH. In the fall of 2020, in order to investigate the virome of a commercial vineyard of the cultivar Assyrtiko in northern Greece, a composite sample was made of leaves and petioles from nine vines exhibiting leafroll disease symptoms. Total RNA was extracted from the composite sample according

to the protocol of White et al. (2008) and subjected to rRNA depletion, library construction (TruSeq Stranded Total RNA kit), and high-throughput sequencing (HTS) in a NovaSeq6000 platform (Illumina) at Macrogen (Korea). The resulting ~42 million 101-nt paired-end reads were analyzed in Geneious Prime 2020, and the assembled de novo contigs were subjected to a local BLASTn search, which revealed the presence of 18 grapevine-infecting viruses and viroids, among which also was a GVH-like contig (GeA-9). GeA-9 was 7,404 nucleotides (nt) long, covering 99.4% of the full virus genome, and shared 98.2% nt identity with a GVH isolate from the United States (MN716768.1). To confirm the presence of GVH, the nine samples of the cultivar Assyrtiko, used initially to produce the composite sample for HTS analysis, were tested individually by reverse transcription polymerase chain reaction, using the primers GVH_F_2504 (5'-CTGCTTCGCTGAACATA TGC-3') and GVH_R_2835 (5'-ATCATTTTGATCGAGAGAGTAGTG-3') that amplify a 331-nt segment of ORF1. GVH was detected in five out of the nine tested samples, and one of these was reamplified and subjected to Sanger sequencing. The fragment of ORF1 obtained by Sanger sequencing (MW460005) was 97.5% identical to the nucleotide sequence of the corresponding GVH-like de novo contig (GeA-9) from HTS analysis, and it shared 97.2% nt identity with GVH sequences reported from Portugal and the United States, respectively (NC_040545.1 and MN716768.1), confirming the presence of GVH in the tested samples. This is the first report of GVH in grapevine in Greece, thus further increasing the number of vitiviruses known to infect Greek vineyards and also expanding the number of geographic locations in which GVH is recorded so far.

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