First Report of Grapevine virus E and Grapevine virus F in Grapevine in Greece

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Grapevine virus E (GVE) and grapevine virus F (GVF) are members of the genus *Vitivirus* (family Betaflexiviridae) (Nakaune et al., 2008; Al Rwahnih et al., 2012) with a worldwide distribution. Even though grapevine viruses A and B, two other vitiviruses, are prevalent in Greek vineyards, no other member of the genus Vitivirus has been reported in Greece so far. In 2017, during a study of the virome of Greek grapevines, one sample (D2.1) from the cultivar Dafnia (Institute of Grapevine, Likovrisi, Attiki) was subjected to high-throughput sequencing (HTS) of total RNA extracted from phloem scrapings using Plant/Fungi Total RNA Purification Kit (Norgen Biotek Corporation, Canada) on an Illumina NextSeq platform (Lifesequencing, S.L., Spain). The run yielded ~51 million 150 bp paired-end reads. De novo assembly of these reads and subsequent BLAST (n/x) analysis of the produced contigs revealed sequences of GVE and GVF, among others. Almost complete genomes from both viruses were reconstructed with 71-98% and 86-89% nucleotide sequence identities to GVE and GVF isolates for which sequences are deposited in databases, respectively. The reconstructed genomes were deposited in GenBank with accession numbers MK490829 for GVE (isolate D2-1/8) and MK490830, MK490831 for GVF (two different GVF variants co-infecting D2.1, namely D2-1/9, D2-1/13). In order to confirm the presence of these two viruses in the D2.1 sample, two sets of primers [GVEup ('5'-ATGGAGTCAAAAGCGATCMG-3') and GVEdo (5'-ACCTGTGACTGAGCATCAAATAC-3') and GVF F 4521 (5'-TGTGTGGGCKAARACATA TG-3') and GVF R 5190 (5'-ATCAGAAAAGATGCTMCTCACCT-3')] were used to amplify a 574-bp and a 670-bp fragments from the coat protein and polymerase gene of GVE and GVF. respectively. Sanger sequencing of the amplicons confirmed the presence of both viruses in the analyzed sample. Seventy additional samples from the Institute's collection were screened for the presence of GVE and GVF and the viruses were identified in five and 12 vines, respectively. To our knowledge, this is the first report of GVE and GVF in grapevine in Greece.

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