First report of grapevine hammerhead viroid - like RNA (GHVd) in grapevine in Greece

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In September 2017, leafroll disease-like symptoms were observed in a commercial vineyard cv. Syrah in the island of Crete. A symptomatic vine (Syr1) was selected and transferred to the Institute of Olive Tree, Subtropical Crops and Viticulture for virus analysis. Total RNA was extracted from phloem scrapings of the affected vine using Plant RNA/DNA Purification Kit (Norgen Biotek Corporation, Canada) and subjected to high-throughput sequencing (HTS) on an Illumina HiSeq2500 platform yielding ~40 million 100 bp paired-end reads. After a first read subtraction against the *Vitis vinifera* genome the *de novo* assembly of the remaining unmapped reads produced 1333 contigs. Ten of the generated sequences were of viral/viroid origin and revealed co-infection by grapevine leafroll-associated virus 3 (GLRaV-3), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine yellow speckle viroid - 1 (GYSVd-1) and hop stunt viroid (HSVd). Moreover, a partial 281 nt sequence of grapevine hammerhead viroid-like RNA (GHVd) was retrieved from the data. GHVd is a recently identified single-stranded circular RNA molecule that is poorly characterized, and the relevant literature is very limited (Wu et al. 2012; Candresse et al. 2017). The presence of

GHVd was confirmed by RT-PCR using published primers Vir-F2 and Vir-R2 (Candresse et al. 2017) and a newly designed primer pair (GHVd F2, 5'-GTG ACT GAC CGT GGA GAA TAG-3', GHVd R2, 5'-CTG TGT AGT GGC TCT GAC GAG-3'), which amplifies GHVd's complete genome. An additional number of 51 randomly selected samples from commercial Cretan vineyards were screened for the presence of GHVd and the viroid-like RNA was detected in one vine cv. Soultanina (Soul1) by using the Vir-F2 and Vir-R2 pair of primers. The amplified 375 bp products of both samples (Syr1, Soul1) were sequenced and deposited in GenBank under accession numbers MK791515 and MK791516. BLASTn analysis of the Greek full-length sequences of GHVd revealed 98.93% identity among themselves and 98.40 to 99.73% identity with the corresponding sequences of the deposited Italian (KR736334) and French (MF093720 and MF093719) isolates. To our knowledge, this is the first report of GHVd in Greece and additional work is needed to elucidate its spread and impact on Greek grapevines.

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