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DISEASE NOTES

First Report of Grapevine rupestris vein feathering virus in grapevine in Germany

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Grapevine rupestris vein feathering virus (GRVFV) is a tentative member of the genus *Marafivirus* in the family *Tymoviridae*. It was described for the first time on a Greek grapevine (*Vitis rupestris*) with symptoms of chlorotic discolorations of leaf veins (El Beaino et al. 2001). Since then, the virus has also been reported in the United States, Canada, Uruguay, Italy, Spain, the Czech Republic, China, and most recently Switzerland, New Zealand, and Korea (Blouin et al. 2017; Cho et al. 2018; Reynard et al. 2017). In September 2017, a grapevine sample from cultivar Syrah from a vineyard from the Rhineland-Palatinate area in Germany was analyzed by high-throughput sequencing. The sequencing library was prepared using the Ribo-Zero Plant Leaf Kit (Illumina) for ribodepletion followed by the TruSeq Stranded Total RNA Library Prep Kit (Illumina). The sample was sequenced (2 × 75 nt) on Illumina Nextseq 500 platform (Liege University). After quality control and elimination of duplicated reads, the 4,521,573 reads were mapped on each of the six whole-genome sequences of GRVFV available in the NCBI database (GenBank accession nos. KY513701, KY513702, MF000325, MF000326, AY706994, and KX828705) using the de novo software SPADES as a plugin in Geneious version 10.1.2. A total of 688 reads mapped to the reference genomes. The closest isolate was KY513702 with a coverage of 6,036 bases (corresponding to 89.5% of genome) scattered along the genome and a pairwise identity of 90.5%. Bioinformatic analysis of the remaining contigs showed the presence of grapevine rupestris stem pitting associated virus, grapevine yellow speckle viroid-1, and hop stunt viroid. The presence of GRVFV in the German vineyards was confirmed by reverse transcription polymerase chain reaction (RT-PCR). In a first set of RT-PCR using the previously reported primers GRVFV_6156F (5′-ACTCWCYATCCCCTTCCAGT-3′) and GRVFV_6600R (5′-GCTGACCATGCCACGAATCA-3′) (Reynard et al. 2017) generating a 445 nucleotide amplification product, the expected product was successfully amplified in three out of 80 randomly collected samples from

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symptomatic and asymptomatic grapevines (two grapevines variety Riesling infected with grapevine fanleaf virus, and the Syrah grapevine, respectively) from different grapevine growing areas in the Rhineland-Palatinate area. The PCR products were cloned and sequenced. The three amplicons shared the same sequence (GenBank accession no. MH131693). BLASTn analysis of the resulting sequences confirmed the presence of the virus, 89% identity being found with the corresponding sequence of the Chass isolate of GRVFV (Reynard et al. 2017). To prevent putative false negative results related to the genetic diversity of the GRVFV, new primers specific to the German isolates (GRVFV-F, 5′-CGCAGCCTCCACCACTCTGAAG-3′; GRVFV-R, 5′-CAGGTAGCCCACAGAGGAC-3′) were designed from the sequences of the PCR products. The expected RT-PCR product of 286 bp was found in 20 out of the 80 samples, from both symptomatic (grapevines variety Riesling with fanleaf disease infected with grapevine fanleaf virus, varieties Dornfelder and Pinot Gris showing stunted growth symptoms of unknown etiology) and asymptomatic grapevines (grapevine varieties Riesling, Dornfelder, and Pinot Gris). It is therefore likely that the symptoms observed in these symptomatic grapevines are owing to other viruses or pathogens or to their interaction with the GRVFV. To our knowledge, this is the first report of GRVFV in Germany.

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