First Report of Grapevine Virus L in Grapevine in Tunisia

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Grapevine virus L (GVL) has recently been identified as a new member of the family *Betaflexiviridae* (genus *Vitivirus*) that infects grapevine (Debat et al. 2019). GVL seems to have a worldwide distribution, having been reported in China, Croatia, New Zealand, and the United States (Debat et al. 2019; Diaz-Lara et al. 2019). In July 2018, in an attempt to monitor the sanitary status of Tunisian vineyards, four grapevine samples of different varieties (grafted on R140 rootstock) showing virus-like symptoms, such as leafroll, yellow vein banding in leaves, shortened internodes, and flattening in wood, were collected from an organic vineyard in Mornag (Northeast of Tunisia) and further analyzed by high-throughput sequencing (HTS). Total RNA was extracted from leaf tissue and sequenced, after ribo-depletion, using RNAseq TrueSeq Illumina methodology on a NextSeq 500 platform (Illumina, San Diego, CA). HTS data analysis was performed by CLC Genomics Workbench 10.1.1 (QIAGEN, Hilden, Germany) and Geneious Prime 2020 software (Biomatters, Auckland, New Zealand). Raw data were subjected to quality control, trimming, and host genome subtraction steps. The resulting reads were used to assemble de novo contigs using CLC. The contigs' similarity to viral sequences was analyzed by BLASTn and BLASTx with a cut-off e-value of 10^{-4} . The analysis showed GVL-related sequences in three of the four generated datasets, corresponding to one sample of cv. Red Globe (four contigs of 5,340, 1,709, 375, and 213 nt) and two samples of local grapevine varieties, cv. Marsaoui (one contig of 7,532 nt) and cv. Razzegui (one contig of 7,409 nt). For recovery of full-length genomes, contigs were

extended by mapping the reads against the recovered contigs and GVL genomic sequences available in the databases using Geneious software. One full-length GVL genome from isolate Red Globe (7,580 nt, MT319081) and two near-full-length GVL genomes from isolates Marsaoui (7,557 nt, MT319082) and Razzegui (7,563 nt, MT319083) were recovered. The nucleotide identity among all three isolates ranged between 98.4 and 99.2%, and their similarity with respect to the closest GVL isolate available in the databases (isolate VL from Croatia, MH681991) was 96.8, 96.9, and 96.8%, respectively. Along with GVL, other sequences of grapevine viruses and viroids were found. The number of reads and contigs, the GVL genome coverage, and the other detected viruses and viroids are in the supplementary material. To confirm the presence of GVL infecting grapevine in Tunisia, RT-PCR analysis was carried out on the HTS-positive samples, using two specific sets of primers. Primers 5'-AGTTGAAGTC TAGGTGCACAC-3' (sense) and 5'-GTACTCAGACTTCCCCGATCTA-3' (antisense) target a 279-bp fragment of the CP/NAB region in the GVL genome (Debat et al. 2019). Newly designed primers based on the HTS-recovered sequences GVL-repF 5'-TGCAACAAGTTACCATGGAGA-3' (sense) and GVL-repR 5'-CATCGAGCACTTTTGGCCAG-3' (antisense) are anticipated to be specific for the amplification of a 273-bp fragment in the replicase region. Indeed, amplicons with the expected sizes were obtained for all three samples using both sets of primers. Sanger sequencing of the amplicons confirmed the identities of the amplicons. Together, these results demonstrated the presence of GVL in Tunisian grapevines. To our knowledge, this is the first report of the occurrence of GVL in Tunisia. Phylogenetic analysis of available full-length GVL sequences shows that the Tunisian isolates cluster together with the Croatian isolate VL, which is in agreement with their geographical origin. Because other vitiviruses have been demonstrated to have an impact on grapevine (Minafra et al. 2017), the identification of GVL in a new geographical area represents potential risk for the grapevine crop. Further studies will determine the biological significance of the presence of this recently identified vitivirus and its prevalence in Tunisian vineyards.

References:

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