Diseases Caused by Viruses

First Report of Grapevine Yellow Speckle Viroid 1 Infecting Grapevine (*Vitis vinifera*) in Canada

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Quebec is the third largest wine grape (*Vitis vinifera* L.) producer in Canada in acreage, tonnage, and wine grape sales (Ben Moussa et al. 2019; Carisse et al. 2017). To evaluate the diversity of viruses infecting grapevine in Quebec, a total of 77 leaf tissue samples (cv. Vidal) were collected from July to October in 2020 in three different vineyards located in Frelighsburg, Hemmingford, and Saint-Jacques-le-Mineur in Quebec, Canada. Double-stranded RNA was extracted from each sample and used for cDNA library preparation with the Nextera XT DNA Library Preparation Kit (Illumina) as described previously (Kesanakurti et al. 2016). High-throughput sequencing (HTS, 2 × 300 bp) was conducted on dual-indexed libraries in a v3 flow cell using the Illumina MiSeq platform (Adkar-Purushothama et al. 2020). The obtained raw FASTQ data were de-multiplexed into 154 separate sequence files, and the adapters and barcode sequences were trimmed. The quality of the sequences was verified using Trimmomatic v.0.32, and the “clean” sequences were analyzed using Virtool and VirFind virus detection pipelines described elsewhere (Ho and Tzanetakis 2014; Rott et al. 2017) to screen for all possible viruses in the databases. Over 100,000 reads per sample were obtained with a percentage of mapped viral reads ranging from 1.47 to 19.43% of total number of reads. Out of 77 samples, 16 revealed the sequence of grapevine yellow speckle viroid 1 (GYSVd-1), for which the length coverage ranged from 98.5 to 99.1%; the depth ranged from 2× to 856×. The GYSVd-1 positive sequence files were subjected to whole

genome assembly on CLC Genomics Workbench v.20.0.4 with the isolate SY-BR from Brazil (KU880715) used as a reference. Seven complete genomes of GYSVd-1 of 366 to 368 nucleotides (nt) in size were deposited (GenBank MW732682 to MW732688). BLASTN analysis of the sequences showed 98 to 100% nt identities with isolate SY-BR. Other viruses and viroids such as grapevine fleck virus, grapevine rupestris stem pitting-associated virus, grapevine rupestris vein feathering virus, and hop stunt viroid were also detected. To confirm GYSVd-1 presence in Quebec vineyards, seven of the 16 HTS-positive grapevine leaf tissue samples were subjected to total RNA extraction, followed by RT-PCR assay as before (Adkar-Purushothama et al. 2015; Sahana et al. 2013); all were positive by RT-PCR. The PCR products were directly Sanger sequenced, and they showed 100% nt identity to the HTS-derived sequences. Three of the seven GYSVd-1 positive grapevines exhibited yellow leaf spots and flecks and tiny yellow leaves, but their mixed infection status makes definitive symptom association difficult to determine. Previously, hop stunt viroid was reported from grapevines in Canada (Fall et al. 2020; Xiao et al. 2019), but to the best of our knowledge, this is the first report of GYSVd-1 infecting grapevines in Canada, specifically in the province of Quebec. Further research is required to assess the GYSVd-1 related yield loss. Monitoring and testing for GYSVd-1 infection is necessary to prevent propagation of infected materials, spread, and potential negative impact for the Canadian grapevine industry.

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