Diseases Caused by Viruses

First Report of Grapevine Red Globe Virus in Grapevines in Washington State

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Grapevine red globe virus (GRGV; genus *Maculavirus*, family *Tymoviridae*) has been reported in grapevines (*Vitis* spp.) from Italy, Greece, France, China, Spain, and Germany and in California, U.S.A. (Cretazzo et al. 2017; Fan et al. 2016; Ruiz-Garcia et al. 2018; Sabanadzovic et al. 2000). During surveys of grapevine nurseries, a total of 241 composite samples, each consisting of four petioles from mature leaves/vine from five asymptomatic grapevines, from 33 grapevine (*Vitis vinifera*) cultivars were collected. Total RNA isolated from these samples using a Spectrum Total RNA isolation kit (Sigma-Aldrich, St. Louis, MO) was subjected to high-throughput sequencing (HTS) on an Illumina HiSeq2500 or NovaSeq 6000 platform in paired-end mode (Genomics Core Facility, Huntsman Cancer Institute, Utah University, Salt Lake City, UT). After trimming raw reads based on quality and ambiguity, the paired-end quality reads of approximately 120 (HiSeq) or 145 (NovaSeq) base pair (bp) length were assembled de novo into a pool of contigs (CLC Genomics Workbench 12). These contigs were subjected to BLASTn analysis against

the nonredundant virus database from GenBank (<https://www.ncbi.nlm.nih.gov/blast>). A total of 49 contig sequences, ranging from 200 to 1,645 bp in length with an average coverage ranging up to 418.7, aligning with GRGV genome, were detected in cultivars Aglianico, Cabernet franc, Pinot gris, and Riesling. BLASTn analysis of contigs greater than 500 bp in length showed sequence identity between 88.5 and 95% with corresponding GRGV sequences reported from other countries. These results indicated the presence of genetically distinct isolates of GRGV. HTS data also revealed coinfection of GRGV in all samples with one or more of the following viruses and/or viroids: grapevine rupestris stem pitting associated virus, grapevine rupestris vein feathering virus, hop stunt viroid, or grapevine yellow speckle viroid-1. To further confirm infection by GRGV, total RNA was extracted from two asymptomatic Pinot gris vines that previously tested positive in HTS using the Spectrum Total RNA isolation kit and subjected to reverse transcription PCR using primers specific to the replicase polyprotein gene of the virus (RG4847F, 5'-TGGTCTGT TGTTCGCATCTT-3'; RG6076R, 5' CGGAAGGGGAAGCATTGATCT-3' Cretazzo et al. 2017). Sequence analysis of the approximately 1,250-bp amplicons (accession no. MT749359) showed 91.2% nt sequence identity with a corresponding sequence of GRGV isolate from Brazil (KX828704.1). To our knowledge, this is the first report of GRGV in Washington State. Together with the report of the occurrence of GRGV in California (Sabanadzovic et al. 2000), these results indicate wide geographical distribution of the virus. Although GRGV can cause asymptomatic infections in grapevines (Martelli et al. 2002), the economic importance of GRGV as single or coinfections with other viruses needs to be examined to assess the potential significance of the virus to grape production and grapevine certification programs.

References:

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