Diseases Caused by Viruses

First Report of Grapevine Red Globe Virus, Grapevine Rupestris Vein Feathering Virus, and Grapevine Syrah Virus-1 Infecting Grapevine in Slovenia

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To investigate the virome status of preclonal grapevine candidates, 82 dormant cuttings of six cultivars (Laški rizling, Refošk, Rebula, Malvazija, Zeleni Sauvignon, and Pokalca) were taken in 2019 from vines in the second cycle of clonal selection in Vipavska dolina, Primorska. Samples were pooled in 12 groups (1× Laški rizling, 1× Pokalca, 2× Rebula, 2× Zeleni Sauvignon, 3× Malvazija, and 3× Refošk) from which small RNA was isolated using a mirVana miRNA Isolation Kit (Ambion, Life Technologies); 12 cDNA libraries were constructed and sequenced on an Ion Proton Sequencer (Ion Torrent, Life Technologies). A VirusDetect pipeline (Zheng et al. 2017) was used for bioinformatics analysis and 3.6 to 13.9 million reads/library were processed. According to the pipeline, grapevine red globe virus (GRGV; *Maculavirus*, *Tymoviridae*) was found in two Refošk libraries. In one, GRGV showed highest coverage of 63.6% and nt identity of 92.71% with GRGV isolate Graciano-T101 (KX171166) from Spain and was represented by 40 contigs with sequence depth of 53.0×. In the other, GRGV showed highest coverage of 45.8% and nt identity of 94.49% with GRGV isolate Graciano-T90 (KX109927) from Spain and was represented by 36 contigs with sequence depth of 22.9×. Grapevine rupestris vein feathering virus (GRVVF; *Marafivirus*, *Tymoviridae*) was found in 11 libraries from six cultivars. In six libraries, GRVVF showed highest coverage of 37 to 85% and nt identity of 88.53 to 94.05% with GRVVF isolate CHASS (KY513702) from Switzerland, and was represented by 34 to 90 contigs with sequence depth of 11.0 to 93.3×; in four, GRVVF showed highest coverage of 27.5 to 64.8% and nt identity of 92.36 to 93.87% with GRVVF isolate

Mauzac (KY513701) from France, and was represented by 30 to 94 contigs with sequence depth of 5.4 to 20.7×; in one, GRVVF showed highest coverage of 30.2% and nt identity of 95.30% with GRVVF isolate SK925 (MH544692) from Slovakia, and was represented by three contigs with sequence depth of 5.6×. Grapevine Syrah virus-1 (GSyV-1; *Marafivirus*, *Tymoviridae*) was found in two libraries. In the Laški rizling library, GSyV-1 showed highest coverage of 44.9% and nt identity of 95.36% with GSyV-1 isolate SK351 (KP221269) from Slovakia and was represented by three contigs with sequence depth of 20.9×. In a Malvazija library, GSyV-1 showed highest coverage of 48.8% and nt identity 92.06% with GSyV-1 isolate SK30 (KP221256) from Slovakia and was represented by 25 contigs with sequence depth of 29.4×. Results of high-throughput sequencing were confirmed in individual samples using reverse transcription PCR (RT-PCR). GRGV was amplified using specific primer pair RG6061F: 5'-CCGAGCTTCTCTCCAAGATCA-3' / RG6801R: 5'-ACTTAA CGTAGGCCACTGGGT-3' (Cretazzo and Velasco 2017). GRVVF was amplified using specific primer pair GRVVFV_6090F: 5'-CATCGTTCTGATCCT CAGCC-3' / GRVVFV_6605R: 5'-AGAGACGCTGACCATGCCAC-3' (Glasa et al. 2019). GSyV-1 was amplified using specific primer pair SY5922F: 5'-CC AATGGGTCGCACTTGTG-3' / SY6295R: 5'-ACTTCATGGTGGTGCCG GTG-3' (Glasa et al. 2015). Three of 13 Refošk samples tested for GRGV, 44 of 78 samples tested for GRVVF (2 Laški rizling, 12 Refošk, 9 Rebula, 7 Malvazija, 8 Zeleni Sauvignon, and 6 Pokalca), and three of eight samples tested for GSyV-1 (one Laški rizling and two Malvazija) were positive. Further amplicon verification was done by bidirectional Sanger sequencing. Sequences were submitted to GenBank: GRGV (MW446914 to MW446916), GRVVF (MW446917 to MW446938), and GSyV-1 (MW446939 to MW446941). Phylogenetic analysis showed that Slovenian GRGV isolates clustered separately from others in NCBI, that some Slovenian GRVVF isolates clustered with those from France or Slovakia and some clustered separately, and that Slovenian GSyV-1 isolates clustered with those from Hungary and Slovakia. This is the first report of GRGV, GRVVF, and GSyV-1 infecting grapevine in Slovenia. Further work is needed on their prevalence in Slovenian vineyards and their impact on production.

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