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DISEASE NOTES

First Report of Grapevine Rupestris Stem Pitting-Associated Virus, Hop Stunt Viroid, and Grapevine Yellow Speckle Viroid 1 Infecting Grapevine (Vitis vinifera) in Bosnia

D. Delić¹⁰ and M. Radulović, University of Banjaluka, Faculty of Agriculture, 78000

COST Action FA1407 (DIVAS)

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and Herzegovina

FOCUS ISSUE Activation, Regulation, and Evolution of MTI and ETI Molecular Plant-Microbe

Banjaluka, Bosnia and Herzegovina; V. Vukosavljević, Faculty of Agronomy, Department of Fruit Growing & Viticulture, Čačak, Serbia; and S. Massart, Plant Pathology Laboratory, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium.

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In Bosnia and Herzegovina (BiH) there is only scarce information on causal agents of grapevine leafroll and infectious degeneration disorders (Karačić et al. 2016). Nextgeneration sequencing (NGS) allows the detection of virus nucleic acids in a single plant or in composite samples, facilitating the identification and the discovery of grapevine viruses. The main objective of this work was to screen the viruses infecting grapevine in BiH using NGS technologies and to confirm the presence of the detected viruses by targeted reverse transcription polymerase chain reaction (RT-PCR). Five grapevine leaf samples collected in May 2016 from commercial vineyards in Derventa (GPS coordinates: 44°54′4″N, 17°56′1″ E) and Prnjavor (GPS coordinates: 44°52'40.595"N, 17°45'7.632"E) showing leaf mottling, yellowing, and malformation were found negative for the presence of grapevine leafroll associated virus 1, 2, and 3 and grapevine fan leaf virus in a double antibody sandwich ELISA test. Total RNA was extracted from each individual plant using a Spectrum Plant Total RNA Kit (Sigma-Aldrich, Germany). The RNA extracts were pooled in a single composite sample (equimolar pooling). The quality of the extract was assessed with a Bioanalyzer (RNA integrity number = 8.7) and the sequencing library 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 on an Illumina Nextseq 500 platform (Liege University). A total of 5,695,352 paired reads (2 $\times 150$ nt) were obtained. Bioinformatics analysis was carried out using Geneious version 9.1 software. Analysis of

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the NGS reads resulted in the identification of grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine Pinot gris virus (GPGV), hop stunt viroid (HpSVd), and grapevine yellow speckle viroid 1 (GYSVd-1). GPGV has been previously reported in one of the pooled samples from the Derventa location in BiH (Delić et al. 2017), whereas GRSPaV, HpSVd, and GYSVd-1 had never been reported previously. To confirm the presence of GRSPaV, HpSVd, and GYSVd-1, RT-PCR was performed using previously reported primers (Eichmeier et al. 2016; Terlizzi et al. 2011; Ward et al. 2011). PCR products of the expected sizes were obtained and directly sequenced using Sanger methodology. The obtained sequences of GRSPaV (GenBank accession no. MH000195), HpSVd (GenBank accession no. MH028395), and GYSVd (GenBank accession no. MH028394) were compared with the sequences in the GenBank database using BLASTn analysis. The sequences shared 92 to 99, 99 to 100, and 94 to 99% identity, respectively, with other GRSPaV, HpSVd, and GYSVd isolates. To our knowledge, this is the first report of GRSPaV, HpSVd, and GYSVd infecting grapevine in BiH. Studies are ongoing to further elucidate their prevalence and evaluate their relationship with disease development to adapt and improve grapevine sanitary propagation protocols.

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