Disease Note

Diseases Caused by Viruses

First Report of Grapevine Leafroll-Associated Virus 4 Infecting Grapevine in Hungary

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Funding: This research was supported by the National Research, Development and Innovation Office (K131679 and 2019-2.1.11-TÉT-2020-00124) and the Slovenian Research and Innovation Agency (ARIS P4-0072). Plant Dis. 108: 2245, 2024; published online as https://doi.org/10.1094/PDIS-03-24-0500-PDN. Accepted for publication 22 April 2024.

In 2020, three vines of Arany Sárfehér, an indigenous cultivar of the Carpathian basin, cultivated in a vineyard at Izsak were selected for virus elimination. To determine the viral status of one of the clones (Sarfeher A1), the virome of the in vitro plantlets, established from the original plant, was determined using small RNA high-throughput sequencing (HTS) (SRA ID: PRJNA1041448). After trimming and quality control of the initial 26.9 million small RNA reads, 25.5 million reads were used for contig assembly using CLC Genomic Workbench (Qiagen). De novo assembled 1,231 contigs were annotated by BLAST against the reference genomes of plant-infecting viruses (accessed on 30.03.2021 at NCBI GenBank). Besides hits to grapevine Pinot gris virus (GPGV) (10 contigs), hop stunt viroid (4 contigs), and grapevine yellow speckled viroid 1 (7 contigs), some contigs showed similarity to NC_016416 (9 contigs), NC_016081 (2 contigs), and NC_016417 sequences (6 contigs) of grapevine leafroll-associated virus 4 (GLRaV-4) strains. 6,112, 2,188, and 4,891 reads resulting in 26.3, 20.1, and 26.7% coverage could be directly mapped to NC_016416, NC_016081, and NC_016417, respectively. To determine and validate which GLRaV-4 strain is present in the sample, RT-PCR was carried out. cDNA synthesis was performed using a random primer version of the RevertAid cDNA synthesis kit (Thermo Scientific). Two primer pairs were designed based on HTS-derived contigs (GLRaV456_11328F: 5′-AATACCCAATTGTTGCRGATAC-3′/GLRaV456_13722R: 5′-TCACAGATGCCTGACATGGTT-3′, and GLRaV456_13268F: 5′-TGGACAATTTAGGTAATGTAGTAGC-3′/GLRaV456_13829R: 5′-GGTCCTGGATCTCTCCAAGATAGGG-3′) that amplified 2,364- and 562-bp fragments specific to the p60 and p23 genes. Amplified products of expected size obtained by Q5 DNA polymerase (New England Biolabs) were cloned into a pJET vector (Thermo Scientific) and sequenced. Sequences of the two overlapping clones were assembled, resulting in a 2,471-nt-long 3′ part of the GLRaV-4 genome. The obtained sequence (GenBank accession no. OR885929) shared 84% nt identity with NC_016416 and 76% nt identity with both NC_016081 and NC_016417, confirming the infection of the analyzed vine with strain 4 of GLRaV-4. The SarfeherHU sequence showed the highest nt identity (91%) to a Slovenian GLRaV-4 (055-SI, KM892778; Štrukelj et al. 2016). To supplement the molecular data with serological tests, asymptomatic leaves were collected in September 2023 from the Sarfeher A1 plant maintained as self-rooted vines in a glasshouse at Kecskemét. Generic GLRaV-4 DAS-ELISA (Bioreba) was performed on two leaves as a mixture and on phloem tissue and confirmed the HTS and RT-PCR results. The GLRaV-4-infected Arany Sárfehér was coinfected with GPGV and viroids but was healthy-looking, without symptoms. To check if GLRaV-4 could be present in other vines, three vines originating from Izsak and three vines of the same cultivar, but grown at Tiszaalpár, were tested using RT-PCR for the presence of GLRaV-4. Out of the six tested plants, two plants regenerated from the clones from Izsak turned out to be infected with the virus, suggesting that more plants of the same cultivar are and could be infected with the virus. According to our knowledge, this is the first report of GLRaV4, supported by sequence data from grapevine in Hungary.

Reference:
Štrukelj, M., et al. 2016. Acta Virol. 60:174.

The author(s) declare no conflict of interest.

Keywords: closteroviridae, ELISA, GLRaV-4, grapevine, HTS, sRNA

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