Diseases Caused by Viruses

First Report of *Grapevine Kizil Sapak Virus* in Grapevine in China

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Grapevine Kizil Sapak virus (GKSV) is a novel member of the family *Betaflexiviridae* classified into the proposed genus *Fivivirus* within the subfamily *Trivirinae*. It was first discovered in the U.S.A. from a grapevine originating from Turkmenistan (Al Rwahnih et al. 2019) and later in France from a grapevine accession from Iran (Marais et al. 2020). In October 2019, an asymptomatic grapevine cv. Crimson Seedless (native to the U.S.A.) was collected from Xinjiang Province in China and analyzed by high-throughput sequencing (HTS). Ribosome-depleted RNA preparations were used for library synthesis followed by HTS on an Illumina HiSeq X-ten platform. A total of 29,141,024 cleaned reads were obtained, and 7,878 contigs were generated using CLC Genomics Workbench 9.5 (QIAGEN). One long contig (7,328 bp) showed 88.2% nucleotide (nt) identity with the sequence of GKSV-127 (MN172165) via Blastx, with an average coverage of 284-X. Bioinformatic analysis of the remaining contigs showed the presence of *Grapevine leafroll-associated virus 4*, *Grapevine rupestris vein feathering virus*, *Grapevine fabavirus*, grapevine yellow speckle viroid-1 (GYSVd-1), GYSVd-2, and *Hop stunt viroid* in the sample. The presence of GKSV was checked by RT-PCR using the primer GKSV-F/R (Al Rwahnih et al. 2019); the 1,240 bp PCR product was cloned using a pTOPO-T vector (Aidlab, China) and sequenced. In pairwise comparison, the obtained nt sequences

shared 92.6 to 95.2% identity to the corresponding HTS sequence, confirming the presence of GKSV in the sample. The complete GKSV genome sequence was obtained as two pieces of overlapping DNA sequence using primers GKSV-20A/20B (5'-TAGTCTGGATTTCCTACCT/5'-CTCCCTAAACTGATTGATG) and GKSV-25A/25B (5'-GCCACTGGTGAATGAAAAGA/5'-CTAAATGAATGGGCAGGTAT) designed based on the HTS-generated sequence. The 5' and 3' termini were determined by rapid amplification of cDNA ends using SMARTer RACE 5'/3' Kit (Takara, Dalian, China). The complete genome of GKSV isolate CS (MW582898) comprised 7,604 nt (without the polyA tail) and shared 77.8 to 89.2% identities with the other nine reported GKSV isolates, among which it shared the highest nt identity (89.2%) with GKSV-127. In phylogenetic analysis based on complete or nearly complete genome sequences of representative members of *Betaflexiviridae*, GKSV-CS clustered with the nine known GKSV isolates, forming a subclade with GKSV-127. To determine the incidence and distribution of GKSV in China, 476 grapevine samples of 75 cultivars were collected from 20 provinces and tested by RT-PCR using primers GKSV-F/R (Al Rwahnih et al. 2019) and Vini-F1/R1 (Marais et al. 2020). The results showed that 0.42% (2 of 476) of the samples tested positive with both primers, including samples GKSV-CS and a 'Black Monukka' grape (native to India) also sampled from Xinjiang. Both PCR products of 'Black Monukka' were cloned and sequenced (MZ311588 to MZ311602) and they showed 85.1 to 88.9% nt identities to the GKSV-CS sequence. This is the first report of GKSV infecting grapevine in China. Although the pathogenicity of GKSV is yet to be determined, it has been found in several countries such as the U.S.A. (Al Rwahnih et al. 2019), France (Marais et al. 2020), and China (this study). Both positive samples in this study were collected from the Nanjiang region in Xinjiang Province, indicating the sporadic occurrence of GKSV in this area.

References:

- Al Rwahnih, M., et al. 2019. Arch. Virol. 164:3145.
Marais, A., et al. 2020. Arch. Virol. 165:1849.

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