



First report of grapevine Pinot gris virus in wild grapevines (*Vitis coignetiae*) in Japan

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Grapevine Pinot gris virus (GPGV) was first identified in Italy from symptomatic *Vitis vinifera* ‘Pinot Gris’ (Giampetruzzi et al. 2012). Since then, GPGV has been detected in most grapevine-growing regions worldwide (Hily et al. 2020). In September 2020, leaf samples were collected from three symptomatic (mosaic or chlorotic mottling of the leaves) and 12 asymptomatic wild *V. coignetiae* growing naturally in Hokkaido, Japan. Total RNA was extracted and pooled into a single sample. This pooled RNA was sequenced using DNBSEQ-G400 (MGI TECH), and 38,507,344 paired reads were obtained. The reads were trimmed and assembled using CLCgenomic WorkBentch (Qiagen). Then the contigs were mapped to the *V. vinifera* genome (GenBank accession No. GCA_000003745.2), and the unmapped contigs were collected. Among these, we found two contigs covering nearly the full-length GPGV genome; one was 7,070 bp long with 78.1% nucleotide sequence identity with GPGV-FEM01 (KU312039.1) and the other was 7,169 bp long with 80.4% nucleotide sequence identity to SRR5332103-GPGV2 (BK011083.1). To confirm the infection of GPGV, we designed a primer pair of GPGV_CPfull_F1 (5'-ATC TGGCTGTGCTGAAAATA-3') and GPGV_CPfull_R1 (5'-ACTACATACTAAATGCACTCTCC-3') to amplify the full-length coat protein (CP) gene by RT-PCR. Amplicons of the expected size (657 bp) were obtained in 5 out of 15 plants. Direct sequencing of amplicons showed three types of nucleotide sequences that were highly homologous to known GPGV sequences with 86%–87% nucleotide identity (87.5% to MN458457.1) and 90%–98% amino acid identity (97.9% to AGV76026.1). Sequences determined in this study were submitted to GenBank under accession

numbers LC601811 to LC601812 (contigs) and LC601601 to LC601603 (viral CP region). Sequences corresponding to LC601601-LC601603 were recovered from two, one, and two infected plants, respectively. To our knowledge, this study is the first to report the detection of GPGV in Japan.

Declarations

Conflicts of interest The authors declared no conflict of interest.

Research involving human participants and/or animals The authors declare that no human participants and animals were involved in this study.

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