First Report of Grapevine Rupestris Vein Feathering Virus in *Vitis vinifera* from Japan

Yoshinao Aoki and Shunji Suzuki[†]

Laboratory of Fruit Genetic Engineering, The Institute of Enology and Viticulture, University of Yamanashi, Kofu, Yamanashi 400-0005, Japan

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The production of wine grapes is gaining widespread popularity and being carried out on approximately 2,200 hectares of land in Japan. Scions grafted onto rootstocks have generally been imported from the EU, U.S.A., New Zealand, and Australia into Japan. Unfortunately, viruses have spread in Japanese vineyards by slipping through the net of plant quarantine. Grapevine rupestris vein feathering virus (GRVfV), which was detected in Greek grapevine accessions, is a member of genus *Marafivirus* in family *Tymoviridae* (El Beaino et al. 2001). GRVfV has been detected in many countries such as the U.S.A., Canada, Australia, New Zealand, Italy, Spain, Switzerland, the Czech Republic, Uruguay, and Pakistan (Blouin and MacDiarmid 2017; Cho et al. 2018; Eichmeier et al. 2016; Jo et al. 2015; Mahmood et al. 2019; Reynard et al. 2017; Wu et al. 2021; Xiao and Meng 2016). Herein we report GRVfV infection in *Vitis vinifera* L. grapevines from Japan. In February 2021, dormant canes from 18 *V. vinifera* cv. Cabernet Sauvignon with leafroll-like disease symptoms, growing in a vineyard located in Kanagawa Prefecture, were collected. No typical vein banding symptom by GRVfV were observed in the grapevines during the growing season. Total RNA was isolated from the canes using an RNeasy Plant Mini Kit and QIAshredder (Qiagen, Valencia, CA), and subjected to cDNA synthesis using a Prime-Script first Strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan). RT-PCR was performed with GRVfV_6156F and GRVfV_6600R primers for GRVfV detection (Reynard et al. 2017). The expected 445 nucleotide (nt) amplification product was obtained from four of 18 grapevines. Sequence analysis of the products revealed 91% identities to corresponding sequences of GRVfV isolates CHASS (KY513702) and Mauzac (KY513701) from Switzerland. Genome walking to determine the whole-genome sequence of the GRVfV isolates from the four grapevines was performed. Briefly, the

upstream and downstream of the 445-nt amplification product were amplified from first-strand cDNA using gene-specific primers designed from the product and CHASS-specific primers. Each amplified fragment was Sanger sequenced. Next, gene-specific primers were designed to obtain the complete genome of GRVfV as 13 overlapping DNA fragments from each of the four grapevine samples. An identical complete genome of 6,704 bp was assembled from the overlapping DNA fragments using MEGA 10 software and named as NA1 isolate (DDBJ accession no. LC619667). Phylogenetic analysis of the NA1 genome and corresponding sequences of GRVfV from other countries showed that NA1 formed a cluster with isolate NZ ChTK0004 from New Zealand (MF000326). In pairwise comparisons, the complete NA1 genome was most identical at 88% and 87%, respectively, to isolates NZ ChTK0004 and Mauzac. The predicted amino acid sequences of NA1 polyprotein shared high homologies (96%) to the corresponding polyprotein sequences of NZ ChTK0004 and Mauzac, suggesting that NA1 is genetically similar to GRVfV isolates from New Zealand and Switzerland. The NA1-infected Cabernet Sauvignon was coinfecting with *Grapevine leafroll-associated virus 3*, *Grapevine virus A*, and *Grapevine rupestris stem pitting-associated virus* according to RT-PCR assay for grapevine virus detection (Nakaune and Nakano 2006). The results underscore the importance of intensifying quarantine measures to prevent introduction of exotic viruses via contaminated wine grape vegetative cuttings.

References:

- Blouin, A. G., and MacDiarmid, R. M. 2017. Plant Dis. 101:2156.
- Cho, I. S., et al. 2018. Plant Dis. 102:1471.
- Eichmeier, A., et al. 2016. PLoS One 11:e0167966.
- El Beaino, T., et al. 2001. Vitis 40:65.
- Jo, Y., et al. 2015. Sci. Rep. 5:15841.
- Mahmood, M., et al. 2019. J. Plant Pathol. 101:1261.
- Nakaune, R., and Nakano, M. 2006. J. Virol. Methods 134:244.
- Reynard, J. S., et al. 2017. Plant Dis. 101:1062.
- Wu, Q., et al. 2021. Plant Dis. 105:515.
- Xiao, H., and Meng, B. 2016. Plant Dis. 100:2175.

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[†]Indicates the corresponding author.
S. Suzuki; suzukis@yamanashi.ac.jp