First Report of Grapevine Latent Viroid Infecting Grapevine (*Vitis vinifera* L.) in Italy

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Grapevine latent viroid (GLVd) is a new viroid recently discovered in grapevines (*Vitis vinifera* L.) of the variety 'Thompson Seedless', located in Xinjiang, China (Zhang et al., 2014), proposed as a new species in the genus *Apscaviroid*. It contains the typical structural elements of the other apscaviroids, i.e. circular genomic RNA able to assume a rod-like conformation, central and terminal conserved regions, poly-purine stretch in the pathogenicity associated P domain. Autonomous replication of GLVd in grapevine and absence of associated symptoms were confirmed by bioassays with infectious *in vitro* transcripts (Zhang et al. 2014). Up to now, the presence of GLVd has been reported only in grapevine of the variety 'Thompson Seedless' grown in China (Zhang et al., 2014) and in *Vitis* sp. collected in South Korea (Genbank accession LC163596.1, unpublished).

In July 2015, a survey based on next generation sequencing (NGS) of small RNAs of the grapevine collection Grinzane-Cavour, located in Piedmont, Italy, was carried out in order to investigate the grapevine virome. Bioinformatic analyses highlighted the presence of GLVd in a pool of 10 plants collected during the survey. A unique contig of 271 bp, a genome coverage of 82.6% and an identity of 98.16% with the GLVd reference genome (KR605505.1) was obtained. This contig represented 822 (0.01%) of total reads, similar to the read percentages observed for other viroids detected by NGS (Candresse et al., 2017).

The presence of GLVd as well as its circularity was confirmed by RT-PCR, using the pair of adjacent primers of opposite polarity GLVd-252F (5'-GCTCTCCAACGCCCTAA-3') and GLVd-251R (5'-ACCATTAGTCCGCACGA-3'), mapping to positions 252-268 and 235-251 of the GLVd reference (KR605505.1), respectively (Zhang et al., 2014), which amplify the full-length monomeric cDNA of the viroid. GLVd was detected in four out of the 10 plants belonging to the sequenced pool: 3 plants from 3 *Vitis vinifera* L. cultivars (Adissi, Rkatsiteli, and Katta Kourgan),

originally from Armenia, Georgia and Uzbekistan, respectively, and 1 plant from *Vitis riparia* (cv. Gloire de Montpellier), originally from North America.

A 330 bp genome sequence was obtained from the cultivar *Vitis vinifera* L. Katta Kourgan by RT-PCR amplification, cloning in the pCR™-Blunt II-TOPO plasmid by Zero Blunt® TOPO® PCR Cloning Kit (Life Technologies, Carlsbad, CA) and Sanger sequencing. The sequence (Genbank accession MG770884) showed 99% identity and a single base insertion (+C226) when compared with the sequence assembled from the Illumina data, possibly reflecting sequence heterogeneity in the GLVd population. The new GLVd genomic sequence showed 97% identity with both the type sequences KR605505.1 (Chinese isolate) and LC163596.1 (Korean isolate), analogous to the sequence identity (97%) between the Chinese and Korean isolates.

To the best of our knowledge, this is the first report of GLVd in Europe. As previously reported, no obvious symptoms were observed in the GLVd infected plants; however, the elicitation of symptoms related to environmental changes or mixed infections with other grapevine-infecting viruses cannot be excluded (Szychowski et al., 1995). Moreover, the recent characterization of viromes associated to apparently healthy host populations has raised the question of the possible biological/ecological role of asymptomatic viral entities (Roossinck & Bazán, 2017). Further studies will be useful to better elucidate these aspects.

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Bibiography

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