First report of Grapevine pinot gris virus (GPGV) and Grapevine rupestris stem pitting-associated virus (GRSPaV) in grapevine in Belgium

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Grapevine pinot gris virus (GPGV) belongs to the genus Trichovirus of the family Betaflexiviridae from the order Tymovirales. It was first discovered in Italy in 2012 (Giampetruzzi et al. 2012), and later in a number of countries including Germany (Reynard et al. 2016) and France (Beuve et al. 2015). Grapevine rupestris stem pitting-associated virus (GRSPaV) is a member of the genus Foveavirus of the family Betaflexiviridae, order Tymovirales (Hily et al. 2018). As grapevine is known to be a host of a wide variety of viruses, a pilot sampling (10 symptomless plants) was carried out in May 2018 and the presence of GPGV evaluated by RT-PCR (data not shown). Subsequently, a leaf sample from a GPGV positive Vitis vinifera ‘Regent’ from a Belgian vineyard (province of Namur) was analyzed by high-throughput sequencing (HTS). The sequencing library was prepared on the template of ribosomal-depleted total RNAs (Ribo-Zero Plant Leaf Kit, Illumina) using the TruSeq Stranded Total RNA Library Prep Kit (Illumina). The sample was sequenced (2 × 150 nt) on the Illumina Nextseq 500 platform (GIGA, Liege University, Liege, Belgium). After quality control and elimination of duplicate reads, 2,934,997 high quality paired reads were assembled de novo using SPAdes as a plugin in Geneious version 10.1.5 (https://www.geneious.com). BLASTn analysis of the contigs against the NCBI reference database showed homologies to two grapevine viruses, GPGV (NC_015782.2) and GRSPaV (NC_001948.1). The nearly full genome sequences (7,344 nt for GPGV, and 8,711 nt for GRSPaV) of both viruses were reconstructed by de novo assembly, and deposited in GenBank under the accession numbers MN228488 (GPGV) and MN228487 (GRSPaV). BLASTn analysis indicated that the closest sequences to the GPGV isolate were KM491305 (France), KU194413 (Canada) and KR528581 (Korea) with 98.6% identity, while the closest sequences to the GRSPaV isolate (98.9% identity) were MG938325, MG938334 and MG938327 (France), all belonging to the molecular clade 3 of group L of GRSPaV (Hily et al. 2018).
estimate the occurrence of GPGV and GRSPaV in the vineyard, 49 samples, all asymptomatic, were randomly selected and tested together with the sequenced sample using reverse transcription polymerase chain reaction (RT-PCR). For the detection of GPGV, the primer pair GPG-14F (5’-AATTGATCCCGTGTAGTGCTG-3’) and GPG-632R (5’-TCCGAGGACGATGAACCTC-3’) (Glasa et al. 2014) was applied (anticipated amplicon size: 618 bp); whereas for the detection of GRSPaV, the primers GRSPV-NGS-Be-s (5’-TCTGCATTAGGCATCATGTG-3’) and GRSPV-NGS-Be-as (5’-GGCCGTTACCAATCTTCTCG-3’) were designed based on the HTS-generated sequences and used (anticipated amplicon size: 420 bp). Among the 50 samples, eight samples tested positive for GPGV, and 12 tested positive for GRSPaV. Interestingly, four samples, including the HTS-sequenced sample, were positive for both viruses. To confirm the identity of the PCR products, amplicons from two samples for each virus were sequenced at Starseq, Mainz, Germany. The sequences of the projected GPGV amplicons (accession numbers MK533603 and MK533604) showed 98.5 and 100% identity to the HTS sequence, respectively, confirming the presence of the virus in the samples. The sequences of the projected GRSPaV amplicons (MK569516 and MK569517) showed 100% identity to each other and 98.5% identity to the isolate 34 clone 1 from France (MG938303), but they diverged from the HTS sequence (80% identity). The results suggest the presence of divergent isolates of GRSPaV that belong to at least two distinct clusters (clade 1 and clade 3 in Hily et al. 2018) in the vineyard. To our knowledge, this is the first report of grapevine viruses in Belgium. Although no detrimental effects were observed on the original plant and the two viruses are very common worldwide, GPGV can be associated with severe symptoms (Giampettruzi et al. 2012). Unveiling the presence of the viruses in Belgium shall contribute to understanding the occurrence of the viruses and developing management measures should they become necessary.

Beuve et al., 2015. Plant Dis., 293
Giampettruzi et al., 2012, Virus Res. 262-268
Glasa et al., 2014. Arch Virol. 2103-2107
Hily et al., 2018. Arch Virol. 3105-3111

Reynard et al., 2016. Plant Dis. 2545