New Disease Reports

First report of *Prunus virus F* infecting sweet cherry cultivars using high-throughput sequencing in Belgium

R. Tahzima [1,2], Y. Foucart [1], S. Massart [2] and K. De Jonghe [1]*

[1] *Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Plant Sciences Unit, Merelbeke, Belgium*; [2] *Université de Liège (ULg) Gembloux Agro-Bio Tech, Department of Plant Pathology, Gembloux, Belgium*

*E-mail: kris.dejonghe@ilvo.vlaanderen.be

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Fruit trees, like sweet cherry (*Prunus avium*) are known to be infected by numerous plant viruses, predominantly as a consequence of their vegetative mode of propagation and extended cropping. *Prunus virus F* (PrVF) is a recently described member of the genus *Fabavirus* (*Secoviridae*) which has a poorly understood pathology (Villamor *et al*., 2017).

During a sweet cherry resistance screening greenhouse experiment (July 2018) against *Little cherry virus 1* (LChV-1) and *Little cherry virus 2* (LChV-2), branches and leaves from 10 non symptomatic trees, representing propagation material from different cultivars grafted on Gisela 5 rootstocks, were collected and pooled as a single composite sample. Total RNA was extracted from 100 mg fresh tissue using the Spectrum Total Plant RNA kit (Sigma-Aldrich, Belgium) and after passing quality control, the sample was sent for library preparation (NEBNext Ultra RNA library kit; New England BioLabs, MA, USA) and high-throughput sequencing (Illumina NextSeq v2, totRNA sequencing; 40M single reads (20M each direction (2x 150bp)) by Admera Health (USA). The reads were quality filtered and submitted to the automated VirusDetect pipeline (Zheng *et al*., 2017). In total, 48 viral contigs mapped to specific PrVF accessions. A reference mapping was then conducted against the closest accessions of RNA1 (NC_039077), and RNA2 (NC_039078), in both cases revealing a 100% coverage (CLC Genomics Workbench 12, Qiagen, Denmark). The obtained RNA1 (GenBank Accession MK834285) and RNA2 (MK834286) genomic fragments were 6150 bp and 3612 bp in length, respectively. The presence of PrVF was confirmed both in the composite sample and individual trees by RT-PCR, using primers PVF1-CAF/PVF1-CAR (James *et al*., 2018), targeting RNA1. RNA2 was detected using primer pair Fab-R2 1808F/Fab-R2 2546R (Villamor *et al*., 2017). RT-PCR products were bidirectionally sequenced (Genewiz, Leipzig, Germany). Phylogenetic analysis revealed the genetic relationship between the Belgian PrVF strain with other strains (Fig. 1).

The PrVF-infected trees also harboured other co-infecting *Prunus* viruses, such as LChV-1 and *Cherry virus A* which is considered a latent virus in *Prunus*. No pathological effect of PrVF could be discerned, as observed previously (Villamor *et al*., 2017). These findings suggest a mixed infection of propagation material with PrVF and other viruses, as previously reported (Šafářová *et al*., 2017; James *et al*., 2018).

This is the first report of PrVF infecting sweet cherry in Belgium. The detection of this graft-transmissible latent virus in trees collected from nurseries may indicate a long-term occurrence of fabaviruses in Belgian germplasm and underlines the need to assess its prevalence and modes of transmission in cherry orchards. It is essential to determine the distribution of PrVF across *Prunus*-growing regions and its potential impact on cherry production in Belgium. The presence of viruses about which there is little or no pathological or epidemiological information poses important technical and regulatory challenges for plant health authorities. Sifting potential high-risk plant viruses from latent ones without required biological information will present the greatest challenge to pathologists and policy makers alike (Massart *et al*., 2017; Adams *et al*., 2018).

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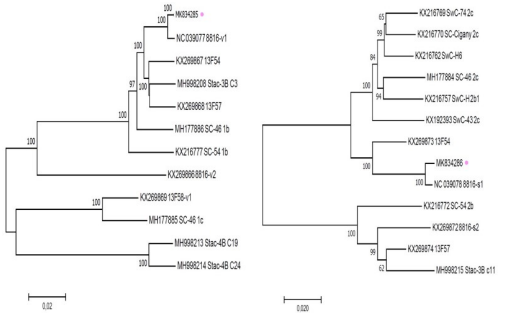

Figure 1

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