nfpareta@doct.uliege.be

P-21

Virome characterization of pome fruit European genetic resources for future breeding and risk analysis

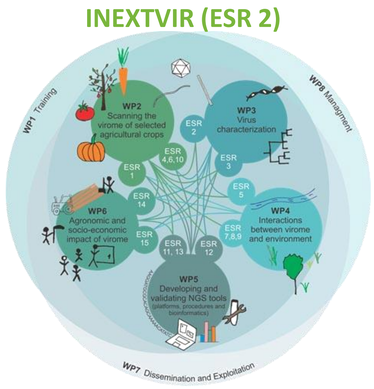
Núria Fontdevila Pareta[1], Marc Lateur[2], Stéphan Steyer[3], Johan Rollin[1,4], Arnaud G. Blouin[1], Sébastien Massart[1]

[1] University of Liège, Gembloux Agro-Bio Tech, Plant Pathology Laboratory, 5030, Gembloux, Belgium
[2] Centre Walloon de Recherches Agronomiques (CRA-W), Plant & Forest Biodiversity & Breeding, 5030, Gembloux, Belgium
[3] Centre Walloon de Recherches Agronomiques (CRA-W), Crops & Forest Health, 5030, Gembloux, Belgium
[4] DNAVision, 6041, Gosselies, Belgium

OVERVIEW OF THE PROJECT

Figure 1 Germplasm collection from the CRA-W (Belgium).

INEXTVIR (ESR 2)



WHY?

Germplasm collections are crucial resources for the breeding programs to develop new cultivars adapted to the customer requirements in a changing environment. However, old collections can be associated with viruses that threaten the survival of some plants and, thus, represent a risk to the industry.

WHAT?

This project is being done in the frame of INEXTVIR, by the Early Stage Researcher 2 (ESR2), and it focuses on the characterization of the virome of European germplasm resources of pome fruits (*Malus Mill.* and *Pyrus L.*), and assessment of the viruses detected in the frame of **pest risk analyses (PRA)**.

METHODOLOGY

HOW?

Double-stranded RNA (dsRNA) extraction used in this study has been based and adapted from the protocol at INRAE Aquitaine [1]. Samples were sequenced with Illumina technology and the data was analyzed following the procedure outlined in Figure 2.

PLANT MATERIAL

Samples were collected in Belgium, Slovenia, and France during spring and summer periods of 2019 to 2021. Sampling strategy consisted of taking one leave at each cardinal point and at two different heights. As a preliminary analysis, five apple and five pear trees from the collection at the CRA-W (Belgium) were analysed.

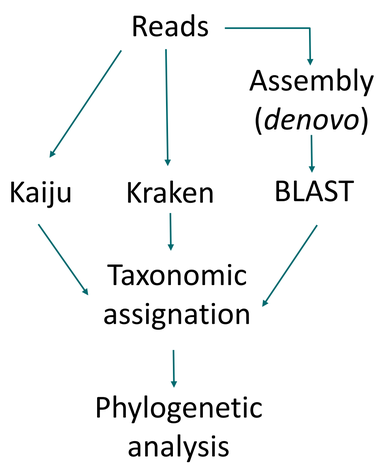


Figure 2 Outline of the bioinformatic analyses and treatment of the generated data.

PRELIMINARY RESULTS

- Apple stem pitting virus (ASPV), apple stem grooving virus (ASGV), apple chlorotic leaf spot virus (ACLSV), apple rubbery wood 1 (ARWV-1), apple luteovirus 1 (ALV-1), and apple hammerhead viroid-like circular RNA (AHVd-like) were detected during the preliminary analysis. This represents the **first detection in Belgium of ARWV-1, ALV-1, and AHVd-like**. To confirm their presence, fragments of their genome were amplified with previously published primers [2-4], and sequenced by Sanger sequencing.
- BLAST analyses of three foveavirus isolates identified returned hits of accessions labelled as ASPV and apple green crinkle-associated virus (AGCaV). Alignments of the polymerase and the coat protein genes, showed that the three foveavirus sequences belong to ASPV, based on their amino acids and nucleotide identities as defined by the International Committee on Taxonomy of Viruses (ICTV) (Figure 3). However, sequences of AGCaV are also within the species threshold for the replicase but not for the CP with ASPV species, raising limit to the current taxonomic assignation system based on the level of homology of two genes. Noteworthy, only ASPV is currently recognized by the ICTV but three accessions present in GenBank are labelled as AGCaV (unclassified foveavirus).
- After optimization of RT-PCR conditions, PCR products amplified with ARWV-1 primers showed that the **concentration of viral particles within the trees is heterogeneous**, given that there was no amplification of ARWV-1 in old leaves but there was an amplification in younger leaves. This possible heterogeneous distribution within the tree must be taken into account during the analysis of other trees and especially during sampling, which should prioritize younger leaves or at least a mix of old and young.

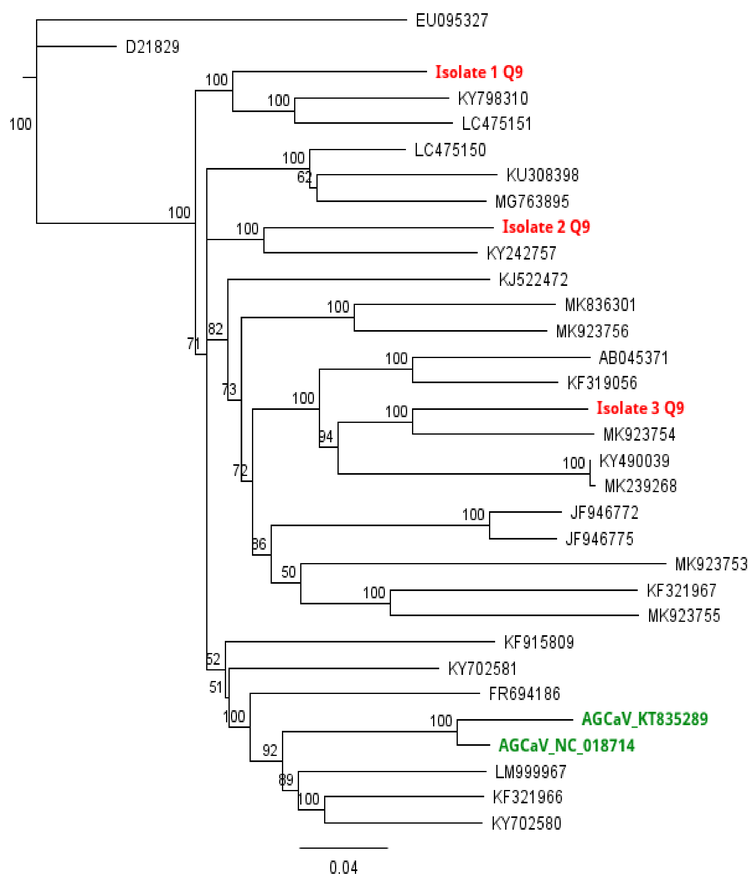


Figure 3 Neighbor-joining consensus tree of complete RdRp region from ASPV and AGCaV. Belgian isolates are highlighted in red and sequences of AGCaV are highlighted in green. AGCaV isolates form a monophyletic branch within sequences of ASPV.

TAKE-AWAY & PERSPECTIVES

The detection of an unknown virus or a new European incurtion will not only require its genome reconstruction (either full or partial), but also a Pest Risk Analyses (PRA) to inform the curators, growers, and phytosanitary authorities about the findings. Initially based on the closest genetically-related characterized viruses, the PRA will then require assays to further biologically characterize the viruses (i.e., determine the host range, symptomatology, transmission, and epidemiology on a large scale).

References

[1] Marais A., *et al.* 2018. In: Pantaleo V., Chiumenti M. (eds) Viral Metagenomics. Methods in Molecular Biology, vol 1746. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-7683-6_4

[2] P. Serra, *et al.* 2018. Virus Res., vol. 249, pp. 8–15. https://doi.org/10.1016/j.virusres.2018.03.001

[3] M. E. Rott, *et al.* 2018. Plant Disease. Vol. 102, no. 7, pp. 1254–1263. https://doi.org/10.1094/PDIS-06-17-0851-RE

[4] H. Liu, *et al.* 2018. Virol J. Vol. 15, no. 85. https://doi.org/10.1186/s12985-018-0998-3

Acknowledgments

This work is financially supported by the European Union's Horizon 2020 Research and Innovation Program under **Grant Agreement No. 813542**. INEXTVIR is a Marie Skodowska-Curie Innovative Training Network (MSCA-ITN).