

Small RNA HTS revealed the presence of CCGaV and AHVd in apple trees in Hungary

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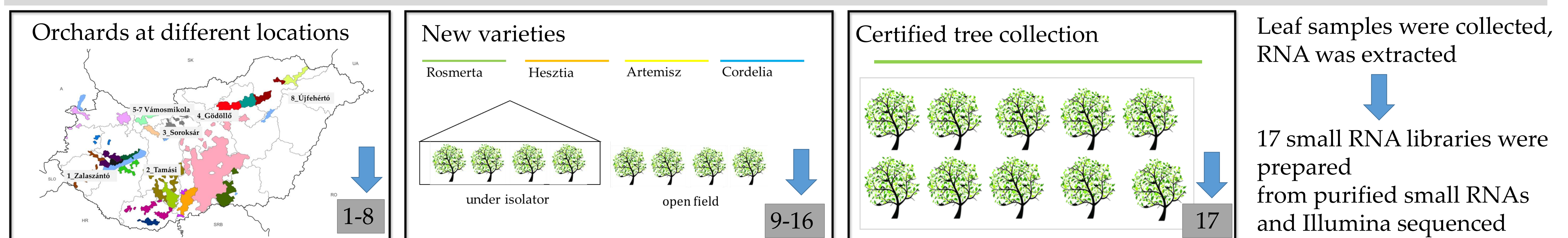
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Decline of fruit trees is a big agronomic problem decreasing crop yield constantly. Fruit trees can be infected by several pathogens, including viruses and viroids. Apple mosaic virus, apple stem pitting virus, apple chlorotic leaf spot virus and apple stem grooving virus are known to infect apple trees and as their infection can lead to crop damage their presence are under strict control. This is why, at the certified stock plantations, presence of these viruses are regularly checked by ELISA, RT-PCR or by biotests using sensitive indicator varieties.

High-throughput sequencing (HTS) revolutionized plant virology as with this method information about all the presenting pathogens in the sample can be disclosed. Small RNA HTS, a special field of this new technology, investigates small RNAs derived during virus infection by the RNA interference based defence mechanism of the host.

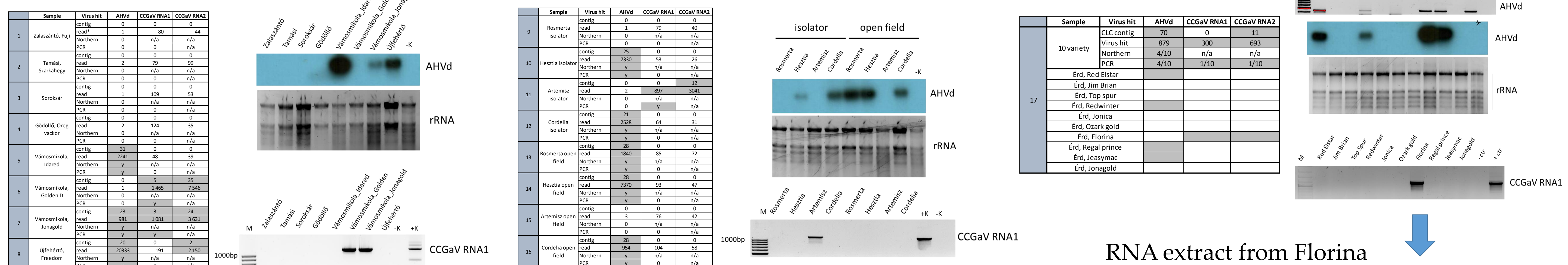
To uncover sanitary status of apple stock collection, new varieties and symptomatic apple orchards, we surveyed apple trees using small RNA HTS.



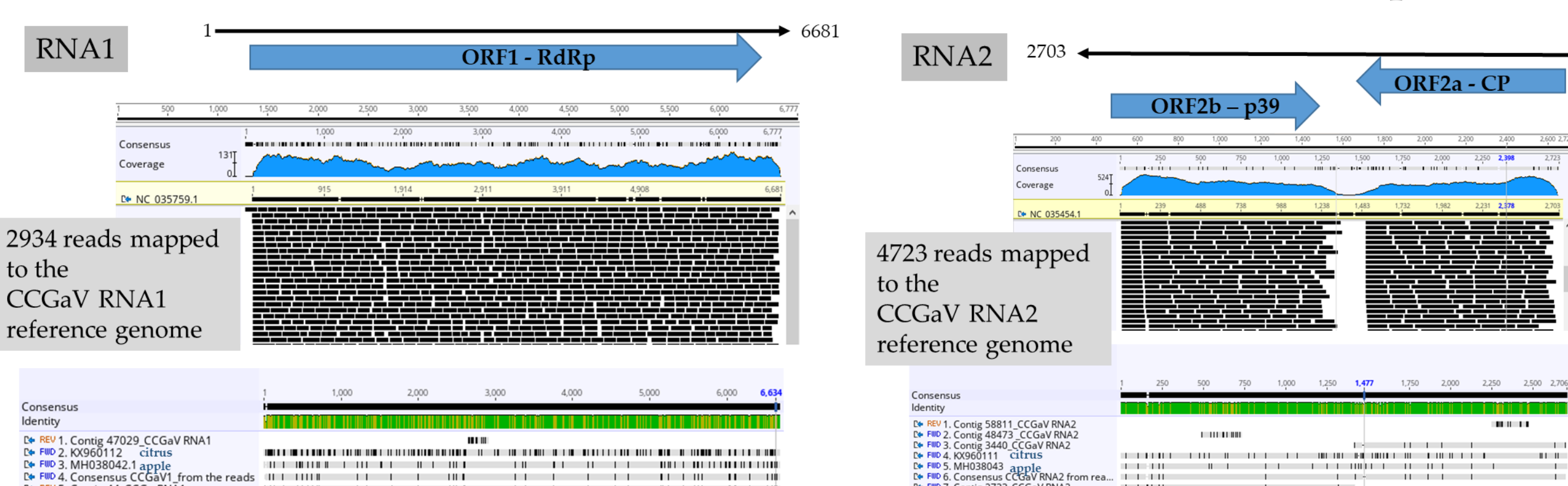
Bioinformatics analysis of the results indicated presence of citrus concave gum-associated virus (CCGaV both RNA1 and RNA2) and apple hammerhead viroid (AHVd) in several samples.



The presence of AHVd were validated both by Northern blot and with RT-PCR using viroid specific radioactive probe and primers. The presence of CCGaV was validated by RT-PCR amplifying part of the RNA1 of the virus genome.



CCGaV, a bipartite, negative stranded phlebo-like RNA virus, was first described in Italy from declining citrus (Navarro et al., 2018). It was further reported recently from apple in USA (Wright et al) and from Brasil. It was suggested that its origin roots back to Europe. Its presence in several plantations in our country can support this theory.



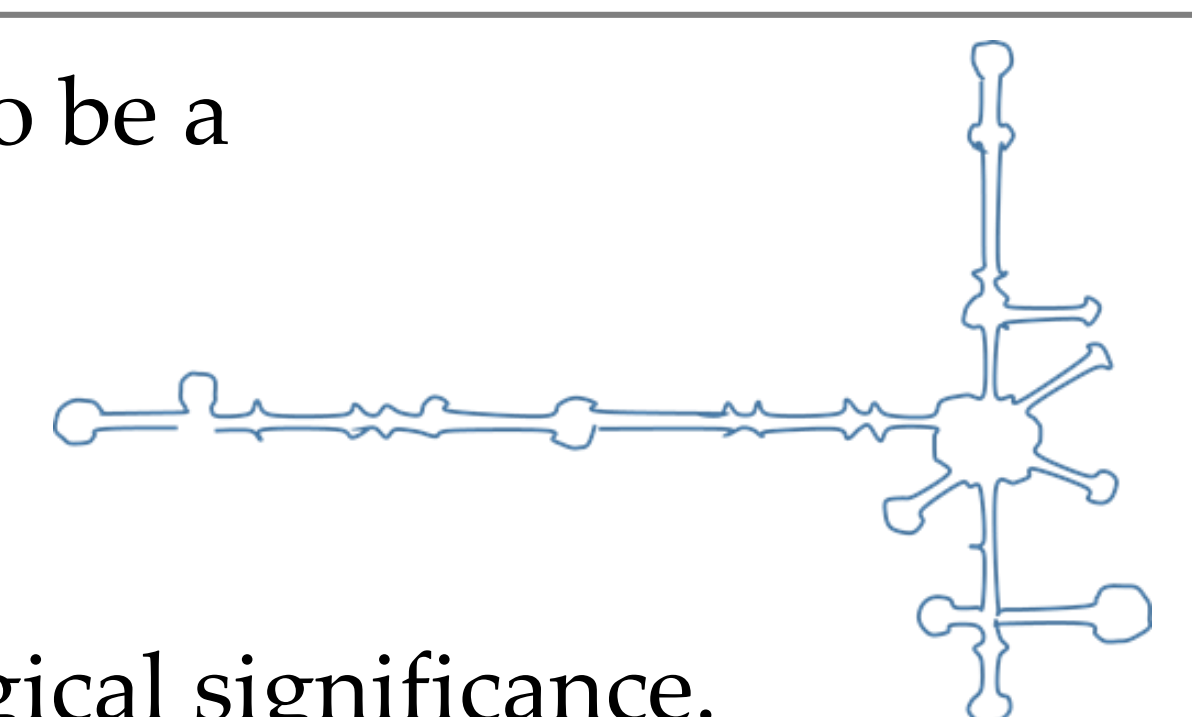
RNA extract from Florina was ribodepleted and RNA sequenced

Both CCGaV RNA1 and RNA2 from Florina variety are more identical to the apple (98,8%) than to the citrus strain (97,1%).

AHVd was first found by a computational algorithms (Zhang et al., 2014), but was further proved to be a real existing viroid (Serra et al., 2018). According to the GenBank records it is present in Canada, US, Australia New-Zealand, Japan, South-Korea and also in Europe: Italy and Spain.

Although it has not been reported from Central-Eastern Europe we found it at several locations.

High incidence of this viroid at the tested plantations raise the question of both its origin and biological significance.



Our results suggest that beside well-described regulated viruses other, non-tested ones could also contribute to tree decline. Moreover, this is a question what should be investigate in the future with sensitive high-throughput methods to prevent their spread by non-tested propagation material.

References:

Navarro et al 2018. Molecular plant pathology, 19, 1075-1089.
Serra et al 2018. Virus research, 249, 8-15.
Wright et al (018) Archives of virology, 163, 3339-3343.
Zhang et al PLOS Pathogens, 10, e1004553.

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