

# Application of next-generation sequencing for simultaneous detection of viruses, viroids and phytoplasmas in grapevine & fruit trees

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Next-generation sequencing (NGS) technologies have now become an integral part of plant health research. In addition, NGS is applied to a greater extent for detection of plant pathogens in the last years. Thus, it is used for diagnostics of viral and virus-associated diseases of grapevines and fruit trees. RNA sequencing combined with metagenomic analysis enables an unbiased analysis of infected plant samples.

Phytoplasma, viral and viroidal diseases cause severe harvest losses in viticulture and orchards.

Grapevine enation disease (GED), causing formation of enations on the underside of basal leaves and growth depression of infected plants, has been reported in Germany in 2006. The etiology of GED still remains unknown, no correlation of reverse transcription-PCR detected virus species and occurrence of disease has been found so far.

Bois noir, European stone fruit yellows, pear decline and apple proliferation

belong to the most prevalent and economically important phytoplasma diseases of grapevine respectively of fruit trees in Europe.

In this study, a NGS protocol (Illumina MiSeq platform) was applied for detection of viral and phytoplasmic infections of grapevine and fruit tree samples. Symptomatic as well as asymptomatic samples were analysed and subjected to a NGS pipeline starting from total RNA extract for generating an untargeted metagenome dataset. Therefore, untargeted and unknown pathogens may be identified.

Besides viruses and phytoplasmas detected by PCR, further viruses and viroids were found to be present. This NGS approach enabled the detection of low titer infections in tissues (samples were partially tested negatively by normal PCR assay) as well as parallel detection of phytoplasmas, viruses and viroids in a single grapevine or fruit tree sample.