

# Endophytic fungi isolated from GTD symptomatic grapevines and testing potential biocontrol agents for treatment

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Grapevine trunk diseases (GTD), caused by different phytopathogenic fungi threaten the vineyards worldwide and are one of the most important diseases in the Hungarian vineyards as well. They are caused by several fungal pathogens and the environmental factors has probable role as well. GTD pathogens can be detected only from the woody tissues of the plants. These fungi can often be found on asymptomatic plants as well. Currently there is no adequate and environmentally friendly protection against GTD pathogens. The aim of our work was to detect the pathogenic and non-GTD pathogenic endosymbiotic fungi from the grapevines, and to search for potential biocontrol agents from the healthy, asymptomatic plants.

Pure fungal cultures isolated from wood tissues were used for morphological and molecular identifications. Different endophytic and GTD pathogen fungi could be isolated from the grapevines from the Tokaj wine region between 2013 and 2014. *Diplodia seriata* and *Diaporthe* species were identified as GTD pathogen fungi from the diseased woody tissue. The presence of *D. seriata* pathogen was 72%, in 2013, while in 2014 it was only 48%. The *Diaporthe* sp. pathogens had identified in 4% and 3% in 2013 and 2014 in the diseased trunks. The identified non-GTD pathogen endophytic fungi were *Alternaria* sp., *Mucor* sp., *Penicillium* sp., *Epicoccum* sp., *Fusarium* sp., *Botrytis* and *Aspergillus* sp. The rate of these fungi was 72% in 2013, while in 52% in 2014.

Several *Trichoderma* species were also isolated from the asymptomatic grapevines. Endophytic, mycoparasitic fungi, like *Trichoderma* species have huge potential against grapevine plant pathogens colonizing woody tissues. Testing new isolates with biocontrol potential and ability to grow under different environmental conditions may provide effective biopesticide to control GTD pathogens. The *Trichoderma* isolates were identified based on their ITS1,2 and *tefl* marker sequences. The ten isolates belonged to three species: *Trichoderma harzianum* (eight isolates), *Trichoderma orientalis* (one isolate) and *Trichoderma viride* (one isolate). Their mycelial growth was determined from average colony diameters on PDA at different temperatures between 5 and 37°C. *T. orientalis* and one of *T. harzianum* isolates showed the highest growth rates within the whole temperature range, and their growth rates were especially high at 30 and 37°C. The mycoparasitic ability was tested in vitro against GTD pathogens (*D. seriata* and *Neofusicoccum parvum*). All *Trichoderma* overgrew the GTD pathogens, and sporulated on their colonies, therefore their Biocontrol Index was 100%. Some GTD symptomatic plants were treated with *Trichoderma* spore suspension in Tokaj vineyard in 2014. The treated trunks were checked monthly. The symptoms decreased on all treated samples, except one. Moreover the *Trichoderma* strains could be isolate again from their woody tissues 10 month following the treatment.

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