

First taxonomical survey of fungi isolated from Grapevine Trunk Diseases in the Tokaj wine region, Hungary

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Grapevine Trunk Diseases (GTD) are amongst the most important diseases in vineyards. GTD have been characterized only recently because of a worrying increase of the frequency of their symptom expression worldwide. Esca-, Petri- and black-foot disease, Eutypa- and Botryosphaeria- dieback are considered as major GTDs, are caused by a variety of pathogens which attack the woody perennial organs of the vine and ultimately lead to the death of the plant. Control of GTD is problematic, no grapevine cultivar is known to be resistant to GTD and there are no highly effective treatments available. The present knowledge of epidemiology, host-pathogen interaction and microbial ecology is still poor. The objective of this study was to isolate and identify fungi associated with symptoms of GTD and from canes of healthy grapevines in vineyards from the Tokaj wine region. The plantation studied is 21 years old of grapevine variety called Furmint. Four rows of grapevine were chosen for the collection of samples. Pure fungal cultures were used for morphological and molecular identifications of fungal species. Chips were cut, disinfected and incubated on potato dextrose agar medium at room temperature. Fungal DNA was obtained from pure fungal colonies after 3 days of growth on 50 ml potato dextrose broth on a rotary shaker (125 rpm). Mycelia were harvested by vacuum filtration. Total genomic DNA was extracted from freeze-dried mycelium and isolated using NucleoSpin Plant II (Macherey-Nagel) according to the protocol, followed the manufacturer's instructions. DNA concentrations were measured by NanoDrop (Thermo Scientific) and used in PCR amplification of the internal transcribed spacer region (ITS) and the 5.8S ribosomal subunit with primers ITS4 and ITS5 [1]. The PCR products were purified by using Nucleospin Gel and PCR Clean-up (Macherey-Nagel). Purified amplification products were sequenced by MWG Biotech Company in Germany. Sequences obtained were assembled and manually corrected. Reference sequences for the Botryosphaeriaceae were obtained from GenBank. Phylogenetic analyses were performed with MEGA. One thousand bootstrap analyses were run to determine confidence levels at the branching points. The principal aim of this work is to assess the GTD types and their occurrence in the Tokaj wine region as well as the identification of the pathogenic fungi (isolated from symptomatic and asymptomatic grapevine trunks) on morphological and genetic basis. In this study, 101 fungal isolates were identified from grapevine woody tissues. The majority of them (72.3%) were determined as member of Botryosphaeriaceae, 66 sample were determined as *Diplodia seriata* , 6 *Botryosphaeria stevensii*. Other fungi, like *Alternaria*, *Mucor*, *Phoma*, *Phomopsis*, *Diaporthe* and *Xylaria* also were identified from grapevine samples. Acknowledgement: The research was supported by COST Action FA1303. [1]White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322.