

Development of an official method to control the quality of mycorrhized forestry material in Hungary

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Abstract: The interest to produce mycorrhized forestry material increased in the Hungarian nurseries in this decade. The demand to certificate mycorrhized plants came from nurserymen. The aim of our work was to establish an official method to certificate mycorrhized propagation materials. The Hungarian certification system checks the identity of the mycorrhiza, and the rate of mycorrhized roots like in other European countries. The identification of mycorrhiza is based on morphological characteristics and DNA-based methods. The adaptation of PCR protocols to identify summer black truffle (*Tuber aestivum*), winter truffle (*Tuber brumale*) and weeping bolete (*Suillus granulatus*) was successful. Strong correlation was found between the results of morphological and PCR-based identifications of these species. The two ways of identification complemented each other well.

The sample plants are chosen randomly before marketing the lot. The quantity of the sample for certification is 10 plants out of 3000. There is a (0-3) scale developed to quantify the rate of the mycorrhized roots.

Zusammenfassung: Das Interesse an der Herstellung von mykorrhiziertem Aufforstungsmaterial stieg in den ungarischen Baumschulen im diesem Jahrzehnt. Das Verlangen mykorrhizierte Pflanzen zu zertifizieren kam von den Baumschulbetreibern. Das Ziel unserer Arbeit war die Erstellung einer offiziellen Methode zur Zertifizierung mykorrhizierten Vermehrungsmaterials. Das ungarische Zertifizierungssystem prüft die Identität der Mykorrhiza und die Mykorrhizierungsrate der Wurzeln wie in anderen europäischen Ländern. Die Identifizierung der Mykorrhizen basiert auf morphologischen Merkmalen und molekularer Methodik. Die Adaptierung der PCR-Protokolle für die Identifizierung von Sommertrüffel (*Tuber aestivum*), Wintertrüffel (*T. brumale*) und Körnchenröhrling (*Suillus granulatus*) war erfolgreich. Eine starke Korrelation wurde zwischen den morphologischen und den molekularen Identifikationen dieser Arten gefunden. Die zwei Identifikationswege ergänzten einander gut.

Die Probenpflanzen werden stichprobenartig vor der Vermarktung ausgewählt. Die Zahl der Proben für die Zertifizierung beträgt 10 von 3000. Eine Skala (0-3) wurde für die Quantifizierung der mykorrhizierten Wurzeln entwickelt.

The Hungarian certification is based on the French and Spanish official methods (FICHER & COLINAS 1996). These two countries gained deep experiences in this field in the last decades. The main provisions of the Hungarian authority method for controlling the quality of mycorrhized plants in containers:

The mycorrhized seedling has to fulfil the same standards as the standard of the conventional (non-mycorrhized) propagation materials (2003./LII.§; 110/2003. X. 21. statutory rule of Agricultural Ministry).

The samples are taken randomly from each lot separately. One lot is considered as the group of plants which has a common origin and was managed in the same way and mycorrhized at the same time with the same method. If the lot is containing less than 100 seedlings the quantity of the sample is 5%, up to 1000 seedlings it is 1%, and over 1000 seedlings 0.3%.

The nurserymen inform the supervisor before marketing the propagation material. The lots are marked by the supervisor and the labelling codes are kept in secret. The favouritism in the course of laboratory assessments can be avoided in this way.

The samples have to be transported to the laboratory in chilled environment. Evaluation of the rate of the mycorrhized root tips and identification of mycorrhizae are performed in the laboratory of plant pathology.

Evaluation of the rate of the mycorrhized root tips

The investigation of the incoming containerised samples is carried out as soon as possible. The plants must be stored in a cool place and watered regularly to avoid the drying up of the root-system until the beginning of the examinations. The roots of the plant before controlling are submerged into a bucket of water. In the next step the substrate is dissolved carefully from the roots.

Washing the roots under running tap water is forbidden. The whole root-system is layed out in a Petri dish filled up with water. A stereomicroscope is used for the evaluation of mycorrhization. The ratio of the mycorrhized root tips of the whole root-system is estimated. As the controller does not know the origin of the samples the favouritism can be avoided.

For certification the following evaluation scale (grades 0-3) was created:
0 = no sign of mycorrhiza, 1 = slightly mycorrhized (1-25%), 2 = well mycorrhized (26-75%), 3 = very well mycorrhized (76-100%).

Identification of the mycorrhiza

Methodological guidance was developed for propagation material supervisors in order to detect certain mycorrhizal fungi in a root-system by morphological examination (AGERER 1991, AGERER & RAMBOLD 2008). This guidance is based on such taxonomic features, which can easily be determined by using a stereomicroscope. The examination of the mantle and the cystidia is necessary for accurate distinguishing of *Tuber* species by using a research microscope. This guidance is illustrated with microphotos and graphs and it is supplemented by a key for identification of mycorrhizae based on Java.

Identifications by PCR analysis were done successfully as well using species specific primers constructed by scientists of the Eötvös University or published in literature (KRETZER & al. 1996, MELLO & al. 2002, DOUET & al. 2004). It may be very useful for verifying the morphological identifications and for correct identification of doubtful materials.

The criteria to certificate the lot

Samples for getting a certificate have to meet the following criteria:

There is no unmycorrhized seedling in the lot (grade 0). More than half of the lot have at least grade 2. There is no other *Tuber* species in the roots.

The theoretical certification systems mentioned above were put into practice in the framework of the MIKOQUAL and QUTAOMEL projects (BRATEK 2008). Furthermore, they were used not only for the examinations of the mycorrhized project materials, but for checking of mycorrhizal nurseries by authorities. The final aim of this project is to work out a Hungarian labelling system for official certification of mycorrhized plants, like it works smoothly in France.

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