

ARTICLE

Detection of the vitalization effect of *Tuber* mycorrhiza on sessile oak by the recently-innovated FMM chlorophyll fluorometer

Ádám Solti^{1*}, Gabriella Tamaskó¹, Sándor Lenk², Attila Barócsi², Zoltán Bratek¹

¹Department of Plant Physiology and Molecular Plant Biology, Eötvös Loránd University, Budapest, Hungary, ²Department of Atomic Physics, Budapest University of Technology and Economics, Budapest, Hungary

ABSTRACT Mycorrhizae enhance the viability of forest trees by the protection against drought and nutrient deficiencies. Here a FluoroMeter Modul (FMM) Chl a fluorometer developed on the Department of Atomic Physics of the Budapest University of Technology and Economics, was used to typify symbiotic relationship between *Quercus* and *Tuber* which has economic impact due to its fruiting body. The actual quantum efficiency photosystem II showed strong correlation with low mycorrhizations up to the level of average mycorrhization in sessile oak seedling population, which refers to strict host control on mycorrhization. F690/F735 ratio of peak fluorescence, which is known to correlate negatively to leaf chlorophyll content, implicated that the higher mycorrhization caused probably stronger leaf area expansion. Therefore, the presence of *Tuber* mycorrhiza on *Quercus* roots enhanced the vitality of oak trees. The portable and relatively low-price FMM fluorometer proved to be an adequate tool for serial *in situ* vitality measurements.

Acta Biol Szeged 55(1):147-149 (2011)

KEY WORDS

chlorophyll a fluorescence
mycorrhiza
Tuber
Quercus
FMM fluorometer

Ectomycorrhiza is a very important symbiotic relationship between plants and fungi enhances the viability and production of forest trees (Nardini et al. 2000). Among some obligate mycorrhiza-forming fungus, Truffles (*Tuber* spp.) has high economic impact due to their fruiting bodies. *Tuber* can form mycorrhiza with various plants including genera *Quercus*, *Carpinus*, *Corylus*, *Tilia*, *Cedrus* and *Pinus* (Chevalier et al. 2002).

Photosynthetic activity is strongly related to vitality of green plants. Mycorrhization is known to protect plants against abiotic stresses (Van Tichelen et al. 2001; Domínguez-Núñez et al. 2006). Chlorophyll (Chl) a fluorescence induction gives information on the efficiency of photosynthetic electron transport. The FMM Chl a fluorometer developed by Barócsi et al. (2000) which differs from PAM-based Chl a fluorometers as it uses a continuous red excitation laser and detects the fluorescence at two different wavelengths (690 and 735 nm). Here, the FMM Chlorophyll a fluorometer was used to typify the symbiotic relationship between *Tuber* and *Quercus*.

Materials and Methods

Plant material

Seedlings of sessile oak (*Quercus petraea* [Matt.] Liebl., 1935), planted in sterile peat-perlite mixture, were inoculated

with summer truffle (*Tuber aestivum* Vitt., 1831) and grown in green houses under controlled conditions. One year after inoculation, three leaves of each of the randomly selected plants were tested. Three hundred root tips of each specimen were measured according to Fischer and Colinas (1996) to calculate mycorrhization.

Chlorophyll fluorescence induction

Fluorescence induction measurements of leaf samples were performed using a FMM Chl a fluorometer (Barócsi et al. 2009). The internal light source is a 635 nm laser diode (QL63H5SA, Roithner Lasertechnik GmbH, Wien, Austria) with 20 mW maximum optical power. The FMM allows the measurement of traditional Kautsky induction kinetic curves detected simultaneously at the two maxima of the Chl a fluorescence in leaves (at the 690 nm red and the 735 nm far-red bands). Data were obtained from leaves adapted to the natural summer-time, full-sun irradiance ($J_{\text{phot}} > 1000 \mu\text{mol m}^{-2} \text{s}^{-2}$). Kautsky kinetics was monitored under 2 min excitation time, where the minimal (F_0') and maximal fluorescence (F_p) values were recognized at both wavelengths, from which the light adapted, steady-state quantum efficiency of photosynthetic electron transport, $F_v/F_p = (F_p - F_0')/F_p$ was calculated at both emission maxima.

Results

Truffle colonised roots were found in more than 80% of

Accepted July 11, 2011

*Corresponding author. E-mail: sadambio@elte.hu

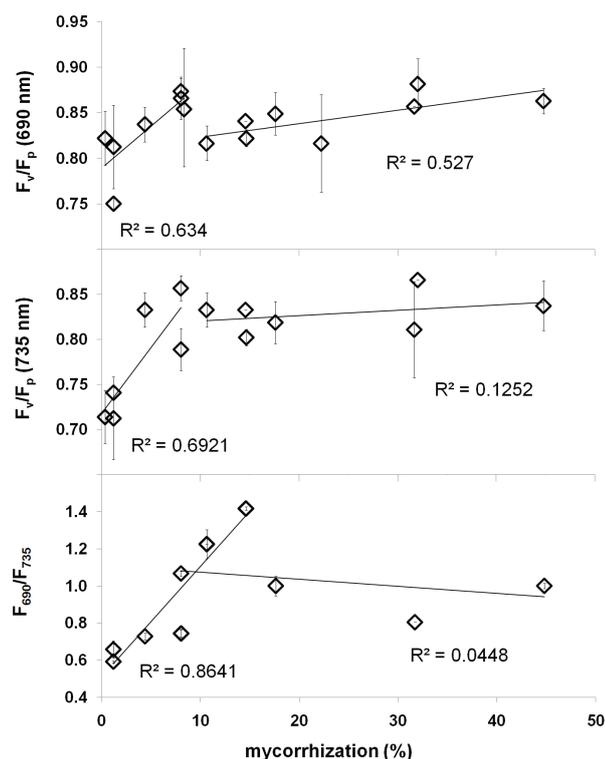


Figure 1. Correlation of mycorrhization with actual quantum efficiency measured at 690 nm (A) and 735 nm (B) and with the red/far red fluorescence ratio (C).

sessile oaks. The percentage of mycorrhization, however, remained relatively low, the average mycorrhization of the roots was $13.7 \pm 11.4\%$. Correlating F_v/F_p values, measured at 690 nm (Fig. 1A), to mycorrhization, below 10% of mycorrhization, the increase of F_v/F_p values showed a strong positive correlation to the mycorrhization, whereas at higher mycorrhization, there was no correlation between these two parameters. In the far red region, F_v/F_p ratios showed a similar pattern (Fig. 1B), thus only at lower mycorrhization were any (positive) correlation found. The F_{690}/F_{735} fluorescence ratio (Fig. 1C) also showed increase with the increasing mycorrhization followed by saturation of its value.

Discussion

Most of the red and far red emission originates from Chl a molecules belonging to the photosystem (PS) II reaction centre and antennae. PSI fluorescence can only affect the far red emission at high temperatures (Agati et al. 2000). At 690 and 735 nm, the similar shape of F_v/F_p as the function of the mycorrhizal frequency means that the controlled greenhouse temperature ($<25^\circ\text{C}$) had little effect on the PSI fluorescence and most of the 735 nm fluorescence also originated from PSII.

Mycorrhization is known to improve the vitality of plants under various stresses (Van Tichelen et al. 2001; Domínguez-Núñez et al. 2006). Up to 10% of mycorrhization, the vitality of sessile oak was improved significantly, which means that even the presence of the mycorrhiza partner at low amount bear an effect. The red/far red fluorescence ratio is known to show inverse correlation to leaf Chl content (Buschmann, 2007). In our experiments, low mycorrhization was correlated with low F_{690}/F_{735} ratio, which refers to higher Chl concentration in leaves of plants with low mycorrhization. The higher Chl concentration of these leaves can be explained by the lower leaf expansion caused either by a relative water or nutrient deficiency. Therefore, the presence of the *Tuber aestivum* mycorrhiza improved the vitality of mycorrhized plants. Percentage of mycorrhization, which is an important factor in the quality of a truffle inoculated plants, is able to be measured by testing photosynthetic activity using the portable FMM fluorometer. However, for a better understanding of this vitalization mechanism, the investigation of some other factors like host species, host developmental stage, and substrate effects can be also important.

Acknowledgements

The work has been supported by the grant of the National Technology Programme entitled QUTAOMEL. This work was done using FMM that is connected to the scientific program of the „Development of quality-oriented and harmonized R+D+I strategy and functional model at BME” project. This project is supported by the New Széchenyi Plan (Project ID: TÁMOP-4.2.1/B-09/1/KMR-2010-0002). A part of this study was presented on the 10th Congress of the Hungarian Society for Plant Biology, August 31 - September 2, 2011, Szeged, Hungary.

References

- Agati G, Cerovic ZG, Moya I (2000) The effect of decreasing temperature up to chilling values on the in vivo F_{685}/F_{735} chlorophyll fluorescence ratio in *Phaseolus vulgaris* and *Pisum sativum*: the role of the photosystem I contribution to the 735 nm fluorescence band. *Photochem Photobiol* 72:75-84.
- Barócsi A, Kocsányi L, Várkonyi S, Richter P, Csintalan Z, Sente K (2000) Two wavelength, multipurpose, truly portable chlorophyll fluorometer and its application in field monitoring of phytoremediation. *Measure Sci Technol* 11:717-729.
- Barócsi A, Lenk S, Kocsányi L, Buschmann C (2009) Excitation kinetics during induction of chlorophyll a fluorescence. *Photosynthetica* 47:104-111.
- Buschmann C (2007) Variability and application of the chlorophyll fluorescence emission ratio red/far-red of leaves. *Photosynth Res* 92:261-271.
- Chevalier G, Gregori G, Frochot H, Zambonelli A (2002) The cultivation of the Burgundy truffle. In Hall I, Wang Y, Danell E, Zambonelli A, eds., *Edible mycorrhizal mushrooms and their cultivation*. Proceedings of the Second International Conference on Edible Mycorrhizal Mushrooms, Christchurch, New Zealand, 3-6 July, 2001., pp. 1-12.
- Domínguez-Núñez JA, Selva-Serrano J, Rodríguez-Barreal JA, Saiz de Omeñaca-González JA (2006) The influence of mycorrhization with

- Tuber melanosporum* in the afforestation of a Mediterranean site with *Quercus ilex* and *Quercus faginea*. *Forest Ecol Management* 231:226-233.
- Fischer, C, Colinas C (1996) Methodology for certification of *Quercus ilex* seedlings inoculated with *Tuber melanosporum* for commercial application. In: Program and Abstracts of the First International Conference on Mycorrhizae. Berkeley, California, U.S.A.
- Nardini A, Salleo S, Tyree MT, Vertovec M (2000) Influence of the ectomycorrhizas formed by *Tuber melanosporum* Vitt. on hydraulic conductance and water relations of *Quercus ilex* L. seedlings. *Ann For Sci* 57:305-312.
- Van Tichelen KK, Colpaert JV, Vangronsveld J (2002) Ectomycorrhizal protection of *Pinus sylvestris* against copper toxicity. *New Phytol* 150:203-213.