Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress

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Abstract
The plants produced by in vitro methods are free of any microflora contrary to natural systems where plants are colonized by symbiotic fungi. The present paper reports the experiments carried out to evaluate the role of arbuscular endomycorrhizal fungi in development of micropropagated strawberries and their photosynthetic activity (measured by chlorophyll fluorescence) under drought conditions.

Mycorrhization strongly affected growth and tolerance to water deficiency of the plants cultivated in greenhouse. Wilting of not-mycorrhized plants was accompanied by drastic increase of Fo and Tfm and decrease of Fm. At the same time, the value of these parameters for mycorrhized plants did not change. Drastic decrease in the value of parameters Fv/Fm, Fv/Fo and Fo/Fm for plants without AMF appeared at the end of dry period. Rise of Fs and decrease Rfd was noted only for not-mycorrhized plants. The plants colonized by fungi, fully recovered their photosynthetic activity when watering was restored.

Introduction
Symbionts called ‘mycorrhizal fungi’ occur in most biomes on earth and are a fundamental reason for plant growth on the planet. The most common mycorrhiza is that formed by the arbuscular mycorrhizal fungi (AMF), which colonize roots of over 80% of the plant kingdom. The plants produced in vitro are devoid of microflora, both beneficial and pathogens. Absence of beneficial microorganisms, provokes negative acclimatization process and poor physiological adaptation to natural conditions. During the last years, the mycorrhizal technology has been used to improve growth of a number of micropropagated horticultural crops as well as to enable host plants to tolerate or withstand the impairing effects of abiotic and biotic stresses (Guillemin et al. 1992, Hooker et al. 1994, Dodd 2000).

Mycorrhizal fungi influence development of a superior root system, enhance water conducting capacity, increase uptake of macro, micro and immobile nutrients. In the presence of mycorrhiza higher photosynthetic rates develops quantified as dioxide carbon assimilation (El-Tohamy et al. 1999, Estrada-Luna et al. 2000, Auge 2001). While there are results reporting involvement of the mycorrhizal fungi in dark phase of photosynthesis, the knowledge about photochemical activity of plants growing in presence of AMF is insufficient.
The present paper reports the effect of arbuscular endomycorrhizal fungi on growth of micropropagated strawberry plants, their tolerance to drought and photosynthetic activity (measured with chlorophyll fluorescence method).

Material and methods

Plant material and inoculation procedure.

Strawberry cultures (cv. Senga Sengana) were initiated and multiplicated according to modified method described by Boxus (1974). Rooting was carried out ex vitro, as described by Borkowska et al. (1999). Before inserting the shoots into substrate, mycorrhizal inoculum was added, in amount of about 200 mg per one cell of multiplates. Inoculum was produced by Societe BIORIZE - Dijon, France and consisted of a mixture of Glomus sp. In control plants no mycorrhizal fungi were added. Multiplates of both treatments were maintained in a growth chamber at 23 - 25 °C under photosynthetic photon flux density (PPFD) ~30 µmol·m⁻²·s⁻¹ from “warm-white” fluorescent lamps (Philips) and photoperiod 16/8 (day/night). Multiplates were covered (not tightly) for 2 weeks. After removing the cover, PPFD increased up to 120 µmol·m⁻²·s⁻¹. The plantlets were watered as needed. Multiplates of both treatments were maintained in a growth chamber at 23 - 25 °C under photosynthetic photon flux density (PPFD) ~30 µmol·m⁻²·s⁻¹ from “warm-white” fluorescent lamps (Philips) and photoperiod 16/8 (day/night). Multiplates were covered (not tightly) for 2 weeks. After removing the cover, PPFD increased up to 120 µmol·m⁻²·s⁻¹. The plantlets were watered as needed. Once a week they were fertilized with solution 12 N + 0 P + 43 K (800 mg/l). After 8 weeks, rooted plantlets were transplanted into pots (5.5 cm in diameter) filled with a mixture of peat-rockwool water repellent (1 : 1) amended with Osmocote Plus, 50 mg/pot. The plantlets rooted in presence of mycorrhizal fungi, received an additional portion of inoculum. Some of the plants were moved to a greenhouse.

After 3 months of growing in the greenhouse, mycorrhized and non-mycorrhized (control) plants were divided into 2 groups. The first group was watered regularly, as needed. The second one, was not watered for 7 days. Afterwards, watering was restored.

Plant growth evaluation and statistical analysis

The plants were characterized several times by fresh weight (FW) of leaves and roots and also by leaf area. The shoot-to-root dry weights was calculated. The first measurement was made at the end of rooting phase - 4 weeks after inserting the microshoots into the substrate. The next two measurements were taken 4 and 8 weeks after the first one. At the end of the experiment, the leaves used for measurements of chlorophyll fluorescence were evaluated individually, in respect to their biomass accumulation and water content.

Ten plants from each treatment were chosen for the measurements. A single plant was treated as a replicate. The results were elaborated statistically by analysis of variance. The significance between means was evaluated by Duncan’ test at P=0.05.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured on fully developed, intact leaves by the fast direct fluorometer PEA (Hansatech, England). Each measurement was done on the same leaf. The leaves were placed into the clip, darkened for 20 min and then illuminated with red light emitting diodes (peak 650 nm, maximum PPFD at leaf surface was 3,000 µmol·m⁻²·s⁻¹, 40 % of full intensity). At each physiological state, a sample was characterized by Fo, Fm, Tfm. The fast fluorescence rise starts at the initial low value Fo (characteristic of open reaction centres) and reaches a maximum value Fm (characteristic of closed reactions centres). The full kinetics of the fluorescence rise is given by value Tfm - rise time from Fo to Fm. Out of these parameters, several expressions were calculated. The basis of calculated expressions is ratio Fv/Fm. This is a useful parameter which has been shown to be proportional to the quantum yield of photochemical reactions, where Fv (variable fluorescence) = Fm – Fo. The second ratio, Fv/Fo is associated with a disruption of water-splitting system (Schreiber et al. 1994). Of these two parameters, the Fm/Fo can be used as an indicator of the physiological state of plants and it is called state change index. The value of this parameter increases with the adaptive process (Strasser 2001). The above parameters refer to the structure and function of PSII. Additional two parameters, Fs (steady state fluorescence) and Rfd (vitality index) referring to the interaction of the light phase reactions with enzymatic dark process (Lichtenthaler et al. 1986), were included. The first measurement was taken at the last day of regular