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Effect of light intensity on growth and chlorophyll fluorescence of *Rhododendron* microcuttings during acclimatisation

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ABSTRACT

Survival, growth, and chlorophyll *a* fluorescence of *in vitro* propagated rhododendron 'Alfred' at two light intensities 75 and 150 μmol m^{-2} s^{-1} PPFD during acclimatisation were evaluated. High survival and better growth were obtained at higher light intensity. Maximum quantum efficiency of PSII (Fv/Fm) of *in vitro* established leaves decreased gradually from 0.70 directly after taking out of *in vitro* culture to 0.57 and 0.53 for plants exposed to 75 and 150 μmol m^{-2} s^{-1} PPFD, respectively for the first weeks, and then progressively increased to 0.76 at the end of acclimatisation period. The Fv/Fm ratio for new *ex vitro* established leaves was significantly higher than for the old ones. High Fs (steady state fluorescence) and low vitality index (Rfd) for *in vitro* established plants were noted at the beginning of acclimatisation and indicate poor functioning of dark phase of photosynthesis. Vitality index of new leaves was about twice as high as the old, *in vitro* formed

ones and reflected their higher photosynthetic potential. The highest vitality index was measured for plants exposed to higher light intensity.

INTRODUCTION

Micropropagation is an important method of young rhododendron plants production. Yearly production of rhododendrons in Poland is presently around 1.7 million of which above 50% are propagated by tissue culture (Muras 2003). The acclimatisation of microplants to *ex vitro* conditions is the most critical stage of micropropagation. Transfer from sterile *in vitro* conditions to non-sterile potting media with no supplied carbon and reduced humidity has been reported to lead to plant mortality and slow growth rate. Poor photosynthetic capacity is one of the main factors responsible for poor survival and slow growth of microcuttings (Grout 1988, Van Huylenbroeck et al. 1998, Debergh et al. 2000).

Different attempts have been made to optimise the growth of microplants *ex vitro*. Among them, supplementary lighting was very effective and stimulated growth rate of many ornamental plants as *Dieffenbachia*, *Ficus benjamina*, *Gerbera*, *Homalomena*, *Nephrolepis exaltata* and *Rosa* (Matysiak 1996, Matysiak and Nowak 2001, Nowak et al. 2002).

During the last years, chlorophyll *a* fluorescence has proven to be a useful instrument to study responses to environmental stresses. Measurements are both non-destructive and non-invasive, and applications range from a method of rapid identification of injuries in leaves without visual symptoms, to a detailed analysis of causes of changes in photosynthetic capacity (Krause and Weis 1991).

The aim of the present experiment was to investigate the effect of light intensity on survival and growth of micropropagated rhododendrons and to evaluate changes in photosynthetic apparatus during acclimatisation.

MATERIAL AND METHODS

Microcuttings of *Rhododendron catawbiense* 'Alfred' were used for experiment. Initial explants for establishment of rhododendron *in vitro* culture were vegetative buds. Single, unrooted *in vitro* cuttings were transplanted into plastic boxes filled with sphagnum peat medium, with pH 4.0. Microcuttings were acclimatised at two levels of photosynthetic photon flux density (PPFD, 75 and 150 μmol m^{-2} s^{-1}). Plants were cultivated at ambient temperature 22°C and 16 h photoperiod under cool white fluorescence tubes. Since the 9^{th} week of culture, microplants were fertigated once a week with 0.1% solution of Peters Professional Foliar Feed fertilizer (27 N + 15 P_2O_5 + 12 K_2O + microelements).

Chlorophyll *a* fluorescence was monitored every 4 weeks with fluorescence monitoring system PEA (Hansatech Instruments Ltd, England). The measurements were done separately on *in vitro* (old) and *ex vitro* (new) established leaves. The leaves were placed into the clip, darkened for 20 min and then illuminated with red light emitting diodes (peak at 650 nm, maximum PPFD at leaf surface was 3000 μmol m^{-2} s^{-1}). At each stage of development, a sample was characterised by Fo, Fm, Fv and Fv/Fm referring to light primary photosynthetic reactions, as well as Fs and Rfd referring to enzymatic dark process of photosynthesis, where Fo, Fm and Fv (Fv = Fm - Fo) – are initial, maximum and variable fluorescence, Fv/Fm – maximum quantum efficiency of PSII, Fs – steady state fluorescence and Rfd – vitality index.

Survival, dry weight of shoots, plant height, number of leaves per plant and mean length of leaves were evaluated after 20 weeks of cultivation. The experiment had one-factorial design with two PPFD levels, each at four replications. Each replication consisted of 10 microplants. The treatments were statistically analysed by analysis of variance and means were compared with Duncan's multiple range test at $p = 0.05$.

RESULTS AND DISCUSSION

Survival of rhododendron 'Alfred' after transfer out of *in vitro* culture was 100% for plantlets cultivated at lower (75 μmol m^{-2} s^{-1}) and 95% for plants cultivated at higher PPFD level (150 μmol m^{-2} s^{-1}), but the differences were not statistically significant (Table 1). Plants exposed to higher PPFD level showed higher biomass accumulation. After 20 weeks of cultivation, shoot dry weight of plants grown at 150 μmol m^{-2} s^{-1} was almost 150% higher than plants cultivated at 75 μmol m^{-2} s^{-1} PPFD. Increasing light intensities also increased plant height, number of leaves, and mean length of leaves of rhododendrons.

Table 1. Survival and growth of micropropagated *Rhododendron catawbiense* 'Alfred' after 20 weeks of acclimatisation at different light intensities

Light intensity [μmol m^{-2} s^{-1}]	Survival [%]	Shoot dry weight [g]	Plant height [cm]	Number of leaves per plant	Mean length of leaves [cm]
75	100 a*	0.09 a	3.8 a	12 a	2.8 a
150	95 a	0.22 b	4.5 b	15 b	3.5 b

* Means designated with the same letter do not differ significantly according to Duncan's multiple range test at $p = 0.05$

The results presented in this study show that *Rhododendron*, known as a shade plant, has to cope with adaptation necessary for survival in the early weeks of the acclimatisation period. A decrease in Fv/Fm ratio takes place during the first weeks after transfering whether the plantlets were grown under low or high light intensities remains irrespective (Fig. 1).

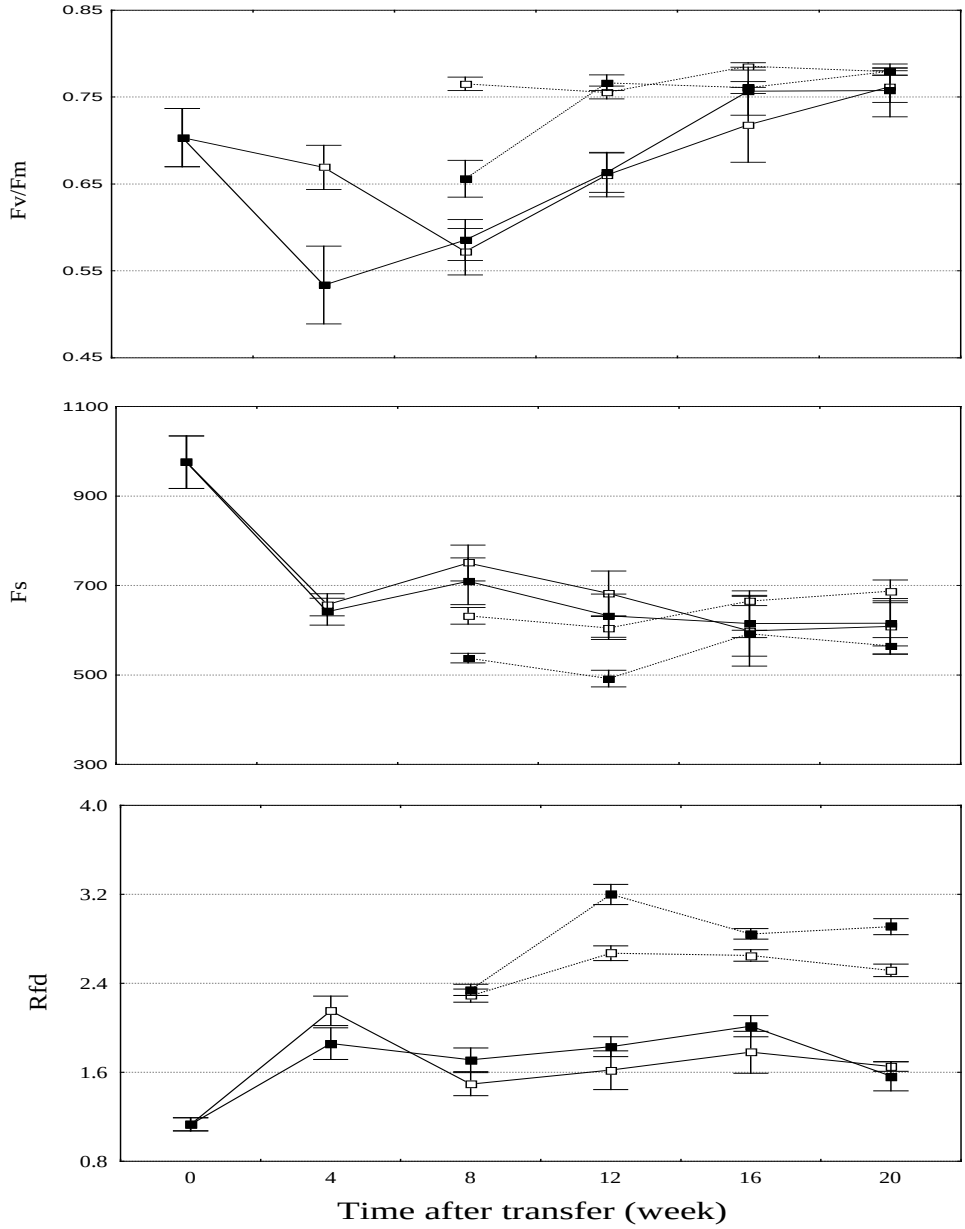

Figure 1. Fluorescence parameters Fv/Fm, Fs and Rfd of micropropagated *Rhododendron catawbiense* 'Alfred' acclimatised at 75 µmol m^{-2} s^{-1} (□) or 150 µmol m^{-2} s^{-1} PPFD (■) for *in vitro* (solid line) and *ex vitro* (broken line) formed leaves. Values are means ± SE (n = 20)

However, this decrease in Fv/Fm was significantly more pronounced as the difference between light conditions in the laboratory and *ex vitro* increased. The Fv/Fm ratio for plants just after taking out of *in vitro* culture was 0.70. Plants exposed to 150 μmol m^{-2} s^{-1} PPFD reached minimal Fv/Fm value (0.53) after 4 weeks of acclimatisation. Similar decreases in Fv/Fm with increasing light intensity have been observed in numerous plants grown in the field (Öquist et al. 1992). The decline of Fv/Fm is linearly correlated with the quantum yield of light-limited oxygen evolution (Björkman and Demmig 1987) and the number of functional PSII reaction centres (Őquist et al. 1992). It is also an indication that changes in climatic conditions at transplantation create stress and photoinhibition. The results demonstrate that photoinhibition can occur directly after transplanting the micropropagated plants, even at low light intensity. Minimal value of Fv/Fm (0.57) for plants exposed to lower light intensity was reached 8 weeks after transplanting. In field grown plants photoinhibition at low light levels has mainly been observed when an additional stress factor (low temperature, drought) was present (Powles 1984, Borkowska 2002). Probably the poorly differentiated chloroplasts of *in vitro* established leaves (Lee et al. 1985) together with water stress, which plants have to deal with at transplanting (Debergh et al. 2000), resulted in a low resistance against photoinhibition and made micropropagated plants more susceptible to changes in light levels. The decrease in Fv/Fm during 8 weeks of acclimatisation was caused both by rise in Fo and decrease in Fm (data not presented). An increase in Fo is characteristic for a destruction of PSII reaction centres (damage to D1 – protein and other reaction centre components) or the impairment of transfer of excitation energy from antenna to the reaction centres, while decrease in the Fm can be related to the denaturation of centres of chlorophyll-protein complexes (Bjőrkman and Demmig 1987). At that moment photoinhibition resulted in photodamage of chlorophyll rather than photoprotection (Demmig and Bjőrkman 1987) due to an excess of excitation energy accumulation in PSII. The resistance against photoinhibition of old leaves increased from the 8^{th} week as new leaves were formed, and this was reflected in an increased Fv/Fm ratio under both low and high light intensities. Plant acclimatised at higher light intensity showed an earlier recovery in Fv/Fm compared to those under the lowest light treatment. After decreasing, the Fv/Fm ratio increased towards the end of acclimatisation and after 20 weeks reached value 0.76 for both light intensities (for non-stressed plants the range of typical Fv/Fm ratio is 0.75-0.85).

The ratio of Fv/Fm of new, *ex vitro* established leaves was significantly higher than old, *in vitro* formed leaves during all acclimatisation period and these values were always above 0.75, which suggests high photochemical efficiency of PSII. However new leaves, just after developing, were still more photoinhibited (as indicated by lower Fv/Fm values) when grown at higher light intensity compared to

low light grown plants, but prolonged exposure resulted in quickly increasing Fv/Fm.

Interaction of the light phase photosynthetic reaction with enzymatic dark process for old and new leaves was also monitored during acclimatisation. The value of steady state fluorescence (Fs) for plants at transplanting time was very high and this value drastically dropped after 4 weeks, irrespective of light conditions. High Fs value indicates low ATP consumption in the Calvin cycle (Krause and Weis 1991). Under *in vitro* culture conditions, low photosynthesis rates are frequently reported. Downregulation of Rubisco activity or starch accumulation due to external carbon source in the medium have been suggested to cause the inhibition of dark reactions of photosynthesis. Decreasing Fs value and increasing vitality index value (Rfd) during first 4 weeks of acclimatisation can suggest switching from mixotrophic metabolism of plantlets *in vitro* to autotrophic growth out of culture. After the initial drastic decrease, steady state fluorescence of old leaves remained at the same level to the end of acclimatisation. New, *ex vitro* formed leaves showed increased ATP consumption in the Calvin cycle in the first weeks of development, as was reflected by higher Fs value. Vitality index (Rfd) of new leaves was about twice as high as the old, *in vitro* formed ones, and reflected their higher photosynthetic potential. Grout (1988) postulated that for a lot of plant species, *in vitro* formed leaves never became fully autotrophic. Those results confirm the work of several other researchers who found significantly higher photosynthetic rates in new leaves compared to *in vitro* formed ones (Lee et al. 1985, Matysiak 1996, Van Huylenbroeck 1998). The highest vitality index (Rfd) was measured for rhododendrons exposed to high light intensity from the 12th week of cultivation. As was shown earlier, rhododendrons exposed to higher light intensity showed higher biomass accumulation.

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WPŁYW INTENSYWNOŚCI ŚWIATŁA NA WZROST I FLUORESCENCJĘ CHLOROFILU MIKROSADZONEK RÓŻANECZNIKÓW W CZASIE AKLIMATYZACJI

Streszczenie: Badano wpływ intensywności światła (75 i 150 μmol m^{-2} s^{-1} PPFD) na przeżywalność, wzrost i fluorescencję chlorofilu *a* mikrosadzonek *Rhododendron catawbiense* 'Alfred' w czasie aklimatyzacji. Wysoką przeżywalność i szybszy wzrost mikrosadzonek uzyskano w warunkach wyższej intensywności światła. Sprawność fotochemiczna PSII mikrosadzonek zmniejszała się w pierwszych tygodniach aklimatyzacji, czego wyrazem było zmniejszenie wartości Fv/Fm z 0,70 do 0,57 i 0,53 dla roślin aklimatyzowanych w warunkach niskiej (75 μmol m^{-2} s^{-1}) i wysokiej (150 μmol m^{-2} s^{-1}) intensywności światła. Sprawność fotochemiczna PSII liści młodych była znacznie większa niż liści starych, powstałych w warunkach *in vitro*. Również funkcjonowanie ciemniowych reakcji fotosyntezy mikrosadzonek bezpośrednio po wyjęciu ze szkła było niewłaściwe, czego wyrazem była wysoka wartość fluorescencji końcowej (Fs) oraz niski współczynnik żywotności (Rfd). Współczynnik żywotności młodych liści (powstałych w warunkach *ex vitro*) był dwukrotnie wyższy niż liści starych powstałych *in vitro*, co wskazuje na ich wyższy potencjał fotosyntetyczny. Współczynnik żywotności różaneczników rosnących przy większej intensywności światła był wyższy niż uprawianych w gorszych warunkach świetlnych.

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