Chlorophyll fluorescence in micropropagated *Rhododendron ponticum* subsp. *baeticum* plants in response to different irradiances

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Abstract

The aim of this study was to investigate acclimation of micropropagated plants of *Rhododendron ponticum* subsp. *baeticum* to different irradiances and recovery after exposure to high irradiance. Plants grown under high (HL) or intermediate (IL) irradiances displayed higher values of maximum electron transport rate (ETR_{max}) and light saturation coefficient (E_k) than plants grown under low irradiance (LL). The capacity of tolerance to photoinhibition (as assessed by the response of photochemical quenching, q_p) varied as follows: HL > IL > LL. Thermal energy dissipation (q_N) was also affected by growth irradiance, with higher saturating values being observed in HL plants. Light-response curves suggested a gradual replacement of q_p by q_N with increasing irradiance. Following exposure to irradiance higher than 1500 μmol m^{-2} s^{-1}, a prolonged reduction of the maximal photochemical efficiency of PS 2 (F_v/F_m) was observed in LL plants, indicating the occurrence of chronic photoinhibition. In contrary, the decrease in F_v/F_m was quickly reverted in HL plants, pointing to a reversible photoinhibition.

Additional key words: electron transport, photochemical quenching, photoinhibition, thermal energy dissipation.

Introduction

Rhododendron ponticum L. subsp. *baeticum* is a shrub of the family *Ericaceae* commonly known as Iberian rose bay. It is restricted to three remote areas of Iberian Peninsula, the Monchique Mountain (Algarve, south of Portugal), the Caramulo Mountain (north of Portugal), and the Aljibe Mountains (northern side of the Strait of Gibraltar, Spain). *In vitro* propagation can offer considerable benefits for the rapid cultivation of endangered species that have limited reproductive capacity and exist in threatened habitats (Fay 1992). *In vitro* propagation of this species was attained (Almeida *et al*. 2005) and the reintroduction in the natural environment at Monchique Mountain is in course. While under the natural habitat *Rhododendron* plants are exposed to Mediterranean conditions and the leaves are sun-acclimated, in *in vitro* culture the nutritional and physical conditions, particularly the low irradiance, are responsible for shade characteristics of its leaves. Therefore they have to cope with excess of energy after transplantation to the soil under natural conditions. Once transferred to *ex vitro*, micropropagated plants are very susceptible to photoinhibition because of lack of well-developed physiological and anatomical systems related with carbon gain and water use efficiency. Therefore, acclimatization of micropropagated plants to *ex vitro* is crucial to overcome different stresses imposed on *in vitro* plants and to guarantee a better growth and development in the greenhouse or in the field. During this process plants undergo morphological and physiological changes that improve their ability to grow in the new environment (Pospíšilová *et al*. 1999). Various studies, which were undertaken to asses the influence of irradiance on acclimatization immediately after transfer of the *in vitro* grown plantlets to *ex vitro* conditions, indicate that photosynthetic acclimation involves a decreased investment in light-harvesting complexes and an increase in

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Abbreviations: E_k - light saturation coefficient; ETR - electron transport rate; F_m - maximum fluorescence; F_0 - initial fluorescence; F_v - variable fluorescence; HL - high irradiance; IL - intermediate irradiance; LL - low irradiance; PS 1 - photosystem 1; PS 2 - photosystem 2; q_p - photochemical quenching; q_N - non-photochemical quenching; α - initial linear slope; Φ_{PS2} - effective quantum efficiency of PS 2.

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PS 2 reaction centers in response to high irradiance (Carvalho *et al*. 2001, Fuentes *et al*. 2005). So, sudden changes in irradiance can have important impacts on the photosynthetic apparatus (especially on PS 2), despite its notable capability for acclimation to a wide range of irradiances. According to Walters (2005), radiation is not only the source of energy but it can cause damage under stress conditions. In fact, photosynthetic acclimation serves not only to maximize photosynthetic gain but also to protect photosynthesis from excess energy, which otherwise can result in photoinhibition and can chronically impair photosynthesis (Osmond 1994). The capacity of electron transport and the pool size of xanthophylls pigments involved in photoprotection are genetically determined (Pearcy *et al*. 1998) but may be modulated by the growing irradiance (Demmig-Adams *et al*. 1995). Nevertheless, despite the notable acclimation capability of a species to a wide range of irradiances, a short-term exposure to a very high irradiance can result in photoinhibition, dynamic or chronic (Osmond 1994). As acclimation to irradiance requires adjustments in PS 2 photochemistry the use of chlorophyll *a* fluorescence analysis is an alternative tool for photoacclimation studies (Kitao *et al*. 2003, Hu *et al*. 2007, Čaňová *et al*. 2008, Pospíšilová *et al*. 2009). Quantum yield of PS 2 photochemistry serves as a good indicator how plants respond to environmental constraints under high irradiance (Kitao *et al*. 2000, 2003, Einhorn *et al*. 2004, Zhang *et al*. 2009. Moreover, the response from ETR and others fluorescence parameters to photosynthetic photon flux density (PPFD) has been employed as a rapid and non-invasive probe for assessing photosynthetic capacity of leaves, its light acclimation state and its capacity to tolerate changes in irradiance (Kosová *et al*. 2005, Osório *et al*. 2006).

Understanding of the plant species potential to acclimate to new environments is of paramount importance to predict and improve performance and survival of micropropagated plants under natural conditions with minor risks of photoinhibition. Thus, the aim of this study was to evaluate the effects of growth irradiance on the performance of photosynthetic apparatus of micropropagated *Rhododendron ponticum* plants and to assess the capability to recover photosynthetic capacity after a sudden increase in irradiance, through chlorophyll *a* fluorescence measurements.

Materials and methods

Micropropagated plants of *Rhododendron ponticum* subsp. *baeticum* (Boissier & Reuter) Handel-Mazzetti were produced *in vitro* as described by Almeida *et al*. (2005). Each rooted plantlet was transplanted into 350 cm^3 plastic pots with a mixture of peat and *Perlite* 3:1 (v/v) and acclimatized in a plant growth chamber (*500E, Aralab*, Lisboa, Portugal) under a photosynthetic photon flux density (PPFD) of 80 μmol m^{-2} s^{-1} for 4 weeks. After this period, plants were transplanted to larger pots (5 dm^3) and two experiments were performed.

In experiment 1, potted plants were grown outdoors on the Algarve University Campus garden in Gambelas (37º 04' N, 7º 97' W), under natural environment, adequate irrigation and fertilization. The three PPFD treatments were attained using semi-natural canopy cover at the garden. Plants were placed in a small stand of trees where PPFD was 1000 ± 75 μmol m^{-2} s^{-1} at the leaf surface for high irradiance (HL1), 300 ± 15 μmol m^{-2} s^{-1} for intermediate (IL1) or 80 ± 10 μmol m^{-2} s^{-1} for low irradiance (LL1). This study was undertaken during spring characterized by a total rainfall of 30 mm, a mean daily maximum air temperature of 22.5 ºC and a mean daily minimum air temperature of 12 °C (data from the Portuguese Meteorological Institute).

In experiment 2, another lot of plants was transferred to a walk-in controlled-environment cabinet (*Fitoclima 16.000 EHVP*, Aralab, Portugal) under day/night temperature and relative humidity of 25/18 ºC and 80/70 %, respectively, and a 12-h photoperiod. Light was provided by incandescent and fluorescent lamps, supplying a PPFD (at the top of the plants) of about 80 μmol m^{-2} s^{-1} (LL2) and 250 μmol m^{-2} s^{-1} (IL2). Plants were watered 3 - 4 times per week to maintain the soil moisture and twice a week were supplemented with a commercial fertilizer solution (*Complesal*®, 12 % N, 4 % P, 6 % K; *Bayer Crop Science*, Carnaxide, Portugal). After 3 weeks, most of the plants under LL2 and IL2 had produced at least 6 leaves and then the irradiance was switched to 500 μmol m^{-2} s^{-1} (HL2). This PPFD was selected according to response of net photosynthetic rate (P_N) to PPFD measured in a minicuvette system (*Walz*, Effeltrich, Germany), which indicate this value as photosynthetic saturating irradiance for those *Rhododendron* plants. To ensure the uniformity of leaves, the analysis was performed in the most recently fully expanded leaves, which had completed leaf expansion under the new growth irradiance (LLH symbolize LL2→HL2 and ILH symbolize IL2→HL2).

The response of chlorophyll fluorescence parameters to PPFD was measured on attached leaves using a portable fluorometer (*PAM-2000, Walz*, Effeltrich, Germany) at room temperature (20 ± 2 ºC). Prior to the first measurement, the leaf was dark-adapted for 30 min. The maximum photochemical efficiency of PS 2 was estimated by the variable to maximum fluorescence ratio (F_v/F_m), calculated from F_0 (basal fluorescence) and F_m (maximum fluorescence), with variable fluorescence $F_v = F_m - F_0$. The leaves were irradiated 10 min with actinic radiation (140 ± 10 μmol m^{-2} s^{-1}) prior to determination of PPDF response curves started by the lowest PPFD and thereafter increasing gradually until the highest PPFD be reached. The leaf clip was covered with a black cloth during measurements to exclude all external

lighting. The leaf was exposed at each PPFD for 6 min and then the steady-state value of fluorescence of light adapted leaves, F_s, was recorded. Subsequently, a saturating flash was imposed to determine the maximum level in the light-adapted state (F'_m). Prior to changing PPFD, the minimum fluorescence in the light, F'_0, was determined using a far-red pulse (720 nm) to excite PS 1, draining PS 2 of electrons. Photochemical efficiency of open PS 2 reaction centers was expressed by the ratio F'_v/F'_m. Effective PS 2 quantum efficiency in light-adapted leaves (Φ_{PS2}) was calculated by the $(F'_m-F_s)/F'_m$ ($= \Delta F/F'_m$) ratio (Genty *et al*. 1989). Electron transport rate is calculated as $ETR = \phi_{PS2} \times E_i \times AF \times 0.5$, where E_i is the incident PPFD, AF is the absorption factor in the leaf and 0.5 is the approximate distribution of excitation energy to PS 2 (Krall and Edwards 1992). The fraction of absorbed irradiance (AF) used was 0.84 based on measurements of 37 C_3 plant species (Björkman and Demmig 1987) and it was assumed that the excitation energy was equally distributed between PS 1 and PS 2 (Bilger *et al*. 1995). Photochemical quenching (q_p), which was used as an estimate of the fraction of open centers, was calculated as the ratio $1 - (F_s - F'_0)/(F'_m - F'_0)$ and thermal energy dissipation at the PS 2 (q_N) as $1 - (F'_m - F'_0)/(F_m - F_0)$ (Bilger and Schreiber 1986).

Four or 5 plants from each treatment (LL1, IL1, HL1) were randomly sampled to test the effect of a short-term high PPFD exposure. At midday, plants were placed for 2 h at full sunlight (1500 ± 140 μmol m^{-2} s^{-1}) and fluorescence parameters and photosynthetic rate were measured before (control) and after the treatment (HL-stress). Recovery took place at dark. The photo-inhibition was assessed by the photochemical efficiency of PS 2 (F_v/F_m) of the leaves after 45 min, 18 h and 24 h at dark, in comparison with F_v/F_m measured at predawn prior the treatment.

Statistical analysis and graphic display were performed with *SPSS*® (*Release 11.5.0*, *SPSS.*, Chicago, IL, USA) and *SigmaPlot*® (*Version 10.00, Systat Software*. San Jose, CA, USA) software packages, respectively. All the determinations were obtained with randomly chosen plants. Comparisons among groups were performed by analysis of variance or Student's *t*-test for unpaired data. For additional pairwise comparisons, Student-Newmans-Keul or Dunnett's test were used. Differences were considered significant at $P \leq 0.05$. Fluorescence parameters *vs*. PPFD fitting was carried out using non-linear regression with the Marquardt-Levenberg algorithm (*SigmaPlot*® *10.0*). The light-response of *Rhododendron* plants was characterized by fitting the model of Platt *et al*. (1980) to ETR *vs*. E_i plots and by estimating the initial linear slope of the curve (α), the maximum electron transport rate (ETR_{max}), the photoinhibition parameter (β) and the light saturation coefficient (E_k). The linear initial slope (α) was determined directly by fitting the model to experimental data, while onset of light saturation (E_k) and maximum electron transport rate (ETR_{max}) were calculated according to Ralph and Gademann (2005). Even thought curves did not show a visible decline in ETR for the higher PPFD applied, the Platt *et al*. (1980) model was still used since the removal of β resulted in over-estimation of ETR_{max} (MacIntyre et al. 2002). The Φ_{PS2} *vs*. PPFD fitting was performed using a double exponential decay function (Rascher *et al*. 2000). The best-fit solutions for q_p, q_N and F'_v/F'_m *vs*. PPFD plots were obtained by using cubic polynomial, exponential rise to maximum and linear regression models, respectively. The fitting quality was very good ($r > 0.90$) in all cases.

Results

Responses of ETR, Φ_{PS2}, q_p and q_N to stepwise increases in irradiance (LCs) were quite similar in HL1 and IL1 plants, both groups being substantially different from the LL1 one. In response to increased PPFD, ETR increases with irradiance until a maximum value (ETR_{max}) is reached (Fig. 1*A*). The initial slope α [approximately 0.30 μmol(electrons) mol^{-1}(photons)] was not affected by growth irradiance. However, HL1 and IL1 plants saturate at considerably higher maximum rates (ETR_{max}) and under higher irradiance levels (E_k) than LL1 ones. Acclimation during HL1 resulted in significant increases of ETR_{max} and E_k, by 27 and 29 %, respectively, compared to LL1 treatment. Photochemical quenching (q_p) and effective PS 2 quantum efficiency in light (Φ_{PS2}) declined in a very similar fashion in HL1 and LL1, but in LL1 were significantly depressed in response to increased PPFD (Fig. 1*B*,*C*). In contrast, q_N increased with PPFD in both LL1 and HL1 plants but saturates quickly in LL1 treatment (Fig. 1D). Moreover, q_p measured *in situ* (Fig. 2*A*), was high and roughly constant (near 0.9) for growth PPFD between 80 and 500 μmol m^{-2} s^{-1}, but considerably lower (*ca*. 0.6) in plants grown at PPFD 1000 μmol m^{-2} s^{-1}. A similar pattern was observed for PS 2 photochemical efficiency as measured by Φ_{PS2}. The differences in Φ_{PS2} cannot be ascribed to changes in intrinsic efficiency of PS 2, since values of F_v/F_m after dark adaptation were consistently high and closed to 0.80 for all plants (Fig. 2*A*). This value is typical of healthy, non-photoinhibited leaves, suggesting that all PS 2 operate normally irrespective of growth irradiance. Besides, dissipation of energy by non-radiative mechanisms in the antenna of PS 2 was more efficient as growth irradiance increased, as illustrated by the enhancement of q_N. Electron transport rate also appeared to be light adapted as the increase in ETR was approximately linear, with only a small decrease at higher growth irradiance (Fig. 2*B*). This finding is in line with results of q_p, q_N and Φ_{PS2}.

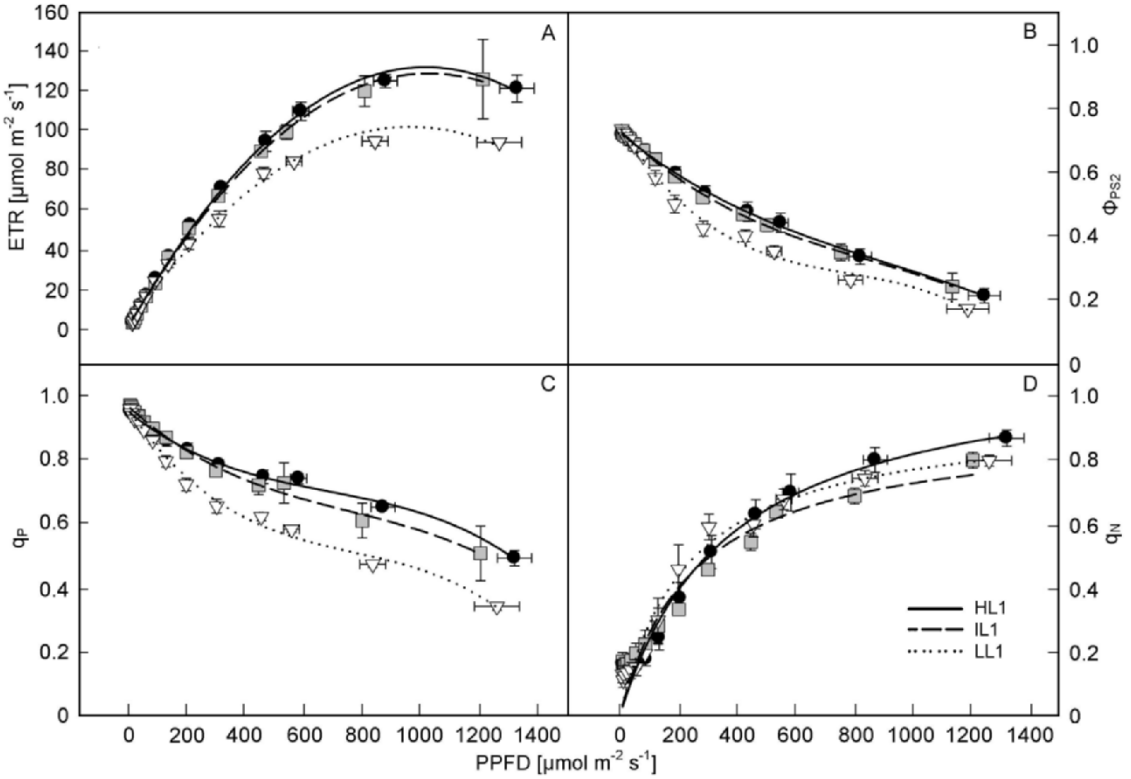

Fig. 1. The response of fluorescence parameters to PPFD in micropropagated plants of *Rhododendron*. *A* - Apparent electron transport rate (ETR); *B* - quantum yield of PS 2 electron transport in the light (ϕ_{PS2}); *C* - photochemical quenching (q_p); *D* - non-photochemical quenching (q_N) of plants grown under high (HL1), intermediate (IL1) and low (LL1) irradiance. Means ± SE from 5 replications.

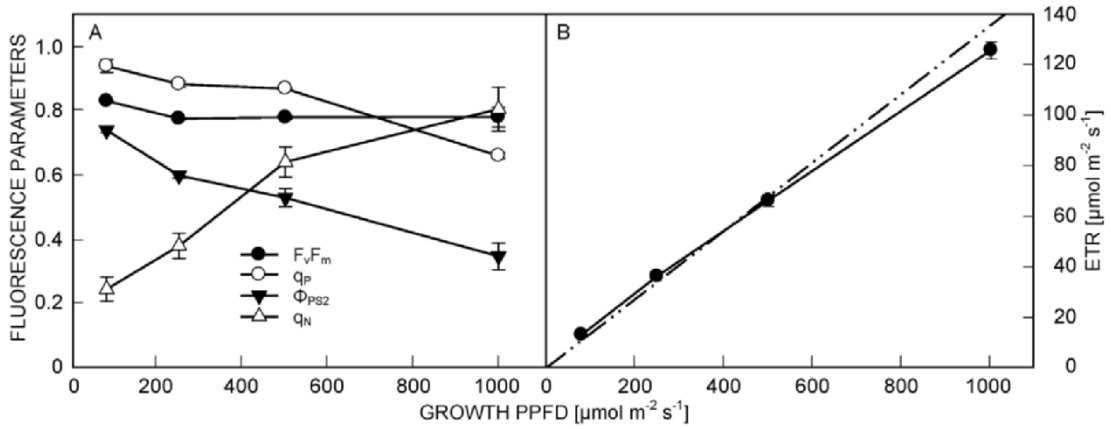

Fig. 2. Effects of growth conditions on photosynthetic function in micropropagated plants of *Rhododendron* assessed by measuring F_v/F_m, ϕ_{PS2}, q_p, q_N (*A*) and ETR (*B*) *in situ*. A regression line (*dash-dot*) for ETR passing through the origin calculated using the first 4 points is also shown. Means ± SE from 5 replications.

There is also evidence that *R. ponticum* plants have a rapid photosynthetic response to an increased PPFD. Leaves grown in LL2 and IL2 before switch to HL2 had a high F_v/F_m value of about 0.80. F_v/F_m decreased continuously on the first 4 - 5 d after the light switch and the extent of this decrease was LLH > ILH. The onset of recovery was evident by day 7, however after 21 d values of F_v/F_m in LLH plants were still lower than those before switch (Fig. 3*B*). The trend of response in F'_v/F'_m, q_p and Φ_{PS2} (Fig. 1 *C-E*) was similar to that of F_v/F_m. In contrast, non-photochemical quenching (q_N) increased considerably in the first hours after exposure to HL2 stabilising thereafter. The trend of decrease in P_N on leaf area basis after the PPFD switch was similar to that of F_v/F_m. P_N decreased during the first days and started to recover after day 7. The final P_N values LLH were significantly

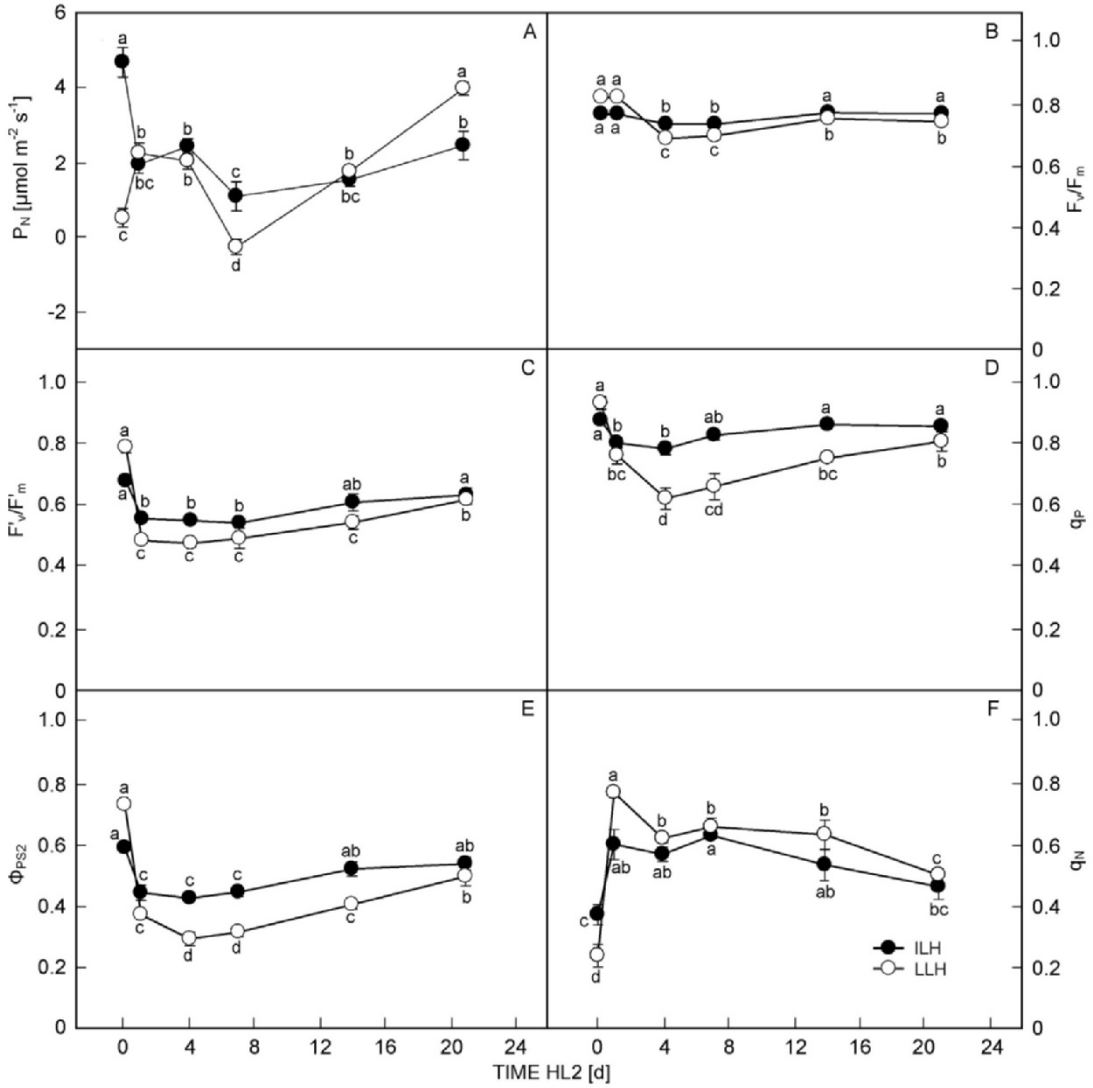

Fig. 3. Net photosynthetic rate (P_N) and fluorescence parameters (F_v/F_m, F'_v/F'_m, q_p, ϕ_{PS2} and q_N) during acclimation at a PPFD of 500 µmol m^{-2} s^{-1} (HL2) in micropropagated plants of *Rhododendron* grown under 80 µmol m^{-2} s^{-1} (LLH denote LL2→HL2) and 250 µmol m^{-2} s^{-1} (ILH denote IL2→HL2). Means ± SE from 5 replications. Values followed by different letters indicate significant difference at $P \leq 0.05$ (one-way *ANOVA*, S-N-K test) between light growth regimes.

Table 1. Changes in net photosynthetic rate (P_N) and chlorophyll fluorescence parameters measured in leaves of plants grown at high (HL1), intermediate (IL1) and low (LL1) irradiance without (control) and after 2 h HL-stress of 1500 ± 140 µmol m^{-2} s^{-1}. Means ± SE from 4 or 5 samples. Values followed by different letters indicate significant difference at $P \leq 0.05$ among HL1, IL1, LL1. *, **, *** indicate significant differences at $P \leq 0.05$, 0.01 and 0.005, respectively, between control and HL-stress.

	P_N [µmol m^{-2} s^{-1}]		Φ_{PS2}		q_p		q_N	
	control	HL-stress	control	HL-stress	control	HL-stress	control	HL-stress
HL1	2.5±0.3a	1.7±0.2^{ns} a	0.586±0.027a	0.103±0.016***a	0.814±0.010a	0.509±0.050***a	0.392±0.043b	0.957±0.008***a
IL1	1.9±0.2a	0.3±0.4**a	0.557±0.017a	0.120±0.010***a	0.747±0.021a	0.477±0.032** a	0.432±0.021b	0.947±0.005***a
LL1	1.1±0.3b	0.1±0.2**	0.342±0.045b	0.042±0.010***b	0.603±0.033b	0.227±0.036***b	0.720±0.061a	0.960±0.005** a

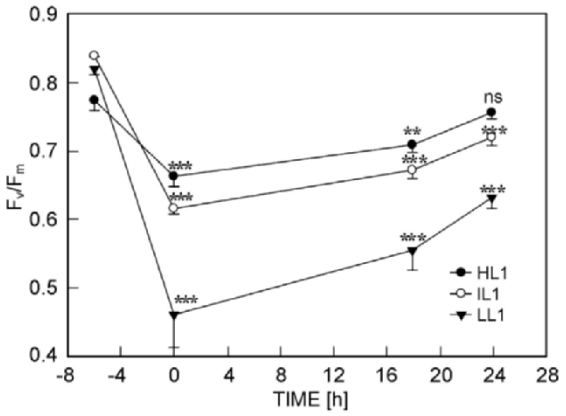

Fig. 4. Effects of 2 h exposure to a strong light (PPFD = 1500 ± 140 μmol m^{-2} s^{-1}) with subsequent recovery of F_v/F_m in micropropagated plants of *Rhododendron* grown under HL1, IL1 and LL1. The data at time -6 h and 0 h correspond to control (predawn) and after 2 h exposure to high irradiance, respectively, while the remaining times correspond to the recovery in dark. Means ± SE from 4 or 5 replications. Asterisks *, **, *** indicate significant differences at $P \leq 0.05$, 0.01 and 0.005, respectively (Dunnet's test).

higher ($P < 0.05$) than those before switch in irradiance.

Photosynthesis was noticeably altered by an exposure of plants for 2 h to strong PPFD (1500 μmol m^{-2} s^{-1}). Reductions of 31, 85 and 92 % in photosynthetic rate (P_N) were observed in HL1, IL1 and LL1 plants, respectively, which cannot be entirely ascribed to stomatal closure (data not shown). After the episode of high irradiance, HL1 and IL1 plants displayed a significantly higher ETR [60 μmol(electrons) m^{-2} s^{-1}] as compared with LL1 [16 μmol(electrons) m^{-2} s^{-1}]. At the same time, Φ_{PS2} and q_p decreased markedly in all groups, but more pronounced under LL1. In order to counterbalance the excess of photon energy, thermal dissipation was enhanced, as is shown by the higher values of q_N after HL-stress (Table 1). Interestingly, plants of all groups exhibited similar values of q_N after the stress. On the other hand, F_v/F_m (Fig. 4) had fallen significantly in all groups, decreases being of the order of *ca.* 14, 27 and 44 % relative to the control in HL1, IL1 and LL1, respectively. The rate of recovery in dark as assessed by F_v/F_m was related with growth irradiance: HL1 plants attained full recovery within 24 h, whereas other groups did not.

Discussion

Photosynthetic acclimation involves distinct strategies for growth under high or low irradiance as it is well documented for several species (Bailey *et al.* 2001, Ralph and Gademann 2005, Hu *et al*. 2007). In the present experiment, *R. ponticum* micropropagated plants acclimated under LL1 displayed lower ETR_{max} and lower E_k values than the HL1 plants (Fig. 1). An interesting feature of results is that E_k was changed proportionally to ETR_{max}, whereas little change was observed among plant groups in α. Although a decrease in α at HL as a result of an imbalance between light absorbed by light-harvesting systems and the energy consumed by Calvin-cycle reactions is generally expected, it is not uncommon that photoacclimation comes for changes in ETR_{max} not accompanied of relevant variation in α. Ensminger *et al*. (2005) attributed these discrepancies to real differences or to methodologies used to assess acclimation. In this study, acclimation changes in α might not be detectable with the chlorophyll fluorescence technique used, since alternative electron sinks are able to maintain an electron flow from PS 2, which may contribute to photoprotection of PS 2. Photoprotective mechanisms underlying such a protective role are photorespiration (Harbinson *et al*. 1990), Mehler-peroxidase reaction (Harbinson *et al*. 1990) and cyclic electron flux around PS 1 (Golding and Johnson 2003). However, light-induced depression in the Φ_{PS2} (Fig. 1*B*) was partially a result of decreased efficiency of energy capture by open PS 2 reaction centres, F'_v/F'_m (data not shown), that may reflect light-induced non-photochemical quenching (Baker 1991). In accordance, a significant rise in q_N was observed in both HL1 and LL1 plants with increasing PPFD (Fig. 1*D*). These results provide evidence that a part of the excitation energy was dissipated as heat, through the activation of non-radiative dissipative processes, such as those associated with the xanthophylls cycle (Demmig-Adams 1995). The finding that q_N reaches its maximum at higher irradiances in HL1 plants indicates that these plants have greater tolerance to photoinhibition than those grown under low PPFD, which is consistent with higher values of q_P (a measure of openness of PS 2 centres) displayed by HL1 plants particularly for elevated PPFDs (Fig. 1*C*). The high values of E_k also indicate that HL plants were well adapted to high radiation conditions and therefore less vulnerable to photoinhibition. In fact, the irradiance level under which *Rhododendron* plants were grown had a great impact in the determination of their susceptibility to photoinhibition, as well as in the type of photoprotective mechanisms developed for its avoidance. The data of chlorophyll fluorescence and photosynthesis (Fig. 4 and Table 1) support the above conclusion: F_v/F_m and P_N significantly decreased in the leaves acclimated to low PPFD when suddenly exposing to a high PPFD (Fig. 4), whereas such decrease was much lower in the leaves acclimated to high PPFD. The noticeable inability of LL plants to achieve full recovery in F_v/F_m over a 24 h period may be interpreted as evidence for PS 2 photodamage, which to be overcome, will require the synthesis of new D1 protein for regenerating the inactivated one. Contrastingly, the complete recovery of F_v/F_m in HL plants points to scenarios of PS 2 down-regulation or dynamic photoinhibition (Osmond 1994). These results are in accordance with those reported in the literature (*e.g.* Kitao *et al*. 2000, Hu *et al*. 2007). The

inhibition of Φ_{PS2}, concurrently with the decreases in P_N and F_v/F_m, and a noteworthy increase in q_N, indicate an enhancement of the thermal dissipation processes in both HL and LL plants. However HL plants showed to be less susceptible to photoinhibition than LL plants despite similar values of q_N in both growing irradiances (Table 1). Moreover, HL plants showed higher ETR, F_v/F_m and q_P than LL plants, which suggest that energy dissipation through electron transport was also important in HL (Kitao *et al*. 2003). In fact, the high values of Φ_{PS2} observed at different growth irradiances denote that the majority of photons absorbed by PS 2 were used in photochemistry, whereas high values for q_p show that PS 2 was maintained in an oxidized state.

The kinetics of changes triggered by a shift of LL and IL to HL observed in *Rhododendron* plants showed that the process of chloroplast acclimation is a long-term one, lasting for about one or more weeks, according to their past history of growing irradiance as postulated by Walters (2005). Plants grown under LLH seem to be more vulnerable to high PPFD exposure than ILH plants. The initial fall in F_v/F_m, more dramatic in LLH plants than in ILH (Fig. 3*B*), can be interpreted as evidence for some degree of irreversible loss in PS 2 photochemical efficiency (*i.e*., chronic photoinhibition), as a consequence of high PPFD (Björkman and Demmig, 1987). The recovery of F_v/F_m was completed in 3 weeks at both light-switched regimes, but it must be emphasized that it was slightly less successful in LLH plants. These conclusions are in agreement with the results from the other fluorescence parameters as well as with photosynthesis data (Fig. 3). Similar results were reported for some species of *Garcinia* by Guo *et al*. (2006).

In conclusion, this research unambiguously demonstrates that micropropagated plants of *R. ponticum* display substantial potential for acclimation. The plants adjust their photosynthetic apparatus to the ambient growth irradiance in a dynamic manner. They are usually able to survive transfer to higher irradiances, but the level of growth irradiance before transfer is crucial for determining the extent of photoinhibition and the type of photoprotective mechanisms developed for its avoidance.

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