Changes in photosynthetic capacity and antioxidant enzymatic systems in micropropagated *Zingiber officinale* plantlets during their acclimation

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Abstract

Ginger (*Zingiber officinale* Rosc.) plantlets were propagated *in vitro* and acclimated under different photosynthetic photon flux densities (60 and 250 µmol m^{-2} s^{-1} = LI and HI, respectively). Increases in chlorophyll (Chl) content and Chl *a/b* ratio were found under both irradiances. *In vitro* plantlets (day 0) exhibited a low photosynthesis, but chloroplasts from *in vitro* leaves contained well developed grana and osmiophillic globules. Photoinhibition in leaves formed *in vitro* was characterized by decrease of photochemical efficiency and quantum efficiency of photosystem 2 photochemistry in HI treatment during acclimation. The new leaves formed during acclimation in both treatments showed a higher photosynthetic capacity than the leaves formed *in vitro*. Also activities of antioxidant enzymes of micropropagated ginger plantlets changed during acclimation.

Additional key words: antioxidative enzymes; chlorophyll content and fluorescence; chloroplast ultrastructure; ginger; dry mass; net photosynthetic rate; photochemical quenching; plant height.

Introduction

In vitro plant micro-propagation has been widely employed in agriculture and forestry for mass propagation of many plant species. However, in many cases, poor survival rates of the *in vitro* plantlets after transplantation from tissue culture vials to greenhouse or open field often limit the commercial applications (Pospíšilová *et al*. 1999). During *in vitro* culture, plantlets grow under strictly controlled environment: a fixed photoperiod and weak photosynthetic photon flux density (PPFD), high air humidity, gradually decreasing medium nutrient supply, as well as a low CO_2 concentration. Due to such a stressful environment, plantlets usually exhibit some abnormalities: low photosynthetic rates, low chlorophyll (Chl) content, and poorly developed cuticle, stomata, and chloroplasts (Van Huylenbroeck *et al*. 1996, Nguyen *et al*. 1999, Pospíšilová *et al*. 1999). After transplantation to *ex vitro* conditions, the plantlets are often subject to changed irradiance, temperature, and other environmental fluctuations. Acclimation is often a critical and necessary period for *in vitro* micro-propagated plantlets. During the acclimation, significant changes in plant anatomy, morphology, physiology, and photosynthesis have been reported (Van Huylenbroeck *et al*. 1998, Amâncio *et al*. 1999, Pospíšilová *et al*. 1999, Carvalho *et al*. 2001, Estrada-Luna *et al*. 2001, Lamhamedi *et al*. 2003). In addition to the techniques for *in vitro* plant hardening before transplanting, some approaches are often used to increase plant survival rate after transplanting, such as natural light shading, enriched CO_2 environment, and antitranspirant application for reducing plant transpiration (Amâncio *et al*. 1999, Van Huylenbroeck *et al*. 2000, Carvalho *et al*. 2001, Osório *et al*. 2005).

Under environmental stresses, activated oxygen species (AOS) production in plants is one of the common plant responses. Even though beneficial effects of AOS as signal molecules regulating plant gene expression to deal with the stress begin to be gradually understood, the damages to plant cell membrane systems caused by AOS are well noticed. As protection against the AOS, plant cells can activate antioxidant and enzymatic scavenging systems such as superoxide dismutase (SOD), catalase (CAT), peroxidase, dehydroascorbate redutase (DHAR), and glutathione reductase (GR) to protect them. Van Huylenbroeck *et al*. (2000) reported that micro-propagated plants develop antioxidant mechanism during acclimation. Under high irradiance significant changes in

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the activity of the antioxidant enzymatic system were observed in micro-propagated *Calathe* (van Huylenbroeck *et al*. 2000) and *Phalaenopsis* (Ali *et al*. 2005) during acclimation.

Ginger (*Zingiber officinale* Rosc.) is an important horticultural crop in tropical Southeast Asia. It produces aromatic and pungent rhizome that is valuable as a spice and herb medicine. Ginger propagates normally by its rhizome with low proliferation rate. Ginger diseases caused by soil-born pathogens often result in heavy yield losses. Ginger rhizome variations and degeneration easily occur due to long-term agamic propagation. It is hard to breed new ginger cultivars through normal crossbreeding because of its poor flowering and no seed (Zhao 2002). *In vitro* culture techniques provide an alternative means for ginger mass propagation and rhizome quality improvement. In ginger, micro-propagation (Hosoki and Sagawa 1977), organ culture (Sharma and Singh 1997), germplasm preservation (Dekker *et al.* 1991), somatic embryogenesis (Kackar *et al*. 1993), somatic embryogenic cell suspension cultures, and protoplast culture (Guo *et al.* 2005, Guo and Zhang 2007) have been reported. However, the previous information is limited to photosynthetic changes and plant performance during acclimation. We investigated the effects of two different PPFDs on growth, photosynthesis, and antioxidant enzymatic scavenging systems of micro-propagated ginger plantlets during acclimation.

Materials and methods

Plants and cultivation: Plantlets of *Z. officinale* Rosc. cv. Chengdu huanglaomen were micro-propagated by shoot-tip culture. The shoot tips (about 4 mm in length) were aseptically cut from sterilized buds under the microscope and transferred to MS (Murashige and Skoog 1962) medium supplemented with 0.2 g m^{-3} naphthylacetic acid (NAA) and 1.0 g m^{-3} benzyladenine (BA). For shoot elongation and root induction, parted shoots were transferred to the MS medium containing 0.6 g m^{-3} NAA and 2.0 g m^{-3} BA. All media were supplemented with 3 % sucrose and 0.7 % agar, and the medium pH was adjusted to 5.8 before autoclaving. The cultures were kept in a growth chamber irradiated with cool-white fluorescent lamps at PPFD of 45±5 µmol m^{-2} s^{-1} with a 16-h photoperiod. Room temperature was maintained at 26±2 °C.

Plant acclimation to two irradiances: *In vitro* plantlets were transplanted to 9-cm diameter pots containing the sterilized mixture of peat and vermiculite (1 : 1, v/v) and then were randomly divided into two groups to explore the effects of different PPDF on plant acclimation. The acclimation took place in a growth room with a day/night temperature scheme of 25±2/18±2 °C and a 16-h photoperiod. Irradiation was provided by four lamps (*SON-T AGRO*, *Philips*) and the PPFD gradient was set by changing the distance of the plantlets to the light sources. The PPFDs the plants received were 60±10 µmol m^{-2} s^{-1} for the low-irradiance (LI) treatment and 250±10 µmol m^{-2} s^{-1} for the high-irradiance (HI) treatment. The plants were watered twice a week with a Long Ashton nutrient solution (Hewitt and Smith 1975).

Experimental scheme: On days 0, 3, 7, and 14 after transplanting, fully expanded leaves were used. The new leaves expanded after transplanting were employed for the measurement only on days 28 and 42. Plant height, dry mass (DM), and Chl content were determined on days 0, 14, and 42 after *ex vitro* transplantation. Photosynthesis measurements were done on days 0, 7, 14, 28, and 42. Chl *a* fluorescence parameters were measured at days 0, 3, 7, 14, 28, and 42, and at day 14 the Chl *a* fluorescence parameters of new leaves were also determined. *In vitro* leaves (day 0) and fully expanded new leaves (day 42) were used for transmission electron microscopic observation. Antioxidant enzyme activities were determined on days 0, 7, 14, 28, and 42. For each parameter the number of replication was as follows: Chl content, photosynthesis-irradiance response curves, and antioxidant enzyme activities, 3; net photosynthetic rate (P_N) and Chl *a* fluorescence, 5; plant height and DM, 8.

Growth parameters: For DM determination, samples were first kept at 110 °C for 15 min and then maintained at 80 °C till constant mass.

$\boldsymbol{P_N}$ was determined using a portable photosynthesis system (*LI-6400*, *LI-COR*, Lincoln, NE, USA) equipped with a LED light source leaf chamber (*6400-02b*). P_N was measured at CO_2 concentration in the inflowing air of 400±10 µmol mol^{-1}, ambient relative humidity 60±5 %, and leaf temperature 25 °C. The PPFDs used for the measurements were 60 and 250 µmol m^{-2} s^{-1} (LI and HI), respectively. P_N-irradiance response curves were determined at the same condition and a gradient of PPFD was set as follows: 0, 60, 100, 150, 200, 250, 400, and 600 µmol m^{-2} s^{-1}.

Chl content and fluorescence: Chl was extracted in 80 % cold acetone (v/v) and the absorbances at 663 and 646 nm were determined with a spectrophotometer *UV-2802-PC* (*UNICO*). The contents of Chl were calculated using the formulae of Lichtenthaler (1987). Chl *a* fluorescence parameters were measured with a fluorometer *PAM-2000* (*Walz*, Effeltrich, Germany). The minimal fluorescence (F_0) was measured in 30-min dark-adapted leaves using weak modulated irradiance (<0.1 µmol m^{-2} s^{-1}, 600 Hz) and then a 0.8-s saturating flash (8 000 µmol m^{-2} s^{-1}, 20 kHz) was applied to determine the maximal fluorescence (F_m) and the photochemical efficiency of photosystem 2, PS2 (F_v/F_m).

Following this, "actinic light" (336 μmol m^{-2} s^{-1}) was applied and the state fluorescence yield (F_s) was determined. F_m' was measured after another saturating pulse, then the quantum efficiency of PS2 photochemistry (Φ_{PS2}) was calculated as follows (Genty *et al.* 1989): $\Phi_{PS2} = (F_m' - F_s)/F_m'$. After the "actinic light" was turned off, far-red irradiation (5 μmol m^{-2} s^{-1}, 3 s) was used to determine F_0', then the photochemical quenching (q_P) and the excitation energy capture efficiency of PS2 reaction centres (F_v'/F_m') were determined according to equations $q_P = (F_m' - F_s)/(F_m' - F_0')$ and $F_v'/F_m' = (F_m' - F_0')/F_m'$ (Genty *et al.* 1989).

Transmission electron microscopy: Fresh leaf pieces (0.2×0.5 cm) were placed in a fixative solution consisting of 3 % glutaraldehyde in 0.2 M phosphate buffer, pH 7.2, and gently vacuumed (½ atmosphere) for 4 h; then kept at 4 °C overnight. The leaf samples were then transferred to 1 % osmium tetroxide fixative for 30–40 min, washed with the buffer, thereafter dehydrated in a graded series of ethanol, and embedded in *Epon* (*Polysciences*, Halifax, Canada). Ultra-thin sections were cut with a diamond knife, deposited on nickel grids, and stained with uranyl acetate and lead citrate. Observations were made with an electron microscope *JEM-1230* (*JEOL*, Japan). Photographs were taken with a *Technical Pan Estar* film (*Morada*, SIS, Germany).

Enzyme extraction and assays: For determination of antioxidant enzyme activities, approximately 0.5 g fresh mass of plant tissue was deep frozen with liquid nitrogen and homogenized in a pre-cooled mortar and pestle. Then to each of the plant homogenates 3 cm^3 pre-cooled extraction buffer was added, containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM ascorbate (ASA), and 1 % (m/v) polyvinylpyrrolidone (PVP). The homogenate was filtered through cheesecloth and centrifuged at 12 000×*g* for 20 min at 4 °C. The supernatant was used for enzyme assay. The protein concentration was determined by the Bradford method. Bovine serum albumin was used as standard. All enzymes were assayed spectrophotometrically at 25 °C.

SOD (EC 1.15.11) activity was determined by the nitro blue tetrazolium (NBT) method (Bryer and Fridovich 1987). The reaction mixture contained 50 mM phosphate buffer, pH 7.8, 10 μM EDTA, 13 mM methionine, 75 μM NBT, 2.0 μM riboflavin, and the required amount of enzyme. The reaction was started by exposing the mixture to cool white fluorescent radiation (70 μmol m^{-2} s^{-1}) for 15 min. After this period the light was switched off and the purple colour was measured at 560 nm. One unit of SOD activity is defined as the amount of enzyme, which causes a 50 % decrease of the SOD-inhibiting NBT reduction.

Activity of POD (EC 1.11.1.7) was determined by monitoring the increase of absorbance at 470 nm, as a result of guaiacol oxidation (Chance 1955). The reaction mixture contained phosphate buffer, pH 7.0, 10 mM guaiacol, 5 mM hydrogen peroxide (H_2O_2), and enzyme extract.

Activity of CAT (EC 1.11.1.6) was measured by monitoring the decrease in absorbance at 240 nm. The reaction mixture was composed of 50 mM phosphate buffer (pH 7.0) and 6 mM H_2O_2 (Aebi 1984).

Activity of APX (EC 1.11.1.11) was assayed in a reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.5 mM EDTA, 0.1 mM H_2O_2, 0.5 mM ascorbate, and enzyme extract. The activity was determined by following the decrease in absorbance at 290 nm (Chance 1955).

Activity of DHAR (EC 1.8.5.1) was determined by following the increase in absorbance at 265 nm due to the reduction of dehydroascorbate (DHA). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM glutathione (GSH), 1 mM DHA, and enzyme extract (Hossain and Asada 1984).

Statistical analyses: Data were analyzed using the statistical analysis systems (SAS). When significant differences occurred, means were separated by the LSD (p=0.05) method.

Results

Growth and Chl: After transplanting, micro-propagated ginger plantlets experienced an acclimation stage to adapt to the fluctuating environmental factors. During this stage, the plantlets showed special growth and photosynthetic characteristics. The plant DM and plant height measured on day 14 after transplanting increased slightly compared to those on day 0 (Table 1). However, these parameters were changed significantly after 42 d of acclimation under both irradiances. The plantlets under HI treatment had significantly higher DM and plant height than the plantlets under LI.

Total Chl content in *in vitro* leaves (the leaves expanded before transplanting) increased during the first two weeks of acclimation, and at the end of acclimation (day 42), the new leaves (leaves formed after transplantation) had significantly higher Chl content than the ones formed before transplantation measured on days 0 and 14 (Table 1). The Chl *a/b* ratio increased during acclimation because content of Chl *a* increased more than that of Chl *b*.

Photosynthesis and Chl *a* fluorescence: Immediately after transplanting (day 0), plantlet photosynthetic activity was low illustrated by the photosynthetic irradiance response curves (Fig. 1). From day 7 on, plant P_N increased gradually, although a plateau stage was readily

Table 1. Changes in plant dry mass (DM) [mg per plant], chlorophyll (Chl) contents [mg kg^{-1}(DM)] and Chl *a/b* in leaves, and plant height [cm] of micro-propagated ginger plantlets acclimated under HI (250±10 μmol m^{-2} s^{-1}) and LI (60±10 μmol m^{-2} s^{-1}) treatments on days 0, 14, and 42 after transplanting. Leaves expanded *in vitro* were used for measurements on days 0 and 14; leaves developed during acclimatization were used for measurements on day 42. For each day and variable, statistical differences among irradiance treatments were analyzed by LSD test. Values followed by different letters are significantly different (p<0.05).

Time [d]	Treatment	Chl *a*	Chl *b*	Chl *a+b*	Chl *a/b*	DM	Height
0		4.1	3.1	7.2	1.3	3.25	4.63
14	HI	7.9a	5.9b	13.8a	1.4a	4.60a	4.88a
	LI	7.7a	4.5a	12.3a	1.7b	4.06a	4.94a
42	HI	43.7b	15.6a	59.3a	2.8b	21.40b	13.00b
	LI	35.6a	14.6a	50.0a	2.4a	15.80a	9.96a

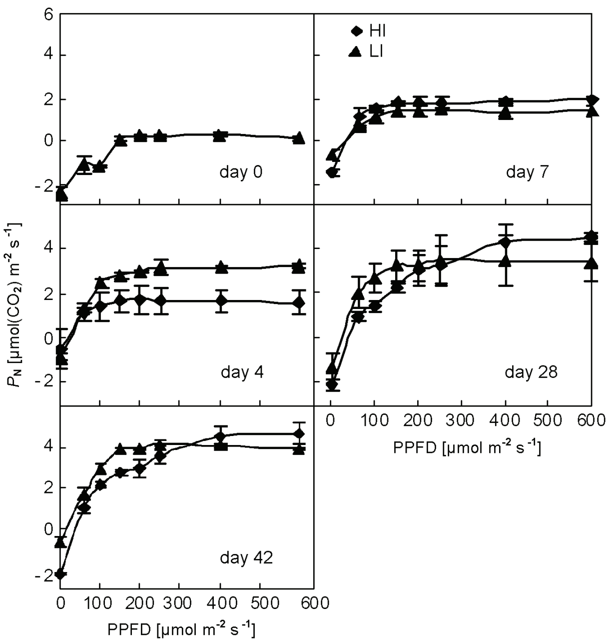

Fig. 1. Photosynthetic response curves of micro-propagated ginger plantlets acclimated under high irradiance, HI (250±10 μmol m^{-2} s^{-1}) and low irradiance, LI (60±10 μmol m^{-2} s^{-1}) treatments at days 0, 7, 14, 28, and 42. Leaves expanded *in vitro* were used for measurements on days 0, 7, and 14 and leaves developed during acclimation were used for measurements on days 28 and 42. Means±S.E. of 3 replicates.

reached at very low PPFD (Fig. 1). On day 14, the increase of photosynthetic capacities in the *in vitro* leaves was only observed under LI. Higher photosynthetic capacities were observed in new leaves and saturation irradiance (SI) increased (day 28 and 42), especially with HI treatment. P_N measured under acclimation was significantly higher in HI treatment than in LI treatment.

Fig. 2 shows that F_v/F_m of the *in vitro* leaves was high on day 0. However, after transplantation, F_v/F_m decreased rapidly and this decrease was mainly due to a reduction in F_m, then a recovery was observed only with LI treatment (Fig. 2*A,C*). F_v/F_m of the newly formed leaves was high in both treatments and remained almost constant during the experimental period (Fig. 2*A*). The same situation was observed in F_m. For F_m, the recovery started from day 3 with LI treatment and from day 7 with HI treatment (Fig. 2*C*). F_0 of *in vitro* leaves declined on day 7 after transplanting under both treatments, but that of HI treatment declined more significantly (Fig. 2*B*).

F_v'/F_m' decreased in the *in vitro* leaves and increased in the new ones under HI, while it remained almost constant in both types of leaves under LI treatment (Fig. 2*D*). Both types of leaves showed higher F_v'/F_m' values under LI than under HI, but at the end of the experiment F_v'/F_m' in new leaves reached a similar level in both treatments.

Φ_{PS2} of the plantlets obtained on day 0 was low compared with those measured afterwards (Fig. 2*E*). After a slight decline (day 3), Φ_{PS2} of *in vitro* leaves increased gradually with LI treatment, while it remained low in HI treatment. In the newly formed leaves, a significant increase of Φ_{PS2} was found between days 14 and 28 under LI whereas a similar increase occurred during the whole experimental period with HI. The maximum value of Φ_{PS2} was observed in leaves developed *ex vitro* under HI treatment on day 42 which was about 200 % of the value of *in vitro* leaves measured on day 0.

In vitro leaf q_P slightly decreased shortly after transplanting (day 3), followed by an increase with LI treatment, but with HI treatment the value remained low (Fig. 2*F*). The newly formed leaves had a higher q_P value than the *in vitro* leaves, and the value of q_P increased under both irradiances although it increased more rapidly under HI treatment. Compared to the plantlets on day 0, q_P measured on day 42 increased by about 190 and 145 % with HI and LI treatments, respectively.

Transmission electron microscopy showed that the leaves developed during acclimation had more starch grains in chloroplasts than the *in vitro* leaves, especially in HI treatment (Fig. 4*A–C*). Another difference was an appearance of osmiophilic globules in chloroplasts from *in vitro* leaves (day 0). Well differentiated grana were observed in chloroplasts of *in vitro* leaves (day 0) as well as leaves developed during acclimation (day 42) (Fig. 4*D–F*).

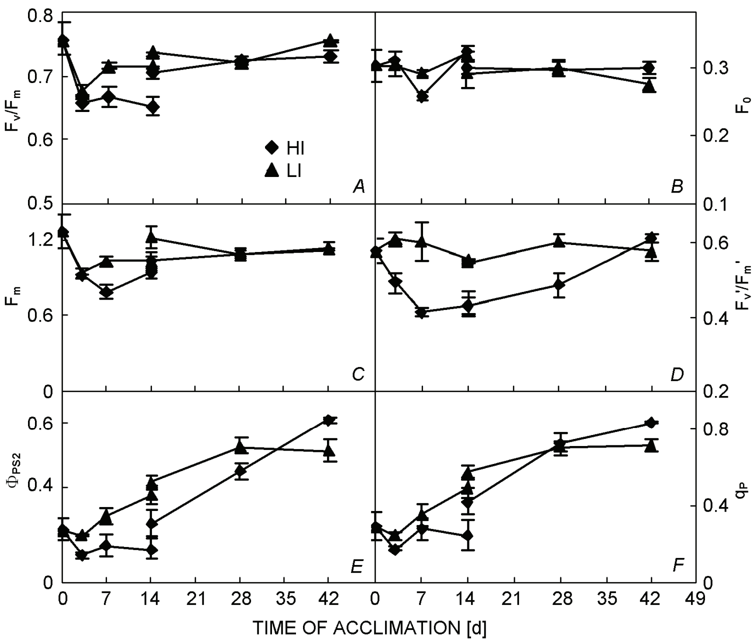

Fig. 2. Changes in chlorophyll (Chl) *a* fluorescence parameters of micro-propagated ginger plantlets during acclimation under high irradiance, HI (250±10 µmol m^{-2} s^{-1}) and low irradiance, LI (60±10 µmol m^{-2} s^{-1}) treatments at days 3, 7, 14, 28, and 42. Leaves expanded *in vitro* were used for measurements on days 0, 7, and 14 and leaves developed during acclimatization were used for measurements on days 14, 28, and 42. Means±S.E. of 5 replicates.

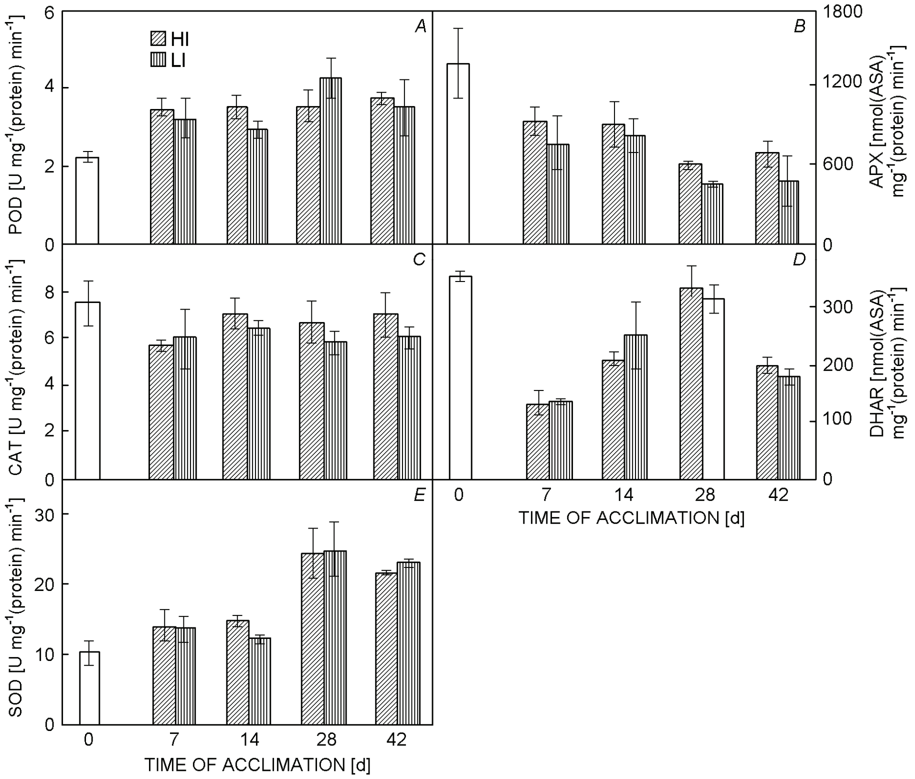

Fig. 3. Changes in the activities of peroxidase, POD (*A*), ascorbate peroxidase, APX (*B*), catalase, CAT (*C*), dehydroascorbate reductase, DHAR (*D*), and superoxide dismutase, SOD (*E*) of the micro-propagated ginger plantlets during acclimation under high irradiance, HI (250±10 µmol m^{-2} s^{-1}) and low irradiance, LI (60±10 µmol m^{-2} s^{-1}) treatments on days 3, 7, 14, 28, and 42. Means±S.E. of 3 replicates.

Enzymatic activities: The POD activity increased during the first 7 d after transplantation and remained stable during the rest period of acclimation. Even though there was no statistically significant difference in POD activity between two irradiance treatments, plants under HI showed a slight higher POD activity than the plants under LI, except on day 28 (Fig. 3*A*). For CAT a decrease in activity was observed on day 7, followed by a recovery under HI treatment. The activity of CAT in plantlets under LI treatment remained stable. The higher values were measured in plantlets under HI treatment from day 14 to day 42 (Fig. 3*C*). SOD activity increased slightly during the first two weeks after transfer, then a significant increase was observed on day 28 followed by a slight decrease on day 42 (Fig. 3*E*). APX activity decreased gradually after transplantation but activity under HI treatment was higher than that under LI treatment during acclimation (Fig. 3*B*). A significant decrease in DHAR activity was observed at day 7 after transfer, then the activity increased at day 28 and decreased at day 42 (Fig. 3*D*). For SOD and DHAR no correlation with PPFD was found (Fig. 3*D,E*).

Discussion

The plantlets produced from tissue culture had low contents of Chl *a* and *a*+*b*, but a high content of Chl *b*, which is propitious for photosynthetic apparatus to absorb photon energy under LI. After being transplanted, plant leaves showed an increase in Chl content and Chl *a/b* (Table 1). Similar results were found in micro-propagated grapevine acclimated at 40 μmol m^{-2} s^{-1} (Amâncio *et al*. 1999), chestnut at 150 μmol m^{-2} s^{-1} (Carvalho *et al*. 2001), and taro in greenhouse (Bai *et al*. 2005). Usually, plants exposed to LI accumulate more Chl-protein complexes of the photosynthetic apparatus to optimize energy harvesting such as in grapevine (Amâncio *et al*. 1999, Carvalho *et al*. 2001) and chestnut (Carvalho *et al*. 2001). However, we did not find a correlation between Chl content and PPFD. In *Calathea* acclimated at three irradiances, there was no such correlation found either (Van Huylenbroeck *et al*. 2000).

The improvement of the photosynthetic competence during acclimation is a common characteristic in various plant species grown *in vitro* (Yue *et al*. 1993, Van Huylenbroeck *et al*. 1998, Pospíšilová *et al*. 1999). Ginger plantlets grown *in vitro* were not photosynthetically active (Fig. 1). This is often observed in micro-propagated plants because they are exposed to LI and low CO_2 concentration (Piqueras *et al.* 1998, Piqueras and Debergh 2000, Van Huylenbroeck *et al*. 2000) and the down-regulation or feedback inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase by high sugar concentrations in medium (Van Huylenbroeck *et al*. 1996, Piqueras *et al*. 1998, Genoud *et al*. 2000). During the first two weeks of acclimation, plants exhibited gradual increases in SI and photosynthetic efficiency (Fig. 2 and Table 1). This is also characterized by increases of q_P and Φ_{PS2} as well as in the recovery of F_v/F_m in LI treatment (Fig. 2). Over this period, the *in vitro* formed leaves are the only photosynthetic product source to sustain the acclimation process of micro-propagated plants. Grout (1988) suggested that based on behaviour of *in vitro* formed leaves, plants can be classified into two groups. In the first group, the *in vitro* leaves are photosynthetically competent and function as normally formed leaves; in the second group, these leaves act as storage organs and never become fully autotrophic. Moreover, the correlation between their performances and *in vitro* culture condition was also reported (Van Huylenbroeck *et al*. 1996, 1998). In our study, the *in vitro* formed leaves did not only play a role as sink and storage organ but developed their photosynthetic competence and

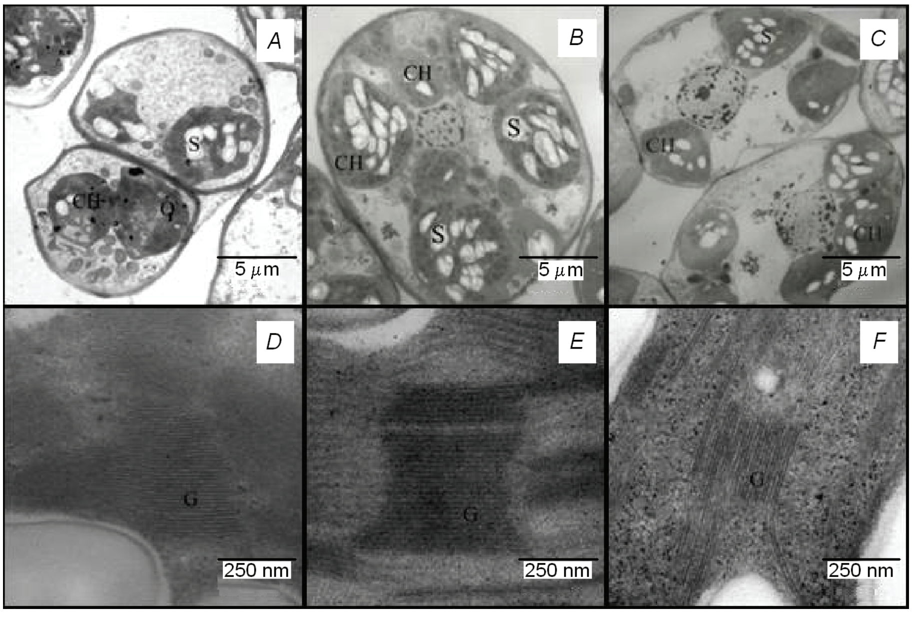

Fig. 4. Ultrastructure of mesophyll cell in leaves from ginger *in vitro* plantlets (day 0) and ginger plantlets acclimated under low irradiance, LI (60±10 μmol m^{-2} s^{-1}) and high irradiance, HI (250±10 μmol m^{-2} s^{-1}) treatments on day 42 under a transmission electron microscope. *A*: mesophyll cell of *in vitro* leaves (day 0); *B*: mesophyll cell of the leaves formed during acclimation under HI (250±10 μmol m^{-2} s^{-1}) treatment on day 42; *C*: mesophyll cell of the leaves formed during acclimation under LI (60±10 μmol m^{-2} s^{-1}) treatment on day 42; *D*: chloroplast ultrastructure of *in vitro* leaves (day 0); *E*: chloroplast ultrastructure of the leaves formed during acclimation under HI (250±10 μmol m^{-2} s^{-1}) treatment on day 42; *F*: chloroplast ultrastructure of the leaves formed during acclimation under LI (60±10 μmol m^{-2} s^{-1}) treatment on day 42. CH – chloroplast; G – granum; O – osmiophillic globule; S – starch grain.

then acted as the functional photosynthetic organ. However, this change was more significant in LI treatment than in the HI one. In HI treatment, only slight increase in P_N was observed and saturation was already reached at very low PPFD, suggesting that the improvement of photosynthetic competence could be limited. Significant increases in SI and photosynthetic efficiency in newly and fully developed leaves were observed on days 28 and 42. Plant growth dramatically increased during this period, which was attributed to greater photoautotrophic competency of plantlets (Table 1). Compared with the plants grown under LI, more pronounced plant growth was observed under HI treatment, because of the higher P_N in HI. The SI was also higher in HI treatment. These facts indicate that plants performed better in HI treatment than LI treatment after the new leaves developed.

Photosynthesis can be evaluated by Chl *a* fluorescence measurements. Immediately after transplanting, a decrease in F_v/F_m (Fig. 2*C*) was observed on day 3, indicating that plants were stressed due to the changes of environmental conditions. Similar plant acclimation phenomenon was also reported for *Calathea* (Van Huylenbroeck *et al*. 1998, 2000) and *Rehmannia glutinosa* (Seon *et al*. 2000). Then a recovery of photosynthetic competence in *in vitro* leaves appeared in LI treatment, which can be indicated by the recovery of F_v/F_m. The photoinhibitory effects were observed in *in vitro* leaves in HI treatment, as was reflected by lower values of F_v/F_m and Φ_{PS2}. When new leaves were fully developed, higher F_v/F_m, Φ_{PS2}, and q_P as well as photosynthetic efficiency were observed in both irradiance treatments. Once new and functional leaves developed, plants showed better overall responses to HI. The changes of q_P were similar to those in Φ_{PS2} (Fig. 2).

Well-developed chloroplast grana can be observed in the *in vitro* developed leaves (Fig. 4*A*), and this is consistent with some of their photosynthetic parameters: F_v/F_m (0.708) at day 0 and P_N at days 7 [0.61 and 1.75 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$ in LI and HI treatments] and 14 [1.29 and 1.66 $\mu mol(CO_2)\ m^{-2}\ s^{-1}$ in LI and HI treatments] (Fig. 3*C*, Table 1). Similar results were reported in coconut and *Gardenia jasminoides* (Serret *et al*. 1996, Triques *et al*. 1997). On the contrary, flattened chloroplasts with irregularly arranged internal membrane were observed in the leaves from *in vitro* grown *Liquidambar styraciflua* plantlets. After acclimation, plantlet chloroplasts had well developed grana and starch granules (Wetzstein and Sommer 1982). The accumulation of starch grains in chloroplasts from leaves developed during acclimation (day 42) were obviously because of the increased photosynthesis (Fig. 4*B,C*). Osmiophillic globules are a characteristic of chloroplast, sometimes related with stress. The particular condition such as weak PPFD, low CO_2 concentration, and high air humidity during *in vitro* culture may cause the appearance of osmiophillic globules in chloroplast from *in vitro* plantlets (day 0) (Fig. 4*E*).

The changes in the antioxidant enzymatic activities of micro-propagated ginger plantlets during acclimation were observed (Fig. 3). During acclimation, micro-propagated plantlets were exposed to a reduced relative humidity and high irradiances, which can lead to water stress and photoinhibition. Both types of environmental stress generate AOS in plant cells. To scavenge those toxic AOS, enzymatic scavenging systems, such as SOD, POD, CAT, APX, and DHAR, are present in plant. SOD catalyzes the reaction from O^{2-} to H_2O_2, then CAT, POD, APX, and DHAR detoxify the H_2O_2 produced. When the accumulation of activated oxygen species under these stresses exceeds the removing capacity of the antioxidant system, significant oxidative damages occur, including peroxidation of membrane lipids, destruction of photosynthetic pigments, and inactivation of photosynthetic enzymes. The changes in SOD, CAT, POD, APX, and DHAR activities observed in our experiment were related, to some extent, to a protective mechanism against the AOS, possibly generated during plant acclimation. The increases in activities of SOD, CAT, POD, and DHAR can be explained by the mild water stress generated during acclimation. Similar trends were observed in micro-propagated *Phalaenopsis*, *Calathea*, and *Spathiphyllum* plantlets (van Huylenbroeck *et al*. 1998, 2000, Ali *et al*. 2005). CAT isoforms are particularly abundant in glyoxysomes and peroxysomes. POD has vacuolar and apoplastic forms. APX isozymes are predominantly located in chloroplasts. The decreased APX activity, along with the acclimation time, differed from those of POD and CAT. The increase of POD, CAT, and APX activities under HI treatment reveals their protection roles against photo-oxidative stress linked to photoinhibition, as previously reported in *Phalaenopsis* and *Calathea* (van Huylenbroeck *et al*. 2000, Ali *et al*. 2005).

We found that tissue-cultured ginger plantlets developed a functional photosynthetic apparatus and antioxidant enzymatic protective system during acclimation. The development of newly formed leaves after plant transplanting is crucial to the plant photosynthesis and growth.

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Aartsma, T.J., Matysik, J. (ed.): **Biophysical Techniques in Photosynthesis. Volume II.** – Springer, Dordrecht 2008. ISBN 978-1-4020-8249-8 (hard-bound), 978-1-4020-8250-4 (e-book). 517 pp., € 234.33.

In 1996, volume 3 of the series Advances in Photosynthesis and Respiration dealing with biophysical techniques used in photosynthesis research appeared. Its editors were well-known scientists in this research field, Jan Amesz and Arnold J. Hoff. Unfortunately, both already passed away to a better world (Jan in 2001 and Arnold in 2002). This is why a second book on this topic was edited by two scientists working at present in the Leiden University of the Netherlands.

The reviewed book, volume 26 of the series, consists of twenty four chapters that describe the most modern procedures and apparatuses used for biophysical research in photosynthesis. Of course, the majority of these methods can be used also in biophysical and biochemical studies on other plant, animal, bacterial, *etc*. topics.

The chapters are divided in five categories. First part entitled "Imaging" contains four chapters dealing with atomic force microscopy, nonlinear optical microscopy, three-dimensional electron microscopy, and magnetic resonance imaging. They are used for studies of proteins of the bacterial photosynthetic apparatus (especially of *Blastochloris*, *Rhodospirillum*, *Phaeospirillum*, and *Rhodobacter*), electron tomography, single particle electron microscopy, water balance and water transport in photosynthesizing organisms, *etc*. Second part, "Structure", provides in five chapters the methods for quantitative studies of photosynthetic proteins and their complexes in membranes and reaction centres, their crystallization, structure, kinetics, and dynamics, electron and X-ray crystallography, use as basis for model building, *etc*. Part 3 consists of four chapters devoted to optical spectroscopy. These methods are used in studies of pigment composition, and dynamics of energy and electron transfer; the recent methods are femtosecond time-resolved infrared and nonlinear optical spectroscopy, picosecond spectral evolution of fluorescence, and single molecule techniques for determination of the electronic structure of pigment-protein complexes.

The part entitled "Magnetic resonance" contains six chapters on high-field, high-frequency, and high time-resolution electron paramagnetic resonance and nuclear magnetic resonance, applications of spin labelling, magic angle spinning, and photochemically induced nuclear polarization. The fifth part contains under the title "Theory" five chapters that deal with calculations of electrostatic energy in proteins using various types of models, excitation energy transfer and optical spectra of photosynthetic organisms, methods of molecular dynamic studies of bio-electronic systems, integral methods for studying bacterial photosynthesis, *etc*.

As is usual in this book series, 13 figures in colour are presented on seven plates (pp. CP1–CP7) in addition to their black-and-white printing in the respective chapters. Also the detailed Index is a traditional part of these books.

Books describing methods applicable in the field of photosynthesis are always welcome by students and researchers. Certainly also this new book will be frequently used and often cited.

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