PHOTOSYNTHETICA 40 (4): 605-614, 2002

Responses of tobacco plantlets to change of irradiance during transfer from *in vitro* to *ex vitro* conditions

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Abstract

Chlorophyll *a* fluorescence kinetics, net photosynthetic rate (P_N), water relations, and photosynthetic pigment contents were studied during acclimation of *in vitro* grown tobacco to higher irradiance (HL; 700 µmol m^{-2} s^{-1}). Plantlets were grown on medium containing sucrose in glass vessels (G-plants) or in *Magenta* boxes (M-plants) with better CO_2 supply in the latter ones. The effect of HL was studied either (*1*) in plantlets grown under original *in vitro* conditions (closed vessels), (*2*) in *in vitro* plantlets exposed to ambient CO_2 concentration (covers removed), or (*3*) in plantlets transplanted to *ex vitro* into pots with sand and nutrient solution. Higher P_N, and fraction of closed photosystem 2 (PS2) centres (1 – q_P), and lower content of xanthophyll cycle pigments were found in M-plants compared to G-plants. HL treatment caused photoinhibition particularly in plants kept in closed vessels. This was indicated by the decrease in the ratio of F_v/F_m and by the increase in non-photochemical quenching, 1 – q_P, and content of xanthophyll cycle pigments. Better CO_2 supply ensured by the removal of closure lead to the moderate reduction of symptoms of photoinhibition, although stomatal conductance (g_s), transpiration rate (E), and P_N were negatively affected. The main reason was the decrease in relative air humidity, which caused similar reduction of P_N, E, and g_s after the transfer of plantlets to *ex vitro*. Nevertheless, plant response to HL seemed not to be affected by any possible root injury caused by transfer to *ex vitro*. The differences in contents of xanthophyll cycle pigments, degree of de-epoxidation, P_N, and quenching parameters between M- and G-plantlets were still significant 7 d after *ex vitro* transfer and HL acclimation.

Additional key words: acclimation; chlorophyll content; fluorescence; net photosynthetic rate; *Nicotiana tabacum*; stomatal conductance; transpiration rate; xanthophyll cycle pigments.

Introduction

Micropropagation has been extensively used for the rapid multiplication of many plant species. Although this technique allows efficient multiplication of many fruit, vegetable, and ornamental plants, it is still confronted with many questions. Micropropagation is achieved through establishment of explants, and their growth *in vitro*, which is followed by transplanting of plantlets to *ex vitro* conditions (glasshouse or field). During *in vitro* cultivation, plantlets grow under special conditions, *i.e.* high air humidity, low irradiance, low air turbulence, insufficient gas exchange, mixotrophic nutrition, large doses of growth regulators, *etc.* These conditions induce the formation of plantlets of abnormal morphology, anatomy, and physiology (for review, see Kozai *et al.* 1991, Pospíšilová *et al.* 1992, 1997, Desjardins 1995). Very important is the retardation of development of cuticule and of functional

Received 14 October 2002, *accepted* 23 December 2002.
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Abbreviations: A – antheraxanthin; Chl – chlorophyll; DEPS – degree of de-epoxidation; E – transpiration rate; F_m – maximum chlorophyll fluorescence; F_v – variable chlorophyll fluorescence; G-plants – plantlets grown in glass vessels; g_s – stomatal conductance; HL – high irradiance (700 µmol m^{-2} s^{-1}); M-plants – plantlets grown in *Magenta* boxes; P_N – net photosynthetic rate; PS2 – photosystem 2; q_N – non-photochemical quenching; q_P – photochemical quenching; RWC – relative water content; V – violaxanthin; Z – zeaxanthin.
Acknowledgement: The authors thank M.Sc. Daniel Haisel, Mrs. L. Hávová, and Mrs. L. Kolčabová for skilled assistance. The authors acknowledge the financial support of Z5 038 910 (Physiological and genetical bases of plant development, cell cycle, morphogenesis, stress reactions and biotechnologies. Genome organisation and function).

stomata, inefficient regulation of gas exchange, and in consequence a susceptibility to wilting.

After *ex vitro* transfer, plantlets are mostly impaired by sudden changes in environment, which can be lethal for them. In the greenhouse, and particularly in the field, air humidity is much lower than in vessels. Even if the water potential of the substrate is higher than the water potential of media with saccharose, the plantlets may quickly wilt because of not restricted water loss from their leaves. In addition, water supply can be limited because of low hydraulic conductivity of roots and root-stem connections (Fila *et al.* 1998). Furthermore, irradiance is much higher and plantlets are very susceptible to excess of photons. The most vulnerable part of the photosynthetic apparatus is photosystem 2 (PS2). At high irradiance the decrease in the quantum efficiency of PS2 electron transport and hence in CO_2 assimilation is often observed (Critchley *et al.* 1999).

Photoinhibition is a common stress affecting both plant growth and productivity. It is often enhanced by the co-existence of other stresses, such as nutrient or water shortage and abnormally low or high temperature (Aro *et al.* 1993a).

The aim of our experiments was to find out how *in vitro* grown plantlets cope with sudden increase of irradiance, which usually accompanies the transfer *ex vitro*. We tried to answer the question whether a better CO_2 supply during *in vitro* growth of plantlets could diminish symptoms of photoinhibition and improve the acclimation to higher irradiance. As the possible damage of root system, caused by the procedure of transplantation, is not usually taken into account, our experiments evaluated also this effect. Therefore the plantlets of two types of pre-cultivation *in vitro* differing in CO_2 supply due to different closure of vessels (M- and G-plants) were divided into three groups. One group of plantlets was exposed to high irradiance (HL) in the original closed vessels, where the differences between M- and G-plantlets in CO_2 concentration were preserved. A second group of M- and G-plantlets were left in the original vessels but with the closures removed. Therefore they had a similar CO_2 supply and their root systems were undisturbed. In the third group, the M- and G-plantlets were removed from the original vessels and were transferred to sand. Thus, the CO_2 concentration and the disturbance of root system were equal and differences existed only in the pre-cultivation stage.

Materials and methods

Plants: Sterilised tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) seeds were sown in Petri dishes on the agar-gelled Murashige and Skoog (1962) medium containing 2 % saccharose. After 3 weeks, plantlets were transferred into two types of cultivation vessels: glass vessels tightly closed with aluminium foil (G-plants), or polycarbonate vessels *Magenta GA-7* (*Sigma*, Diesenhofen, Germany) (M-plants) covered with closures with microporous vents (diameter 10 mm, pore size 0.22 μm, *Sigma*, St. Louis, USA). Exchange of CO_2 and water vapour between vessels and the surrounding air was about 100 % more in *Magenta* boxes than in glass vessels (Solárová *et al.* 1996). Plants were grown at a 16/8-h photoperiod, irradiance of 100-120 μmol m^{-2} s^{-1}, and day/night temperature of 25/20 °C. Temperature inside vessels reached 27 °C during the light period. Relative humidity inside both types of vessels was more than 90 %. CO_2 concentration in the cultivation chamber was 350 μmol(CO_2) mol^{-1}(air), but inside cultivation vessels it was rather low during the light period (for details, see Solárová 1989). After 5 weeks of pre-cultivation, both M- and G-plants were divided into three groups and were transferred into a chamber, where irradiance was 700 μmol m^{-2} s^{-1} (HL) and the relative air humidity was about 40 %. In one group, M- and G-vessels were kept closed (MC, GC); in another, the plastic closures and the aluminium foil were removed (MO, GO); and in a third group the plantlets were exposed to air for the next two days. The third group of the plantlets was immediately transplanted to pots with coarse sand saturated with water and nutrient solution. They were kept in open air under the same conditions for next seven days (M, G).

Chlorophyll (Chl) *a* fluorescence kinetics were measured on the adaxial surface of detached leaves after 25-min dark acclimation with the *PAM Chlorophyll Fluorometer* (*Walz*, Effeltrich, Germany) at room temperature and ambient CO_2 concentration. Measuring irradiance was 0.35 μmol m^{-2} s^{-1}, actinic irradiance 200 μmol m^{-2} s^{-1}, and 700-ms saturated flashes of "white light" (2 500 μmol m^{-2} s^{-1}) were applied at 300 s intervals. Data sampling, control, and calculation were served by the *DA 100 Data Acquisition System* (*Walz*, Effeltrich, Germany) (for detail, see Pospíšilová *et al.* 1998). The nomenclature of van Kooten and Snel (1990) and Osmond *et al.* (1993) was used throughout this work.

Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were measured using the gas exchange system *LCA-4* (*ADC Bio Scientific*, Hoddesdon, UK) at a temperature of 25 °C, irradiance of 750 μmol m^{-2} s^{-1}, relative humidity of 50 %, and CO_2 concentration of 350 μmol mol^{-1}. Water-loss curves were measured gravimetrically, as a decrease of fresh leaf mass on leaves originally fully saturated with water.

Contents of photosynthetic pigments were determined in acetone extracts of leaf discs (area 3.8 cm^2) by HPLC (*Spectra-Physics*, San Jose, USA) using a reverse phase column (*Sepharon SGX C18*, *Tessek*, Prague, Czech Republic). The solvent system was acetonitrile: methanol : water (80 : 12 : 6) followed by 100 % methanol, and the gradient was run from 8 to 12 min. The flow rate was 1 $mm^3\ s^{-1}$, the detection wavelength was 445 nm.

The whole experiment was repeated twice. In each experimental set, at least 25 plants of each variant were used for determination of each parameter. The second to fourth leaves from the top were used. All analytical methods were verified and average accuracy was ±5 %. Data were analysed by *ANOVA* or Fisher's comparative test.

Results

Chl fluorescence kinetics: After exposure to HL, the ratio of variable to maximum fluorescence (F_v/F_m) decreased in all *in vitro* plantlets. The effect of HL was more dependent on CO_2 supply during HL treatment (opened or closed vessels) than on CO_2 supply during pre-cultivation (M- or G-plants). 18 h at HL was sufficient to induce significant photoinhibition, particularly in closed vessels, where lower F_v/F_m ratios were observed (Fig. 1*A*).

After transplantation (*i.e.*, *ex vitro*), the F_v/F_m ratio was more dependent on type of pre-cultivation and the duration of HL treatment. The most pronounced photoinhibition was observed in G-plants after 18 h, whereas in M-plants it was observed after 24 h. Restoration of F_v/F_m ratio started after 48 h and reached the initial values after 168 h in M-plants, while in G-plants F_v/F_m it stayed significantly lower (Fig. 1*B*).

No significant differences were found in non-photochemical quenching (q_N) between M- and G-plants under original *in vitro* condition. M-plants in opened vessels showed considerable but transient increase of q_N after 18 h of HL, and then decreased nearly to initial value, while in G-plants from opened vessels q_N almost did not change during HL treatment. In both closed-vessel plants q_N significantly increased within the first 6 h of HL and that value persisted until the end of experiment (Fig. 1*E*).

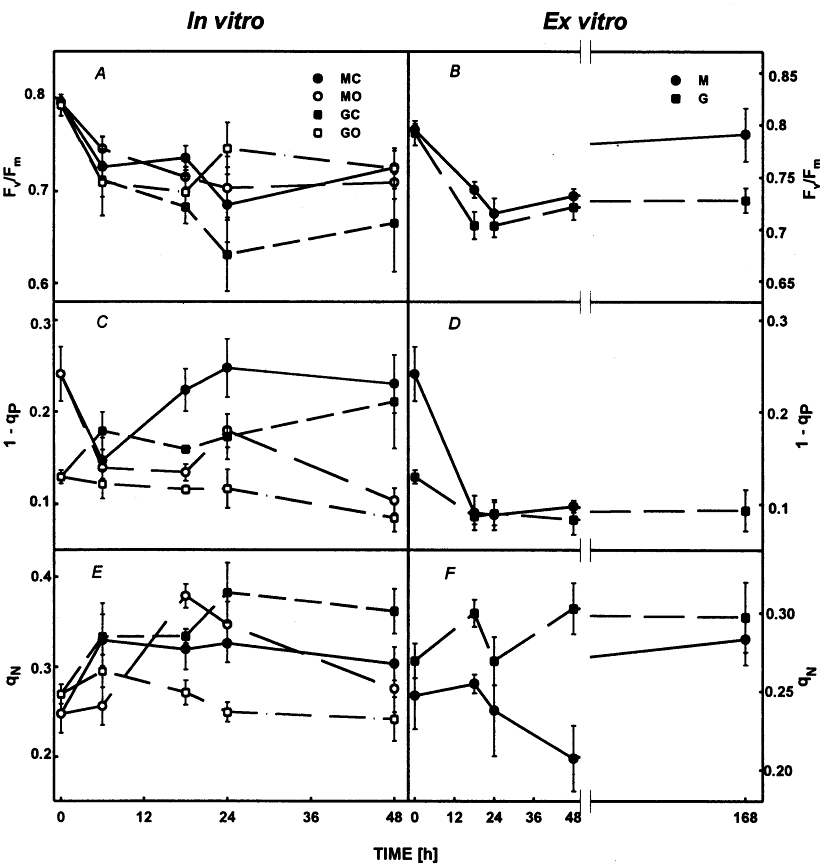

Fig. 1. Chlorophyll *a* fluorescence kinetic parameters [variable to maximum fluorescence ratio (F_v/F_m)], fraction of closed reaction centres photosystem 2 of (1 – q_P), and non-photochemical quenching (q_N) in leaves of tobacco plantlets grown *in vitro* under high irradiance (HL) (*A*, *C*, *E*) and during transfer from *in vitro* to *ex vitro* conditions (*B*, *D*, *F*). Means ± SE. MC = *Magenta* closed, MO = *Magenta* opened, GC = glass closed, GO = glass opened.

In the transplanted plants (*ex vitro*), the difference between the two types of pre-cultivation was not significant after 168 h of HL, although q_N was slightly higher in G-plants (Fig. 1*F*). The significant difference in q_N between the two types was observed after 48 h of HL, when M-plants showed lower q_N compared to G-plants.

Significant differences were observed between M- and G-plants in Q_A reduction (the fraction of closed reaction centres of PS2, $1 - q_P$) under original *in vitro* conditions. $1 - q_P$ was significantly higher in M-plants than in G-plants (Fig. 1*C*). The exposure to HL caused in closed M-plants a steep increase in $1 - q_P$, which started after 6 h and reached the highest values after 24 h of HL. In G-plants from closed vessels, the increase of $1 - q_P$ was slower, although the values were similar after 48 h to those of M-plants. The lowest $1 - q_P$ values were observed for both types in opened vessels after 48 h (Fig. 1*C*).

Immediately after transplantation, values of $1 - q_P$ were significantly higher in M-plants than in G-plants. This difference disappeared within 18 h of HL, when values of $1 - q_P$ decreased to the same level. After 168 h the fraction of closed PS2 centres was lower in M-plant than in G-plants (Fig. 1*D*).

Pigment contents: During *in vitro* pre-cultivation the content of Chl *a+b* was almost the same in M- and G-plants. However, significantly higher contents of Chl *a+b* were found in M-plants than in G-plants during acclimation to HL (Fig. 2*A*).

After transfer to *ex vitro*, total Chl content of G-plants decreased within 6 h of HL, but in the M-plants it remained virtually unchanged (Fig. 2*B*). The difference between the two types of pre-cultivation remained apparent even after 168 h of acclimation to HL.

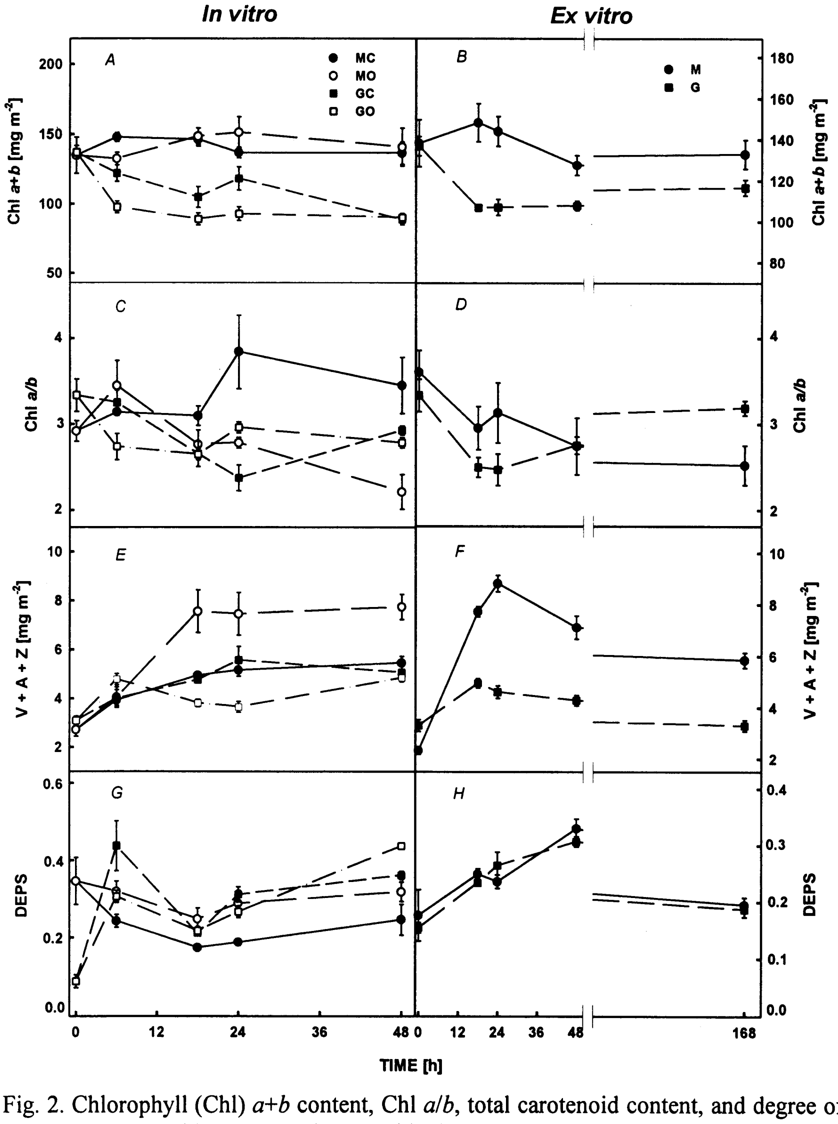

Fig. 2. Chlorophyll (Chl) *a+b* content, Chl *a/b*, total carotenoid content, and degree of de-epoxidation of xanthophyll cycle pigments [DEPS = (zeaxanthin + 0.5 antheraxanthin)/(zeaxanthin + antheraxanthin + violaxanthin)] in leaves of tobacco plantlets grown *in vitro* in HL (*A, C, E, G*) and during transfer from *in vitro* to *ex vitro* conditions (*B, D, F, H*). Means ± SE. MC = *Magenta* closed, MO = *Magenta* opened, GC = glass closed, GO = glass opened.

The ratio of Chl *a*/*b* was higher in G-plants under original *in vitro* condition. The highest value of Chl *a*/*b* was found in M-plants from closed vessels after 24 h of HL, due to lower content of Chl *b*. The final values after 48 h in HL were the lowest in M-plants from opened vessels (Fig. 2*C*).

After *ex vitro* transfer, the decrease of the ratio of Chl *a*/*b* was observed as an effect of HL in both types of plantlets. Higher ratio of Chl *a*/*b* was found in G-plants than in M-plants after 168 h under HL (Fig. 2*D*).

Significant differences in total content of xanthophyll cycle pigments [violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z)] and their individual representations were found between M- and G-plants during pre-cultivation *in vitro* (Figs. 2*E* and 3*A*). The initial content of V+A+Z was slightly lower in M-plants than in G-plants. The main difference between both types was found in V content, which was originally significantly higher in G-plants (Fig. 3*A*). The increase of total xanthophylls started after 6 h of HL in all types of plants with the peak values reached after 18 h of HL in open M-plants. The increase in individual xanthophyll cycle pigments, especially in A and Z in the M-plants in opened vessels, was due to HL (Fig. 3*A*).

In plantlets transferred to *ex vitro*, the V+A+Z content increased particularly in M-plants during 18 h in HL. In transplanted G-plants only moderate and temporary increase occurred (Figs. 2*F* and 3*B*), although these plants showed higher V+A+Z content *in vitro*. After 168 h in HL, M-plants still showed higher contents of V+A+Z. The highest content of Z was found after 48 h treatment in both plant types (Fig. 3*B*).

Significant differences in degree of de-epoxidation (DEPS) were observed between M- and G-plants during *in vitro* pre-cultivation. The initial value of DEPS was higher in M-plants than in G-plants (Fig. 2*G*), which was caused by high content of V measured in G-plants (Fig. 3*A*). The most apparent changes in DEPS occurred within the first 6 h of HL, when the lowest DEPS was found in closed M-plants. After this only small changes were observed, although significant differences were proved after 48 h of HL among plantlets.

Degree of DEPS in *ex vitro* transplanted plants increased gradually during 48 h of HL, and then decreased; the initial levels were reached after 168 h of HL (Fig. 2*H*).

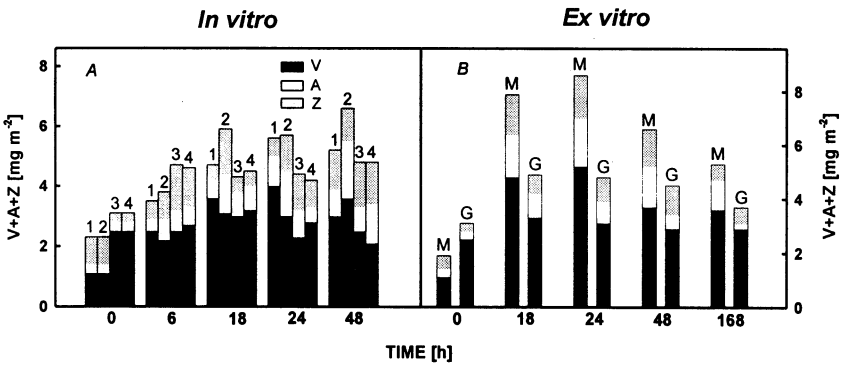

Fig. 3. Contents of individual xanthophyll cycle pigments [violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z)] in leaves of tobacco plantlets grown *in vitro* in HL (*A*) and during transfer from *in vitro* to *ex vitro* conditions (*B*). Means ± SE. 1 = *Magenta* closed, 2 = *Magenta* opened, 3 = glass closed, 4 = glass opened, M = *Magenta*, G = glass.

Gas exchange and relative water content: The differences in P_N, E, g_s, and relative water content (RWC) between closed and opened vessels were statistically significant (Fig. 4). P_N was higher in M-plants compared to G-plants under original *in vitro* condition. Decrease in P_N was found as the effect of HL after 6 h in all plantlets. Whereas P_N increased during following exposure to HL to initial values in both types of plants kept in the closed vessels, in plants in the opened vessels P_N significantly declined (Fig. 4*A*).

After *ex vitro* transfer, P_N rapidly decreased within 18 h of HL to about 43 % compared to *in vitro* M- and G-plants. Contrary to M-plants, P_N of transplanted G-plants reached the initial values after 168 h in HL (Fig. 4*B*).

During *in vitro* pre-cultivation E was similar in M- and G-plants (Fig. 4*C*). Due to HL, a more pronounced decrease was observed in plantlets in opened vessels, while plantlets in closed vessels were not seriously affected.

Despite of the fact that the differences in E between M- and G- plants after *ex vitro* transplantation were not statistically significant in individual sample periods, E was slightly higher in G-plants than in M-plants during the whole acclimation to HL (Fig. 4*D*).

Significant differences in g_s were found between opened and closed vessels during HL treatment in both the types of plants. Significantly higher g_s was found in closed vessels than in the opened ones (Fig. 4*E*). In both *ex vitro* transplanted plants, g_s markedly decreased after 18 h of HL compared to *in vitro* grown plantlets, and remained very low during HL acclimation (Fig. 4*F*).

RWC varied from 96 to 98 % during *in vitro* pre-cultivation in all types of plantlets (Fig. 4*G*). During HL treatment RWC decreased significantly in dependence on duration of irradiation, particularly in plants in opened vessels. G-plants in closed vessels maintained a similar value during the whole treatment. After 48 h of HL the lowest values (about 40 %) were observed in M-plants from opened vessels. RWC of *ex vitro* transplanted plants varied in the range 98-73 % during 7 d of HL in both plant types (Fig. 4*H*). The small differences between M- and G-plants were not statistically significant.

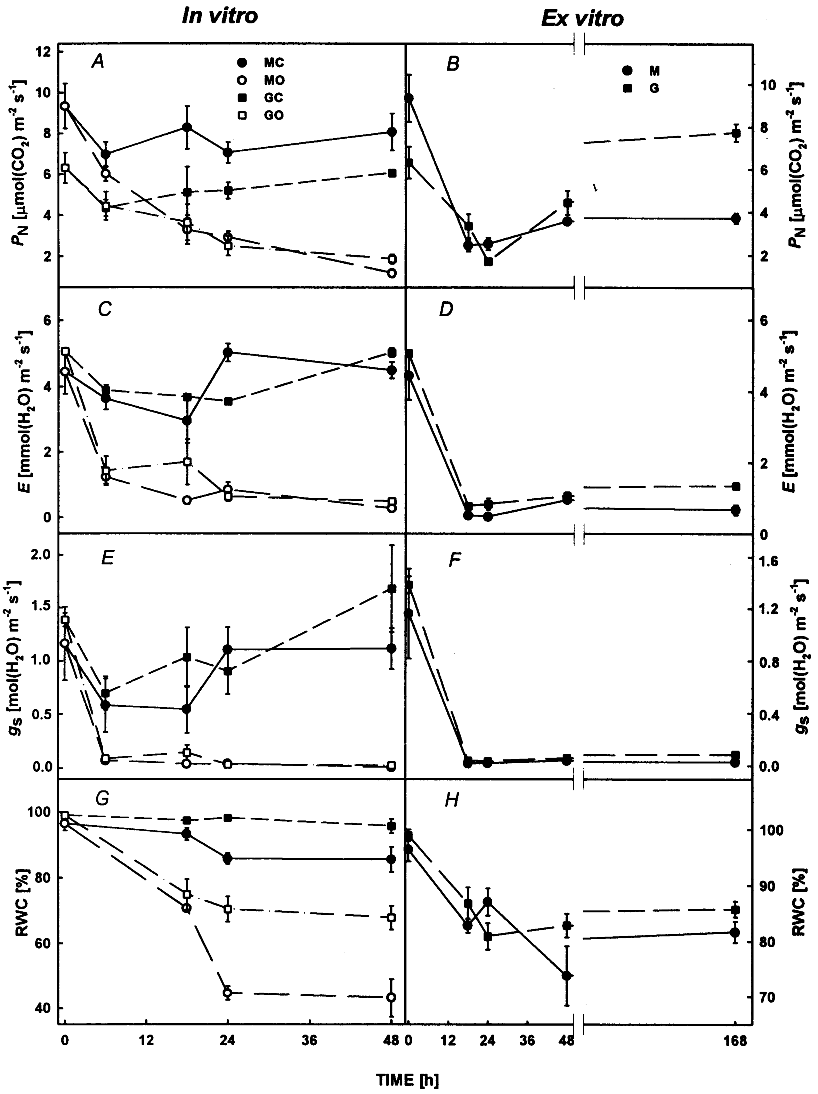

Fig. 4. Net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and relative water content (RWC) in leaves of tobacco plantlets grown *in vitro* in HL (*A, C, E, G*) and during transfer from *in vitro* to *ex vitro* conditions (*B, D, F, H*). Means ± SE. MC = *Magenta* closed, MO = *Magenta* opened, GC = glass closed, GO = glass opened.

Discussion

The effect of HL on micropropagated plants in *in vitro* conditions: Tobacco plantlets cultivated in *Magenta* boxes grew more quickly than plantlets in glass vessels. They had more and thicker leaves and higher P_N, and a lower content of xanthophyll cycle pigments. Nevertheless, values of F_v/F_m and RWC, and contents of Chl *a* and Chl *b* were almost identical in both types of cultivation vessels. This was in agreement with previous experiments (Haisel *et al.* 1999).

The decrease of maximal photochemical efficiency of open PS2 centres (F_v/F_m) is often considered as an indicator of photoinhibition (Krause *et al.* 1995). Some authors observed photoinhibition during *in vitro* growth under relatively low irradiance, *e.g.* 200 µmol m^{-2} s^{-1} in fully photoautotrophic cultures (Tichá *et al.* 1995, 1998) contrary to *ex vitro* grown plants, where photoinhibition was observed mostly under much higher irradiance, *e.g.* 1 600 μmol m^{-2} s^{-1} (Aro *et al.* 1994). Nevertheless, photoinhibition is often promoted by water stress (*e.g.* Jagtap *et al.* 1998, Lu and Zhang 1998) or by high or low temperature (Öquist *et al.* 1992b) and is dependent on plant species. In our experiments, significant decrease of the ratio of F_v/F_m of both M- and G-plants *in vitro* indicated a temporary photodamage caused by exposure to HL.

Our experiments also showed that M- and G-plants probably used different mechanisms to cope with a relative excess of photons during *in vitro* pre-cultivation. M-plants preferentially increased the fraction of closed reaction centres of PS2 ($1 - q_P$), while G-plants released redundant energy probably non-photochemically. Osmond *et al.* (1993) reported that HL caused increase in q_N and decrease in $1 - q_P$ at the same time, which characterised more the situation in G-plants in our experiments. Closed reaction centres are probably inactivated due to permanent excitation and changes in D1 protein content (Aro *et al.* 1993b). D1 protein, a key component of PS2 reaction centre, turns over far more rapidly than any other protein in the photosynthetic membrane (Mattoo *et al.* 1989). If the rate of repair of PS2 does not keep pace with its rate of damage, then photoinhibition is observed as a decrease in photosynthetic capacity (Andersson and Barber 1996). We suggest that M-plants may have faster turnover of D1 protein than G-plants. Nevertheless, in both types of plantlets the fraction of closed reaction centres of PS2 and q_N increased after 48 h of HL, when cultivation vessels remained closed by original closures (see Fig. 1), while their removal (*i.e.* better CO_2 supply) caused the decrease in both the $1 - q_P$ and q_N. This showed that CO_2 supply played more important role in acclimation to HL than previous *in vitro* pre-cultivation.

Some authors suggest a close association between q_N and xanthophyll cycle pigments (*e.g.* Demmig-Adams 1990, Fäber *et al.* 1997), *e.g.* the decrease in q_N is connected with inhibition of xanthophyll cycle (Lokstein *et al.* 1994). The increase in contents of V+A+Z pigments and sequential conversion of V to Z was proved to be stimulated by HL (Björkman and Demmig-Adams 1995, Demmig-Adams *et al.* 1998, Gray *et al.* 1998). This was observed also in our experiments, where V+A+Z was found to increase in all plants after HL treatment. Nevertheless, plant pre-cultivation was important, particularly for the relative content of individual xanthophylls (see Fig. 3), while a higher CO_2 supply stimulated more the conversion of V to Z in M-plants.

Photosynthetic gas exchange and water relations of *in vitro* grown plantlets were studied by numerous authors (*e.g.* Donnelly and Vidaver 1984, Kozai 1991, Pospíšilová *et al.* 1997). Our experiments proved that the effect, which prevailed over the other factors, was a sudden decrease in air humidity caused by removal of covers from original cultivation vessels and subsequent uncontrolled water loss (Fig. 4). The used types of *in vitro* pre-cultivation played some role only during cultivation itself and seemed to have minimal effect on HL acclimation.

In our experiments, contents of Chl *a* and Chl *b* were in the beginning similar in *in vitro* M- and G-plants. Chl content decreased by HL only in G-plants, independently of the CO_2 supply. This also corresponded with decrease of F_v/F_m ratio, occurring particularly in closed G-plants. Serret *et al.* (2001b) reported that different type of closure may alter the photoinhibitory impairment during micropropagation. This indicated that G-plants might be more susceptible to photoinhibition caused by HL than M-plants.

The effect of HL on *in vitro* plantlets transferred to *ex vitro*: In our experiment, the decrease of F_v/F_m due to HL in transplanted plantlets was similar as under *in vitro* conditions, and the original values were reached after 7 d of acclimation particularly in M-plants. Exposure of *Calathea louisae* and *Spathiphyllum floribundum* plantlets to HL immediately after transplantation caused photoinhibition and Chl photobleaching (van Huylenbroeck 1994, van Huylenbroeck *et al.* 1995). Similarly, photoinhibition was observed in *Rosa hybrida*, but only in the first week after transplantation, and especially in plantlets transplanted into medium with decreased osmotic potential by addition of mannitol (Sallanon *et al.* 1998).

Better acclimation after transfer to *ex vitro* was often reported in plant cultures where saccharose was present in medium during *in vitro* pre-cultivation (Lima da Silva *et al.* 1995, Kadleček 1997, Serret *et al.* 2001a). Contrary to this, Trillas *et al.* (1995) observed better adaptation to HL in transplanted photoautotrophic plants than in plants growing previously on saccharose supplemented medium.

P_N in *Solanum tuberosum* and *S. floribundum* plants decreased in the first week after transplantation and increased thereafter (Baroja *et al.* 1995, van Huylenbroeck and Debergh 1996). Two weeks after *ex vitro* transplantation of *Nicotiana tabacum*, P_N was higher than in plantlets grown *in vitro* (Pospíšilová *et al.* 1998).

Stomatal control was critical for survival and directly affected the physiological performance of the plantlets in our experiments. Immediately after transplantation, visible wilting was observed and correspondingly low RWC was found. The g_s was high prior to the transplantation. However, already 12 h after transplantation the stomata were able to close and control water loss partially. This was indicated by a decrease in g_s, and in consequence of E and P_N. Similar results in *Zea mays* and *N. tabacum* were found by Saccardy *et al.* (1998) and

Pospíšilová *et al.* (1999b, 2000), respectively. The root damage during transplantation was probably not serious, because similar changes in water regime were observed in plantlets exposed to lower relative humidity after removal of closures from cultivation vessels without affecting their roots.

Increase in Chl content within a few days after transplantation *ex vitro* was found in several plant species, such as *Nicotiana* (Synková 1997, Pospíšilová *et al.* 1998), *Prunus* and *Amygdalus* (Trillas *et al.* 1995), and oil palm (Rival *et al.* 1997). Photomixotrophically grown *Nicotiana* showed an abrupt decrease in Chl *a* and Chl *b* contents during the first week after transplantation, which was later followed by a slow increase (Kadleček *et al.* 1998). In our experiments a similar situation was found, particularly in G-plants.

The ratio of Chl *a*/*b* decreased *ex vitro* in both types of plants during acclimation. Similar results were described by Demmig-Adams *et al.* (1998). Logan *et al.* (1998) observed in *Cucurbita pepo* an increase in Chl *a*/*b* due to HL, whereas in *Vinca major* cultivated under the same conditions no change was found. Bailey *et al.* (2001) suggested in *Arabidopsis thaliana* three separate phases in the response of Chl *a*/*b* to growth irradiance. They observed the increase at low and high irradiance and a plateau at intermediate irradiance. These results corresponded with changes in LHC2.

Ex vitro transplanted plants showed some differences in the way of elimination of excessive energy, which were dependent on *in vitro* pre-cultivation. G-plants seemed to use more non-photochemical processes in HL elimination, because these plants had significantly higher q_N than M-plants during the first two days. Faster turnover of D1 protein could be a protective mechanism against HL in M-plants (Öquist *et al.* 1992a). Düring (1998) and Pastenes *et al.* (1998) observed also decrease of q_N due to water stress.

Pospíšilová *et al.* (1999a) reported that content of xanthophyll cycle pigments and degree of DEPS during acclimation of transplanted *N. tabacum* plants at low irradiance (200 µmol m^{-2} s^{-1}) was not changed. On the contrary, during acclimation to high irradiance (700 µmol m^{-2} s^{-1}) in our experiments, V+A+Z content and DEPS were much higher than during *in vitro* cultivation. Logan *et al.* (1998) found similar increase in *C. pepo* and *Vinca major* after abrupt change in irradiance in the field. When tobacco plantlets were acclimated in two phases, first in the greenhouse (irradiance of 30-90 µmol m^{-2} s^{-1}) for 35 d and then in the open air (200-1 400 µmol m^{-2} s^{-1}), no photoinhibition was found and plants were adapted to HL (Kadleček 1997). Fast ability to recovery after photoinhibition was found, zeaxanthin was removed from reaction centres of PS2, epoxidated back to violaxanthin, and turnover of D1 run (Thiele *et al.* 1995). We found similar results after 168 h of HL that showed that plants were able to adapt to HL within seven days.

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