Effect of abscisic acid on photosynthetic parameters during *ex vitro* transfer of micropropagated tobacco plantlets

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Abstract

The aim of this research was to determine whether exogenous abscisic acid (ABA) applied immediately after *ex vitro* transfer of *in vitro* grown plants can improve their acclimatization. Tobacco (*Nicotiana tabacum* L.) plantlets were transferred into pots with Perlite initially moistened either by water or 50 μM ABA solution and they were grown under low (LI) or high (HI) irradiance of 150 and 700 μmol m^{-2} s^{-1}, respectively. Endogenous content of ABA in tobacco leaves increased considerably after ABA application and even more in plants grown under HI. Stomatal conductance, transpiration rate and net photosynthetic rate decreased considerably 1 d after *ex vitro* transfer and increased thereafter. The gas exchange parameters were further decreased by ABA application and so wilting of these plants was limited. Chlorophyll (*a*+*b*) and β-carotene contents were higher in ABA-treated plants, but the content of xanthophyll cycle pigments was not increased. However, the degree of xanthophyll cycle pigments deepoxidation was decreased what also suggested less stress in ABA-treated plants. No dramatic changes in most chlorophyll *a* fluorescence parameters after *ex vitro* transfer suggested that the plants did not suffer from restriction of electron transport or photosystem damage.

Additional key words: carotenoids, chlorophyll contents, chlorophyll fluorescence, net photosynthetic rate, *Nicotiana tabacum*, stomatal conductance, transpiration rate, xanthophyll cycle pigments.

Introduction

The main problem during *ex vitro* transfer is high rate of water loss from shoots of plantlets taken out of the cultivation vessels. Even if the water potential of the substrate (soil or sand with nutrient solution) is usually higher than the water potential of media with sucrose, the plantlets may quickly wilt. The cause is unrestricted rate of transpiration due to the retardation in development of cuticle, epicuticular waxes and functional stomatal apparatus (for recent review see, *e.g.*, Pospíšilová *et al*. 2007). Few weeks of growth under a shade and gradually lowering air humidity are usually prerequisite for successful establishment of vigour plants. In some plant species, the leaves formed *in vitro* are unable to develop further under *ex vitro* conditions and they are replaced by newly formed leaves.

After *ex vitro* transfer stomata density decreased or increased and the decrease in stomata density was sometimes compensated by an increase in stomatal size and accompanied by changes in stomata shape from ring-shaped to elliptical (Tichá *et al*. 1999). Ability of stomata to close in response to external stimuli which is usually low in *in vitro* grown plants (especially in those grown in tightly closed vessels) usually increased after *ex vitro* transfer and stomatal conductance and stomatal transpiration rate decreased to values found in naturally grown plants within several weeks, but the cuticular

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Abbreviations: ABA - abscisic acid; Car - carotenoids; Chl - chlorophyll; DEPS - degreee of XCP deepoxidation; F_m - maximum chlorophyll fluorescence; F_v - variable chlorophyll fluorescence; PS - photosystem; q_{NP} - non-photochemical quenching; q_P - photochemical quenching; RWC - relative water content; WUE - water use efficiency; XCP - xanthophyll cycle pigments; Φ_{PS2} - quantum yield of PS 2 photochemistry.
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The article is dedicated to our colleagues Dr. Jiří Čatský and Dr. Zdeněk Šesták passing away last year.
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transpiration rate decreased more slowly (*e.g.* Fila *et al*. 1998, Pospíšilová *et al*. 1998, 1999, Haisel *et al*. 1999, Bhatia and Asnwath 2004).

Net photosynthetic rate (P_N) in plantlets is affected by irradiance, CO_2 concentration in the vessels and sugar type and concentration in the medium (*e.g*. Fuentes *et al*. 2005). P_N usually decreased in the first days after transplantation and increased thereafter (*e.g*. Van Huylenbroeck and Debergh 1996, Kadleček *et al.* 1998, 2001, Seon *et al*. 2000, Estrada-Luna *et al*. 2001, Guan *et al*. 2008). Substantial increase in P_N was measured when new leaves were fully developed (3 - 4 weeks after *ex vitro* transfer) (*e.g*. Díaz-Pérez *et al*. 1995, Fila *et al*. 1998, Pospíšilová *et al*. 1998, Van Huylenbroeck *et al*. 1998, 2000, Amâncio *et al*. 1999, Slavtcheva and Dimitrova 2001). In *Picea glauca*, *in vitro* hardening considerably increased P_N after *ex vitro* transfer (Lamhamedi *et al*. 2003).

Chlorophyll (Chl) *a* and Chl *b* contents usually increased after transplantation (Trillas *et al*. 1995, Rival *et al*. 1997, Synková 1997, Pospíšilová *et al*. 1998, Van Huylenbroeck *et al*. 2000, Osório *et al*. 2005) and also improvement of chloroplast ultrastructure was observed (Wettstein and Sommer 1982).

Sudden increase in irradiance after *ex vitro* transfer might be dangerous. Exposure of *Calathea louisae* and *Spathiphyllum floribundum* plantlets to high irradiance immediately after transplantation caused photoinhibition and even Chl photobleaching (Van Huylenbroeck 1994, Van Huylenbroeck *et al.* 1995, 2000) but no photoinhibition was observed in plants acclimatized under low irradiance (Van Huylenbroeck 1994). Similarly, photoinhibition characterized by decrease in variable to maximum fluorescence ratio (F_v/F_m) was observed in *Rosa hybrida* and *Zingiber officinale* plantlets in the first week after *ex vitro* transfer (Sallanon *et al*. 1998, Genoud *et al*. 1999, Guan *et al*. 2008). Immediately after *ex vitro* transfer, reversible photoinhibition was observed in *Castanea sativa* and *Vitis vinifera* (Carvalho *et al*. 2001).

In addition to fluorescence parameters, the increased content of xanthophyll cycle pigments [violaxanthin + antheraxanthin + zeaxanthin] and particularly the degree of their deepoxidation [DEPS = (zeaxanthin + 0.5 antheraxanthin)/(zeaxanthin + antheraxanthin + violaxanthin)] may be indicators of defence against photoinhibition (*e.g*. Kadleček *et al.* 2001, 2003). In tobacco plantlets, the xanthophyll cycle pigment contents and degree of their deepoxidation were not changed markedly during acclimation under shade (Pospíšilová *et al*. 1999, 2000) but temporary increased during acclimation at high irradiance (Semorádová *et al*. 2002).

Involvement of abscisic acid (ABA) in the regulation of stomatal opening is generally accepted. ABA controls stomatal conductance and hence CO_2 transport into leaf mesophyll. However, non-stomatal effects of ABA on photosynthesis are still under debate. ABA application increased Chl and carotenoid (Car) contents in wheat genotypes under moderate water stress (Agarwal *et al*. 2005). ABA also affects expression of many genes including those encoding small and large subunit of Rubisco (*rbsS* and *rbsL*), *cab* genes encoding proteins of light-harvesting pigment-protein complexes, or *psbA* gene encoding D1 protein (Giraudat *et al*. 1994, Bray 2002). The application of ABA to barley seedlings resulted in partial protection of the photosystem (PS) 2 against photoinhibition. This was accompanied with higher photochemical quenching in ABA-treated leaves, considerable increase in the amount of total Car and xanthophylls and activity of xanthophyll cycle (Ivanov *et al.* 1995). Similarly, Jia and Lu (2003) found that long-term ABA treatment increased resistance to photoinhibition in maize associated with maintained photosynthetic rate and enhanced xanthophyll cycle activity.

Addition of ABA to the medium or to the substrate immediately after transplantation of *in vitro* grown *Nicotiana tabacum* or *Tagetes erecta* plants alleviated their wilting (Pospíšilová 1996, Pospíšilová *et al*. 1998, Aguilar *et al*. 2000). The stabilization of water status is prerequisite of plantlet survival, but their further growth requires sufficient photosynthetic rate under correspondingly high irradiance. Therefore the aim of these experiments was to determine if ABA application can alleviate photoinhibition which can be induced by sudden increase in irradiance. The following hypotheses were tested: *1*) exogenous ABA can either close stomata or inhibit their opening, reduce transpiration rate and so partially eliminate initial wilting and *2*) exogenous ABA readily absorbed by plants can substitute endogenous ABA synthesized from neoxanthin or violaxanthin during water stress and thus a higher pool of xanthophyll cycle pigments remains for dissipation of excess energy absorbed in thylakoids and thus photoinhibition might be alleviated.

Materials and methods

Tobacco regenerants from axillary buds grown *in vitro* for about 2 months (three sub-cultures) in ventilated *Magenta* boxes were transferred into pots with *Perlite* initially moistened either by water or 50 μM ABA solution. Further the plants were irrigated only with water when necessary. Plants were grown in controlled conditions (16-h photoperiod, day/night temperature 25/20°C, air humidity about 45 %) either under low irradiance of 150 μmol m^{-2} s^{-1} (LI) or high irradiance of 700 μmol m^{-2} s^{-1} (HI). All parameters were measured before *ex vitro* transfer and one or seven days after *ex vitro* transfer. In every treatment six plants and three mature leaves on each plant were usually measured.

Relative water content (RWC) was measured

gravimetrically in leaf discs (0.5 cm^2) water-saturated by immersing into holes of fully moistened polyurethane foam under dark according to Čatský (1960).

For determination of the endogenous abscisic acid accurately weighted plant material was ground to powder in liquid nitrogen and extracted overnight by modified Bieleski (1964) solution. Extract was centrifuged and purified by using C_{18} and MCX SPE (solid phase extraction) column. In the next step the extract was purified by HPLC and the proprietary fraction was collected. This purified fraction was methylated by diazomethane and analysed by GC-MS/MS (ion trap) with quantitation in MS/MS mode and confirmation of the identity in a full-spectra MS mode.

Net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) were measured on upper, middle and lower attached leaves using the commercial gas exchange system *LCA-4* (*ADC Bio Scientific*, Hoddesdon, UK) with leaf chamber *LC4/PLC4BT-1/E* at a temperature of 25 °C, irradiance of 750 μmol m^{-2} s^{-1}, CO_2 concentration of 350 μmol mol^{-1}, and relative humidity of about 30 %.

Contents of photosynthetic pigments were determined in acetone extracts of leaf discs by HPLC (*ECOM, s.r.o.*, Prague, Czech Republic) using a reverse phase column (*Watrex Nucleosil 120-5-C18*, 5 μm particle size, 125 × 4 mm, *ECOM, s r.o.*, Prague, Czech Republic). The solvent system was acetonitrile : methanol : water (80:12:10) followed by methanol : ethylacetate (95:5), and the gradient was run from 2 to 6 min. The flow rate was 1 cm^3 min^{-1}, the detection wavelength 445 nm. Data were captured by PC-software *Clarity* (*DataApex*, Prague, Czech Republic).

Chlorophyll (Chl) *a* fluorescence kinetics was 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). The nomenclature of Van Kooten and Snel (1990) was used throughout this work.

Results and discussion

One week after *ex vitro* transfer no evident differences in the growth of plants induced by different irradiances or ABA application were observed (Fig. 1). As was expected, content of endogenous ABA increased considerably after ABA application. Further, it was always higher in plants grown under higher irradiance (HI) than in those grown under lower irradiance (LI) showing occurrence of stress in plants grown under HI (Fig. 2). Increase in ABA content in tobacco plantlets grown *ex vitro* under HI was found by Hofman *et al*. (2002), but only in those plantlets grown *in vitro* under very low irradiance. Hronková *et al*. (2003) found transient increase in endogenous ABA content after ABA application to medium of *in vitro* grown tobacco plants. In their experiments, endogenous ABA content after *ex vitro* transfer was dependent on air humidity.

Stomatal conductance (g_s) and transpiration rate (E) were high in *in vitro* grown plantlets, which was in agreement with our previous experiments and data in literature. E and g_s of all variants were considerably lower after *ex vitro* transfer than in *in vitro* grown plants (Fig. 3). In the first day, this decrease was mostly induced by water stress even if the values of relative water content (RWC) remained rather high (86 - 94 %). However, tobacco is isohydric plant with sensitive regulation of water loss (Haisel *et al*. 2008). In *Doritaenopsis*, RWC remained high when plantlets acclimatized under 90 % relative humidity and 20 °C, but decreased considerably

Fig. 1. Tobacco (*Nicotiana tabaccum* L. cv. SR1) plants 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer.

under relative humidity 70 or 50 % and temperature either 15 or 35 °C (Jeon *et al*. 2006). The above mentioned data suggested that tobacco plantlet grown in ventilated *Magenta* boxes were at least partially able to regulate their gas exchange and so prevent uncontrolled wilting. Also in pepper plants leaf stomata that developed *in vitro* were functional *ex vitro* (Estrada-Luna *et al*. 2001). The improvement of stomatal function in carnation plantlets grown in ventilated vessels in comparison with those grown in non-ventilated vessels was found by Majada *et al*. (1998).

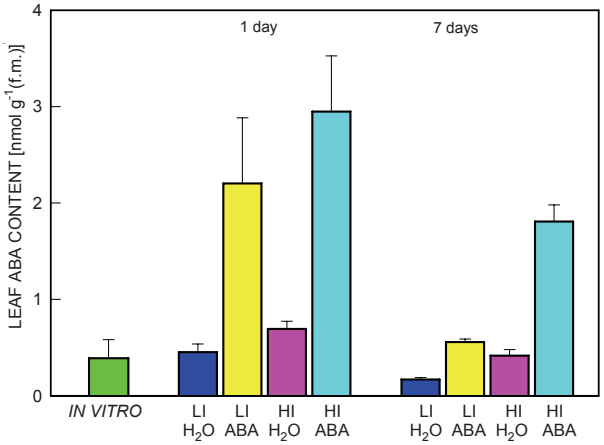


Fig. 2. Endogenous ABA content in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer. Means ± SE, n = 3.

The values of g_s and E measured 7 d after *ex vitro* transfer were higher than those measured 1 d after *ex vitro* transfer but considerably lower than those in *in vitro* grown plants and represented acclimation of plants to new environmental conditions. The observed decrease in g_s and E after *ex vitro* transfer was in agreement with our previous experiments with tobacco plantlets (Pospíšilová *et al*. 1998) and with the decrease in g_s and E during acclimatization of many other species (for review see Pospíšilová *et al*. 2007). In grapevine, the decrease in g_s after *ex vitro* transfer was compensated by an increase in mesophyll conductance to CO_2 diffusion (Fila *et al*. 2006).

ABA application to *in vitro* grown plants reduced water loss from their leaves (Colón-Guaspp *et al*. 1996, Hartung and Abou-Mandour 1996, Pospíšilová 1996). *Tagetes erecta* plantlets cultivated in ventilated containers exhibited better control of water loss and higher content of ABA that those grown in sealed containers but addition of ABA into cultivation medium in sealed containers produced plantlets that had similar control of water loss as plantlets cultivated in ventilated containers (Aguilar *et al*. 2000). After *ex vitro* transfer, ABA application decreased both parameters under LI in agreement with our previous experiments (Pospíšilová *et al*. 1998, 2000). Effect of ABA on *ex vitro* growth under HI was not measured previously and ABA application decreased g_s and E only after 7 d but not after 1 d (Fig. 3).

Net photosynthetic rate (P_N) of *in vitro* grown plantlets was rather high which showed development of functional photosynthetic apparatus in ventilated *Magenta* boxes. P_N decreased in the first day after *ex vitro* transfer less in those grown under LI than in those grown under HI. However, 7 d after *ex vitro*

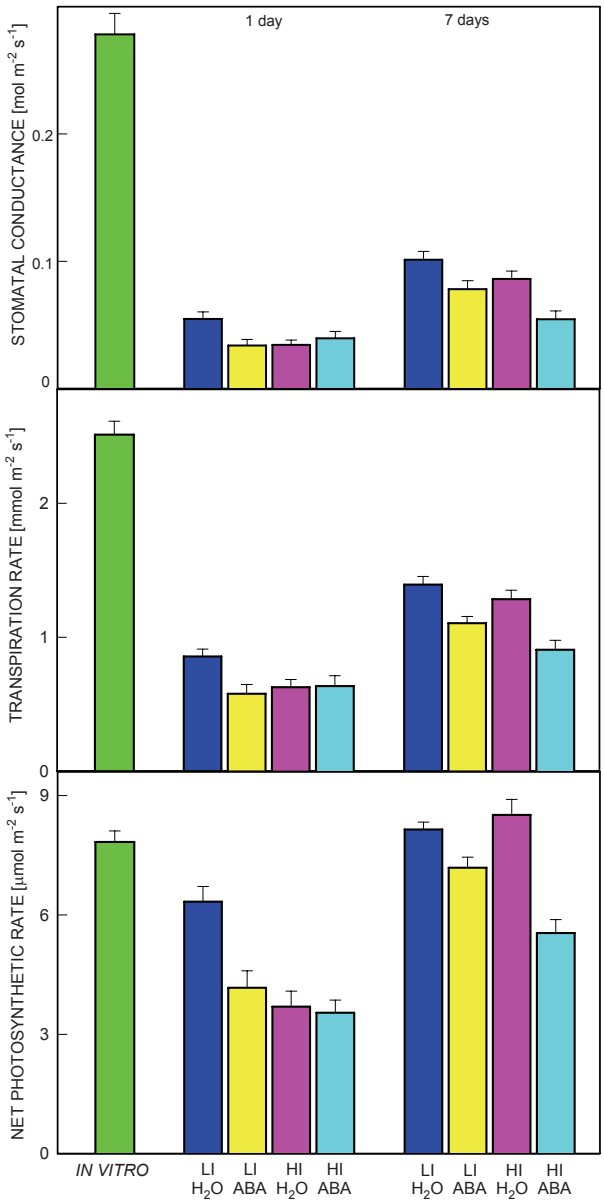


Fig. 3. Stomatal conductance (*A*), transpiration rate (*B*) and net photosynthetic rate (*C*) in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer. Means ± SE, n = 18.

transfer, P_N increased to values similar as in *in vitro* grown plants and negative effects of HI remained only in ABA-treated plants (Fig. 3). In contrast, P_N of *Fragaria ananasa* was higher when the plants were grown under irradiance of 600 than 200 μmol m^{-2} s^{-1} (Zhou *et al*. 2005). In *Solanum tuberosum* (Baroja *et al.* 1995), *Spathiphyllum floribundum* (Van Huylenbroeck and Debergh 1996), *Rehmania glutinosa* (Seon *et al*. 2000), *Capsicum annuum* (Estrada-Luna *et al*. 2001), *Ocimum basilicum* (Siddique and Anis 2008) and *Zingiber officinale* (Guan *et al*. 2008) P_N also decreased in the first days after transplantation and increased thereafter. In present experiments we measured persistent leaves, however, significant increase in P_N to values higher than during *in vitro* growth was usually found only when new leaves were fully developed (*e.g*. Díaz-Pérez *et al*. 1995, Fila *et al*. 1998, Pospíšilová *et al*. 1998, Van Huylenbroeck *et al*. 1998, 2000, Amâncio *et al*. 1999, Slavtcheva and Dimitrova 2001). After ABA application P_N decreased as a result of decreased stomatal conductance (Fig. 3).

Water use efficiency (WUE = P_N/E) or intrinsic water use efficiency (WUEi = P_N/g_s) increased considerably after *ex vitro* transfer and 1 d after *ex vitro* transfer WUE and WUEi were higher in plants grown under LI than in those grown under HI. In most cases, positive effect of ABA application was observed (Table 1). Similarly, ABA pre-treatment increased WUE in control and water-stressed bean, sugar beet and maize seedlings grown in sand with nutrient solution (Pospíšilová and Baťková 2004, Stuchlíková *et al*. 2007).

Table 1. Water use efficiency (WUE = P_N/E) or intrinsic water use efficiency (WUEi = P_N/g_s) in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer.

Treatment	Duration	WUE [μmol(CO_2) mmol(H_2O)]	WUEi [μmol(CO_2) mol(H_2O)]
In vitro		3.14	29.93
LI H_2O	1 d	7.37	120.46
LI ABA	1 d	7.61	122.24
HI H_2O	1 d	6.03	107.54
HI ABA	1 d	6.71	104.99
LI H_2O	7 d	5.84	84.70
LI ABA	7 d	6.52	94.67
HI H_2O	7 d	6.53	97.65
HI ABA	7 d	6.10	105.26

Chlorophyll (Chl) *a*+*b* content slightly decreased 1 d after *ex vitro* transfer only in control plants grown under LI. Slightly higher Chl *a*+*b* content was observed under HI than under LI but only 1 d after *ex vitro* transfer. Less water stress due to lower transpiration rate after ABA application was reflected in increased chlorophyll content in all variants. The differences were important 7 d after *ex vitro* transfer when Chl *a*+*b* content in H_2O treated plants was lower than during *in vitro* growth (Fig. 4). In previous experiments, Chl *a*+*b* content was higher in new leaves 2 weeks after *ex vitro* transfer than during *in vitro* growth and further increased by ABA application (Pospíšilová *et al*. 1998). Chl *a*/*b* ratio did not changed considerably after *ex vitro* transfer. This ratio was higher under LI than under HI a mostly slightly decreased after ABA treatment (Fig. 4). Chl *a* and Chl *b* contents in *Ocimum basilicum* decreased 7 d after *ex vitro* transfer but increased during further acclimatization (Siddique and Anis 2008). In *Anoectochilus formosanus* cultivated *ex vitro* under 3 irradiances (60, 180 and 300 μmol m^{-2} s^{-1}) for 30 d, the highest Chl *a* and Chl *b* contents were found under intermediate irradiance, and Chl *a*/*b* ratio at the highest irradiance (Pandey *et al*. 2006).

Changes in contents of β-carotene and lutein were very similar to those in Chl *a*+*b* content. Positive effect of HI was also observed only 1 d after *ex vitro* transfer and ABA application increased β-carotene and lutein content in all variants (Fig. 5). However, positive effect of ABA application was observed only under LI 7 d after *ex vitro* transfer. The positive effect of ABA application

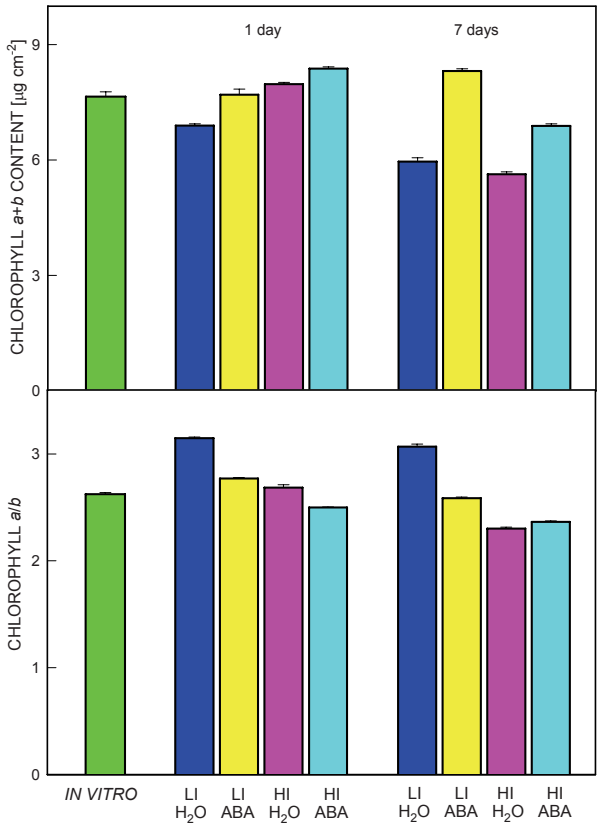


Fig. 4. Chlorophyll *a*+*b* content and chlorophyll *a*/*b* ratio in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer. Means ± SE, *n* = 6.

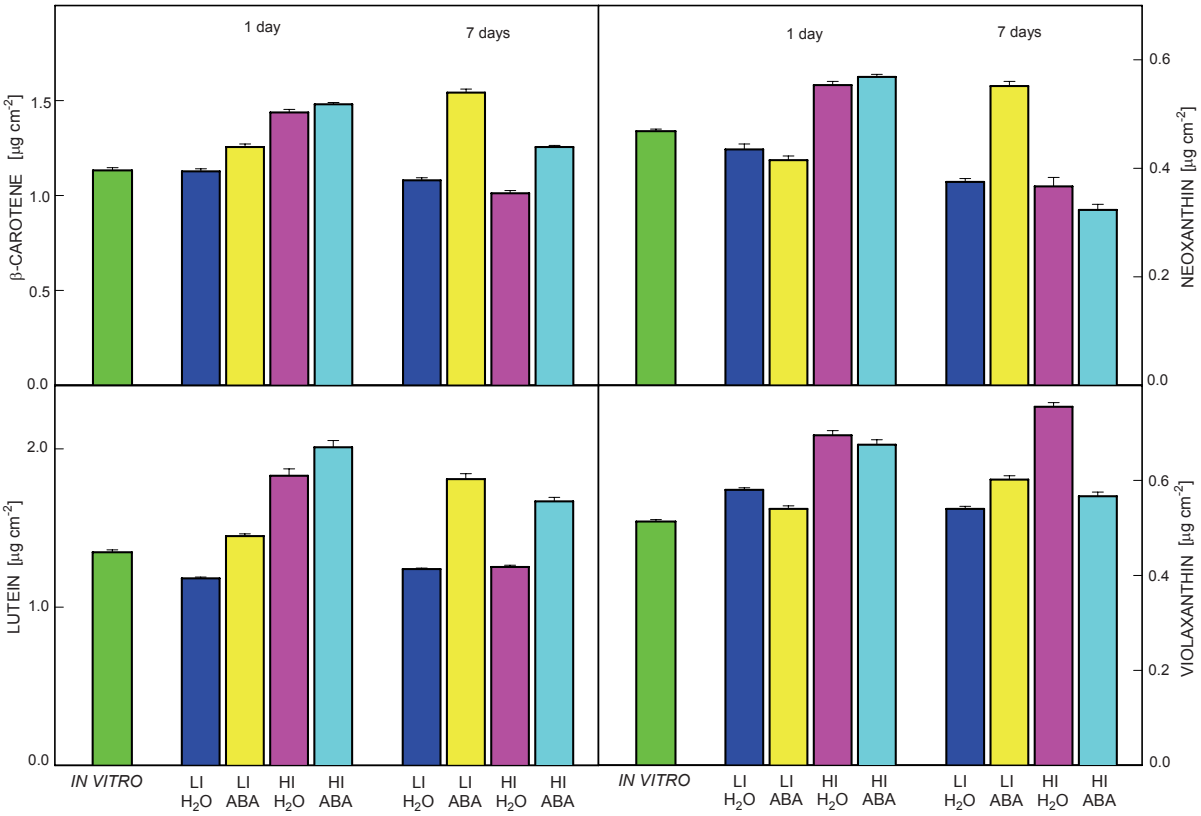


Fig. 5. Contents of β-carotene, lutein, neoxanthin a violaxanthin in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer. Means ± SE, n = 6.

on Chl and Car retention during water stress was observed in barley, bean, maize, sugar beet, tobacco and wheat (Mizrahi *et al*. 1974, Agarwal *et al*. 2005, Haisel *et al*. 2006)

Pool of xanthophyll cycle pigments (violaxanthin + antheraxanthin + zeaxanthin) was not changed considerably in plants grown under LI after *ex vitro* transfer but increased considerably in plants grown under HI (Fig. 6). Neoxanthin content was higher under HI than under LI but only 1 d after *ex vitro* transfer. Pool of Xan as well as ABA precursors neoxanthin and violaxanthin were mostly slightly decreased after ABA application. The exception was under LI 7 d after *ex vitro* transfer (Fig. 5)

The degree of deepoxidation of xanthophyll cycle pigments which is connected with harmless dissipation of light energy (DEPS) was always higher under HI than under LI similarly as was found by Pandey *et al*. (2006) in *Anoectochilus*. It decreased after ABA application in all variants which showed less stress in these plants.

Parameters of chlorophyll fluorescence kinetics showed that any dramatic and statistically significant changes did not occur in photosynthetic apparatus of plants after transfer from *in vitro* to *ex vitro* conditions (Fig. 7). The only slight decrease in maximum photochemical efficiency (F_v/F_m) was found 7 d after transfer in LI grown ABA treated plants (Fig. 7). Non-photochemical quenching seemed to be the most affected parameter after transfer to *ex vitro*. It decreased apparently 7 d after *ex vitro* transfer in comparison with *in vitro* grown plantlets, but the effect of ABA was not statistically significant. All above mentioned means that transplanted tobacco plantlets did not experience any severe stress that affected their photosynthetic apparatus. It is also in agreement with our previous results when tobaco plantlets acclimatized to *ex vitro* conditions under shade in greenhouse (Pospíšilová *et al*. 1999, 2000). On the contrary, during acclimatization of tobacco at high irradiance (700 μmol m^{-2} s^{-1}) F_v/F_m temporarily decreased after transplantation and the decrease was more marked in plantlets cultivated *in vitro* in tightly closed glass vessels than in those cultivated in *Magenta* boxes with more permeable lids (Semorádová *et al*. 2002), which were also used in experiments presented in this paper. Similarly in *Gardenia jasminoides* or *Calathea orbifolia*, occurrence of photoinhibition was dependent on conditions during previous *in vitro* cultivation (Serret *et al*. 2001a,b, Yang and Yeh 2008). In *Doritaenopsis*

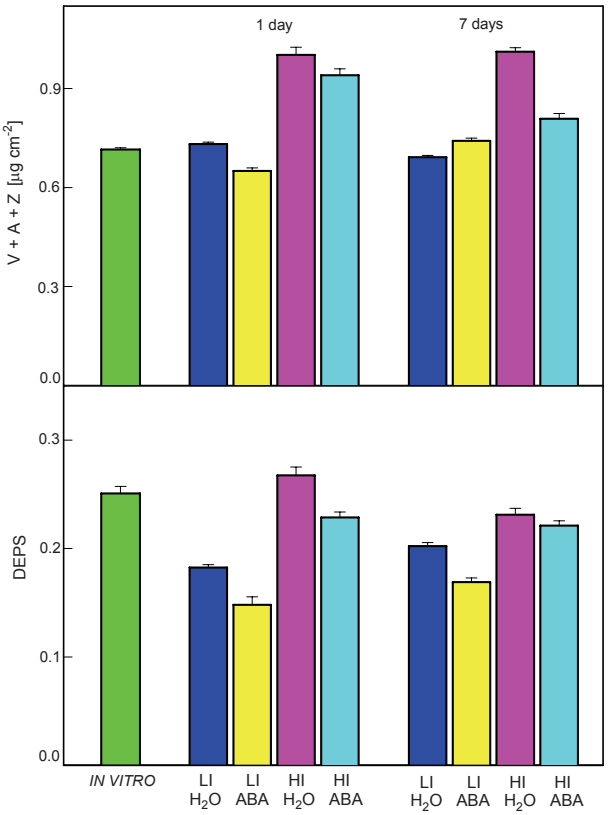
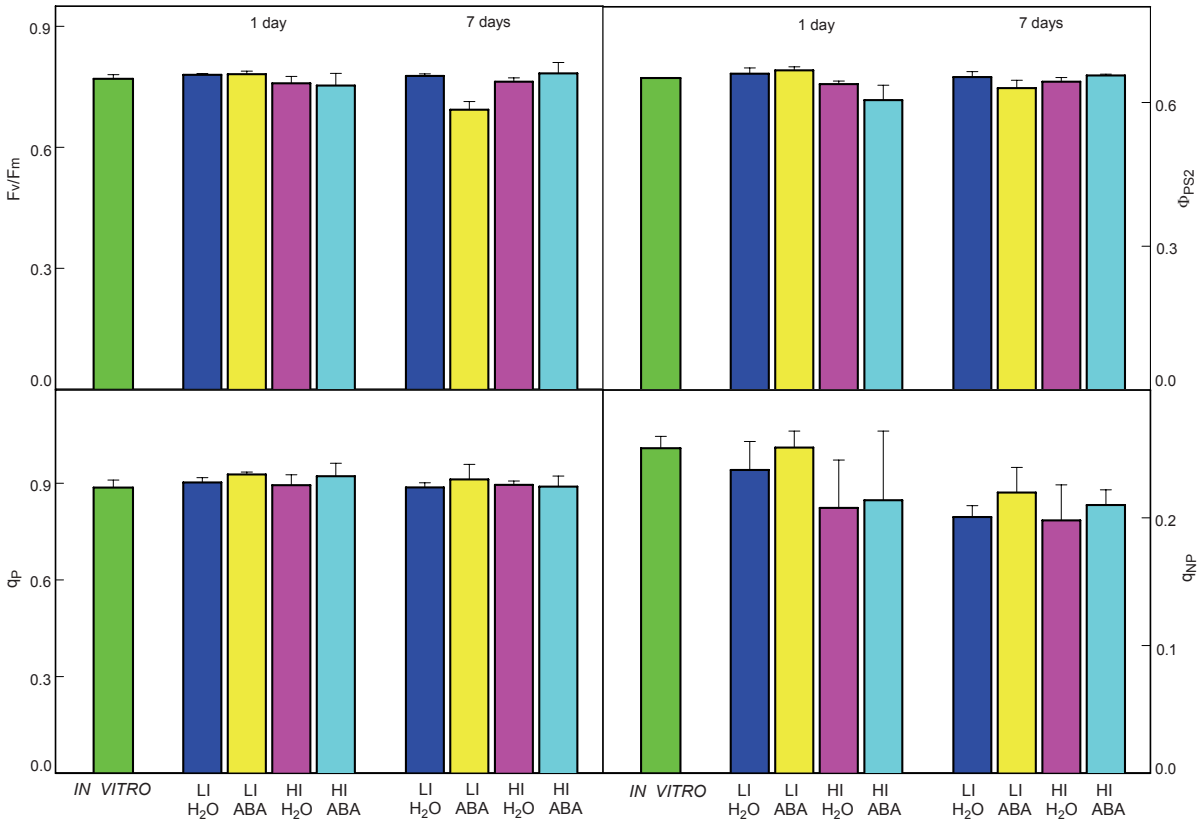


Fig. 6. Contents of xanthophyll cycle pigments [violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z)] and degree of their deepoxidation [DEPS = (Z + 0.5 A)/Z + A + V)] in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer. Means ± SE, n = 6.

Fig. 7. Chlorophyll *a* fluorescence parameters (variable to maximum fluorescence ratio, F_v/F_m, actual efficiency of photosystem 2, Φ_{PS2}, photochemical quenching, q_p and nonphotochemical quenching, q_{NP}) in *in vitro* grown tobacco plants and 1 and 7 d after *ex vitro* transfer and cultivated under low or high irradiance (LI = 150, HI = 700 μmol m^{-2} s^{-1}) and treated by 50 μM ABA or H_2O immediately after transfer. Means ± SE, n = 5.



plantlets, photoinhibition occurred after *ex vitro* transfer only in combination with sudden decrease in air humidity or low temperature (Jeon *et al*. 2006). Slight increase in irradiance was beneficial for *ex vitro* acclimatization of strawberry (Zhou *et al*. 2005) and *Anoectochilus* (Pandey *et al*. 2006), but higher increase in irradiance caused photoinhibition (Pandey *et al*. 2006). When *Nicotiana tabacum* plantlets were acclimatized in two phases, first in the greenhouse (low irradiance of 30 - 90 μmol m^{-2} s^{-1}) and then in the open air (irradiance of 200 - 1400 μmol m^{-2} s^{-1}), no photoinhibition was found during growth in the greenhouse, but F_v/F_m decreased transiently after transfer to the open air (Kadleček *et al.* 1998, 2001).

Conclusions

When we sum up the results obtained we can say that the first hypothesis that application of ABA can reduce transpiration rate and so partially eliminate initial wilting was confirmed. As concern the possible role of ABA for alleviation of photoinhibition we can say that the applied ABA was rapidly absorbed by plants, however, increased pool of xanthophyll cycle pigments after ABA application was not found. However, lower degree of their deepoxidation showed less stress in plants after ABA application and so probably less need of their synthesis.

References

Agarwal, S., Sairam, R.K., Srivastava, G.C., Meena, R.C.: Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. - Biol. Plant. **49**: 541-550, 2005.

Aguilar, M.L., Espadas, F.L., Coello, J., Maust, B.E., Trejo, C., Robert, M.L., Santamaría, J.M.: The role of abscisic acid in controlling leaf water loss, survival and growth of micropropagated *Tagetes erecta* plants when transferred directly to the field. - J. exp. Bot. **51**: 1861-1866, 2000.

Amâncio, S., Rebordão, J.P., Chaves, M.M.: Improvement of acclimatization of micropropagated grapevine: photosynthetic competence and carbon allocation. - Plant Cell Tissue Organ Cult. **58**: 31-37, 1999.

Baroja, M.E., Aguirreolea, J., Sánchez-Díaz, M.: CO_2 exchange of *in vitro* and acclimatizated potato plantlets. - In: Carre, F., Chagvardieff, P. (ed.): Ecophysiology and Photosynthetic *in Vitro* Cultures. Pp. 187-188. CEA, Saint-Paul-lez-Durance 1995.

Bieleski, R.L.: The problem of halting enzyme action when extracting plant tissues. - Anal. Biochem. **9**: 431-442, 1964.

Bhatia, P., Ashwath, N.: Comparative performance of micropropagated and seed-grown tomato plants. - Biol. Plant. **48**: 625-628, 2004.

Bray, E.A.: Abscisic acid regulation of gene expression during water-deficit stress in the era of the *Arabidopsis* genome. - Plant Cell Environ. **25**: 153-161, 2002.

Carvalho, L.C., Osório, M.L., Chaves, M.M., Amâncio, S.: Chlorophyll fluorescence as an indicator of photosynthetic functioning of *in vitro* grapevine and chestnut plantlets under *ex vitro* acclimatization. - Plant Cell Tissue Organ Cult. **67**: 271-280, 2001.

Čatský, J.: Determination of water deficit in disks cut out from leaf blades. - Biol. Plant. **2**: 76-78, 1960.

Colón-Guasp, W., Nell, T.A., Kane, M.E., Barrett, J.E.: Effects of abscisic acid on *ex vitro* acclimatization of *Aronia arbutifolia* (L.) Pers. - J. amer. Soc. hort. Sci. **121**: 101-104, 1996.

Díaz-Pérez, J.C., Sutter, E.G., Shackel, K.A.: Acclimatization and subsequent gas exchange, water relations, survival and growth of microcultured apple plantlets after transplanting them in soil. - Physiol. Plant. **95**: 225-232, 1995.

Estrada-Luna, A.A., Davies, F.T., Jr., Egilla, J.N.: Physiological changes and growth of micropropagated chile ancho pepper plantlets during acclimatization and post-acclimatization. - Plant Cell Tissue Organ Cult. **66**: 17-24, 2001.

Fila, G., Badeck, F.-W., Meyer, S., Cerovic, Z., Ghashghaie, J.: Relationship between leaf conductance to CO_2 diffusion and photosynthesis in micropropagated grapevine plants, before and after *ex vitro* acclimatization. - J. exp. Bot. **57**: 2687-2695, 2006.

Fila, G., Ghashghaie, J., Hoarau, J., Cornic, G.: Photosynthesis, leaf conductance and water relations of *in vitro* cultured grapevine rootstock in relation to acclimatisation. - Physiol. Plant. **102**: 411-418, 1998.

Fuentes, G., Talavera, C., Desjardins, Y., Santamaria, J.M.: High irradiance can minimize the negative effect of exogenous sucrose on the photosynthetic capacity of *in vitro* grown coconut plantlets. - Biol. Plant. **49**: 7-15, 2005.

Genoud, C., Coudret, A., Amalric, C., Sallanon, H.: Effect of micropropagation conditions of rose shootlets on chlorophyll fluorescence. - Photosynthetica **36**: 243-251, 1999.

Giraudat, J., Parcy, F., Bertauche, N., Gost, F., Leung, J., Morris, P.-C., Bouvier-Durand, M., Vartanian, N.: Current advances in abscisic acid action and signalling. - Plant mol. Biol. **26**: 1557-1577, 1994.

Guan, Q.Z., Guo, Y.H., Siu, X.L., Li, W., Zhang, Z.X.: Changes in photosynthetic capacity and antioxidant enzymatic systems in micropropagated *Zingiber officinale* plantlets during their acclimation. - Photosynthetica **46**: 193-201, 2008.

Haisel, D., Pospíšilová, J., Synková, H., Čatský, J., Wilhelmová, N., Plzáková, Š.: Photosynthetic pigments and gas exchange of *in vitro* grown tobacco plants as affected by CO_2 supply. - Biol. Plant. **42**: 463-468, 1999.

Haisel, D., Pospíšilová, J., Synková, H., Schnablová, R., Baťková, P.: Effects of abscisic acid or benzyladenine on pigment contents, chlorophyll fluorescence, and chloroplast ultrastructure during water stress and after rehydration. - Photosynthetica **44**: 606-614, 2006.

Haisel, D., Vaňková, R., Synková, H., Pospíšilová, J.: The impact of *trans*-zeatin *O*-glucosyltransferase gene over-

expression in tobacco on pigment content and gas exchange. - Biol. Plant. **52**: 49-58, 2008.

Hartung, W., Abou-Mandour, A.A.: A beneficial role of abscisic acid for regenerates of *Ruta graveolens* ssp. *divaricata* (Tenore) Gams suffering from transplant shock. - Angew. Bot. **70**: 221-223, 1996.

Hofman, P., Haisel, D., Komenda, J., Vágner, M., Tichá, I., Schäfer, C., Čapková, V.: Impact of *in vitro* cultivation conditions on stress responses and on changes in thylakoid membrane proteins and pigments of tobacco during *ex vitro* acclimation. - Biol. Plant. **45**: 189-195, 2002.

Hronková, M., Zahradníčková, H., Šimková, M., Šimek, P., Heydová, A.: The role of abscisic acid in acclimation of plants cultivated *in vitro* to *ex vitro* conditions. - Biol. Plant. **46**: 535-541, 2003.

Ivanov, A.G., Krol, M., Maxwell, D., Huner, N.P.A.: Abscisic acid induced protection against photoinhibition of PSII correlates with enhanced activity of the xanthophyll cycle. - FEBS Lett. **371**: 61-64, 1995.

Jeon, M.-W., Ali, M.B., Hahn, E.-J., Paek, K.-Y.: Photosynthetic pigments, morphology and leaf gas exchange during *ex vitro* acclimatization of micro-propagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. - Environ. exp. Bot. **55**: 183-194, 2006.

Jia, H., Lu, C.: Effects of abscisic acid on photoinhibition in maize plants. - Plant Sci. **165**: 1403-1410, 2003.

Kadleček, P., Rank, B., Tichá, I.: Photosynthesis and photoprotection in *Nicotiana tabacum* L. *in vitro*-grown plantlets. - J. Plant Physiol. **160**: 1017-1024, 2003.

Kadleček, P., Tichá, I., Čapková, V., Schäfer, C.: Acclimatization of micropropagated tobacco plantlets. - In: Garab, G. (ed.): Photosynthesis: Mechanisms and Effects. Vol. V. Pp. 3853-3856. Kluwer Academic Publishers, Dordrecht 1998.

Kadleček, P., Tichá, I., Haisel, D., Čapková, V., Schäfer, C.: Importance of *in vitro* pretreatment for *ex vitro* acclimatization and growth. - Plant Sci. **161**: 695-701, 2001.

Lamhamedi, M.S., Chamberland, H., Tremblay, F.M.: Epidermal transpiration, ultrastructural characteristics and net photosynthesis of white spruce somatic seedlings in response to *in vitro* acclimatization. - Physiol. Plant. **118**: 554-561, 2003.

Majada, J.P., Luz Centeno, M., Feito, I., Fernández, B., Sanchez-Tames, R.: Stomatal and cuticular traits on carnation tissue culture under different ventilation conditions. - Plant Growth Regul. **25**: 113-121, 1998.

Mizrahi, Y., Scherings, S.G., Arad, S.M., Richmond, A.E.: Aspects of the effects of ABA on the water status of barley and wheat sedlings. - Physiol. Plant. **31**: 44-50, 1974.

Osório, M.L., Gonçalves, S., Osório, J., Romano, A.: Effects of CO_2 concentration on acclimatization and physiological responses of two cultivars of carob tree. - Biol. Plant. **49**: 161-167, 2005.

Pandey, D.M., Yu, K.W., Wu, R.Z., Hahn, E.-J., Paek, K.-Y.: Effects of different irradiances on the photosynthetic process during *ex-vitro* acclimation of *Anoectochilus* plantlets. - Photosynthetica **44**: 419-424, 2006.

Pospíšilová, J.: Hardening by abscisic acid of tobacco plantlets grown *in vitro*. - Biol. Plant. **38**: 605-609, 1996.

Pospíšilová, J., Baťková, P.: Effects of pre-treatments with abscisic acid and/or benzyladenine on gas exchange of French bean, sugar beet, and maize leaves during water stress and after rehydration. - Biol. Plant. **48**: 395-399, 2004.

Pospíšilová, J., Haisel, D., Synková, H., Čatský, J., Wilhelmová, N., Plzáková, Š., Procházková, D.: Photosynthetic pigments and gas exchange of *in vitro* grown tobacco plants during *ex vitro* acclimation. - Plant Cell Tissue Organ Cult. **61**: 125-133, 2000.

Pospíšilová, J., Synková, H., Haisel, D., Čatský, J., Wilhelmová, N., Šrámek, F.: Effect of elevated CO_2 concentration on acclimation of tobacco plantlets to *ex vitro* conditions. - J. exp. Bot. **50**: 119-126, 1999.

Pospíšilová, J., Synková, H., Haisel, D., Semorádová, Š.: Acclimation of plantlets to *ex vitro* conditions: effects of air humidity, irradiance, CO_2 concentration and abscisic acid. - Acta Hort. **748**: 29-38, 2007.

Pospíšilová, J., Wilhelmová, N., Synková, H., Čatský, J., Krebs, D., Tichá, I., Hanáčková, B., Snopek, J.: Acclimation of tobacco plantlets to *ex vitro* conditions as affected by application of abscisic acid. - J. exp. Bot. **49**: 863-869, 1998.

Rival, A., Beulé, T., Lavergne, D., Nato, A., Havaux, M., Puard, M.: Development of photosynthetic characteristics in oil palm during *in vitro* micropropagation. - J. Plant Physiol. **150**: 520-527, 1997.

Sallanon, H., Berger, M., Genoud, C., Coudret, A.: Water stress and photoinhibition in acclimatization of *Rosa hybrida* plantlets. - *In Vitro* cell. dev. Biol. Plant **34**: 169-172, 1998.

Semorádová, Š., Synková, H., Pospíšilová, J.: Responses of tobacco plantlets to change of irradiance during transfer from *in vitro* to *ex vitro* conditions. - Photosynthetica **40**: 605-614, 2002.

Seon, J.-H., Cui, Y.-Y., Kozai, T., Paek, K.-Y.: Influence of *in vitro* growth conditions on photosynthetic competence and survival rate of *Rehmania glutinosa* plantlets during acclimatization period. - Plant Cell Tissue Organ Cult. **61**: 135-142, 2000.

Serret, M.D., Trillas, M.I., Araus, J.L.: The effect of *in vitro* culture conditions on the pattern of photoinhibition during acclimation of gardenia plantlets to *ex vitro* conditions. - Photosynthetica **39**: 67-73, 2001a.

Serret, M.D., Trillas, M.I., Matas, J., Araus, J.L.: The effect of photoautotrophy on photosynthesis and photoinhibition of gardenia plantlets during micropropagation. - Photosynthetica **39**: 245-255, 2001b.

Siddique, I., Anis, M.: An improved plant regeneration system and *ex vitro* acclimatization of *Ocimum basilicum* L. - Acta physiol. Plant. **30**: 493-499, 2008.

Slavtcheva, T., Dimitrova, V.: Gas exchange of *in vitro* and *ex vitro* grown grapevine plants. - Photosynthetica **39**: 29-33, 2001.

Stuchlíková, K., Hejnák, V., Šafránková, I.: The effect of abscisic acid and benzylaminopurine on photosynthesis and transpiration rates of maize (*Zea mays* L.) under water stress and subsequent rehydration. - Cereal Res. Commun. **35**: 1593-1602, 2007.

Synková, H.: Sucrose affects the photosynthetic apparatus and the acclimation of transgenic tobacco to *ex vitro* culture. - Photosynthetica **33**: 403-412, 1997.

Tichá, I., Radochová, B., Kadleček, P.: Stomatal morphology during acclimatization of tobacco plantlets to *ex vitro* conditions. - Biol. Plant. **42**: 469-474, 1999.

Trillas, M.I., Serret, M.D., Jorba, J., Araus, J.L.: Leaf chlorophyll fluorescence changes during acclimatization of the root-stock GF677 (peach × almond) and propagation of *Gardenia jasminoïdes* E. - In: Carre, F., Chagvardieff, P. (ed.): Ecophysiology and Photosynthetic *in vitro* Cultures. Pp. 161-168. CEA, Saint-Paul-lez-Durance 1995.

Van Huylenbroeck, J.M.: Influence of light stress during the acclimation of *in vitro* plantlets. - In: Struik, P.C.,

Vredenberg, W.J., Renkema, J.A., Parlevliet, J.E. (ed.): Plant Production on the Threshold of a New Century. Pp. 451-453. Kluwer Academic Publishers, Dordrecht 1994.

Van Huylenbroeck, J.M., Debergh, P.C.: Impact of sugar concentration *in vitro* on photosynthesis and carbon metabolism during *ex vitro* acclimatization of *Spathiphyllum* plantlets. - Physiol. Plant. **96**: 298-304, 1996.

Van Huylenbroeck, J.M., Huygens, H., Debergh, P.C.: Photoinhibition during acclimatization of micropropagated *Spathiphyllum* "Petite" plantlets. - *In Vitro* cell. dev. Biol. Plant **31**: 160-164, 1995.

Van Huylenbroeck, J.M., Piqueras, A., Debergh, P.C.: Photosynthesis and carbon metabolism in leaves formed prior and during *ex vitro* acclimatization of micropropagated plants. - Plant. Sci. **134**: 21-30, 1998.

Van Huylenbroeck, J.M., Piqueras, A., Debergh, P.C.: The evolution of photosynthetic capacity and the antioxidant enzymatic system during acclimatization of micropropagated *Calathea* plants. - Plant Sci. **155**: 59-66, 2000.

Van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. - Photosynth. Res. **25**: 147-150, 1990.

Yang, S.-H., Yeh, D.M.: *In vitro* leaf anatomy, *ex vitro* photosynthetic behaviors and growth of *Calathea orbifolia* (Linden) Kennedy plants obtained from semi-solid and temporary immersion systems. - Plant Cell Tissue Organ Cult. **93**: 201-207, 2008.

Zhou, Y.-H., Guo, D.-P., Zhu, Z.-J., Qian, Q.-Q.: Effect of *in vitro* rooting environments and irradiance on growth and photosynthesis of strawberry plantlets during acclimatization. - Plant Cell Tissue Organ Cult. **81**: 105-108, 2005.