| Phyton (Austria) Special issue: "Free Radicals" | **Vol. 37** | Fasc. 3 | (283)-(290) | 1. 7. 1997 |
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# Effects of Light Intensity on Photosynthesis and Toxic $O_2$ Scavenging Enzymes during Acclimatization of Micropropagated *Calathea*

By

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Key words: Acclimatization, chlorophyll fluorescence, antioxidative enzymes, light, photosynthesis.


## Summary

VAN HUYLENBROECK J.M., PIQUERAS A. & DEBERGH P.C. 1997. Effects of light intensity on photosynthesis and toxic $O_2$ scavenging enzymes during acclimatization of micropropagated *Calathea*. - Phyton (Horn, Austria) 37 (3): (283) - (290).

Directly after transplantation, micropropagated *Calathea louisae* GAGNEP. 'Maui Queen' plantlets did not show any positive net photosynthetic activity (= heterotrophic metabolism), and photoinhibition exacerbated with increasing light intensity as was shown by the decrease in $F_v/F_m$. Recovery from light stress was observed during the second week after transfer, when plants became fully autotrophic. An inverse relation between light intensity and the total chlorophyll over carotenoid ratio was found. Also changes in activities of antioxidative enzymes were observed. Catalase and glutathione reductase increased during the first three weeks of acclimatization, while an increase in guaiacol peroxidase and ascorbate peroxidase was observed later on in the acclimatization. Both superoxide dismutase and guaiacol peroxidase activities increased with increasing light intensity, while catalase activity was reduced. The results suggest an adaptive response of the plants to stress conditions generated by changes in climatic conditions at transplantation.


## Introduction

When compared to ex vitro, tissue cultured plants are grown under aberrant environmental conditions of high relative humidity, altered gas

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composition and low light intensity (PPFD < 30 μmol $m^{-2}$ $s^{-1}$) in the culture vessel. These conditions, together with the external carbon supply in the medium, cause morphological (e.g. changes in cuticula and stomatal behaviour) and physiological changes (e.g. heterotrophy or mixotrophy). At transition to the greenhouse, the plants have to adapt to normal growing conditions and to develop a normal physiology (PREECE & SUTTER 1991). Apart from relative humidity, changes in light intensity directly after transfer may play an important role and possibly explain some of the difficulties during acclimatization. Exposure to sudden high light intensities directly after transplanting (especially during winter and early spring, when periods of cloudy, dark weather can be interrupted by bright periods of full sunlight) and excessive radiation can possibly lead to photoinhibition and cause damage to the young plantlets (CHAVES 1994). Higher plants have a wide array of different ecophysiological and metabolic strategies to shield the photosynthetic apparatus and other cell components from the adverse effects of high light stress and the toxic activated oxygen species generated in the chloroplasts (ASADA 1995, FOYER & al. 1994, KRAUSE 1988). However, in literature almost no information is available on the development of these different protective mechanisms in micropropagated plantlets and how they respond to stress situations generated by sudden changes in growing conditions during acclimatization. Therefor, we studied the effect of light intensity on photosynthesis and the development of an active oxygen scavenging system during the acclimatization of micropropagated *Calathea* 'Maui Queen' plantlets.

## Materials and Methods

*Calathea louisae* GAGNEP. 'Maui Queen' was micropropagated according to DUNSTON & SUTTER 1984, and cultures were maintained at a photosynthetic photon flux density (PPFD) at plant level 30 μmol $m^{-2}$ $s^{-1}$. For acclimatization, rooted shoots were transplanted and placed in a growth room (21 ± 1°C, RH 80-90%, $CO_2$-concentration 350 ppm, photoperiod 16 h). PPFD during acclimatization was, depending on the treatment, 40, 120 or 360 μmol $m^{-2}$ $s^{-1}$.

Net $CO_2$ gas exchange rates were measured as described in VAN HUYLENBROECK & al. 1995, using a portable ADC-LCA3 photosynthesis measuring system and a modified Parkinson Leaf Chamber (PP-Systems, Hertfordshire, United Kingdom). Chlorophyll fluorescence was measured with a pulse-amplitude-modulation fluorometer (Model PAM-2000, Walz, Effeltrich, Germany) as described earlier in detail (VAN HUYLENBROECK & al. 1995). Minimal fluorescence ($F_o$) was determined after 15 min dark adaptation, while maximal fluorescence ($F_m$) was measured after a saturation pulse. The fluorescence ratio $F_v/F_m$, with $F_v=F_m-F_o$ being the variable fluorescence, was then calculated.

Pigments were extracted in darkness using the methanol:chloroform (1:3 v/v) procedure of QUAIL & al. 1976. Chlorophyll a and b as well as total carotenoids in the chloroform layer were calculated according to WELLBURN 1994.

For enzyme determination approximately 1 g fresh weight of plant tissue was homogenized in 3 ml pre-cooled extraction buffer consisting of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 2.5 mM DMSF, 8% (w/v) insoluble PVP and 0.2% Triton X-100. The homogenate was filtered through cheesecloth (Miracloth) and centrifuged 3 times during 5 min at 10 000 g at 4°C. The protein concentration was determined according to LOWRY & al. 1951 using BSA as a standard. Dehydroascorbate reductase (DHAR, EC 1.8.5.1) was measured according to FOYER

& HALLIWELL 1977. The reaction mixture contained 50 mM phosphate buffer (pH 6.8) and 2.5 mM glutathione. The reaction was started with dehydroascorbic acid (0.5 mM). The absorption coefficient for ascorbate at 265 nm is 14 mM $cm^{-1}$. Control rates in the absence of enzyme extract were subtracted. Glutathione reductase (GR, EC 1.6.4.2) activity was determined according to FOYER & HALLIWELL 1976. The assay contained 50 mM Tris buffer (pH 7.8), 1.0 mM EDTA and 0.12 mM NADPH (absorption coefficient at 340 nm: 6.22 mM $cm^{-1}$). The reaction was started with 0.8 mM GSSG. Control rates obtained in the absence of GSSG and of enzyme extract were subtracted. Ascorbate peroxidase (APO, EC 1.11.1.7) activity was determined as described by NAKANO & ASADA 1981. The reaction mixture contained 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA, 0.1 mM hydrogen peroxide and 0.5 mM ascorbate. The hydrogen peroxide dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (absorption coefficient 2.8 mM $cm^{-1}$). Guaiacol peroxidase (POD) activity was measured according to CHANCE & MAEHLY 1955 using a reaction mixture with 50 mM phosphate buffer (pH 7) and 5 mM guaiacol (absorption coefficient at 436 nm: 25.5 mM $cm^{-1}$). The reaction was started with 20 mM hydrogen peroxide. Catalase (CAT, EC 1.11.1.6) activity was measured as described by AEBI 1984. The decomposition of $H_2O_2$ was monitored by the decrease in absorbance at 240 nm. For the assay a 50 mM phosphate buffer (pH 7.8) and 10 mM $H_2O_2$ were used. Superoxide dismutase (SOD, EC 1.15.11) activity was determined by the ferricytochrome c method using xanthine/xanthine oxidase as the source of superoxide radicals, and a unit of activity was defined according to MCCORD & FRIDOVICH 1969.

## Results

At transplantation (day 0), *Calathea* 'Maui Queen' showed almost no positive net photosynthetic activity, while dark respiration was about -0.6 µmol $CO_2$ $m^{-2}$ $s^{-1}$ (Fig. 1).

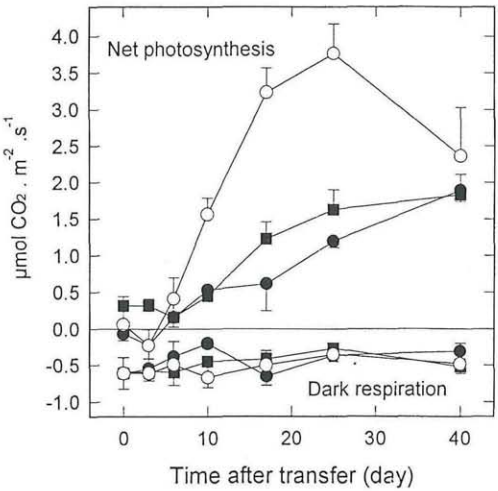


Fig. 1. Net photosynthesis and dark respiration of micropropagated *Calathea* 'Maui Queen' plantlets acclimatized at 40 (●), 120 (■) or 360 (○) µmol $m^{-2}$ $s^{-1}$. Values are means ± SE (n=6). (If not visible, SE smaller than data label).

In the days following transplantation, a decrease in photosynthesis was observed. From day 6 on, a positive net photosynthesis took place. During the next 10 days photosynthesis increased for all treatments to reach a steady state level. At the end of the experiment (after 40 days) plants exposed to 360 µmol $m^{-2}$ $s^{-1}$ showed a decreased photosynthetic activity, while it still increased in plants grown at the lowest light intensity. Chlorophyll fluorescence analysis showed a decrease in $F_v/F_m$ directly after transplantation, followed by a continued recovery (Fig. 2).

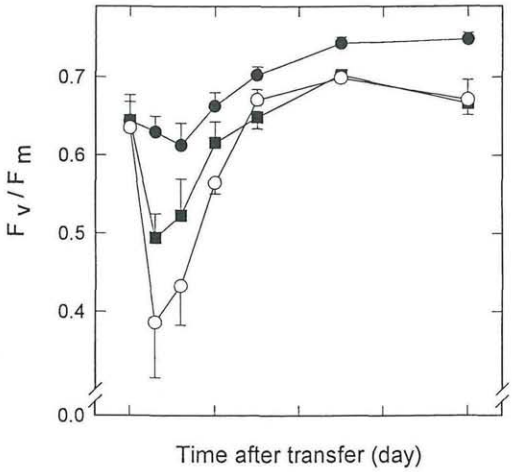


Fig. 2. Changes in $F_v/F_m$ during acclimatization of micropropagated *Calathea* 'Maui Queen' plantlets at 40 (●), 120 (■) or 360 (○) μmol $m^{-2}$ $s^{-1}$. Values are means ± SE (n=6). (If not visible, SE smaller than data label).

This decrease of $F_v/F_m$ was more pronounced with increasing light intensity. Although plants acclimatized at higher light intensities showed an earlier (day 4) recovery compared to those grown under the lowest light treatment, $F_v/F_m$ remained significantly lower in these treatments over the whole acclimatization period. Pigment analysis revealed no direct relation between total pigment content and light regime. However, in newly developed leaves chlorophyll content was almost three times higher compared to in vitro ones (475 vs. 1145 μg $g^{-1}$ FW). The chlorophyll a to b ratio increased during acclimatization (Fig. 3A). The maximal ratio was reached faster in plants grown at 120 and 360 μmol $m^{-2}$ $s^{-1}$. The chlorophyll (a+b)/carotenoids ratio was almost constant during the whole experimental period for plants acclimatized at 40 μmol $m^{-2}$ $s^{-1}$ . However, a sharp decrease during the first ten days of acclimatization was found in plants grown at 120 and 360 μmol $m^{-2}$ $s^{-1}$. Finally an inverse relation between light intensity and the chlorophyll (a+b)/carotenoids ratio was observed (Fig. 3B).

SOD and guaiacol POD activities were significantly higher for plants grown at 360 μmol $m^{-2}$ $s^{-1}$ compared to those at 40 μmol $m^{-2}$ $s^{-1}$, although the measured levels were only about 10 to 15% higher (Table 1). On the contrary, the CAT activity decreased significantly with increasing PPFD. When followed in time, SOD activity decreased during the first three weeks, followed by an increase towards the end of the experimental period (Table 1).

APO suffered a slight reduction during the first two weeks, followed by a stable recovery. DHAR activity was high at transplantation compared to the rest of the acclimatization period. CAT activity increased significantly during the first 10 days after transfer from in vitro, to reach a steady state level. A similar increase, followed by a steady state level, was found for GR. Guaiacol POD activity remained constant during the first month after transfer, whereafter a strong increase in activity was observed (Table 1).

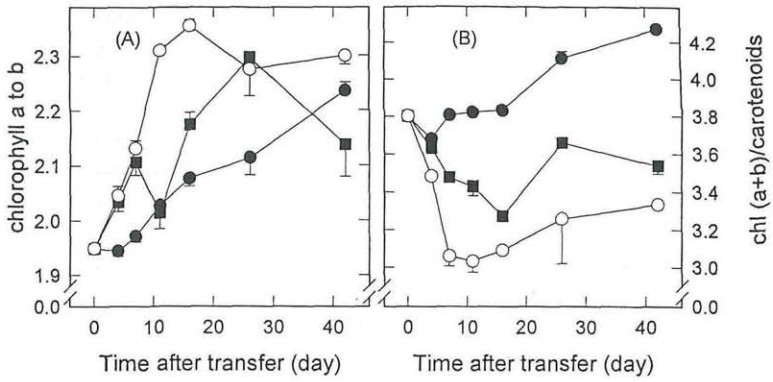


Fig. 3. Time course of (A) chlorophyll a to b and (B) chlorophyll (a+b)/carotenoids ratios during acclimatization of micropropagated *Calathea* 'Maui Queen' plantlets at 40 (●), 120 (■) or 360 (○) µmol $m^{-2}$ $s^{-1}$. Values are means ± SE (n=4). (If not visible, SE smaller than data label).

Table 1. Effects of light intensity (40, 120 and 360 µmol $m^{-2}$ $s^{-1}$) on the changes in activity of SOD (U $mg^{-1}$ protein), APO and DHAR (nmol $mg^{-1}$ protein $min^{-1}$), CAT (U $mg^{-1}$ protein $min^{-1}$), GR and guaiacol POD (nmol $mg^{-1}$ protein $min^{-1}$) during acclimatization of *Calathea* 'Maui Queen'. Data are means ± SE.

| Factor | | SOD | APO | DHAR | CAT | GR | Guaiacol POD |
|---|---|---|---|---|---|---|---|
| PPFD | 40 | 5.7 a | 492 a | 39.7 a | 6.1 b | 14.6 a | 70.7 a |
| | 120 | 5.7 a | 544 a | 38.5 a | 5.2 ab | 15.6 a | 85.8 b |
| | 360 | 6.7 b | 553 a | 45.5 a | 4.8 a | 15.4 a | 82.4 b |
| DAY | 0 | 8.1 | 534 abc | 59.4 b | 3.2 a | 9.7 a | 73.2 a |
| | 4 | 5.7 ab | 436 a | 39.7 a | 4.0 ab | 12.8 ab | 80.5 a |
| | 7 | 7.6 bc | 487 ab | 43.5 a | 4.8 bc | 14.1 ab | 78.7 a |
| | 11 | 5.2 a | 442 a | 37.2 a | 6.2 de | 16.9 bc | 74.6 a |
| | 16 | 5.0 a | 599 bc | 44.0 ab | 7.4 e | 12.1 ab | 74.5 a |
| | 26 | 5.4 a | 633 c | 27.5 a | 6.0 cd | 20.6 c | 76.2 a |
| | 42 | 6.7 abc | 588 bc | 34.4 a | 6.0 cd | 20.8 c | 103.2 b |
| PPFD | | * | NS | NS | * | NS | * |
| DAY | | * | * | * | * | * | * |
| PPFD x DAY | | NS | NS | NS | NS | NS | NS |

Mean separation in columns by LSD, P=0.05 (a,b,c,d), only when ANOVA significant
NS, * Non-significant or significant P=0.05, respectively, using analysis of variance

## Discussion

Based on photosynthesis and fluorescence measurements, three different periods can be described in the ex vitro acclimatization process of *Calathea* 'Maui Queen' plantlets. The first period (day 0 to 4) is characterized by a heterotrophic metabolism. Heterotrophy is frequently observed during micropropagation, due to depletion of $CO_2$ in the culture vessels (KOZAI 1991) and feedback inhibition of the Calvin cycle by exogenous sugar supply resulting in deactivation of Rubisco

(GROUT 1988). As observed by GROUT & ASTON 1978 a negative $CO_2$ balance persisted up to two weeks after cauliflower plants had been transferred to soil. During this period a strong decrease in $F_v/F_m$ was observed. This decrease in $F_v/F_m$ was significantly more pronounced as the difference between light conditions in the laboratory and the greenhouse increased. Similar decreases of $F_v/F_m$ with increasing light intensity have been observed in numerous plants grown in the field (ARO & al. 1994, GREER & LAING 1988, ÖQUIST & al. 1992). The decline of $F_v/F_m$ is linearly correlated with the quantum yields of light-limited oxygen evolution (BJÖRKMAN & DEMMIG 1987) and the number of functional PSII reaction centers (ÖQUIST & al. 1992). Our results demonstrate that photoinhibition can occur directly after transplanting of micropropagated plants, even at very low light intensities. In field grown plants photoinhibition at low light levels has mainly been observed when an additional stress factor (low temperature, drought) was present. Probably the poorly differentiated chloroplasts of in vitro formed leaves (LEE & al. 1985), together with water stress plants have to cope with at transplanting (PREECE & SUTTER 1991), resulted in a low resistance against photoinhibition and made micropropagated plantlets more susceptible to changes in light levels.

During the next two weeks (period 2) a switch from heterotrophic to autotrophic metabolism was observed. A functional photosynthetic apparatus develops, as reflected by the increase of net photosynthesis. In addition to this metabolic switch, photoinhibitory effects of increased light intensities diminished, as evidenced by the recovery of $F_v/F_m$. During this adaptation period new ex vitro formed leaves and functional roots were fully developed and plants acquired complete autotrophic metabolism. This moment marks the beginning of the third period in which plants are completely acclimatized. Prolonged exposure of ex vitro formed leaves to 120 and 360 $\mu mol\ m^{-2}\ s^{-1}$ still had an inhibitory effect on photosynthetic activity (lower $F_v/F_m$, decreasing net photosynthesis at day 40 for plants grown at 360 $\mu mol\ m^{-2}\ s^{-1}$) compared to lower light intensity. These results are in agreement with observations in micropropagated *Liquidambar* (LEE & al. 1985) and *Spathiphyllum* (VAN HUYLENBROECK & al. 1995).

During the acclimatization of *Calathea* chlorophyll a/b increased, as was also observed by DONNELLY & VIDAVER 1984 for *Rubus*. The results show a light intensity dependent increase of chlorophyll a to b ratio in earlier stages of acclimatization. Moreover, the reverse relationship between light intensity and chlorophyll (a+b)/ carotenoids ratio reflects a flexible response of the photosynthetic apparatus functionality to different light environments during the acclimatization. The photoprotective role of carotenoids against photooxidative damage is well documented and an increase in the amount of carotenoids in the light-harvesting antenna during acclimation to high light is frequently observed (YOUNG 1991).

The observed increases in activities of different superoxide and hydrogen peroxide scavenging enzymes along the acclimatization of *Calathea* indicate a progressive development of these protective systems against oxidative stress. However, increasing light intensity only slightly enhanced SOD and guaiacol POD

activities, while APO, DHAR and GR activities, all involved in the ascorbate-glutathione redox chain, did not significantly differ. CAKMAK & MARSCHNER 1992 also found no influence of light intensity on the enzymatic activities of this pathway in beans, when sufficient magnesium is supplied. On the other hand, in wheat seedlings significant higher activities of antioxidative enzymes were found when exposed to high light intensities (MISHRA & al. 1995). CAT activity significantly decreased under higher light intensities. These results are in agreement with previous observations that CAT synthesis and degradation are light sensitive (HERTWIG & al. 1992).

Our results indicate that *Calathea* plants are very sensitive to sudden changes in light intensity (difference between laboratory conditions and the greenhouse). Especially the two first weeks after transfer plants suffered from increased light intensity. Changes in carotenoids and $O_2$-scavenging enzymes reflect that tissue cultured plants can develop defense mechanisms within two to three weeks to overcome these stress conditions. However, for growers we recommend to acclimatize plantlets first at low light conditions and to increase light intensity more gradually in order to avoid photoinhibition and possible photo-oxidative damage.

## References


AEBI H. 1984. Catalase in vitro. - Meth. Enz. 105:121-126.

ARO E.-M., MCCAFFERY S. & ANDERSON J.M. 1994. Recovery from photoinhibition in peas (*Pisum sativum* L.) acclimated to varying growth irradiances: Role of D1 protein turnover. - Plant Physiol. 104:1033-1041.

ASADA K. 1995. Mechanisms for scavenging reactive molecules generated in chloroplasts under light stress. - In: BAKER N.R. & BOWYER J.R. (eds), Photoinhibition of photosynthesis: from molecular mechanisms to the field, 129-142. - Bios Scientific Publishers, United Kingdom.

BJÖRKMAN O. & DEMMIG B. 1987. Photon yield of $O_2$ evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. - Planta 170:489-504.

CAKMAK I. & MARSCHNER H. 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. - Plant Physiol. 98: 1222-1227.

CHANCE B. & MAEHLY A.C. 1955. Assay of catalase and peroxidase. - Meth. Enz. 2: 764-755.

CHAVES M.M. 1994. Environmental constraints to photosynthesis in ex vitro plants. - In: LUMSDEN P.J., NICHOLAS J.R. & DAVIES W.J. (eds.). Physiology, growth and development of plants in culture, 1-18. - Kluwer Academic Publishers, The Netherlands.

DONNELLY D.J. & VIDAVER W.E. 1984. Pigment content and gas exchange of red raspberry in vitro and ex vitro. - J. Amer. Soc. Hort. Sci. 109: 177-181.

DUNSTON S. & SUTTER E. 1984. In vitro propagation of prayer plants. - Hort Science 19:511-512.

FOYER C.H. & HALLIWELL B. 1976. The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. - Planta 133: 21-25.

— & — 1977. Purification and properties of dehydroascorbic reductase from spinach leaves. - Phytochem. 16:1347-1350.

— , LELANDAIS M. & KUNERT K.J. 1994. Photooxidative stress in plants. - Physiol. Plant. 92: 696-717.

GREER D.H. & LAING W.A. 1988. Photoinhibition of photosynthesis in intact kiwifruit (*Actinidia deliciosa*) leaves: effect of light during growth on photoinhibition and recovery. - Planta 175: 355-363.

GROUT B.W.W. 1988. Photosynthesis of regenerated plantlets in vitro, and the stresses of transplanting. - Acta Hort. 230: 129-135.

— & ASTON J. 1978. Transplanting of cauliflower plants regenerated from meristem culture. II. Carbon dioxide fixation and the development of photosynthetic ability. - Hort. Res. 17: 65-71.

HERTWIG B., STREB P. & FEIERABEND J. 1992. Light dependence of catalase synthesis and degradation in leaves and the influence of interfering stress conditions. - Plant Physiol. 100: 1547-1553.

KOZAI T. 1991. Photoautotrophic micropropagation. - In Vitro Cell. Dev. Biol. 27P: 47-51.

KRAUSE G.H. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. - Physiol. Plant. 74: 566-574.

LEE N., WETZSTEIN Y. & SOMMER H.E. 1985. Effects of quantum flux density on photosynthesis and chloroplast ultrastructure in tissue-cultured plantlets and seedlings of *Liquidambar styraciflua* L. towards improved acclimatization and field survival. - Plant Physiol. 78: 637-641.

LOWRY D.H., ROSEBROUGH N.J., FARR A.L. & RANDALL R.J. 1951. Protein measurement with the folin phenol reagent. - J. Biol. Chem. 193: 265-275.

MCCORD J.M. & FRIDOVICH I. 1969. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). - J. Biol. Chem. 244: 6049-6055.

MISHRA N.P., FATMA T. & SINGHAL G.S. 1995. Development of antioxidative defense system of wheat seedlings in response to high light. - Physiol. Plant. 95: 77-82.

NAKANO Y. & ASADA K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. 22: 867-880.

ÖQUIST G., ANDERSON J.M., MCCAFFERY S. & CHOW W.S. 1992. Mechanistic differences in photoinhibition of sun and shade plants. - Planta 188: 422-431.

PREECE J.E. & SUTTER E.G. 1991. Acclimatization of micropropagated plants to the greenhouse and field. - In: DEBERGH P.C. & ZIMMERMAN R.H. (eds.), Micropropagation, 71-93. - Kluwer Academic Publishers, The Netherlands.

QUAIL P.H., GALLAGHER E.A. & WELLBURN A.R. 1976. Membrane-associated phytochrome: non-coincidence with plastid membrane marker profiles on sucrose gradients. - Photochem. Photobiol. 24: 495-498.

VAN HUYLENBROECK J.M., HUYGENS H. & DEBERGH P.C. 1995. Photoinhibition during acclimatization of micropropagated *Spathiphyllum* "Petite" plantlets. - In Vitro Cell. Dev. Biol. 31P: 160-164.

WELLBURN A.R. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. - J. Plant Physiol. 144: 307-313.

YOUNG A.J. 1991. The protective role of carotenoids in higher plants. - Physiol. Plant. 83: 702-708.