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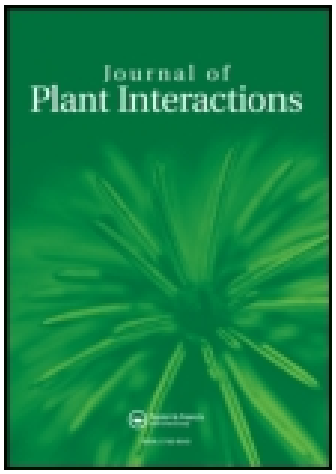

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RESEARCH ARTICLE

Photosynthetic and biochemical characterization of in vitro-derived African violet (*Saintpaulia ionantha* H. Wendl) plants to ex vitro conditions

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African violet (*Saintpaulia ionantha* H. Wendl) is one of the most easily and commonly tissue-cultured ornamental plants. Despite this, there are limited reports on photosynthetic capacity and its impact on the plant quality during acclimatization. Various growth, photosynthetic and biochemical parameters and activities of antioxidant enzymes and dehydrins of micropropagated plants were assessed under three light intensities (35, 70, and 100 µmol m^{-2} s^{-1} photosynthetic photon flux density – PPFD). Fresh and dry plant biomass, plant height, and leaf area were optimal with high irradiance (70–100 µmol m^{-2} s^{-1} PPFD). Chlorophyll and carotenoid contents and net photosynthesis were optimal in plants grown under 70 µmol m^{-2} s^{-1} PPFD. Stomatal resistance, malondialdehyde content, and F_v/F_m values were highest at low light irradiance (35 µmol m^{-2} s^{-1} PPFD). The activities of three antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase, increased as light irradiance increased, signaling that high light irradiance was an abiotic stress. The accumulation of 55, 33, and 25 kDa dehydrins was observed with all light treatments although the expression levels were highest at 35 µmol m^{-2} s^{-1} PPFD. Irradiance at 70 µmol m^{-2} s^{-1} PPFD was suitable for the acclimatization of African violet plants. Both low and high irradiance levels (35 and 100 µmol m^{-2} s^{-1} PPFD) induced the accumulation of antioxidants and dehydrins in plants which reveals enhanced stress levels and measures to counter it.

Keywords: acclimatization; antioxidants; dehyrdrins; light intensity; micropropagation

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; GSH, glutathione; F_0, minimal fluorescence yield of the dark-adapted state; F_m, maximal fluorescence yield of the dark-adapted state; F_v, variable fluorescence; F_v/F_m, maximal quantum yield of PSII photochemistry; PPFD, photosynthetic photon flux density; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase

Introduction

African violet (*Saintpaulia ionantha* H. Wendl), a member of the Gesneriaceae family, is a popular commercial ornamental plant and holds good export potential as a pot flower in many countries. Micropropagated plants are associated with several physiological and anatomical abnormalities during in vitro growth such as low photosynthetic capacity, nonproper functioning of stomata, and malfunctioning of housekeeping systems mainly due high humidity inside the culture vessel (Hazarika 2003). After ex vitro transfer, these plantlets might easily be impaired by sudden changes in environmental conditions, and so a period of acclimatization to correct abnormalities is needed (Pospíšilová et al. 1999). As a consequence, plants raised in vitro are grown in environmentally controlled growth chambers or greenhouses and factors such as light, temperature, and humidity are gradually enhanced for their step-by-step acclimatization (Pospíšilová et al. 1999; Hazarika 2003; Dewir et al. 2005). In order to increase growth and to reduce the mortality of plantlets at the acclimatization stage, research has focused on the control of environmental factors which optimizes the survival of plantlets (Hazarika 2003; Dewir et al. 2004, 2005).

Light intensity is one important factor that can be controlled at the acclimatization stage because it can lead to the depression of photosynthetic efficiency (photoinhibition) mainly due to oxidative damage to photosystem II, as occurs in the natural environment (Powles 1984). At high light intensity, an increase in photosynthetic carbon fixation can occur, which varies depending on light intensity and may lead to different susceptibilities to photoinhibition (Powles 1984). However, above a certain threshold, carbon fixation becomes saturated and photosynthesis is incapable of using all the energy absorbed by the plants. Under conditions of excess light absorption, the chloroplast lumen becomes acidic in nature, which reduces the electron transport chain, and excitation energy accumulates within the chloroplast which can lead to the generation of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide (Asada 1999). The accumulation of ROS may lead to

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lipid peroxidation and to reduced accumulation of enzymatic antioxidants which exist as a defense system in plants (Asada 1999). It is also believed that dehydrins (DHNs) can accumulate in acclimatizing plants because, as observed in light-induced expression of DHNs during seedling de-etiolation in sunflower (Natali et al. 2007). Some DHNs have been implicated as antioxidants that scavenge free radicals (Hara et al. 2003) and may act as protectants of osmoregulation.

In a bid to better understand the photosynthetic response and its impact on plant quality of acclimatized African violet plants derived from micropropagation, this study examined multiple photosynthetic parameters. In addition, lipid peroxidation, the activity of stress-related enzymes, and DHN proteins of acclimatized plants was assessed in response to three light intensities in order to clarify their role in overcoming light-induced stress during the process of acclimatization.

Materials and methods

Chemicals and reagents

All chemicals were of tissue culture grade, except for those used in enzyme-related analyses, which were of HPLC grade. All reagents and chemicals were obtained from Sigma-Aldrich (Poole, UK), unless otherwise stated.

Plant material

In vitro shoots of African violet (*Saintpaulia ionantha* H. Wendl cv. 'Diana blue') were regenerated from leaf segments after 3 weeks of culture on Murashige and Skoog (MS) medium (Murashige & Skoog 1962) containing 0.2 mg L^{-1} 6-benzyladenine and 0.1 mg L^{-1} Kinetin. The regenerated shoot clumps were cultured for 4 weeks onto MS medium without plant growth regulators for their elongation and rooting. These plantlets were gently removed from the medium and the roots were washed under tap water and used as experimental plant material in this study.

Light intensity treatments during acclimatization

Micropropagated plantlets of African violet were transplanted into culture pots (plastic coffee cups with three holes at the bottom) filled with sterilized compost (John Innes, No. 1, Westland Ltd., Berkshire, UK) and grown in a Sanyo growth cabinet for 4 weeks under three levels of PPFD (35, 70, and 100 µmol m^{-2} s^{-1}) under a 16-h photoperiod using halide lamps (Sanyo). The environment in the growth cabinet was adjusted to 25 ± 2°C air temperature and 50% relative humidity (RH). Growth parameters (fresh weight, dry weight, number of leaves/plantlet, shoot and root length, and leaf area) were determined after 4 weeks in the growth chamber. Plants were irrigated twice a week and a compound fertilizer (19: 19: 19; N: P_2O_5: K_2O) at 1 g L^{-1} was applied once after 3 weeks.

Photosynthetic parameters

Determination of chlorophyll and carotenoid contents

The contents of chlorophyll (Chl) *a*, Chl *b* and total carotenoids in the fully expanded young leaves of 4-week-old plants were determined by a UV-Jenway 7315 spectrophotometer (Staffordshire, UK). Chlorophyll was extracted from leaf tissue by grinding in a mortar with liquid nitrogen to a fine powder and adding 100 mg to a 2-mL Eppendorf tube. One mL of 80% acetone was added and the powder was homogenized by inverting for 10 min in ice using a shaker and the absorbance was measured at 470, 649, and 665 nm. Chlorophyll and carotenoid concentrations were calculated from spectrophotometric data using the formulae of Lichtenthaler and Wellburn (1983).

Fluorescence of organs and photosystem II

Chlorophyll fluorescence parameters were measured on the abaxial surface of the intact leaves. Plants were kept for 30 min in the dark prior to measurement. Modulated fluorescence was measured using a portable chlorophyll fluorimeter (PEA, Hansatech Instrument Ltd., version 1.21, Norfolk, UK). Minimal fluorescence (F_0) was measured for 30 min in dark-adapted leaves using light of <0.1 µmol m^{-2} s^{-1} and maximal fluorescence (F_m) was measured after a 1 s saturating pulse (>3500 µmol m^{-2} s^{-1}) in the same leaves. Maximal variable fluorescence ($F_v = F_m - F_0$) and the photochemical efficiency of PSII (F_v/F_m) were calculated for dark-adapted leaves (Dewir et al. 2005). Four plants were randomly selected and measurements were made on fully expanded young leaves using a standard leaf chamber. There were four single-leaf replications within each treatment.

Stomatal resistance

Stomatal resistance of plant leaves (resistance to loss of water vapor through the stomata; Scm^{-1}) was estimated by an AP4 Porometer (Delta-T Devices Ltd., Cambridge, UK; Monteith et al. 1988). Four plants were randomly selected and measurements were made using fully expanded young leaves in a standard leaf chamber. There were four single-leaf replications within each treatment.

Photosynthetic rate and transpiration rate

Photosynthetic rate and transpiration rate were measured with a portable LCi photosynthesis system (ADC BioScientific Ltd., Herts, UK). Photosynthetic measurements were carried out under the following conditions: CO_2 concentration of the in-flow air was 350 µmol mol^{-1} and RH was 60%. Leaf temperature was 25°C. Four plants (4 weeks old) were randomly selected and measurements were made on fully expanded young leaves using a standard leaf chamber. There were four single-leaf replications within each treatment.

Biochemical parameters

Antioxidant enzyme assay

To determine the activities of antioxidant enzymes, 0.5 g of fully expanded young leaves was homogenized under liquid nitrogen with 1.5 mL of respective extraction buffer using pre-chilled mortar and pestle. The homogenate was filtered through four layers of cheesecloth and centrifuged at 22,000 × *g* for 20 min at 4°C. The supernatant, which was re-centrifuged at 22,000 × *g* for 20 min at 4°C, was used for the assays indicated next.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977) and data are expressed as units ml^{-1}. Catalase (CAT; EC 1.11.1.6) activity was determined according to Clairbone (1985). CAT activity was calculated from a standard curve prepared using purified catalase (0–2000 U mL^{-1}). Glutathione peroxidase (GPx; EC 1.11.1.9) activity was determined according to Tappel (1978). GPx activity was calculated in pmol mL^{-1} min^{-1}.

Determination of total glutathione

Total glutathione was determined in fully expanded young leaves using a method based on that of Owens and Belcher (1965). Leaf tissues weighed, chopped into small pieces (1–2 mm) and homogenized using a Potter homogenizer at a ratio of 1 g of tissue to 4 ml of homogenization buffer (250 mM Tris-HCl, pH 7.4, containing 1 mM EDTA). The homogenate was centrifuged in 1.5-ml Eppendorf tubes at 13,000 × *g* for 10 min at 4°C, after which the supernatant was mixed at a 1:1 ratio with DTNB solution (100 mM potassium phosphate, pH 7.4, containing 10 mM DTNB and 5 mM EDTA) and placed on ice until use. The assay mix was prepared by mixing 0.6 U of GR with the assay buffer. Samples (40 µL) were transferred to a 96-well plate, then 210 µL of assay mix was added. After equilibration for 1 min, the reaction was started by adding 60 µL of 1 mM NADPH (Melford, Ipswich, UK) dissolved in assay buffer and the absorbance was recorded at 412 nm over 5 min using a VersaMax™ plate reader. In each run there was a blank (buffer) and a standard (20 µM reduced glutathione) to calibrate the results. Samples were measured in triplicate and total glutathione was calculated according to Owens and Belcher (1965) as:

$$\text{Total glutathione } (\mu\text{mol g}^{-1}\text{ fresh weight}) = [A_{412}(\text{sample}) - A_{412}(\text{blank})/A_{412}(\text{standard})] \times 20\,\mu\text{M} \times \text{DF},$$

where DF = dilution factor and ΔA = the rate of change of absorbance.

Determination of lipid peroxidation

The thiobarbituric acid reactive substance (TBARS) assay was used to assess the level of lipid peroxidation in the fully expanded young leaves as a consequence of oxidative stress (Hodges et al. 1999). Leaf tissues were chopped into small pieces (about 1–2 mm^2), and homogenized at a ratio of 1 g of tissue to 4 ml of RIPA buffer (50 mM Tris-HCl, pH 6.5, containing 150 mM NaCl, 0.1% SDS and 1% Triton X-100). The homogenates in 1.5-mL Eppendorf tubes were then centrifuged at 13,000 × *g* for 10 min at 4°C. An equal volume of ice-cold 10% trichloroacetic acid (TCA) was added to the supernatant and the mixture was centrifuged at 13,000 × *g* for 5 min at 4°C. The supernatant was then mixed at a 1:1 ratio with of 0.67% (w/v) thiobarbituric acid in 1.5-mL Eppendorf tubes. The mixture was incubated at 80°C for 30 min, and after cooling, 290 µL was transferred to a 96-well plate. The absorbance was then measured at 532 nm using a VersaMax™ plate reader. The concentration of malondialdehyde (MDA) was determined by reference to a standard curve produced using 0–50 µM 1, 1, 3, 3-tetramethoxypropane. Measurements were carried out in triplicate and MDA content was expressed as µmol g^{-1} fresh weight.

Western blotting for detection of DHNs

A modified protocol from the protocol described by Rihan et al. (2014) was used to detect DHNs. Total proteins from the fully expanded young leaves were extracted as described by Ni et al. (1996). Samples from –80°C were thawed on ice, placed in a mortar, and 2 mL of extraction buffer (100 mM potassium phosphate, 1 mM dithiothreitol, 1 mM EDTA, 1% Triton X-100, 10% glycerol, pH 7.8) was added to 1 g of tissues and ground with a pestle. One mL of the liquid slurry was transferred to a microfuge tube (1.5 mL) on ice. The supernatant was centrifuged twice at 13,000 × *g* for 15 and 10 min, respectively at 4°C. Total protein content was evaluated using the Pierce BCA assay kit following the manufacturer's instructions. The clear supernatant containing proteins was separated using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis; Laemmli 1970) and visualized using Coomassie blue staining solution. Unstained gels were used for western blotting and the proteins were transferred to a PVDF membrane (pore size 0.2 µm) (Millipore, Cat. No ISE000010, UK) by electro-blotting at 100 V for 35 min. 1×Tris glycine transfer buffer was prepared by dissolving 3.05 g L^{-1} Tris-HCl and 14.4 g L^{-1} glycine in 20% (v/v) methanol (Towbin et al. 1979). After transferring the proteins onto the PVDF membrane, the membrane was blocked using PBST (phosphate buffer saline + 0.05 Tween 20%) + 5% Marvel (a low fat skimmed milk powder) (Iceland Ltd., UK) for 1 h at RT with mild shaking. After incubation, the membrane was incubated to hybridize with an antibody raised against the K-segment of DHNs (primary antibodies obtained from Dr Michael Wisniewski's Laboratory, USDA-ARS, Appalachian Fruit Research Station, USA). Primary antibodies were diluted 1:1000 in a total volume of 20 mL PBST supplemented with 4% of Marvel semi-skimmed milk and the membrane was incubated at 4°C overnight with very gentle shaking. The membranes were then washed three times with PBST while agitating to wash off excess primary antibody, for 5 min each wash, and

the membrane was then incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (PBS diluted 1:20,000) (Abcam) for 1 h at RT with mild shaking to detect primary antibodies attached to the DHN protein. The membranes were washed three times with PBS for 5 min each and then incubated with ECL detection reagent (Luminata crescendo western HRP substrate; Millipore, WBLUR0100, UK) in the dark for 5 min. The image from the membrane was captured using a UVP gel documentation system.

Experimental design and data analysis

All experiments were set up in a completely randomized design. Data on explants forming callus and explants forming shoots were expressed as percentages. All data were subjected to ANOVA and Duncan's multiple range test (DMRT) using SAS software (version 6.12; SAS Institute Inc., Cary, NC, USA).

Results and discussion

Growth and photosynthetic characteristics

PPFD at 70–100 μmol m^{-2} s^{-1} favored the growth of African violet plantlets (Table 1). The shoot and root fresh weights and the shoot and root dry weights of plantlets acclimatized under 70–100 μmol m^{-2} s^{-1} were significantly greater than low PPFD (35 μmol m^{-2} s^{-1}). Shoot length, number of leaves and leaf area of plantlets cultured under 70–100 μmol m^{-2} s^{-1} were also significantly higher than those of plantlets cultured at low PPFD. A high PPFD at 140 μmol m^{-2} s^{-1} had severely negative effect on plantlets, which turned yellow and eventually died (data not shown). The highest stomatal resistance was recorded at 35 μmol m^{-2} s^{-1}. Chlorophyll and carotenoid contents, net photosynthetic rate and transpiration rate were higher at moderate PPFD than at low PPFD. Micropropagated plants are characterized by increased transpiration rate due to a lack of a thick cuticle (Pospíšilová et al. 1999). However, an incremental increase in Chl content has been reported in many plant species after ex vitro transplantation (Pospíšilová et al. 1988). The increase in photosynthetic activity recorded here showed that African violet plants are able to respond to increases in PPFD but this could represent a risk of ROS formation if the energy captured cannot be fixed chemically.

The F_v/F_m value, a measure of the intrinsic or maximum efficiency of PSII i.e. the quantum efficiency if all PSII centers were open, decreased significantly in African violet plantlets acclimatized at 100 μmol m^{-2} s^{-1}. The decrease in this parameter indicates the down regulation of photosynthesis or photoinhibition (Matysiak 2004; Dewir et al. 2005). Kozai and Sekimoto (1988) reported that high PPFD was essential for improving the efficiency of photosynthesis. However, the response of plantlets to PPFD is species-dependent. Increasing PPFD to a high level (300 – 500 μmol m^{-2} s^{-1}) could improve photosynthesis if other conditions necessary for a maximum photosynthetic rate were sustained (Lee et al. 1985), while a moderate PPFD level, 70 μmol m^{-2} s^{-1}, was optimal for the acclimatization of *Spathiphyllum* plantlets (Dewir et al. 2005). The exposure of *Calathea louisae* and *Spathiphyllum floribundum* plantlets to high irradiance (300 μmol m^{-2} s^{-1}) immediately after transplantation caused photoinhibition and even Chl photobleaching (Van Huylenbroeck et al. 1995). Thus, 70 μmol m^{-2} s^{-1} may be more optimal for acclimatization of African violet plantlets than higher levels of PPFD. At this light intensity level, the plants had short petioles, wide and large leaves,

Table 1. Growth characteristics of micropropagated African violet as affected by light intensity 4 weeks after transplanting in a growth chamber.

Growth characteristics		PPFD (μmol m^{-2} s^{-1})		
		35	70	100
Fresh weight (g)	Shoot	2.0 b*	4.2 a	5.0 a
	Root	0.31 b	0.66 a	0.68 a
	Total	2.31 b	4.87 a	5.68 a
Dry weight (g)	Shoot	0.061 c	0.135 b	0.166 a
	Root	0.025 b	0.069 a	0.085 a
	Total	0.086 c	0.205 b	0.250 a
Shoot length (cm)		4.0 b	4.3 ab	5.0 a
Root length (cm)		1.3 c	5.9 a	4.9 b
No. of leaves $plantlet^{-1}$		14.8 a	16.2 a	17.0 a
Leaf area (cm^2) $plantlet^{-1}$		37.7 c	77.6 b	105.1 a
Chlorophyll *a* + *b* (mg g^{-1} FW)		0.247 a	0.253 a	0.120 b
Carotenoids (mg g^{-1} FW)		0.090 b	0.103 a	0.033 c
Net photosynthetic rate (mol CO_2 m^{-2} s^{-1})		74.16 b	355.39 a	375.41 a
Stomatal resistance (mol H_2O m^{-2} s^{-1})		4.26 a	2.52 b	2.09 b
Transpiration rate (mol H_2O m^{-2} s^{-1})		0.39 c	0.78 b	1.00 a
F_v/F_m		0.80 a	0.78 a	0.75 b
MDA content (μmol g^{-1} FW)		0.87 a	0.46 b	0.42 b
GSH (μmol g^{-1} FW)		14.18 c	22.05 b	22.27 a

*Means followed by different letters within rows by Duncan's multiple range test are significantly different at $P \leq 0.05$.

compact texture and overall better quality of pot plants than at other light intensities (Figure 1).

Biochemical parameters

Lipid peroxidation and glutathione content

In the present study, MDA content in acclimatized African violet plantlets at 35 µmol m^{-2} s^{-1} was significantly higher than at 70 or 100 µmol m^{-2} s^{-1} (Table 1). The high content of MDA at low PPFD, which was nearly twice that the level at higher PPF, indicating the occurrence of oxidative stress. MDA, a decomposition product of polyunsaturated fatty acid hydroperoxides, is a suitable biomarker for lipid peroxidation which is an effect of oxidative damage (Dewir et al. 2006; Turan & Ekmekçi 2011). Low light intensity (100 µmol m^{-2} s^{-1}) increased the MDA content in cucumber leaves (Zhang et al. 2011). Increased MDA content has also been associated with increased oxidative stress during acclimatization of *Phalaenopsis* under high irradiance (300 µmol m^{-2} s^{-1}; Ali et al. 2005). GSH content was significantly increased at 100 µmol m^{-2} s^{-1} compared with 70 or 35 µmol m^{-2} s^{-1} (Table 1). GSH not only serves as a substrate in the ascorbate–glutathione cycle, but also act as an antioxidant to mitigate stresses (Alscher et al. 1997). The enhanced GSH level under high PPFD may be an adaptive response of African violet plants to stress. This is consistent with the increase in GPx activity, a GSH-related enzyme, at 100 µmol m^{-2} s^{-1} (Figure 2). Photoinhibition in chestnut (*Castanea sativa*) under high irradiance (300 µmol m^{-2} s^{-1}) was accompanied with an increase in the content of reduced glutathione (Carvalho & Amâncio 2002). These results indicate that African violet plantlets suffered stress under high (100 µmol m^{-2} s^{-1}) or low (35 µmol m^{-2} s^{-1}) PPFD during acclimatization. However, it should be noted that African violet plants require a light intensity

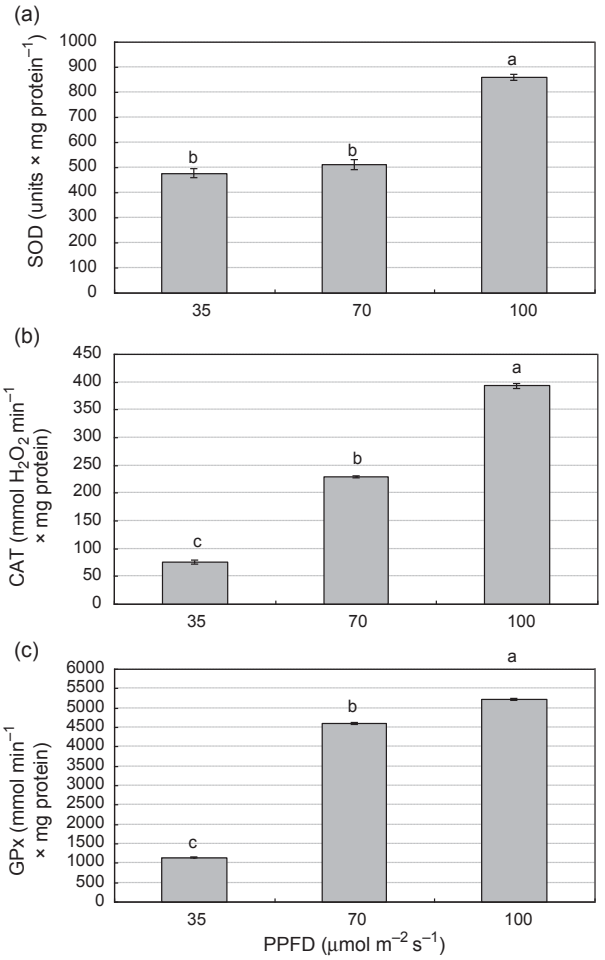

Figure 2. Activity of antioxidant enzymes in *Saintpaulia ionantha* H. Wendl. as affected by three light intensities (35, 70, and 100 µmol m^{-2} s^{-1}) 4 weeks after acclimatization. (a) Superoxide dismutase (SOD); (b) Catalase (CAT); (c) Glutathione peroxidase (GPx).

Note: Values are means ± standard error (n = 3). Different letters above bars indicate significant differences between means (Duncan's multiple range test; $P < 0.05$).

Figure 1. Acclimatized *Saintpaulia ionantha* H. Wendl plants under different light intensities (35, 70, and 100 µmol m^{-2} s^{-1}) after 4 weeks in a growth chamber.

of 100–150 µmol m^{-2} s^{-1} for their growth in natural environment (Thomas 2012).

Activity of antioxidant enzymes

The activity of SOD, CAT, and GPx increased significantly as PPFD increased, peaking at 100 µmol m^{-2} s^{-1}. CAT and GPx showed the lowest activity at 35 µmol m^{-2} s^{-1} but SOD activity at 35 and 70 µmol m^{-2} s^{-1} did not differ significantly (Figure 2). Light provides the energy needed for growth and photosynthesis but different light intensities can be a limiting factor for the acclimatization of tissue-cultured plants (Matysiak 2004; Ali et al. 2005; Dewir et al. 2005). Optimal light intensity for acclimatization is species-dependent and thus plant may undergo light stress during acclimatization. Plants activate a defense mechanism to effectively scavenge harmful ROS. ROS scavenging is essential for plants to cope with changes in the growing environments to maintain their photosynthetic function (Waraich et al. 2012). The induction of these enzymes is synchronized under some environmental stresses (Dewir et al. 2014). During acclimatization of *Phalaenopsis* plantlets to ex vitro conditions, SOD and CAT activities in leaves increased more under high (300 µmol m^{-2} s^{-1}) than under intermediate (160 µmol m^{-2} s^{-1}) or low (60 µmol m^{-2} s^{-1}) irradiance (Ali et al. 2005). During ex vitro acclimatization of *Spathiphyllum* and *Calathea* plants, the changes in antioxidative enzyme activities after ex vitro transfer were dependent on irradiance (40–360 µmol m^{-2} s^{-1}): SOD activity did not change at low irradiance (40 µmol m^{-2} s^{-1}) but increased at high irradiance (360 µmol m^{-2} s^{-1}); CAT activity increased more after transplantation to low than at high irradiance (Van Huylenbroeck et al. 2000). Several reports indicated that plants grown under low PPFD were less able to protect themselves from ROS due to a lower capacity to dissipate excess light energy and a lower capacity to scavenge ROS compared with plants grown at higher PPFD or full sun which involves gradual increases in activities of antioxidant enzymes (Mishra et al. 1995; Logan et al. 1998; Burritt & Mackenzie 2003; Ali et al. 2005). In the present study, the up/down regulation of antioxidant enzymes activities indicates that African violet plantlets underwent light stress at both low and high PPFD levels. However, under low light intensity, SOD rather than CAT or GPx played a major role in eliminating harmful ROS during the acclimatization of African violet plantlets.

DHNs

DHNs are late embryogenic abundant proteins which accumulate during seed development and are associated with acquisition of desiccation tolerance in developing seeds (Dure et al. 1981). In recent years, various studies have reported accumulation of DHNs in diverse species and tissues in response to abiotic stresses (Close 1997; Kosova et al. 2007). Accumulations of DHNs are meant for membrane stability and confer freezing, drought and salt stress tolerance in *Arabidopsis* and rice (Cheng et al. 2001; Puhakainen et al. 2004). In the present study, we evaluated the effects of light treatments on DHN accumulation during acclimatization of African violet though western blotting analysis. Analysis of the DHN protein fraction extracted from leaves of African violet revealed the presence of three major bands with molecular weight of approximately 55, 33, and 25 kDa (Figure 3). All the these three bands expressed with all light treatments i.e. 30, 70, and 100 PPFD treatments, however the expression levels were higher with treatment 35 PPFD. Densitometric analysis showed that the relative intensity of 55 kDa band with 35 PPFD treatment. Our results show that accumulation of varied DHNs during light induced stress during ex vitro acclamatization of African violet plants and probably

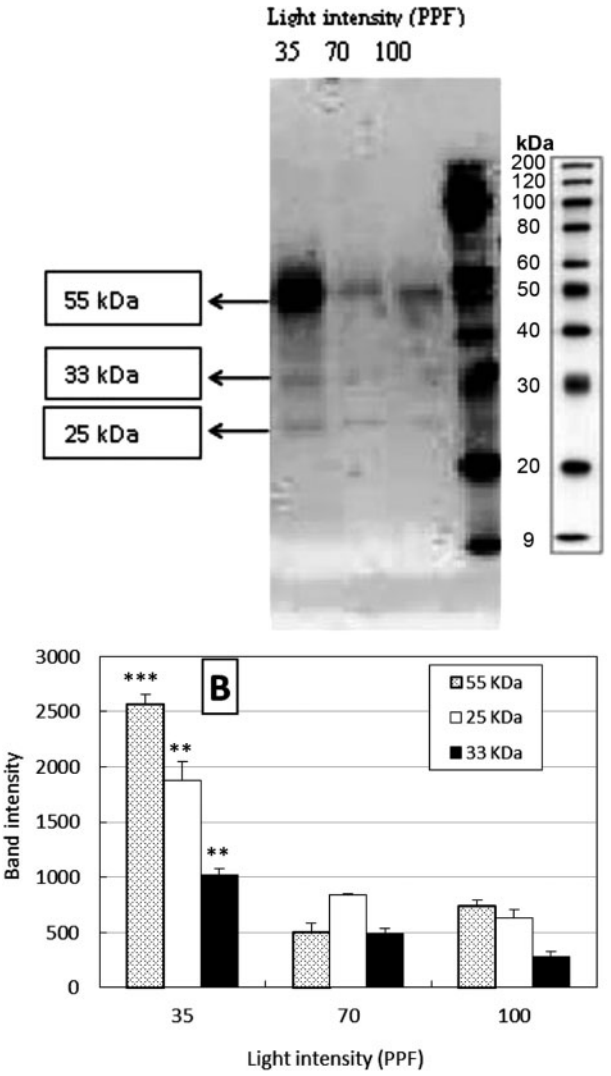

Figure 3. Western blot to detect dehydrins in micropropagated *Saintpaulia ionantha* H. Wendl. as affected by three light intensities (35, 70, and 100 µmol m^{-2} s^{-1}) 4 weeks after acclimatization.

Note: Values are mean of band intensity (KDa) ± standard error. ** and *** = significant differences between means at $P \leq 0.01$ and $P \leq 0.001$, respectively. LSD 0.05 values 253.90, 159.95, and 331.39 for 55, 33, and 25 KDa, respectively.

DHNs proteins also involved in safeguarding the membranes during light induced stress. We did not find reports on accumulation of DHNs in relation light induced stress, however, based on line of work of Hara et al. (2003) who have demonstrated the role DHNs in transgenic tobacco in inhibition of lipid peroxidation and we are of opinion that DHNs might be playing a role in overcoming lipid peroxidation in African violet plants during ex vitro acclimatization. However, further investigations are necessary to clear the role of DHNs during ex vitro acclimatization of tissue cultured plants.

In this study, the effect of different light irradiance (35, 70, and 100 μmol m^{-2} s^{-1} PPFD) on ex vitro acclimatization of micropropagated *Saintpaulia* plants was evaluated. The biomass of plants, plant height, leaf area, chlorophyll and carotenoid contents, and net photosynthesis were optimal in plants irradiated at 70 μmol m^{-2} s^{-1} PPFD. Both low and high irradiance levels (35 and 100 μmol m^{-2} s^{-1} PPFD) induced the accumulation of antioxidants and DHNs in plants which reveals enhanced stress levels. These results clearly depict that 70 μmol m^{-2} s^{-1} PPFD is suggested for the ex vitro acclimatization of micropropagated *Saintpaulia* plants. The plants acclimatized at this light intensity level had short petioles, compact texture and overall better quality of pot plants.

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