

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

Yaser Hassan Dewir, Mohammed El-Sayed El-Mahrouk, Hanady Salim Al-Shmgani, Hail Z. Rihan, Jaime A. Teixeira da Silva & Michael P. Fuller

To cite this article: Yaser Hassan Dewir, Mohammed El-Sayed El-Mahrouk, Hanady Salim Al-Shmgani, Hail Z. Rihan, Jaime A. Teixeira da Silva & Michael P. Fuller (2015) Photosynthetic and biochemical characterization of in vitro-derived African violet (*Saintpaulia ionantha* H. Wendl) plants to ex vitro conditions, Journal of Plant Interactions, 10:1, 101-108, DOI: [10.1080/17429145.2015.1018967](https://doi.org/10.1080/17429145.2015.1018967)

To link to this article: <https://doi.org/10.1080/17429145.2015.1018967>



© 2015 The Author(s). Published by Taylor & Francis.



Published online: 19 Mar 2015.



Submit your article to this journal [↗](#)



Article views: 1944



View related articles [↗](#)



View Crossmark data [↗](#)



Citing articles: 6 View citing articles [↗](#)

RESEARCH ARTICLE

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

Yaser Hassan Dewir^{a,b*}, Mohammed El-Sayed El-Mahrouk^{b,c}, Hanady Salim Al-Shmgani^{c,d}, Hail Z. Rihan^{c,e},
Jaime A. Teixeira da Silva^f and Michael P. Fuller^c

^aPlant Production Department, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia; ^bDepartment of Horticulture, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt; ^cSchool of Biological Sciences, Faculty of Science and Technology, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK; ^dCollege of Education for Pure Sciences/Ibn Al-Haitham, Baghdad University, Baghdad, Iraq; ^eHorticulture Department, Faculty of Agriculture, Damascus University, Damascus, Syria; ^fP. O. Box 7, Ikenobe 3011-2, Kagawa-ken 761-0799, Japan

(Received 12 January 2015; accepted 10 February 2015)

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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density – PPF). Fresh and dry plant biomass, plant height, and leaf area were optimal with high irradiance (70–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF). Chlorophyll and carotenoid contents and net photosynthesis were optimal in plants grown under 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF. Stomatal resistance, malondialdehyde content, and F_v/F_m values were highest at low light irradiance (35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF). 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF. Irradiance at 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF was suitable for the acclimatization of African violet plants. Both low and high irradiance levels (35 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) induced the accumulation of antioxidants and dehydrins in plants which reveals enhanced stress levels and measures to counter it.

Keywords: acclimatization; antioxidants; dehydrins; 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; PPF, 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 house-keeping 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 (photo-inhibition) 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

*Corresponding author. Email: ydewir@hotmail.com

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 $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$) under a 16-h photoperiod using halide lamps (Sanyo). The environment in the growth cabinet was adjusted to $25 \pm 2^\circ\text{C}$ 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: P₂O₅: K₂O) 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 $<0.1 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and maximal fluorescence (F_m) was measured after a 1 s saturating pulse ($>3500 \mu\text{mol m}^{-2} \text{ s}^{-1}$) 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: CO₂ concentration of the in-flow air was $350 \mu\text{mol mol}^{-1}$ 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 $22,000 \times g$ for 20 min at 4°C . The supernatant, which was re-centrifuged at $22,000 \times g$ 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 $\text{pmol mL}^{-1} \text{min}^{-1}$.

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 $13,000 \times g$ 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:

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

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 $13,000 \times g$ 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 $13,000 \times g$ for 5 min at 4°C . The supernatant was then mixed at a 1:1 ratio with 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 $\mu\text{mol g}^{-1}$ 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 $13,000 \times g$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were significantly greater than low PPFD (35 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Shoot length, number of leaves and leaf area of plantlets cultured under 70–100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were also significantly higher than those of plantlets cultured at low PPFD. A high PPFD at 140 $\mu\text{mol m}^{-2} \text{s}^{-1}$ had severely negative effect on plantlets, which turned yellow and eventually died (data not shown). The highest stomatal resistance was recorded at 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$. 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) could improve photosynthesis if other conditions necessary for a maximum photosynthetic rate were sustained (Lee et al. 1985), while a moderate PPFD level, 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) immediately after transplantation caused photoinhibition and even Chl photobleaching (Van Huylenbroeck et al. 1995). Thus, 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			
	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 <i>a</i> + <i>b</i> (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 ₂ O m ⁻² s ⁻¹)	4.26 a	2.52 b	2.09 b	
Transpiration rate (mol H ₂ O 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 ($\mu\text{mol g}^{-1}$ FW)	0.87 a	0.46 b	0.42 b	
GSH ($\mu\text{mol g}^{-1}$ 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 $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ was significantly higher than at 70 or $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 1). The high content of MDA at low PPF, 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 ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) 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 ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$; Ali et al. 2005). GSH content was significantly increased at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared with 70 or $35 \mu\text{mol m}^{-2} \text{s}^{-1}$ (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 PPF 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 $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2). Photoinhibition in chestnut (*Castanea sativa*) under high irradiance ($300 \mu\text{mol m}^{-2} \text{s}^{-1}$) 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 ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) or low ($35 \mu\text{mol m}^{-2} \text{s}^{-1}$) PPF during acclimatization. However, it should be noted that African violet plants require a light intensity

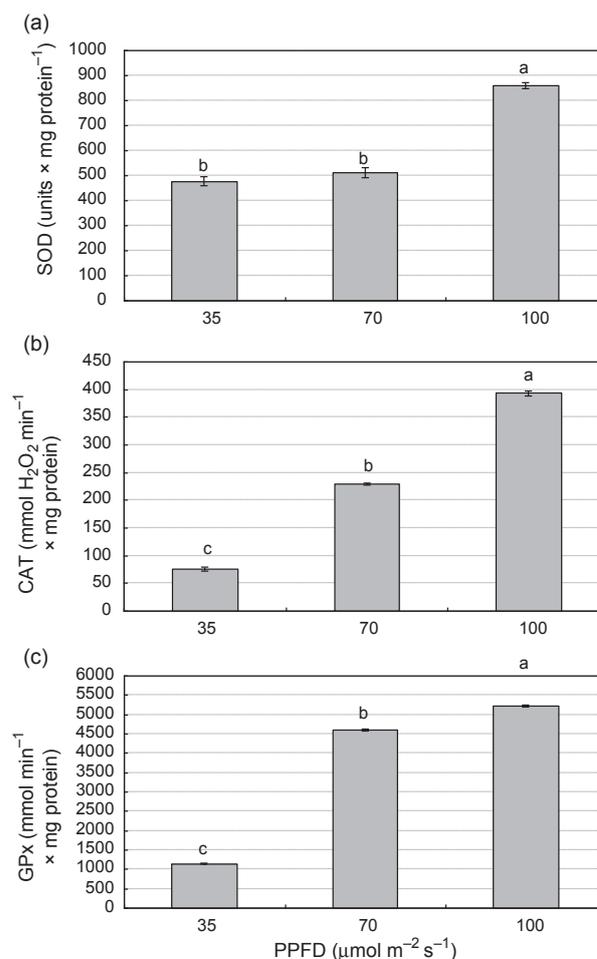


Figure 2. Activity of antioxidant enzymes in *Saintpaulia ionantha* H. Wendl. as affected by three light intensities (35, 70, and $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) 4 weeks after acclimatization. (a) Superoxide dismutase (SOD); (b) Catalase (CAT); (c) Glutathione peroxidase (GPx).

Note: Values are means \pm 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 $100 \mu\text{mol m}^{-2} \text{s}^{-1}$) after 4 weeks in a growth chamber.

of 100–150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$. CAT and GPx showed the lowest activity at 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but SOD activity at 35 and 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$) than under intermediate (160 $\mu\text{mol m}^{-2} \text{s}^{-1}$) or low (60 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$): SOD activity did not change at low irradiance (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$) but increased at high irradiance (360 $\mu\text{mol m}^{-2} \text{s}^{-1}$); 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 through 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 acclimatization of African violet plants and probably

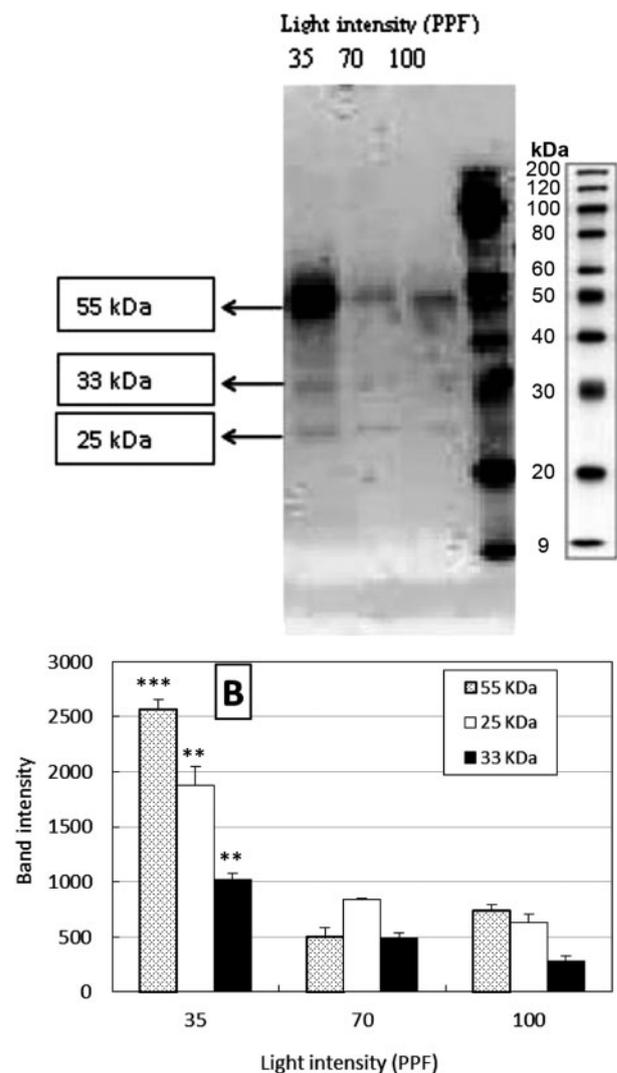


Figure 3. Western blot to detect dehydrins in micropropagated *Saintpaulia ionantha* H. Wendl. as affected by three light intensities (35, 70, and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) 4 weeks after acclimatization.

Note: Values are mean of band intensity (KDa) \pm 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) 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 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF. Both low and high irradiance levels (35 and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF) induced the accumulation of antioxidants and DHNs in plants which reveals enhanced stress levels. These results clearly depict that 70 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF 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.

Acknowledgments

This project was supported by King Saud University, Deanship of Scientific Research, College of Food & Agriculture Sciences, Agriculture Research Center. We would like to thank Prof. Timothy J. Close (Riverside University) for providing K-segment consensus peptide. We are also grateful to Angela Harrop (Plymouth University) for technical help.

References

- Ali MB, Hahn EJ, Paek KY. 2005. Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated *Phalaenopsis* plantlet. *Environ Exp Bot.* 54:109–120.
- Alscher RG, Donahue JL, Cramer CL. 1997. Reactive oxygen species and antioxidant: relationship in green cells. *Physiol Plant.* 100:224–233.
- Asada K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annu Rev Plant Physiol Plant Mol Biol.* 50:601–639.
- Burritt DJ, Mackenzie S. 2003. Antioxidant metabolism during acclimation of *Begonia xerythrophylla* to high light levels. *Ann Bot.* 91:783–794.
- Carvalho LC, Amâncio S. 2002. Antioxidant defence system in plantlets transferred from *in vitro* to *ex vitro*: effects of increasing light intensity and CO₂ concentration. *Plant Sci.* 162:33–40.
- Cheng ZQ, Targolli J, Su J, He CK, Li F, Wu R. 2001. Transgenic approaches for generating rice tolerant to dehydration stress. In: Khush GS, Brar DS, Hardy B, editors. *Rice genetics IV*. Manila: IRRI Science Publishers; p. 423–439.
- Clairbone A. 1985. Catalase activity. *Handbook of methods for oxygen radical research*. Boca Raton: CRC Press. p. 283–284.
- Close TJ. 1997. Dehydrins: a commonality in the response of plants to dehydration and low temperature. *Physiol Plant.* 100:291–296.
- Dewir YH, Chakrabarty D, Ali MB, Hahn EJ, Paek KY. 2005. Effects of hydroponic solution EC, substrates, PPF and nutrient scheduling on growth and photosynthetic competence during acclimatization of micropropagated *Spathiphyllum* plantlets. *Plant Growth Regul.* 46:241–251.
- Dewir YH, Chakrabarty D, Ali MB, Hahn EJ, Paek KY. 2006. Lipid peroxidation and antioxidant enzyme activities of *Euphorbia millii* hyperhydric shoots. *Environ Exp Bot.* 58:93–99.
- Dewir YH, Hahn EJ, Paek KY. 2004. Factors affecting *ex vitro* rooting and growth of *Spathiphyllum* plantlets in the microponic culture system. *Kor J Hort Sci Technol.* 22:42.
- Dewir YH, Indoliya Y, Chakrabarty D, Paek KY. 2014. Biochemical and physiological aspects of hyperhydricity in liquid culture system. In: Paek K-Y, Murthy HN, Zhong J-J, editors. *Production of biomass and bioactive compounds using bioreactor technology*. Berlin: Springer Science + Business Media; p. 693–703.
- Dure L III, Greenway SC, Galau GA. 1981. Development biochemistry of cotton seed embryogenesis and germination changing mRNA populations as shown by *in vitro* and *in vivo* protein synthesis. *J Biochem.* 20:4162–4168.
- Giannopolitis CN, Ries SK. 1977. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 54:309–314.
- Hara M, Terashima S, Fukaya T, Kuboi T. 2003. Enhancement of cold tolerance and inhibition of lipid peroxidation by citrus dehydrin transgenic tobacco. *Planta.* 217:290–298.
- Hazarika BN. 2003. Acclimatization of tissue-cultured plants. *Curr Sci.* 85:1704–1712.
- Hodges M., DeLong JM., Forney CF, Prange RK. 1999. Improving the thiobarbituric acid reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta.* 207:604–611.
- Kosova K, Vitamvas P, Prasil IT. 2007. The role of dehydrins in plant response to cold. *Biol Plant.* 51:601–617.
- Kozai T, Sekimoto K. 1988. Effects of the number of air changes per hour of the closed vessel and the photosynthetic photon flux on the carbon dioxide concentration inside the vessel and the growth of strawberry plantlets *in vitro*. *Environ Control Biol.* 26:21–29.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 227:680–685.
- Lee N, Wetzstein HY, Sommer HE. 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.* 78:637–664.
- Lichtenthaler HK, Wellburn AR. 1983. Determination of total carotenoids and chlorophyll *a* and *b* of leaf extracts in different solvents. *Biochem Soc Trans.* 11:591–592.
- Logan BA, Demmig-Adams B, Adams WW, Grace SC. 1998. Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. acclimated to four growth PPFs in the field. *J Exp Bot.* 49:1869–1879.
- Matysiak B. 2004. Effect of light intensity on growth and chlorophyll fluorescence of *Rhododendron* microcuttings during acclimatization. *Folia Hort.* 16:107–114.
- Mishra NP, Fatma T, Singhal GS. 1995. Development of antioxidative defense system of wheat seedlings in response to high light. *Physiol Plant.* 95:77–82.
- Monteith JL, Campbell GS, Potter EA. 1988. Theory and performance of a dynamic diffusion porometer. *Agric For Meteorol.* 44:27–38.

- Murashige T, Skoog F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol Plant*. 15:473–495
- Natali L, Giordani T, Lercari B, Maesrini P, Cozza R, Pangaro T, Vernieri P, Martinelli F, Cavallini A. 2007. Light induces expression of dehydrin-encoding gene during seedling de-etiolation in sunflower (*Helianthus annuus* L.). *J Plant Physiol*. 164:263–273.
- Ni M, Dehesh K, Tepperman JM, Quail PH. 1996. GT-2: *In vivo* transcriptional activation activity and definition of novel twin DNA binding domains with reciprocal target sequence selectivity. *Plant Cell*. 8:1041–1059.
- Owens CWI, Belcher RV. 1965. Colorimetric micro-method for the determination of glutathione. *Biochem J*. 94:705–711.
- Pospíšilová J, Solarova J, Catsky J, Ondrej M, Opatrny Z. 1988. The photosynthetic characteristics during micropropagation of tobacco and potato plants. *Photosynthetica*. 22:205–213.
- Pospíšilová J, Tichá I, Kadleček P, Haisel D, Plzánková Š. 1999. Acclimatization of micropropagated plants to *ex vitro* conditions. *Biol Plantarum*. 42:481–497.
- Powles SB. 1984. Photoinhibition of photosynthesis induced by visible light. *Annu Rev Plant Physiol*. 124:15–44.
- Puhakainen T, Hess MW, Makela P, Svensson J, Heino P, Palva ET. 2004. Overexpression of multiple dehydrin genes enhances tolerance to freezing stress in *Arabidopsis*. *Plant Mol Biol*. 54:743–753.
- Rihan HZ, Al-Issawi M, Al Shamari M, Woldie W, Kiernan M, Fuller M. 2014. The effect of molybdenum on the molecular control of cold tolerance in cauliflower (*Brassica oleracea* var. botrytis) artificial seeds. *Plant Cell Tiss Org Cult*. 118:215–228.
- Tappel AL. 1978. Glutathione peroxidase and hydroperoxides. *Methods Enzymol*. 52:506–513.
- Thomas PA. 2012. Growing African violets. Available from: <http://extension.uga.edu/publications/detail.cfm?number=C660#Conditions>
- Towbin H, Staehelin T, Gordon J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA*. 76:4350–4354.
- Turan Ö, Ekmekçi Y. 2011. Activities of photosystem II and antioxidant enzymes in chickpea (*Cicer arietinum* L.) cultivars exposed to chilling temperatures. *Acta Physiol Plant*. 33:67–78.
- Van Huylbroeck JM, Huygens H, Debergh PC. 1995. Photoinhibition during acclimatization of micropropagated *Spathiphyllum* “Petite” plantlets. *In Vitro Cell Dev Biol*. 31:160–164.
- Van Huylbroeck JM, Piqueras A, Debergh PC. 2000. The evolution of photosynthetic capacity and the antioxidant enzymatic system during acclimatization of micropropagated *Calathea* plants. *Plant Sci*. 155:59–66.
- Waraich EA, Ahmad R, Halim A, Aziz T. 2012. Alleviation of temperature stress by nutrient management in crop plants: a review. *J Soil Sci Plant Nutr*. 12:221–244.
- Zhang XL, Jia XF, Yu B, Gao Y, Bai JG. 2011. Exogenous hydrogen peroxide influences antioxidant enzyme activity and lipid peroxidation in cucumber leaves at low light. *Sci Horti-Amsterdam*. 129:656–662.