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Effects of hydroponic solution EC, substrates, PPF and nutrient scheduling on growth and photosynthetic competence during acclimatization of micropropagated *Spathiphyllum* plantlets

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Abstract

In vitro regenerated shoots of *Spathiphyllum* from bioreactor were hydroponically cultured for 30 days. The response of plant growth and photosynthesis to different substrates, photosynthetic photon flux (PPF), nutrient scheduling and electrical conductivity (EC) of hydroponic solution were studied. The best plant growth response was observed in perlite based substrates with moderate PFF (70–100μmol m^{-2} s^{-1}). Highest fresh weight, dry weight, shoot length, root length, root number and photosynthetic characteristics (chlorophyll, carotenoids and Fv/Fm) was observed in continuous immersion system. Plant growth responses, photosynthetic rate, stomatal conductance and transpiration rate were also found to be affected by EC levels. The optimum EC of a balanced nutrient solution was recorded as 1.2 dS m^{-1}. Photosynthetic activity was also characterized in terms of photochemical efficiency using measurements of chlorophyll fluorescence. Fv/Fm (it is a measure of the intrinsic or maximum efficiency of PSII i.e. the quantum efficiency if all PSII centers were open) also decreased significantly in plants grown under higher EC level; a decrease in this parameter indicates down regulation of photosynthesis or photoinhibition. Antioxidant defense enzymes such as catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monodehydroascorbate reductase (MDHAR) significantly elevated in the leaves and roots of plantlets at higher EC levels. This increase could reflect a defense response to the cellular damage provoked by higher EC levels in the nutrient solution.

Introduction

For many species the major drawback of micropropagation is the acclimatization process. Plants produced in nutrient-rich substrate under sterile conditions are fragile. Transfer and acclimatization to the *ex vitro* environment is the final but frequently most hazardous step in a successful micropropagation system (Preece and Sutter 1991). The specific *in vitro* environment, with artificial medium usually supplied with sugar(s), the growth of plantlets in small air-tight vessels with high air humidity, low gas exchange and thus a CO_2-shortage during almost the whole photoperiod, ethylene production and relatively low photosynthetic photon flux density (PPFD), induces disturbances in

plant development and photosynthetic performance (Kozai 1991; Pospísilová et al. 1997). After the transfer from *in vitro* to *ex vitro*, plants have to correct the abnormalities and to acclimatize to the new environments in the greenhouse or in the field. Recently modification of the *in vitro* production phases to more closely resemble *ex vitro* conditions contributed significantly to enhance plantlets survival during acclimatization process. A microponic culture system, combining micropropagation with hydroponics, has been applied successfully in a number of species, as one-step acclimatization before transferring to soil (Hahn et al. 2000; Piao et al. 2004; Wu et al. 2005). The enhanced plantlet growth, rooting and *ex vitro* acclimatization rates in microponic culture were proven to facilitate efficient transition from *in vitro* to *ex vitro* acclimatization. Yet, research concerning different physical and chemical microenvironment conditions like substrates, PPF, EC of the solution, is necessary for practical use of this technique.

In the present study we investigated the effects of different physical (substrate) and chemical (PPF, nutrient scheduling and EC of the solution) micro-environmental conditions on growth, photosynthesis and antioxidant enzymatic scavenging systems during the *ex vitro* establishment of micropropagated *Spathiphyllum*.

Material and methods

Plant material

In vitro plantlets of *S. cannifolium* were cultured onto MS (Murashige and Skoog 1962) liquid medium (3% sucrose with 8.88 μM BA, 4.9 μM IBA) in a 5 l balloon type bubble bioreactor (BTBB) and kept at 25 °C and a 35 μmol m^{-2} s^{-1} PPF (16 h day^{-1}) for 6 weeks (Unpublished results). Shoots were individually separated and used as an experimental material for the experiments.

Hydroponic culture: experiment on substrate

Shoots (3–5 cm) at the 3–4 leaf stage after harvested from the bioreactor were transplanted into plug trays (3.3 cm × 5.1 cm; 72 cells per tray) filled with perlite or peatmoss or vermiculite. Plug trays were placed in an individual tank to supply the plants with nutrient solution. The nutrient solution contained NH_4^+–N, NO_3^-–N, P, K, Ca and Mg at 8.0, 1.5, 4.5, 4.0, 2.0 and 1.0 mM, respectively as macronutrients. Micronutrients of the solution were composed of Fe, B, Mn, Zn, Cu and Mo at 1.95, 0.3, 0.3, 0.45, 0.05 and 0.08 μM, respectively. The EC and pH were maintained at 1.2 dS m^{-1} and 5.8, respectively. The nutrient solution was supplied using ebb and flood method. The ebb and flood system was programed to immerse the plantlets in medium for 4 times per day and 30 min every time. The environments in the growth chamber were adjusted to a 25 ± 2 °C, 40–50% relative humidity with a 16 h photoperiod (35 μmol m^{-2} s^{-1}) using a halide lamps and high pressure sodium lamps. Air at 0.1 vvm (air volume/culture volume/min) was supplied into the nutrient solution. The plantlets were covered with a clear plastic lid for the first week.

Experiment on PPF

The EC and pH were maintained at 1.2 dS m^{-1} and 5.8, respectively. The nutrient solution was supplied using ebb and flood method. The ebb and flood system was programed to immerse the plantlets in medium for 4 times per day and 30 min every time. The environments in the growth chamber were adjusted to a 25 ± 2 °C and 40–50% relative humidity. Cultures were maintained at different levels of PPF (35, 70, 100 and 150 μmol m^{-2} s^{-1}) with a 16 h photoperiod using metal halide and high pressure sodium lamps. PPF was measured (top of the plant canopy) with a data logger (LI-6400; LI-COR, Lincoln, Nebr.).

Experiment on mode of nutrient supply

The EC and pH were maintained at 1.2 dS. m^{-1} and 5.8, respectively. The environments in the growth chamber were adjusted to a 25 ± 2 °C and 40–50% relative humidity. Cultures were maintained at 100 μmol m^{-2} s^{-1} PPF with a 16 h photoperiod using metal halide and high pressure sodium lamps. In the continuous immersion type, plantlets were submerged in liquid during the whole period, whereas, the ebb and flood system was programed to immerse the plantlets in medium for 12 or 6 or 4 times per day and 30 min every time. In

continuous immersion system, the nutrient solution was continuously monitored during the entire experimental period (30 d) to maintain a constant nutrient and water status in the root zone.

Experiment on solution EC

The EC of the nutrient solution was adjusted to 0.6, 1.2, 1.8 and 2.4 dS m^{-1} using an EC controller (HM-20E, and CM-20E, TOA, Tokyo, Japan). EC was maintained by replacing the nutrient solution with fresh solution. Nutrients were supplied using continuous immersion system. The environments in the growth chamber were adjusted to a 25 ± 2 °C, 40–50% relative humidity with a 16 h photoperiod (100 μmol m^{-2} s^{-1} PPF) using a halide lamps and high pressure sodium lamps. Air at 0.1 vvm was supplied into the nutrient solution. The plantlets were covered with a clear plastic lid for the first week.

Growth and photosynthesis

Growth responses were measured after 30 d of culture in terms of fresh weight and dry weight of shoots and roots. The dry weight was determined after drying for 48 h at 70 °C. Chlorophyll contents were measured according to Lichtenthaler (1987). Rate of photosynthesis, stomatal conductance and transpiration were measured *in situ* at 30 d after planting with a portable photosynthesis system (LI-6400, LI-COR Co., Lincoln Nebr.). Photosynthetic measurements were made at the following conditions: CO_2 concentration of the in-flow air was at 350 μmol mol^{-1} and RH at 60%. Leaf temperature was 25 °C. Three plants were randomly selected and measurements were taken on three young fully expanded leaves using a standard leaf chamber. Measurements were made in triplicate; there were three single-leaf replications within each treatment. Chlorophyll fluorescence parameters were measured in the abaxial side of freshly detached discs. Plants were kept for 30 min in the dark prior to measurement. Modulated fluorescence was measured using a PAM chlorophyll fluorometer (PAM-200, Heinz Walz, Effeltrich, Germany) connected with leaf clip holder (2030-B, Walz) and DA-200 (Walz) program for collection of data. Minimal fluorescence (Fo) was measured in 30 min dark adapted leaves using light of < 0.1 μmol m^{-2} s^{-1} and maximal fluorescence (Fm) was measured after a 1 s saturating pulse (> 3500 μmol m^{-2} s^{-1}) in the same leaves. Maximal variable fluorescence (Fv = Fm−Fo) and the photochemical efficiency of PSII (Fv/Fm) were calculated for dark adapted leaves.

Leaf relative water content (RWC)

Leaf relative water content was determined on a leaf discs (1.0 cm) and calculated from the following relationship: $(W_{fresh} - W_{dry})/(W_{turgid} - W_{dry}) \times 100$, where W_{fresh} is the weight of freshly harvested sample, W_{turgid} is the turgid weight after saturating the sample with distilled water for 24 h at 4 °C, and W_{dry} is the oven-dry (70 °C for 48 h) weight of the sample (Weatherley 1950).

Leaf osmotic potential (LOP)

Leaf osmotic potential was measured in the fully expanded leaves using WP4 Dewpoint Potentia Meter (Decagon Devices, Inc. Pullman WA, USA). Leaf discs (1.0 cm) were cut from the center of leaf by using a perforator. Leaf discs were placed in a disposable sample cup (Aqua lab., Pullman, WA, USA) and 10 leaf discs from each treatment were used. The leaf discs were ground immediately and readings (-MPa) were taken as LOP.

Assay of free proline content

Free praline content was measured as described by (Bates et al. 1973). Leaves (0.5 g) were weighted, homogenized in 3 ml of 3% 5-sulfosalyicilic acid in a pre-chilled mortar and pestle in liquid nitrogen then centrifuged for 10 min at 4000 rpm; 2 ml of the supernatant were taken for proline estimation by the ninhydrin reagent (0.125 g ninhydrin, 3 ml of galacial acetic acid, 2 ml 6 M H_3PO_4) and incubated for one hour at 100 °C. The reaction was stopped by ice-cold bath for 15 min. About 4 ml of toluene was used for extraction. The absorbance at 520 nm was determined and proline concentration estimated from a standard curve.

Antioxidants enzyme assay

For determination of antioxidant enzyme activities, 0.5 g of leaves and roots were homogenized in 1.5 ml of respective extraction buffer in a pre-chilled mortar and pestle by liquid nitrogen. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant re-centrifuged at 15,000 rpm for 20 min at 4 °C for determination of antioxidant enzyme activities. The preparation was applied to a column of sephadex G-25, equilibrated with the same buffers and kept in an ice bath until the assays were completed. Protein concentration of the enzyme extract was determined according to Bradford (1976).

For APX activity, samples were homogenized in 100 mM sodium phosphate buffer (pH 7.0) containing 5 mM ascorbate, 10% glycerol and 1 mM EDTA. Specific APX activity was determined by following the decrease of absorbance at 290 nm (Chen and Asada 1989); using extinction coefficient, 2.8 mM^{-1} cm^{-1}. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.3 mM H_2O_2 and the suitable volume of enzyme extract.

DHAR activity was assessed according to Doulis et al. (1997). The 1.0 ml reaction mixture contained 90 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, 5.0 mM glutathione (GSH), and 10 μl crude extract. The reaction was initiated by the addition of 0.2 mM dehydro ascorbate (DHA) and activity was determined at 25 °C by following the reduction of DHA at 265 nm for 4 min (extinction coefficient, 10 mM^{-1} cm^{-1}).

MDHAR activity was determined according to Hossain et al. (1984). The 1.0 ml reaction mixture contained 90 mM potassium phosphate buffer (pH 7.0), 0.0125% Triton X-100, 0.2 mM NADH, 2.5 mM L-ascorbic acid, and 30 μl crude extract. The reaction was initiated with the addition of 0.25 units of ascorbate oxidase (AO) and decrease in absorbance was recorded at 340 mm due to the oxidation of NADH to NAD^+ for 4 min (extinction coefficient, 14 mM^{-1} cm^{-1}). One unit of ascorbate oxidase is defined by the manufacturer (units as defined by Sigma Chem. Co.) as the amount that causes the oxidation of 1μmol of ascorbate to monodehyadroascorbate per minute.

For determination of catalase (CAT), peroxidase (POD), glutathione reductase (GR) and glutathione *S* transferase (GST), samples were homogenized in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA. GR (EC 1.6.4.2) activity was determined by the reduction of 5,5′-dithiobis (2-nitrobenzoic acid) at 412 nm following Barata et al. (2000). The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.5), 1 mM oxidized glutathione and 0.1 mM NADPH. The reaction was initiated by the addition of enzyme extract and increase in absorbance was recorded up to 5 min. Catalase (CAT) (EC 1.11.1.6) activity was monitored by the decomposition of H_2O_2 (extinction coefficient, 39.4 mM^{-1} cm^{-1}) at 240 nm according to the method of Aebi (1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0) and plant extract in 0.75 ml volume. The reaction was initiated by adding 10 mM H_2O_2. Peroxidase (EC 1.11.1.7) activity was determined by monitoring the formation of tetraguaiacol (extinction coefficient, 6.39 mM^{-1} cm^{-1}) at 436 nm following the method of Pütter (1974). About 3.18 ml of reaction mixture contained 100 mM potassium phosphate buffer (pH 7.0), 0.3 mM guaiacol and plant extract. The reaction was initiated by adding 0.1 mM H_2O_2. GST (EC 2.2.2.18) activity was determined following the modified method of Droter et al. (1985). The reaction mixture contained 100 mM sodium phosphate buffer (pH 6.25), 0.8 mM CDNB (1-chloro-2,4-dinitrobenzene dissolved in 100% ethyl alcohol) and 2.0 mM reduced glutathione and required amount of enzyme extract in a final volume of 1 ml at 25 °C. The reaction was initiated by the addition of enzyme and conjugation of GSH to CDNB was catalyzed by GST and an increase in absorbance was monitored at 340 nm for 5 min (extinction coefficient, 9.6 mM^{-1} cm^{-1}).

Statistics

Data were subjected to Duncan's multiple range test using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

Results and discussions

Experiment on substrate

The effect of different substrates was compared and the growth parameters were presented in Table 1.

Table 1. Growth characteristics of *Spathiphyllum* as affected by growing substrates 4 weeks after planting in the hydroponic culture system.

Growth characteristics		Peatmoss	Perlite	Vermiculite
Fresh weight (mg)	Shoot	254ab[A]	287a	216b
	Root	41b	72a	60a
	Total	295ab	359a	277b
Dry weight (mg)	Shoot	30b	38a	26b
	Root	5b	7a	5b
	Total	35b	45a	31b
Shoot/root ratio (DW)		6.0b	5.4a	5.2a
Shoot Length (mm)		53b	63a	51b
Leaf no.		6.0ab	6.5a	5.5b
Leaf area (cm^2)		1.2b	1.7a	1.0b
Root length (mm)		47b	66a	48b
Roots per plantlet		3.1b	4.2a	3.2b
Chlorophyll content (mg g^{-1} FW)		0.90a	0.88a	0.87a
Carotenoids (mg g^{-1} FW)		0.21a	0.22a	0.20a
Fv/Fm		0.77a	0.78a	0.76a

[A]Mean separation within rows by Duncan's multiple range test at 5% level.

There was significant difference in total fresh weight, dry weight, shoot length, root length and roots per plantlets among peatmoss, perlite and vermiculite (Figure 1a). However, Fv/Fm, chlorophyll and carotenoid contents remained unchanged. The best response was observed in perlite based substrates. Perlite is used throughout the world as a component of soilless growing mixes where it provides aeration and optimum moisture retention for superior plant growth. Past studies have shown that outstanding yields are achieved with perlite hydroponic systems (DeBoodt and Verdonck 1972).

Experiment on PPF

The plantlet growth was greatest in the treatment with moderate PPF. The shoot and root fresh

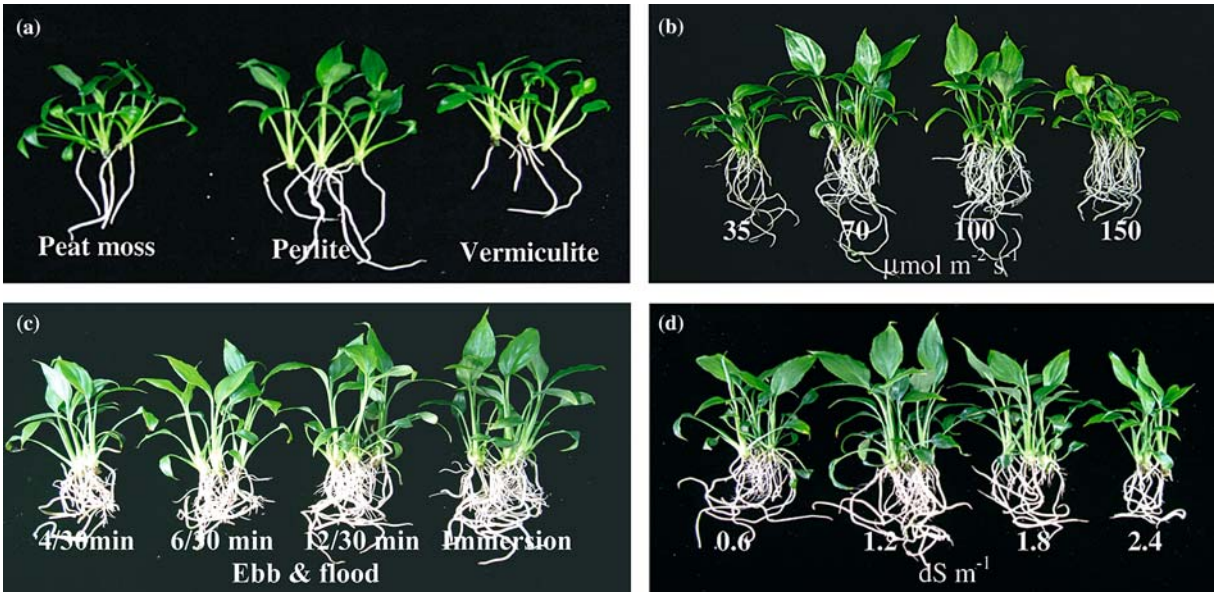

Figure 1. *Spathiphyllum* plantlets as affected by substrates (a), PPF (b), nutrient scheduling (C) and EC of the nutrient solution (d) 4 weeks after planting in the hydroponic culture system.

weights and the shoot and root dry weights of plantlets cultured under 70–100 μmol m^{-2} s^{-1} PPF were greater than other treatments. The shoot length, number of leaves and leaf area of plantlets cultured under 70–100 μmol m^{-2} s^{-1} PPF were greater than those of plantlets cultured with high or low PPF (Table 2); Figure 1b. Net CO_2 assimilation rate, transpiration rate and stomatal conductance were higher in moderate PPF than in high or low PPF treatment (Figure 2a). Chlorophyll content did not change even under the relatively low PPF values (35 μmol m^{-2} s^{-1}) and high (150 μmol m^{-2} s^{-1}) PPF values. However, the slight overall increase in chlorophyll content at moderate PPF corresponds well with the net CO_2 assimilation rates recorded at 70–100 μmol m^{-2} s^{-1}. *Spathiphyllum* plantlets in hydroponics could not utilize high PPF to its full extent. However, the effect of high PPF could only be seen on photosynthesis as well as on the shoot length suppression and root elongation. The increase in rates of net CO_2 assimilation with high PPF can be explained on the basis of increases in available energy. Kozai and Sekimoto (1988) reported that high PPF was essential for improving the efficiency of photosynthesis. Lee et al. (1985) also showed that increasing PPF to a high level (300–500 μmol m^{-2} s^{-1}) could improve photosynthesis if the other conditions necessary for a maximum photosynthetic rate were also obtained.

Experiment on mode of nutrient supply

We have also tested the mode of nutrient supply on growth of *Spathiphyllum* plantlets in hydroponic system. There are two aspects to this: the interval between nutrient supply and the length of the nutrient supply (continuous or temporary immersion). Table 3 shows that the most efficient nutrient supply method is continuous immersion system. Highest fresh weight, dry weight, shoot length, root length, root number and photosynthetic characteristics (chlorophyll, carotenoids, Fv/Fm, net photosynthetic rate, stomatal conductance and transpiration rate) were observed in this system (Figure 1c, 2b). Supply of nutrients many times (12 times) a day also resulted in good growth of plantlets. From this result, it is clear that the *Spathiphyllum* plantlets need more water and nutrients for its vegetative growth. Actually, the mode of nutrient supply is not well studied in the past; each grower follows some basic guidelines in combination with his personal experience. A general recipe for all plants and hydroponics does not exist. Research on nutrient supply must focus on finding a more efficient use of water and avoiding nutrient deficiencies. Therefore, the plant requirements and all the related influencing factors must be studied and understood.

Table 2. Growth characteristics of *Spathiphyllum* as affected by PPF 4 weeks after planting in the hydroponic culture system.

Growth characteristics		PPF (μmol m^{-2} s^{-1})			
		35	70	100	150
Fresh weight (mg)	Shoot	292c[A]	560a	585a	455b
	Root	71d	270c	386a	331b
	Total	363c	830b	971a	790b
Dry weight (mg)	Shoot	39c	64a	73a	52b
	Root	7c	26b	31a	25b
	Total	46c	90ab	104a	77b
Shoot/root ratio (DW)		5.6c	2.5b	2.3ab	2.1a
Leaf no.		6.5a	6.7a	6.5a	6.6a
Leaf area (cm^2)		1.2b	2.0a	2.1a	1.2b
Shoot length (mm)		63b	90a	85a	64b
Root length (mm)		66c	116a	124a	97b
Roots per plantlet		4.4b	7.2a	7.4a	6.4a
Chlorophyll content (mg g^{-1} FW)		0.87ab	1.05a	1.05a	0.81b
Carotenoids (mg g^{-1} FW)		0.20c	0.28a	0.29a	0.24b
Fv/Fm		0.79a	0.78ab	0.77ab	0.76b

[A]Mean separation within rows by Duncan's multiple range test at 5% level.

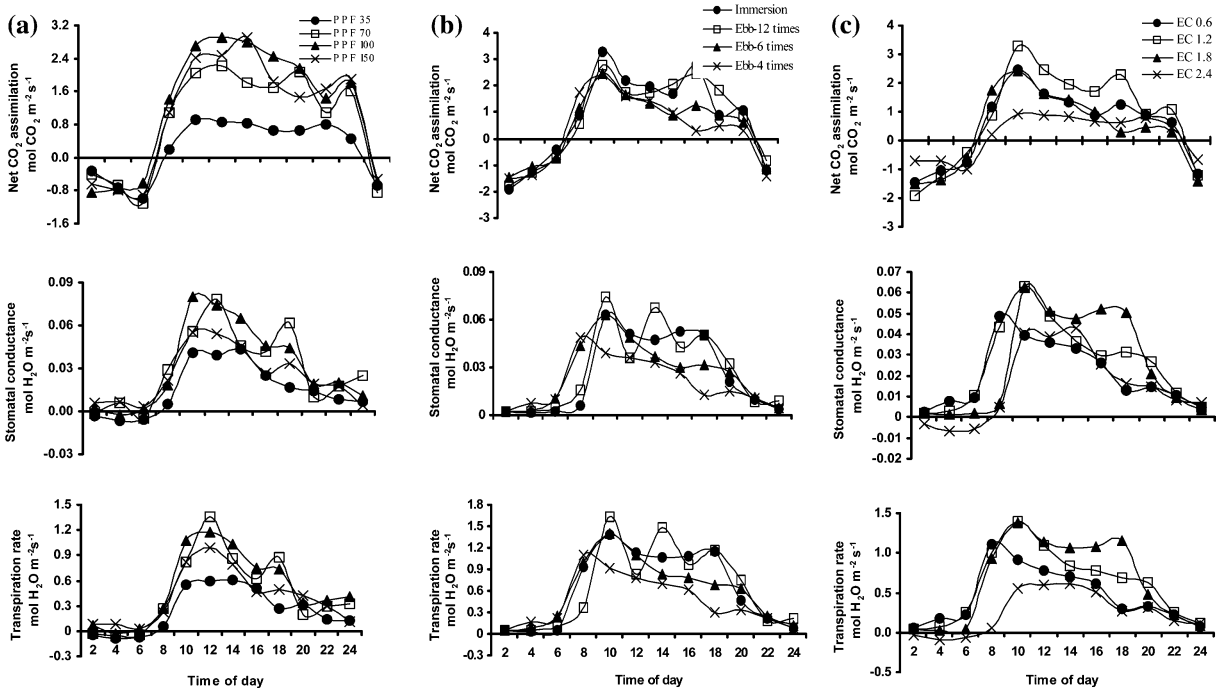

Figure 2. Net photosynthetic rate, stomatal conductance and transpiration rate in *Spathiphyllum* plantlets as affected by PPF (a), nutrient scheduling (b) and EC of the nutrient solution (c) 4 weeks after planting in the hydroponic culture system.

Experiment on solution EC

The effect of EC was compared in perlite and in 100 μmol m^{-2} s^{-1} PPF. Plantlets shoot length; total shoot and root fresh and dry weight were highest at EC 1.2 (Table 4). Photosynthetic rate, stomatal conductance and transpiration rate were also found to be affected by EC levels (Figure 2c). An EC of 1.2 dS m^{-1} resulted in the highest photosynthetic rate, stomatal conductance and

Table 3. Growth characteristics of *Spathiphyllum* as affected by nutrient scheduling 4 weeks after planting in the hydroponic culture system.

Growth characteristics		Temporary immersion (30 min)			Continuous Immersion
		4 times	6 times	12 times	
Fresh weight (mg)	Shoot	597c[A]	724b	940a	1051a
	Root	397d	544c	695b	826a
	Total	994d	1267c	1635b	1877a
Dry weight (mg)	Shoot	74d	95c	119b	138a
	Root	32b	38b	49a	55a
	Total	107d	134c	168b	193a
Shoot/root ratio		2.3a	2.7a	2.5a	2.7a
Leaf No.		6.4b	6.3b	7.1a	7.1a
Leaf area (cm^2)		2.2b	2.5b	3.4a	3.6a
Shoot length (mm)		86c	88bc	94ab	100a
Root length (mm)		124b	125b	129ab	138a
Roots per plantlet		7.3c	7.6bc	9.3a	8.8ab
Chlorophyll content (mg g^{-1} FW)		1.23b	1.39b	1.50ab	1.70a
Carotenoids (mg g^{-1} FW)		0.31b	0.32b	0.35ab	0.39a
Fv/Fm		0.775a	0.760a	0.748a	0.777a

[A]Mean separation within rows by Duncan's multiple range test at 5% level.

Table 4. Growth characteristics of *Spathiphyllum* as affected by EC of the nutrient solution 4 weeks after planting in the hydroponic culture system.

Growth characteristics		EC (dS m^{-1})			
		0.6	1.2	1.8	2.4
Fresh weight (mg)	Shoot	715c[A]	1011a	852b	608c
	Root	395b	799a	339bc	271c
	Total	1110b	1810a	1191b	879c
Dry weight (mg)	Shoot	91b	137a	106b	69c
	Root	29b	53a	23c	19c
	Total	121b	190a	128b	88c
Shoot/root ratio (DW)		3.1b	2.6a	4.6c	3.6b
Leaf no.		7.5a	7.4a	6.5b	4.7c
Shoot length (mm)		93b	109a	91b	87b
Root length (mm)		120a	137a	94b	101 b
Roots per plantlet		6.8b	8.9a	6.3bc	5.2c
Chlorophyll content (mg g^{-1} FW)		1.79a	1.76a	1.76a	1.51b
Carotenoids (mg g^{-1} FW)		0.38ab	0.41a	0.43a	0.31b
Fv/Fm		0.766b	0.790a	0.763b	0.741c
Survival rate		100	100	100	95

[A]Mean separation within rows by Duncan's multiple range test at 5% level

transpiration rate, compared to other EC levels. Increased EC level resulted in reduced stomatal conductance and photosynthetic rate. Photosynthetic activity was also characterized in terms of photochemical efficiency using measurements of chlorophyll fluorescence. Fv/Fm (it is a measure of the intrinsic or maximum efficiency of PSII i.e. the quantum efficiency if all PSII centers were open) also decreased significantly in plants grown under higher EC level; a decrease in this parameter indicates down regulation of photosynthesis or photoinhibition. Total chlorophyll and carotenoid contents also decreased under high EC of the nutrient solution. Research by Piao et al. (2004) has shown that a high EC of 2.2 dS m^{-1} significantly decreases photosynthetic capacity. They also reported that higher susceptibility to photoinhibition in the plants grown under the higher EC levels was associated with more PSII reaction centers being inactivated due to higher proportion of the non-QB-reducing PSII reaction centers. The optimum EC of a balanced nutrient solution is generally known to be 1.5 dS m^{-1}; however, optimum EC level was found to vary with plant species, season, growth stages and the quality of water. Schwarz (1985) reported that physiological disorders such as Ca deficiency could occur at high EC level. Growth depression may also originate from inhibited nutrient uptake, transport and use in the plants at high EC.

To understand how water status of *Spathiphyllum* leaves was affected by EC treatment, we monitored RWC and LOP of the leaves. Results on RWC and LOP on leaf tissue are reported in Figure 3. RWC decreased under high EC of the nutrient solution, while, LOP increased with increasing EC concentration. This response could be due to the fact that species have a number of tolerance and avoidance mechanisms under stress. RWC affects the physiology of the cell in several ways, including changes in intercellular organelle positions, transport channels, enzyme biochemistry; as well as cell wall shrinkage (Nilsen and Orcutt 1996; Lambers et al. 1998; Hall 2001; Lawlor and Cornic 2002). Clearly, these changes impact on cellular metabolism, including photosynthesis (Lawlor and Cornic 2002). Lawlor (2002) and Lawlor and Cornic (2002) reported that decreased leaf RWC progressively reduces stomatal conductance.

Figure 4 shows antioxidant enzyme activities (CAT, APX, POD, GR, MDHAR, DHAR and GST) in leaf and root extracts of *Spathiphyllum* plantlets cultured in hydroponic under different EC of the nutrient solution. The CAT and POD activity significantly elevated in the leaves and roots of plantlets at higher EC levels. The rate of increase in GR activity in leaves was found to be dependent on EC levels. Contrary to GR, glutathione-*S*-transferase (GST) activity was

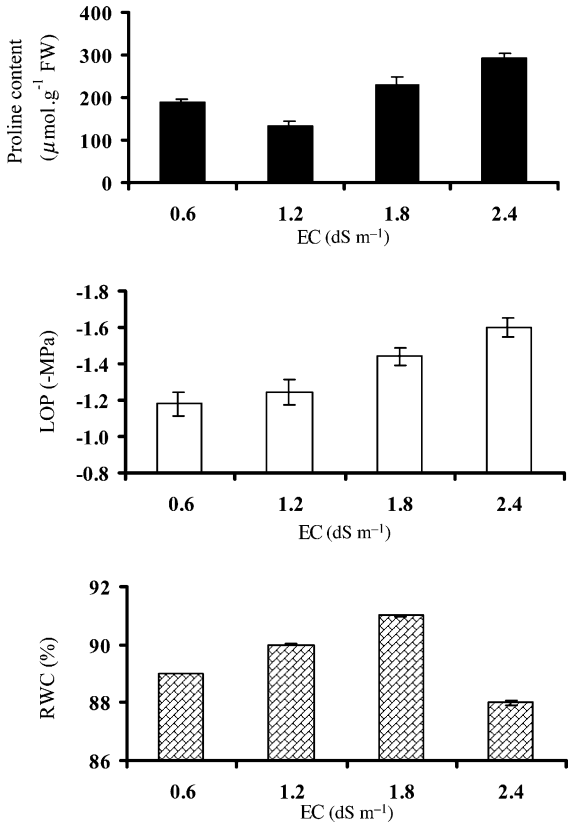

Figure 3. Leaf osmotic potential (LOP), leaf relative water content (RWC) and proline content in *Spathiphyllum* plantlets as affected by EC of the nutrient solution 4 weeks after planting in the hydroponic culture system.

unchanged in the leaves and roots of plants cultured in all the EC levels. High EC levels also increased specific APX activity. The highest level was obtained in plants cultured in nutrient solution of 2.4 EC. Treatment of *Spathiphyllum* plants with nutrient solution of higher EC levels slightly increased MDHAR and DHAR in leaves and roots, respectively. Quantitative estimation of proline among the plantlets grown under different EC levels were depicted in Figure 3. Plantlets grown under higher EC levels accumulated significantly higher amounts of proline.

EC level in the external medium is known to induce wide range of responses in plants viz., readjustment of transport and metabolic processes, and growth. High EC levels also induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes, thus making it difficult for roots to extract water from their surrounding media and thus exerts many symptoms similar to those observed under water deficit. Salt damage to plants is produced by a combination of several causes, including mainly osmotic injury and specific ion toxicity (Munns et al. 1995; Nandwal et al. (2000) that affect a wide variety of physiological and metabolic processes in plants (Silveira et al. 2001). In the last few decades an increasing body of evidence has suggested that salt stress is associated with oxidative stress, through altering antioxidant molecule levels and inducing antioxidative enzymes (Gossett et al. 1994; Meneguzzo et al. 1998, 1999; Hernandez et al. 2000). Antioxidant defense enzymes such as CAT, APX, POD, GR and MDHAR are the systems designed to minimize the concentrations of superoxide and hydrogen peroxide. H_2O_2 is eliminated by catalase and peroxidases, which include both enzymic and non-enzymic H_2O_2 degradation (Peltzer et al. 2002). Catalase dismutates H_2O_2 into water, whereas POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Blokhina et al. 2003). The antioxidants such as ascorbate and glutathione are involved in scavenging AOS primarily via the Halliwell–Asada pathway, which scavenges H_2O_2, while MDHAR and GR are involved in the regeneration of ascorbate (Horemans et al. 2000). Accumulation of protective solutes like proline and glycine betaine is a unique plant response to environmental stresses (Sakamoto and Murata 2002). Proline has a prominent role as an osmoticum and because of its zwitterionic and high hydrophilic characters, it acts as a compatible solute also (Hayashi et al. 2000). Our data indicate that high EC levels in the external medium induced an oxidative stress in *Spathiphyllum*, despite the concomitant increase in antioxidant enzymes. This increase could reflect a defense response to the cellular damage provoked by higher EC levels in the nutrient solution. Moreover, this increase in antioxidant activities, which was not strong enough to eliminate all the deleterious effects provoked by salts, only alleviated the impact of stress, thus allowing plant growth to occur.

It is clear from our study that optimal EC level, nutrient scheduling, substrate in combination with a moderate PPF were important for *ex vitro* growth of micropropagated *Spathiphyllum* plantlets grown hydroponically. This experiment also confirmed a direct relation of photosynthesis to

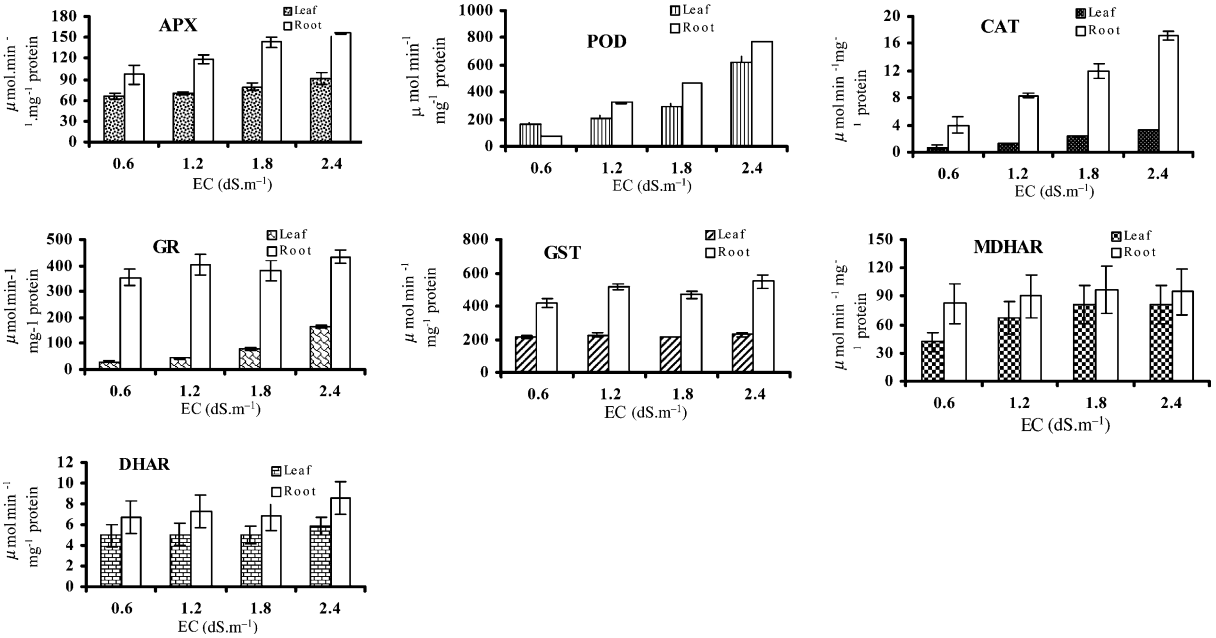

Figure 4. Antioxidant scavenging enzymes in leaves and roots of *Spathiphyllum* plantlets as affected by EC of the nutrient solution 4 weeks after planting in the hydroponic culture system.

growth, as affected by different EC levels of nutrient solution. High EC levels resulted down regulation of photosynthesis in response to stress.

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