Regeneration of *Anthurium andraeanum* from Leaf Explants and Evaluation of Microcutting Rooting and Growth under Different Light Qualities

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Abstract. This study established a new method for regenerating *Anthurium andraeanum* Lind. and evaluated effects of different wavelengths from light-emitting diodes (LEDs) on rooting and growth of adventitious shoots. Callus occurred in leaf explants of *A. andraeanum* ‘Alabama’ and ‘Sierra’ cultured on a modified Murashige and Skoog (MS) basal medium supplemented with four concentrations of N-phenyl-N’-1,2,3-thiadiazol-5-ylurea (TDZ). Adventitious shoots were induced from callus pieces ($\approx 1\text{ cm}^3$) cultured on the modified MS medium containing 6-benzyladenine (BA) with kinetin (KN), BA, and/or KN with 3-indolebutyric acid (IBA) or α -naphthalene acetic acid (NAA). Results showed that 1.82 μM TDZ induced 83.3% and 77.8% of leaf explants of ‘Alabama’ and ‘Sierra’ to produce callus and 24.9 and 24.7 adventitious shoots were produced per callus piece of ‘Alabama’ and ‘Sierra’ cultured on the modified MS medium containing 0.89 μM BA, 2.32 μM KN, and 0.98 μM IBA, respectively. Adventitious shoots were cut and rooted in the modified MS medium containing 0.98 μM IBA and grown under the same light level but with different light qualities. All adventitious shoots rooted; root numbers, root lengths, root fresh and dry weights, and leaf area of plantlets grown under red plus blue light were comparable to those grown under conventional fluorescent white light. Shoot height was the greatest in monochromatic blue light followed by red light. Shoot fresh and dry weights of plantlets grown under red plus blue light, however, were significantly greater than those grown under the other light qualities. Plantlets grown under red plus blue light had 22.7% greater total dry weight and more balanced root-to-shoot ratio than those grown under fluorescent white light. These results suggested the use of complex of red plus blue LED could be an option for improving growth of *A. andraeanum* plantlets in vitro.

Anthurium is the largest genus in the family Araceae, consisting of ≈ 1000 species (Croat, 1992). *Anthurium* flowers are valued for their exotic shape, colorful spathes, and spadices (Henny and Chen, 2003). Several species have been used commercially as cut flowers and as potted flowering foliage plants (Kamemoto and Kuehnle, 1996). With the introduction of interspecific hybrids (Henny, 1999; Henny et al., 1988; Kamemoto and Kuehnle, 1996), anthurium production has significantly increased as it took ninth position in the cut flower and fourth place in

potted plant in the Dutch auctions in 2005 (Evans, 2006). The increased production of anthuriums is also attributed to the availability of tissue-cultured liners (Chen and Henny, 2008). Micropropagation speeds the introduction of hybrid cultivars. New cultivars reach sufficient numbers to become commercially available in 2 to 3 years through micropropagation compared with the 5 to 7 years needed through traditional propagation methods. Micropropagation also helps to eliminate systemic diseases in starting materials, dramatically reduces greenhouse space required for maintaining stock plants, and provides growers with healthy and uniform liners on a year-round schedule (Chen and Henny, 2008).

In vitro propagation of *Anthurium* includes shoot multiplication from pre-existing meristems (Kunisaki, 1980; Teixeira da Silva et al., 2005) or regenerated through organogenesis (Kuehnle and Sugii, 1991; Martin et al., 2003;

Pierik et al., 1974; Teng, 1997; Viégas et al., 2007) and somatic embryogenesis (Hamidah et al., 1997; Kuehnle et al., 1992). Explant source used for in vitro propagation includes axillary buds, shoot tips, leaf, petiole, anther, and roots (Gantait and Mandal, 2010; Winarto et al., 2011). Culture media rely largely on MS basal medium (Murashige and Skoog, 1962) or modified MS media. The most widely used cytokinins are BA, KN, and occasionally TDZ with auxins of either 2,4-dichlorophenoxyacetic acid or NAA. Although anthurium micropropagation has been intensively studied, many reported methods appeared to be ineffective; some require a prolonged time for regeneration and others only produce a limited number of adventitious shoots (Beyramizade et al., 2008; Gantait and Mandal, 2010; Vargas et al., 2004). Additionally, little information is available regarding the effects of light quality on rooting and growth of regenerated adventitious shoots.

Light quality has a pronounced effect on plant growth and development (Briggs and Olney, 2001; Clouse, 2001; Kevin, 2000). The light source generally used for tissue culture is broad-spectrum fluorescent lamps, which have peak wavelengths from 380 to 750 nm (Kim et al., 2004). LEDs have many advantages over conventional fluorescent lamps, including specific wavelength, low thermal output, adjustable light intensity and quality, high photoelectric conversion efficiency as well as small size and extended longevity (Brown et al., 1995; Bula et al., 1991). Such advantages make LEDs adaptable for supporting plant growth in controlled environments such as plant tissue culture rooms and growth chambers (Yeh and Chung, 2009). Application of LEDs for tissue culture showed that plantlet growth of *Cymbidium* (Tanaka et al., 1998), *Lilium* (Lian et al., 2002), strawberry (Nhut et al., 2003), and chrysanthemum (Kim et al., 2004) was affected by different wavelengths. Recently, LEDs were also used for in vitro culture of *Spathiphyllum* and *Zantedeschia*, two genera of the Araceae family, in which 80% red wavelength plus 20% blue wavelength supplied through LED lamps resulted in *Spathiphyllum* plantlets with higher total fresh weight compared with those grown under the other wavelengths (Nhut et al., 2005). For *Zantedeschia*, however, plantlets grown under fluorescent lamps had the highest chlorophyll content and dry weight compared with those grown under different wavelengths of LEDs (Jao et al., 2005).

The objectives of this study were to establish a new method of regenerating *A. andraeanum* cultivars from leaf explants and to study different light wavelengths (red, blue, yellow, and red + blue) emitted from LEDs for rooting and growth of adventitious shoots under in vitro culture conditions.

Materials and Methods

Plant materials. Young leaves were excised from healthy and disease free *A. andraeanum* ‘Alabama’ and ‘Sierra’ stock

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plants maintained in a shaded greenhouse under a maximum photosynthetic photon flux density of $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. After washing with running tap water for 15 min, the leaves were surface-sterilized in 70% ethanol for 30 s and soaked in a 20% Clorox solution (1.2% NaOCl) for 20 min. The Clorox solution was poured off, and leaves were rinsed three times with sterile distilled water. Explants were produced by cutting the sterilized leaves into 1-cm² pieces in sterile petri dishes.

Medium preparation. A modified half-strength MS (Murashige and Skoog, 1962) with 3% (w/v) sucrose and 0.6% (w/v) agar was used as a basal medium. The pH of the medium was adjusted to 5.8 with 0.1 M NaOH before autoclaving at 121 °C for 20 min. When the medium temperature dropped to 50 °C, filter-sterilized stock solutions of TDZ, BA, KN, IBA, and NAA were added into the autoclaved basal medium based on the need for callus or adventitious shoot induction. The medium was aliquoted to petri dishes or culture vessels at 20 mL each.

Callus induction. Leaf explants were transferred onto petri dishes containing the modified MS basal medium supplemented with 0.23, 0.91, 1.82, or 2.73 μM TDZ. Leaf explants were placed with the adaxial surface up. Petri dishes were sealed with parafilm. There were four explants per dish and 12 dishes per treatment. The explants were cultured in the dark at 25 °C for callus induction.

Shoot induction. A large number of callus clumps were produced from leaf explants cultured with 1.82 μM TDZ. The callus clumps were cut into $\approx 1\text{-cm}^3$ pieces and cultured on the MS basal medium containing 0.89 μM BA with 2.32 μM KN, 0.98 μM IBA, or 1.07 μM NAA; 0.89 μM BA with 2.32 μM KN and 0.98 μM IBA or with 2.32 μM KN and 1.07 μM NAA as well as 0.93 μM KN with either 0.98 μM IBA or 1.07 μM NAA. The use of the cytokinins and auxins at the selected concentrations was based on a preliminary experiment conducted for adventitious shoot induction. There were four callus pieces per culture vessel and 10 vessels per treatment, which were cultured under a 12-h photoperiod provided by cool-white fluorescent tubes with a photon flux density of $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 25 °C.

Adventitious shoot rooting and growth under different light qualities. Adventitious shoots of ‘Alabama’ induced from MS medium containing 0.89 μM BA, 2.32 μM KN, and 0.98 μM IBA at the three-leaf stage were cut, and resultant microcuttings were stuck in a rooting medium in culture vessels. The rooting medium was modified MS containing 0.98 μM IBA only. There were four shoots per vessel. All microcuttings were grown under a $40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light level with different wavelengths: 380 to 750 nm (white) provided by cool-white fluorescent tubes and 658 nm (red), 460 nm (blue), 585 nm (yellow) and 658 + 460 (red + blue at 1:1 ratio) provided by LED light sources (Table 1). The LED light source was aligned so that 600 LEDs (60 × 10 cm) formed a rectangular light-emitting set, manufactured by Zhejiang Lianhe Saifu

experimental apparatus Technology Co., Ltd. (Ningbo, Zhejiang Province, China). The LED sets were mounted on the ceiling of growth chambers (Fig. 1). Growth chamber temperature was set at 25 °C with a 12-h photoperiod. There were eight culture vessels per light quality. After plants were grown under different wavelengths for 60 d, plantlet height, number of roots, root length and diameter, leaf area, shoot and root fresh and dry weights, and root/shoot ratio were recorded from randomly selected five vessels per treatment. Mean individual leaf area (total leaf area of a plantlet divided by the total number of leaves) of each plantlet was measured with a leaf area meter (Skye Co., U.K.).

Data collection and analysis. A completely randomized design was used for the experiments of callus and adventitious shoot induction and also microcutting growth. Each petri dish or cultural vessel was considered an experimental unit. Explants or calluses that responded to the induction were recorded per petri dish or culture vessel and frequencies for callus formation and shoot numbers per callus piece were determined. All data, i.e., callus and adventitious shoot induction and growth parameters of plantlets, were subjected to analysis of variance using SAS (SAS Institute, Cary, NC), and mean separations were determined using Duncan’s multiple range

test at $P \leq 0.05$ (Duncan, 1955). Additionally, Pearson’s correlation analysis was conducted to determine if there were any associations between leaf area and root numbers or total dry weight and root numbers.

Results

Callus induction. Leaf explants cultured on the modified MS medium containing different concentrations of TDZ expanded and became dark green in 3 weeks. Yellow callus occurred at the cut edges 2 months later (Fig. 2A) and proliferated into a callus mass (Fig. 2B). All treatments were able to induce callus formation, but frequencies varied from 41.7% to 83.3% for ‘Alabama’ and 33.3% to 77.8% for ‘Sierra’ (Table 2). Regardless of the cultivar, the highest callus formation frequency occurred in medium containing 1.82 μM TDZ.

Shoot induction. When callus pieces induced by 1.82 μM TDZ were cultured on shoot induction medium, callus proliferated, became green, and produced compact callus clumps in 4 weeks. Two weeks later bud primordia appeared (Fig. 2C) followed by the formation of multiple adventitious shoots (Fig. 2D). Shoot formation frequencies were 100% for both cultivars irrespective of treatments, but shoot numbers per callus piece

Table 1. Parameters of light spectral energy distribution of florescent lamps and light-emitting diodes used for evaluating rooting and growth of adventitious shoots of *A. andraeanum* ‘Alabama’.

Light treatment	Peak wavelength (nm)	Spectral half width (nm)	Light quantity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
Fluorescent (W)	380–750	—	40
100% red light (R)	658	5	40
100% blue light (B)	460	5	40
100% yellow (Y)	585	5	40
Red + blue (R + B) ^a	658 + 460	5	40

^aRed and blue at 1:1 ratio.

Fig. 1. Light-emitting diodes (LEDs) mounted on the ceiling of growth chambers for evaluating rooting and growth of adventitious shoots of *A. andraeanum* ‘Alabama’ where R = red LED with a peak wavelength of 658 nm, B = blue LED with a peak wavelength of 460 nm, Y = yellow LED with a peak wavelength of 585 nm, and R + B = red + blue LEDs having wavelengths of 658 nm and 460 nm at 1:1 ratio.

Fig. 2. Morphogenesis of *A. andraeanum* 'Alabama'. (A) Light yellowish callus produced from leaf explants cultured on a modified Murashige and Skoog basal medium containing $1.82 \mu\text{M}$ N-phenyl-N'-1,2,3-thiadiazol-5-ylurea. (B) Yellowish callus from leaf explants proliferated into callus mass. (C) A callus piece differentiated on shoot induction medium with the appearance of bud primordia. (D) Multiple shoots occurred. (E) *A. andraeanum* 'Alabama' plantlets after growing in rooting medium in vitro for 60 d under different light wavelengths: white light (380–750 nm) provided by florescent tubes and red (658 nm); blue (460 nm); yellow (585 nm); and red + blue (658 nm + 460 nm at 1:1 ratio) provided by light-emitting diode light sources.

Table 2. Frequency of callus formation from leaf explants of *A. andraeanum* 'Alabama' and 'Sierra' cultured on a modified Murashige and Skoog basal medium^z supplemented with four concentrations of TDZ.^y

TDZ concn (μM)	Frequency of callus formation (%) ^x	
	Alabama	Sierra
0.23	$41.7 \pm 8.3 \text{ c}^w$	$33.3 \pm 8.3 \text{ c}$
0.91	$58.3 \pm 8.3 \text{ b}$	$50.0 \pm 14.4 \text{ b}$
1.82	$83.3 \pm 8.3 \text{ a}$	$77.8 \pm 11.1 \text{ a}$
2.73	$66.7 \pm 8.3 \text{ b}$	$58.3 \pm 8.3 \text{ b}$

^zBasal medium comprises half-strength Murashige and Skoog mineral salts and vitamins, 3% (w/v) sucrose, and 0.6% (w/v) agar.

^yTDZ = N-phenyl-N'-1,2,3-thiadiazol-5-ylurea.

^xFour leaf explants per petri dish and 12 petri dishes per treatment.

^wDifferent letters within a column indicate significant differences at $P \leq 0.05$ level by Duncan's multiple range test.

were treatment-dependent. Mean shoot numbers per callus piece were 22.3 and 24.9 for 'Alabama' when cultured on medium containing BA with KN and BA with KN and

IBA, respectively (Table 3), which were significantly higher than the other treatments. The same treatments also resulted in the higher shoot numbers for 'Sierra', 21.5 and 24.7, respectively. The other treatments showed significantly lower shoot formation frequencies for 'Sierra' except for the treatment of BA with KN and NAA, which resulted in a shoot number that was insignificantly different from that of BA with KN treatment.

Effects of light qualities on rooting and subsequent growth of microcuttings. All microcuttings rooted (Fig. 2E), but root numbers differed significantly. Mean root number of microcuttings grown under red + blue light was 4.5, but it was not significantly different from the root number of 3.75 produced under fluorescent white light (Table 4). The lowest root number was 1.25 produced under yellow light followed by blue (2.5) and red (3.25) lights, which were significantly lower than that produced under red + blue light. Yellow light resulted in shorter roots (6.82 cm) with the smallest diameter (0.093 cm). Root lengths

of plantlets grown under the other light wavelengths (white, red, blue, and red + blue) were similar. Roots with the greatest diameter (0.149 cm) were plantlets grown under white light, which was significantly different from those produced under red + blue (0.123 cm), red (0.122 cm), and blue light (0.108 cm). Plantlet canopy height was 6.83 cm when grown under blue light followed by those grown under red light (4.77 cm), red + blue (4.13 cm), yellow (4.05 cm), and white light (3.18 cm). Mean individual leaf areas of plantlets produced under white and red + blue light were similar (3.141 and 3.467 cm^2 , respectively); both were significantly greater than leaf area produced under the other light qualities.

Root fresh and dry weights of plantlets produced under white and red + blue lights were significantly greater than those produced under the other lights, in which blue and yellow lights produced the lower root fresh and dry weights than red light (Table 5). The highest shoot fresh and dry weights were plantlets grown under red + blue light followed by fluorescent white light. Red, blue, and yellow lights produced the lowest shoot fresh and dry weights. As a result, total fresh and dry weights of plantlets produced under red + blue light were the greatest, 0.91 and 0.081 g, respectively, compared with the next highest of 0.74 and 0.066 g produced under fluorescent white light. Root/shoot ratio of plantlets produced under red + blue light was 0.55, which was significantly lower than those produced under red light, comparable to those produced under white and blue light but higher than those produced under yellow light. Pearson's correlation analysis showed that correlation coefficients (r) between leaf area and root numbers were 0.87 and between total dry weight and root numbers were 0.91 among plantlets produced under the five light sources. After transplanting to a soilless substrate, plants initially produced under red + blue light established rapidly and had significantly higher net photosynthetic rates than those initially produced under the other light sources (data not shown). All regenerated plants were true to type without somaclonal variation.

Discussion

The present study established a method of regenerating *Anthurium* 'Alabama' and 'Sierra' through indirect shoot organogenesis in which callus should be induced from leaf explants cultured on the half-strength MS medium supplemented with $1.82 \mu\text{M}$ TDZ, and adventitious shoots should be induced from callus pieces cultured on medium supplemented with $0.89 \mu\text{M}$ BA with $2.32 \mu\text{M}$ KN and $0.98 \mu\text{M}$ IBA. Shoots should be rooted in the MS medium supplemented with $0.98 \mu\text{M}$ IBA. This regeneration protocol is effective because up to 25 adventitious shoots were produced per callus piece in 4–5 months. It is also simple and different from previously reported methods. Among the 40 cited publications for *Anthurium* regeneration (Gantait

Table 3. Number of adventitious shoots per callus piece (1 cm³) of *A. andraeanum* 'Alabama' and 'Sierra' cultured on a modified Murashige and Skoog basal medium^z supplemented with different growth regulators.

Growth regulator ^y	Alabama ^x	Sierra ^x
0.89 μ M BA + 2.32 μ M KN	22.3 $\pm$ 2.1 a ^w	21.5 $\pm$ 1.5 ab
0.89 μ M BA + 0.98 μ M IBA	15.8 $\pm$ 1.8 c	12.7 $\pm$ 1.1 c
0.89 μ M BA + 1.07 μ M NAA	13.2 $\pm$ 1.5 cd	11.2 $\pm$ 1.3 cd
0.89 μ M BA + 2.32 μ M KN + 0.98 μ M IBA	24.9 $\pm$ 2.3 a	24.7 $\pm$ 1.9 a
0.89 μ M BA + 2.32 μ M KN + 1.07 μ M NAA	19.9 $\pm$ 1.8 b	19.6 $\pm$ 1.6 b
0.93 μ M KN + 0.98 μ M IBA	10.6 $\pm$ 1.1 d	9.0 $\pm$ 1.2 d
0.93 μ M KN + 1.07 μ M NAA	10.1 $\pm$ 1.2 d	8.5 $\pm$ 1.5 cd

^zBasal medium comprises half-strength Murashige and Skoog mineral salts and vitamins, 3% (w/v) sucrose, and 0.6% (w/v) agar.

^yBA = 6-benzyladenine; KN = kinetin; IBA = indole-3-butyric acid; and NAA = α -naphthalene acetic acid.

^xFour callus pieces per culture vessel and 10 vessels per treatment.

^wDifferent letters within a column indicate significant differences at $P \leq 0.05$ level by Duncan's multiple range test.

Table 4. Effects of light quality on root and shoot growth of *A. andraeanum* 'Alabama' plantlets.^z

Treatment ^y	Root number	Root length (cm)	Root diam (cm)	Plantlet ht (cm)	Leaf area (cm ²)
W	3.75 ab ^x	7.44 ab	0.149 a	3.18 d ^x	3.141 a
R	3.25 bc	7.57 ab	0.122 b	4.77 b	0.715 b
B	2.5 c	8.07 a	0.108 c	6.83 a	0.470 b
Y	1.25 d	6.82 b	0.093 d	4.05 c	0.130 b
R + B	4.5 a	8.12 a	0.123 b	4.13 c	3.467 a

^zData collected from plantlets of five randomly sampled culture vessels.

^yW = white light having a wavelength range from 380 to 750 nm provided by florescent tubes, R = red light-emitting diode (LED) with a peak wavelength of 658 nm, B = blue LED with a peak wavelength of 460 nm, Y = yellow LED with a peak wavelength of 585 nm, and R + B = red + blue LEDs having wavelengths of 658 nm and 460 at 1:1 ratio.

^xDifferent letters within a column indicate significant differences at $P \leq 0.05$ level by Duncan's multiple range test.

Table 5. Effects of light quality on fresh weight, dry weight, and root to shoot ratio of *A. andraeanum* 'Alabama' plantlets.^z

Treatment ^y	Fresh wt (g)			Dry wt (g)			Root/shoot ratio
	Root	Shoot	Total	Root	Shoot	Total	
W	0.35 a ^x	0.39 b	0.74 b	0.027 a	0.039 b	0.066 b	0.68 ab
R	0.27 b	0.31 bc	0.58 bc	0.023 b	0.023 c	0.041 c	0.78 a
B	0.15 c	0.30 bc	0.45 cd	0.009 c	0.023 c	0.032 cd	0.40 cd
Y	0.09 c	0.28 c	0.37 d	0.006 c	0.022 c	0.028 d	0.29 d
R + B	0.36 a	0.54 a	0.91 a	0.028 a	0.052 a	0.081 a	0.55 bc

^zData collected from plantlets of randomly sampled five culture vessels.

^yW = white light having a wavelength range from 380 to 750 nm provided by florescent tubes, R = red light-emitting diode (LED) with a peak wavelength of 658 nm, B = blue LED with a peak wavelength of 460 nm, Y = yellow LED with a peak wavelength of 585 nm, and R + B = red + blue LEDs having wavelengths of 658 nm and 460 at 1:1 ratio.

^xDifferent letters within a column indicate significant differences at $P \leq 0.05$ level by Duncan's multiple range test.

and Mandal, 2010), only one protocol used TDZ in callus induction; the remainder used other cytokinins. TDZ is a highly stable cytokinin and is resistant to degradation by cytokinin oxidase (Mok et al., 1987). It can also elicit both auxin and cytokinin responses (Gill and Saxena, 1993). TDZ alone induced callus formation or somatic embryogenesis of other plants, including geranium (Gill et al., 1993), tobacco (Gill and Saxena, 1993), peanut (Gill and Ozias-Akins, 1999), and *A. andraeanum* cv. Valentino (Yu et al., 2009).

Light quality significantly affected in vitro rooting and root growth of adventitious shoots of *A. andraeanum* 'Alabama'. Microcuttings grown under monochromatic red, blue, or yellow lights produced significantly lower numbers of roots than those grown under red + blue light (Table 4; Fig. 2E). Root fresh weights of plantlets grown under red +

blue and fluorescent white light sources were significantly greater than those of plantlets grown under monochromatic LED lights. Although there is increased application of LEDs on plant micropropagation, limited information is available on LED effects on rooting and root growth. Because all microcuttings were rooted in the same medium and grown under the same light intensity, the difference in rooting, particularly in root number, is likely attributable to the difference in shoot growth under different light qualities. High correlations were actually identified between leaf area and root numbers as well as total dry weight and root numbers. This is because root formation relies substantially on photosynthetic products of leaves (Ahkami et al., 2008; Davis and Potter, 1989; Eliasson, 1978).

Shoot growth was also greatly affected by light quality. Plantlets grown under

monochromatic blue light were two times taller than those grown under fluorescent white light (Table 4). This result agrees with the report of Heo et al. (2002) that stem of marigold was tallest in monochromatic blue light but differs from the work of Puspa et al. (2008) in which shoot elongation and internode length of grape were greatest under red LED light. Nevertheless, the present study showed that shoot fresh and dry weights as well as total fresh and dry weights of plantlets produced under red + blue were the greatest. Thus, red + blue LED light source appears to be superior to conventional fluorescent white light as well as monochromatic red, blue, and yellow LEDs for biomass accumulation of *Anthurium* 'Alabama' in vitro. The superiority is likely because spectral energy distribution of red + blue coincides with that of chlorophyll absorption (Goins et al., 1997), thus increasing net photosynthetic rate and biomass accumulation. The highest total root and shoot fresh weights were also achieved when *Spathiphyllum* (Nhut et al., 2005) and upland cotton (Li et al., 2010) were cultured under red + blue light.

In conclusion, this established new method of regenerating *A. andraeanum* cultivars may provide an additional option for effective propagation of this important aroid genus. Evaluation of microcutting rooting and subsequent growth of plantlets of 'Alabama' showed that red + blue LED light source improves plant growth as demonstrated by a 22.7% increase in total dry weight compared with the conventionally used fluorescent white light source. Because the use of fluorescent lamps consumes 65% of the total electricity in a tissue culture laboratory, it is the highest non-labor cost (Yeh and Chung, 2009). Fluorescent lamps also produce unwanted heat in culture rooms; therefore, the use of LED light sources could not only improve plantlet growth, but also save non-labor costs in micropropagation.

Literature Cited

- Ahkami, A.H., S. Lischewski, K.T. Haensch, S. Porfirova, J. Hofmann, H. Rolletschek, M. Melzer, P. Franken, B. Hause, U. Druege, and M.R. Hajirezaei. 2008. Molecular physiology of adventitious root formation in *Petunia* hybrid cuttings: Involvement of wound response and primary metabolism. *New Phytol.* 181: 613–625.
- Beyramizade, E., P. Azadi, and M. Mii. 2008. Optimization of factors affecting organogenesis and somatic embryogenesis of *Anthurium andraeanum* Lind. 'Tera'. *Propag. Orn. Plants* 8:198–203.
- Briggs, W.R. and M.A. Olney. 2001. Photoreceptors in plant photomorphogenesis to date, five photochromes, two cryptochrome, one phototropin and one superchrome. *Plant Physiol.* 125:85–88.
- Brown, C.S., A.C. Schyerger, and J.C. Sager. 1995. Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *J. Amer. Soc. Hort. Sci.* 120:808–813.
- Bula, R.J., T.W. Morrow, T.W. Tibbitts, D.J. Barta, R.W. Ignatius, and T.S. Martin. 1991.

- Light-emitting diodes as a radiation source for plants. *HortScience* 120:808–813.
- Chen, J. and R.J. Henny. 2008. Role of micro-propagation in the development of foliage plant industry, p. 206–218. In: Teixeira da Silva, J.A. (ed.) *Floriculture, ornamental and plant biotechnology*. Vol. V. Global Science Books, London, UK.
- Clouse, S.D. 2001. Integration of light and brassinosteroid signals in etiolated seedling growth. *Trends Plant Sci.* 6:443–445.
- Croat, T.B. 1992. Species diversity of Araceae in Colombia: Preliminary survey. *Ann. Mo. Bot. Gard.* 79:17–28.
- Davis, T.D. and J.R. Potter. 1989. Relations between carbohydrate, water status and adventitious root formation in leafy pea cuttings rooted under various levels of atmospheric CO₂ and relative humidity. *Physiol. Plant.* 77:185–190.
- Duncan, D.B. 1955. Multiple range and multiple F test. *Biometrics* 11:1–42.
- Eliasson, L. 1978. Effects of nutrients and light on growth and root formation in *Pisum sativum* cuttings. *Physiol. Plant.* 43:13–18.
- Evans, A. 2006. *Anthurium* sets sail to conquer all continents. *FlowerTech* 9:6–8.
- Gantait, S. and N. Mandal. 2010. Tissue culture of *Anthurium andraeanum*: A significant review and future prospective. *Intl. J. Bot.* 6:207–219.
- Gill, R., J.M. Gerrath, and P.K. Saxena. 1993. High frequency direct embryogenesis in thin layer cultures of hybrid seed geranium (*Pelargonium × hortorum*). *Can. J. Bot.* 71:408–413.
- Gill, R. and P. Ozias-Akins. 1999. Thidiazuron-induced highly morphogenic callus and high frequency regeneration of fertile peanut (*Arachis hypogaea* L.) plants. *In Vitro Cell. Dev. Biol. Plant* 35:445–450.
- Gill, R. and P.K. Saxena. 1993. Somatic embryogenesis in *Nicotiana tobacum*: Induction by thidiazuron of direct embryo differentiation from leaf disc. *Plant Cell Rpt.* 12:154–159.
- Goins, G.D., N.C. Yorio, M.M. Sanwo, and C.S. Brown. 1997. Photomorphogenesis, photosynthesis and seed yield of wheat plants grown under red light emitting diodes (LEDs) with and without supplemental blue light. *J. Expt. Bot.* 48:1407–1413.
- Hamidah, M., A.G.A. Karim, and P. Debergh. 1997. Somatic embryogenesis and plant regeneration in *Anthurium scherzerianum*. *Plant Cell Tiss. Org. Cult.* 48:189–193.
- Henny, R.J. 1999. ‘Red Hot’ *Anthurium*. *HortScience* 34:153–154.
- Henny, R.J. and J. Chen. 2003. Cultivar development of ornamental foliage plants, p. 245–290. In: Janick, J. (ed.) *Plant breeding reviews*. Vol. 23. John Wiley and Sons, Inc., Hoboken, NJ.
- Henny, R.J., R.T. Poole, and C.A. Conover. 1988. ‘Southern Blush’ *Anthurium*. *HortScience* 23:922–923.
- Heo, J.W., C.W. Lee, D. Chakrabarty, and K.Y. Paek. 2002. Growth responses of marigold and salvia bedding plants as affected by monochromatic or mixture radiation provided by a light-emitting diode (LED). *Plant Growth Regulat.* 38:225–230.
- Jao, R.C., C.C. Lai, W. Fang, and S.F. Chang. 2005. Effects of red light on the growth of *Zantedeschia* plantlets *in vitro* and tuber formation using light-emitting diodes. *HortScience* 40:436–438.
- Kamemoto, H. and A.R. Kuehnle. 1996. *Breeding anthuriums in Hawaii*. University of Hawaii Press, Honolulu, HI.
- Kevin, W. 2000. ‘Photo-manipulation-boxes’: An instrument for the study of plant photobiology. *Plant Photobiol.* 26:3–15.
- Kim, S.J., E.J. Hahn, J.W. Heo, and K.Y. Paek. 2004. Effects of LEDs on net photosynthetic rate, growth and leaf stomata of chrysanthemum plantlets *in vitro*. *Sci. Hort.* 101:143–151.
- Kuehnle, A.R., F. Chen, and N. Sugii. 1992. Somatic embryogenesis and plant regeneration in *Anthurium andraeanum* hybrids. *Plant Cell Rpt.* 11:438–442.
- Kuehnle, A.R. and N. Sugii. 1991. Callus induction and plantlet regeneration in tissue cultures of Hawaiian anthuriums. *HortScience* 26:919–921.
- Kunisaki, J.T. 1980. *In vitro* propagation of *Anthurium andraeanum* Lind. *HortScience* 12:508–509.
- Li, H., Z. Xu, and C. Tang. 2010. Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets *in vitro*. *Plant Cell Tiss. Org. Cult.* 103:155–163.
- Lian, M.L., H.N. Murthy, and K.Y. Paek. 2002. Effects of light emitting diodes (LEDs) on the *in vitro* induction and growth of bulblets of *Lilium* oriental hybrid ‘Pesaro’. *Sci. Hort.* 94:365–370.
- Martin, K.P., D. Joseph, J. Madassery, and V.J. Philip. 2003. Direct shoot regeneration from lamina explants of two commercial cut flower cultivars of *Anthurium andraeanum* Hort. *In Vitro Cell. Dev. Biol. Plant* 39:500–504.
- Mok, M.C., D.W.S. Mok, J.E. Turner, and C.V. Mujer. 1987. Biological and biochemical effects of cytokinin-active phenylurea derivatives in tissue culture systems. *HortScience* 22:1194–1196.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473–497.
- Nhut, D.T., T. Takamura, H. Watanabe, K. Okamoto, and M. Tanaka. 2003. Responses of strawberry plantlets cultured *in vitro* under superbright red and blue light-emitting diodes (LEDs). *Plant Cell Tiss. Org. Cult.* 73:43–52.
- Nhut, D.T., T. Takamura, H. Watanabe, K. Okamoto, and M. Tanaka. 2005. Artificial light source using light-emitting diodes (LEDs) in the efficient micropropagation of *Spathiphyllum* plantlets. *Acta Hort.* 692:137–142.
- Pierik, R.L.M., H.H.M. Steegmans, and J.A.J. van der Meys. 1974. Plantlet formation in callus tissues of *Anthurium andraeanum* Lind. *Sci. Hort.* 2:193–198.
- Puspa, R.P., K. Ikuo, and M. Ryosuke. 2008. Effect of red- and blue-light-emitting diodes on growth and morphogenesis of grapes. *Plant Cell Tiss. Org. Cult.* 92:147–153.
- Tanaka, M., T. Takamura, H. Watanabe, M. Endo, T. Yanagi, and K. Okamoto. 1998. *In vitro* growth of *Cymbidium* plantlets cultured under super bright and blue light-emitting diodes (LEDs). *J. Hort. Sci. Biotechnol.* 73:39–44.
- Teixeira da Silva, J.A., F. Murasei, and M. Tanaka. 2005. Growth vessel and substrate affect *Anthurium* micropropagation. *Plant Tiss. Cult.* 15:1–6.
- Teng, W.L. 1997. Regeneration of *Anthurium* adventitious shoots using liquid or raft culture. *Plant Cell Tiss. Org. Cult.* 49:153–156.
- Vargas, T.E., A. Mejias, M. Oropeza, and E. de Garcia. 2004. Plant regeneration of *Anthurium andraeanum* cv. Rubrun. *Electron. J. Biotechnol.* 7:285–289.
- Viêgas, J., M.T.R. da Rocha, I. Ferreira-Moura, M.G.S. Corrêa, J.B. da Silva, N.C. dos Santos, and J.A. Teixeira da Silva. 2007. *Anthurium andraeanum* (Linden ex André) culture: *in vitro* and *ex vitro*. *Floricult. Ornament. Biotech.* 1:61–65.
- Winarto, B., F. Rachmawati, D. Pramanik, and J.A. Teixeira da Silva. 2011. Morphological and cytological diversity of regenerants derived from half-anther cultures of anthurium. *Plant Cell Tiss. Org. Cult.* 105:363–374.
- Yeh, N. and J.P. Chung. 2009. High-brightness LEDs-energy efficient lighting sources and their potential in indoor plant cultivation. *Renew. Sustain. Energy Rev.* 10:1–6.
- Yu, Y., L. Liu, J. Liu, and J. Wang. 2009. Plant regeneration by callus-mediated protocorm-like body induction of *Anthurium andraeanum* Hort. *Agr. Sci. China* 8:572–577.