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 (≈1 cm²) cultured on the modified MS medium containing 6-benzyladenine (BA) with kinetin (KN), BA, and/or KN with 3-indolebutyric acid (IBA) or α-naphthelene 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., 1998; 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 maturity 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 plants. Leaf explants were excised from healthy and disease free *A. andraeanum* ‘Alabama’ and ‘Sierra’ stock plants. Growth and development of adventitious shoots under in vitro culture conditions.

**Materials and Methods**

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plants maintained in a shaded greenhouse under a maximum photosynthetic photon flux density of 300 μmol·m⁻²·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 1-cm² 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 μmol·m⁻²·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 μmol·m⁻²·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 selected 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 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

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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. andræanum "Alabama"

<table>
<thead>
<tr>
<th>Light treatment</th>
<th>Peak wavelength (nm)</th>
<th>Spectral half width (nm)</th>
<th>Light quantity (μmol·m⁻²·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescent (W)</td>
<td>380-750</td>
<td>--</td>
<td>40</td>
</tr>
<tr>
<td>100% red light (R)</td>
<td>658</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>100% blue light (B)</td>
<td>460</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>100% yellow (Y)</td>
<td>585</td>
<td>5</td>
<td>40</td>
</tr>
<tr>
<td>Red + blue (R + B)</td>
<td>658 + 460</td>
<td>5</td>
<td>40</td>
</tr>
</tbody>
</table>

*Red and blue at 1:1 ratio.

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Fig. 1. Light-emitting diodes (LEDs) mounted on the ceiling of growth chambers for evaluating rooting and growth of adventitious shoots of A. andræanum ‘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.
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 cm², 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 μM TDZ, and adventitious shoots should be induced from callus pieces cultured on medium supplemented with 0.89 μM BA and 0.98 μM IBA. Shoots should be rooted in the MS medium supplemented with 0.98 μM 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, 1987...
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 and elicited both auxin and cytokinin responses (Gill et al., 2010), only one protocol used TDZ + BA as the basic medium supplemented with different growth regulators.

**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 supplemented with different growth regulators.

<table>
<thead>
<tr>
<th>Growth regulator</th>
<th>Alabama</th>
<th>Sierra</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.89 μM BA + 7.32 μM KN</td>
<td>22.3 ± 2.1 a</td>
<td>21.5 ± 1.5 ab</td>
</tr>
<tr>
<td>0.89 μM BA + 0.98 μM IBA</td>
<td>15.8 ± 1.8 c</td>
<td>12.7 ± 1.1 c</td>
</tr>
<tr>
<td>0.89 μM BA + 1.07 μM NAA</td>
<td>13.2 ± 1.5 cd</td>
<td>11.2 ± 1.3 cd</td>
</tr>
<tr>
<td>0.89 μM BA + 2.32 μM KN + 0.98 μM IBA</td>
<td>24.9 ± 2.3 a</td>
<td>24.7 ± 1.9 a</td>
</tr>
<tr>
<td>0.89 μM BA + 2.32 μM KN + 1.07 μM NAA</td>
<td>19.9 ± 1.8 b</td>
<td>19.6 ± 1.6 b</td>
</tr>
<tr>
<td>0.93 μM KN + 0.98 μM IBA</td>
<td>10.6 ± 1.1 d</td>
<td>9.0 ± 1.2 d</td>
</tr>
<tr>
<td>0.93 μM KN + 1.07 μM NAA</td>
<td>10.1 ± 1.2 d</td>
<td>8.5 ± 1.5 cd</td>
</tr>
</tbody>
</table>

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

**Table 4.** Effects of light quality on fresh weight, dry weight, and root to shoot ratio of *A. andraeanum* ‘Alabama’ plantlets.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root number</th>
<th>Root length (cm)</th>
<th>Root diam (cm)</th>
<th>Plantlet ht (cm)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>3.75 ab</td>
<td>7.44 ab</td>
<td>0.149 a</td>
<td>3.18 d</td>
<td>3.141 a</td>
</tr>
<tr>
<td>R</td>
<td>3.25 bc</td>
<td>7.57 ab</td>
<td>0.122 a</td>
<td>4.77 b</td>
<td>0.715 b</td>
</tr>
<tr>
<td>B</td>
<td>2.5 c</td>
<td>8.07 a</td>
<td>0.108 c</td>
<td>6.83 a</td>
<td>0.470 b</td>
</tr>
<tr>
<td>Y</td>
<td>1.25 d</td>
<td>8.62 b</td>
<td>0.093 d</td>
<td>4.05 c</td>
<td>0.130 b</td>
</tr>
<tr>
<td>R + B</td>
<td>4.5 a</td>
<td>8.12 a</td>
<td>0.123 b</td>
<td>4.13 c</td>
<td>3.467 a</td>
</tr>
</tbody>
</table>

*Different letters within a column indicate significant differences at P ≤ 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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root number</th>
<th>Root length (cm)</th>
<th>Root diam (cm)</th>
<th>Plantlet ht (cm)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>0.35 a</td>
<td>0.39 b</td>
<td>0.74 b</td>
<td>0.027 a</td>
<td>0.039 b</td>
</tr>
<tr>
<td>R</td>
<td>0.27 b</td>
<td>0.31 bc</td>
<td>0.58 bc</td>
<td>0.023 b</td>
<td>0.023 c</td>
</tr>
<tr>
<td>B</td>
<td>0.15 c</td>
<td>0.30 bc</td>
<td>0.45 cd</td>
<td>0.009 c</td>
<td>0.023 c</td>
</tr>
<tr>
<td>Y</td>
<td>0.09 c</td>
<td>0.28 c</td>
<td>0.37 d</td>
<td>0.006 c</td>
<td>0.022 c</td>
</tr>
<tr>
<td>R + B</td>
<td>0.36 a</td>
<td>0.54 a</td>
<td>0.91 a</td>
<td>0.028 a</td>
<td>0.052 a</td>
</tr>
</tbody>
</table>

*Different letters within a column indicate significant differences at P ≤ 0.05 level by Duncan’s multiple range test.

and 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 unnecessary 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**


