Effects of Red and Blue Light-Emitting Diodes on Callus Induction, Callus Proliferation, and Protocorm-Like Body Formation from Callus in Cymbidium Orchid

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The effects of light generated by red and blue light-emitting diodes (LEDs) on callus induction from protocorm-like body (PLB) segments, callus proliferation and PLB formation from callus in Cymbidium orchid were investigated. The cultures were placed in 'LED PACK 4' incubators under different ratios of red and blue LED light (100% red LEDs, 75% red LEDs+25% blue LEDs, 50% red LEDs+50% blue LEDs, 25% red LEDs+75% blue LEDs, and 100% blue LEDs) at 45 μmol m⁻² s⁻¹ with a 16-h photoperiod or put under plant growth fluorescent (PGF) light with the same light intensity and photoperiod. Among the treatments, 100% red LEDs was the most effective for callus induction from PLB segments. Callus proliferation was best in the 75% red LEDs+25% blue LEDs treatment but was not significantly different from that in PGF light. The highest PLB formation from callus was obtained in 25% red LEDs+75% blue LEDs. The results suggested that LEDs are the effective light source for callus induction, callus proliferation and PLB formation from callus in Cymbidium orchid.

Keywords: callus, Cymbidium, light-emitting diodes, orchid, protocorm-like body

INTRODUCTION

Light-emitting diodes (LEDs) have attracted attention since the late 1980s with their many attractive feathers including small mass and volume, solid-state construction, specific narrow-bandwidth wavelength emissions and longevity, making them a promising light source for intensive plant culture systems. Furthermore, the wavelength specificity and narrow bandwidth of LEDs have recently been exploited in many areas of photobiological research (Bula et al., 1991; Barta et al., 1992; Brown et al., 1995). LEDs have been used for studies on photosynthesis in kudzu (Tennessee et al., 1994), chlorophyll biosynthesis in wheat (Tripathy and Brown, 1995), and photomorphogenesis and photosynthesis of wheat (Goins et al., 1997). Some plant species, such as lettuce (Bula et al., 1991; Hoenecke et al., 1992), pepper and cucumber (Schuerger and Brown, 1994; Brown et al., 1995) have been successfully cultured under LEDs. The effects of LEDs on plantlets cultured in vitro were reported in potato (Miyashita et al., 1995), Cymbidium (Tanaka et al., 1998), Rehmannia glutinose (Hahn et al., 2000) and strawberry (Nhut et al., 2003). However, there is very little information on the effects of LEDs on cultured plant cells and tissues.

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Cymbidium, one of the most commercially important orchids in the world, is used for cut flowers and potted plants. There are many reports on micropropagation of Cymbidium using shoot-tips or protocorm-like bodies (PLBs) as explants (Morel, 1960, 1964; Wimber 1963; Sagawa et al., 1966; Ueda and Toritaka, 1968; Wang, 1988). However, there are very few reports on callus cultures in Cymbidium (Steward and Mapes, 1971; Begum et al., 1994; Chang and Chang, 1998). We have successfully induced subculturable callus from PLB segments of Cymbidium orchid hybrid for the first time (unpublished work). In the research presented here, the effects of red and blue LEDs on callus induction from PLB segments, callus proliferation, and PLB formation from callus in Cymbidium were examined.

MATERIALS AND METHODS

Plant material and culture condition. Protocorm-like bodies (PLBs) of Cymbidium Twilight Moon 'Day Light' derived from shoot-tip culture were subcultured every two months on modified Vacin and Went medium (1949) supplemented with 0.1 mg l⁻¹ 1-naphthaleneacetic acid (NAA), 0.1 mg l⁻¹ kinetin and solidified with 8 g l⁻¹ Bacto agar (Difco laboratories, USA). Longitudinally bisected segments of PLBs were used as explants for callus induction.

Modified Vacin and Went culture medium (1949) supplemented with 1 ml l⁻¹ Nitsch microelements (Nitsch and Nitsch, 1967), 2 g l⁻¹ tryptone, 20 g l⁻¹ sucrose, and solidified with 8 g l⁻¹ Bacto agar was used as the basal medium. All media were adjusted to pH 5.3 with 1 N NaOH or HCl prior to autoclaving at 100 K Pa for 17 min. Cultures were kept at 25°C, under a 16-h photoperiod with a light intensity of 45 µmol m⁻² s⁻¹ provided by plant growth fluorescent lamps (Homo Lux, Matsushita Electric Industrial Co., Japan), unless otherwise stated.

Light-emitting diode (LED) irradiation device. The apparatuses used for LED light experiments were 37×47×37 cm white acrylic plastic boxes named 'LED PACK 4' equipped with LED boards mounted on the ceiling. Two types of LED boards were developed: the first one was comprised of 600 individual red LEDs (wavelength : 660 nm, material : GaN, GL5UR3K1 = 3cd, 5 mm in diameter, Sharp Electric Ltd., Tokyo, Japan) and 600 individual blue LEDs (wavelength : 450 nm, material : GaAlAs, NLPB 500= 1cd, 5 mm in diameter, Nichia Chemicals Ltd., Tokushima, Japan) and the second one was comprised of 1200 individual red LEDs. The intensity of LEDs in each box was maintained at 45 µmol m⁻² s⁻¹ with a 16-h photoperiod. The ratios of red and blue LEDs as well as the intensity of light were adjusted using PA36-2A Regulated DC Power Supply (Kenwood TMI Corp., Japan) and LI-250 Light Meter (LI-COR Inc., Nebraska, USA).

Effects of red and blue LEDs on callus induction from PLB segments. To examine the effects of red and blue LEDs on callus induction from PLB segments, 10 longitudinally bisected PLB explants were cultured in 100-ml Erlenmeyer flasks containing 40 ml of basal medium supplemented with 0.1 mg l⁻¹ NAA and 0.01 mg l⁻¹ N-phenyl-N'-1,2,3-thiadiazol-5-yl urea (TDZ) under different LED light conditions (100% red LEDs, 75% red LEDs+25% blue LEDs, 50% red LEDs+50% blue LEDs, 25% red LEDs+75% blue LEDs, and 100% blue LEDs) as well as plant growth fluorescent (PGF) light. A total of 40 explants were used for each treatment, and the experiment was repeated four times. After about one month of culture, the percentages of explants forming big callus (++), about 4-5 mm in size), small callus (+, less than 4 mm in size), callus with PLBs (small callus and PLBs formed from the same explant), PLBs, and dead explants were recorded.

Effects of red and blue LEDs on callus proliferation. Calli induced from PLB segments on basal medium supplemented with 0.1 mg l⁻¹ NAA and 0.01 mg l⁻¹ TDZ, under PGF light
were subcultured five times on the same medium for use in this experiment. Five callus pieces (about 40 mg) were transferred to 100-ml Erlenmeyer flasks containing 40 ml of the same medium and subjected to the light conditions described above. Thirty explants were used for each treatment, and the experiment was repeated three times. Callus growth after 4 weeks of culture was evaluated using the final fresh weight divided by the initial fresh weight.

**Effects of red and blue LEDs on PLB formation from callus.** Five callus pieces (about 40 mg) from five-time subcultured calli were cultured in 100-ml Erlenmeyer flasks containing 40 ml of basal medium without plant growth regulator under each of the light conditions described above. Thirty explants were used for each treatment, and the experiment was repeated three times. After about two months of culture, the number of PLBs per explant, and fresh and dry weights of PLB clusters regenerated from each callus were recorded.

**Data analysis.** The data were subjected to analysis of variance and significantly different means were identified using Duncan's multiple range test.

**RESULTS AND DISCUSSION**

**Effects of red and blue LEDs on callus induction from PLB segments**

Longitudinally bisected segments of PLBs were cultured on callus induction medium under different light conditions. Explants formed big callus (+ +), small callus (+), callus along with PLBs, PLBs or died at different frequencies within about one month (Table 1). The highest callus induction from PLB segments was observed under 100% red LEDs (Fig. 1), followed by PGF light, 75% red LEDs + 25% blue LEDs and 50% red LEDs + 50% blue LEDs. Callus formation tends to decrease as the percentage of red LEDs decreases along with an increase in the percentage of blue LEDs. The opposite effect was observed in PLB formation from cultured PLB segments, and PLB segments formed PLBs lowest under 100% red LEDs. Mortality of explants in culture was not significantly different among the treatments.

In *Phalaenopsis*, we also observed red LEDs were suitable for callus formation from PLB segments (Tanaka et al., 2001). Kadkade and Jopson (1978) reported that a narrow bandwidth fluorescent red light (660 nm) promoted callus production from embryo tissue in Douglas fir (*Pseudotsuga menziesii*).

**Table 1** Effects of red and blue LEDs on callus induction from PLB segments of *Cymbidium Twilight Moon ‘Day Light’*.

<table>
<thead>
<tr>
<th>Light conditions</th>
<th>Percentage of explants forming callus and/or PLB%</th>
<th>Dead (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Callus (+ +)</td>
<td>Callus (+)</td>
</tr>
<tr>
<td>PGF</td>
<td>45.0b*</td>
<td>2.5a</td>
</tr>
<tr>
<td>100% red LEDs*</td>
<td>45.0b*</td>
<td>2.5a</td>
</tr>
<tr>
<td>75% red LEDs + 25% blue LEDs</td>
<td>2.5a</td>
<td>2.5a</td>
</tr>
<tr>
<td>50% red LEDs + 50% blue LEDs</td>
<td>2.5a</td>
<td>2.5a</td>
</tr>
<tr>
<td>25% red LEDs + 75% blue LEDs</td>
<td>2.5a</td>
<td>2.5a</td>
</tr>
<tr>
<td>100% blue LEDs</td>
<td>2.5a</td>
<td>2.5a</td>
</tr>
</tbody>
</table>

* Callus (+ +), big callus about 4-5 mm in size was induced; callus (+), small callus less than 4 mm in size was induced; callus + PLBs, callus (+) and PLBs formed from the same explant; PLBs, PLBs were formed.
* PGF, plant growth fluorescent light.
* LEDs, light-emitting diodes.
* Means within a column followed by the same letters are not significantly different at *P* < 0.05 according to Duncan's multiple range test.
Effects of red and blue LEDs on callus proliferation of *Cymbidium* Twilight Moon 'Day Light'.

<table>
<thead>
<tr>
<th>Light conditions</th>
<th>Callus growth</th>
<th>Morphogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF</td>
<td>9.6a</td>
<td>callus</td>
</tr>
<tr>
<td>100% red LEDs</td>
<td>7.7b</td>
<td>callus</td>
</tr>
<tr>
<td>75% red LEDs + 25% blue LEDs</td>
<td>9.9a</td>
<td>callus</td>
</tr>
<tr>
<td>50% red LEDs + 50% blue LEDs</td>
<td>7.8b</td>
<td>callus</td>
</tr>
<tr>
<td>25% red LEDs + 75% blue LEDs</td>
<td>6.2c</td>
<td>callus</td>
</tr>
<tr>
<td>100% blue LEDs</td>
<td>6.0c</td>
<td>callus</td>
</tr>
</tbody>
</table>

*Means followed by the same letters are not significantly different at P<0.05 according to Duncan's multiple range test.*

Effects of red and blue LEDs on callus proliferation

Callus pieces excised from calli subcultured five times were transferred to the same medium under different light treatments. In general, callus proliferated well in all treatments with approximately six- to ten-fold fresh weight increases (Table 2). Callus growth was best under 75% red LEDs + 25% blue LEDs (Fig. 2B) and PGF light; the growth under the former
treatment was slightly, but not significantly, better than under the latter. Use of 100% red LEDs stimulated callus growth, but small parts of some explants turned brown (Fig. 2A). The small amount of blue light in 75% red LEDs + 25% blue LEDs might mitigate the adverse impacts of red LEDs and thus improved conditions for callus growth. When red LEDs were decreased from 75% to 0% and blue LEDs increased from 25% to 100%, the growth of callus decreased.

There have been some reports about the effects of different light conditions on the growth of callus cultures, but with conflicting results. Both red and white fluorescent light favored the growth of olive callus while green light and dark supported a weaker growth (Lavee and Messer, 1969). Kadkade and Jopson (1978) found that narrow band fluorescent light wavelengths at 550 and 660 nm enhanced Douglas fir callus growth compared to darkness, while near-UV (371 nm) inhibited callus growth. On the contrary, Weis and Jaffe (1969) reported the enhancement of tobacco callus growth under continuous blue light. Studying the effects of different light sources provided by narrow-bandwidth-emitting fluorescent lamps on tobacco callus, Seibert et al. (1975) found that near-UV light (371 nm) and blue light region stimulated tobacco callus growth, while red light did not appear to affect callus growth. In this study, red LEDs were found to stimulate Cymbidium orchid callus growth; however, the addition of a small amount of blue LED light to red LEDs produced the best results.

**Effects of red and blue LEDs on PLB formation from callus**

Callus pieces continued to proliferate after transfer to the plant growth regulator-free basal medium, turned green, and began to form PLBs about 15 days later in most light treatments (Table 3). Under 100% red LEDs, calli continued to proliferate and then turned brown or died without forming any PLBs (Fig. 3A). This suggested that red LEDs inhibit the formation of PLBs from Cymbidium orchid callus. In contrast, blue LEDs might play an important role in stimulating PLB formation from callus as PLBs formed very well in blue LED treatments. However, the addition of a small amount of red LED light to blue LEDs (25% red LEDs + 75% blue LEDs treatment) improved PLB formation from callus, producing the highest number of PLBs per explant and the highest fresh and dry weights of the PLB cluster (Table 3; Fig. 3B). The proposed explanation is that PLB formation from callus involves a short period of callus proliferation followed by PLB formation from callus; the small amount of red LED light in this light treatment might partially promote callus proliferation. As the ratio of red to blue LEDs was reduced, PLB formation from callus was improved. PLB formation from callus under PGF light was also good. In general, PLBs formed in different treatments were not markedly different and converted into normal plantlets when transferred to fresh basal medium under PGF light.

**Table 3** Effects of red and blue LEDs on PLB formation from callus of *Cymbidium* Twilight Moon 'Day Light'.

<table>
<thead>
<tr>
<th>Light conditions</th>
<th>Number of PLBs per explant</th>
<th>Fresh weight of PLB cluster (mg)</th>
<th>Dry weight of PLB cluster (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF*</td>
<td>89.6b</td>
<td>1541.2b</td>
<td>136.3b</td>
</tr>
<tr>
<td>100% red LEDs*</td>
<td>0e</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>75% red LEDs + 25% blue LEDs</td>
<td>73.6d</td>
<td>1135.2d</td>
<td>99.2d</td>
</tr>
<tr>
<td>50% red LEDs + 50% blue LEDs</td>
<td>84.4c</td>
<td>1409.6c</td>
<td>124.1c</td>
</tr>
<tr>
<td>25% red LEDs + 75% blue LEDs</td>
<td>98.0a</td>
<td>1691.5a</td>
<td>151.0a</td>
</tr>
<tr>
<td>100% blue LEDs</td>
<td>92.0b</td>
<td>1473.0bc</td>
<td>127.0c</td>
</tr>
</tbody>
</table>

* PGF, plant growth fluorescent light.
* LEDs, light-emitting diodes.
* Means within a column followed by the same letters are not significantly different at P < 0.05 according to Duncan's multiple range test.
Until now, there have been only few studies on the role of light in the development of cultured plant cells and tissues. In *Phalaenopsis*, blue LEDs proved to be effective for PLB formation from cultured PLB segments (Tanaka et al., 2001). Weis and Jaffe (1969) found that continuous fluorescent blue light as well as white light enhanced organogenesis of tobacco callus, while red light and far-red light had almost no effect. Similarly, shoot formation from tobacco callus were reportedly stimulated in the fluorescent blue light region, while the red and far-red light regions did not stimulate shoot initiation (Fridborg and Eriksson, 1975; Seibert et al., 1975). According to Kaldenhoff et al. (1994), plantlet regeneration in cell-suspension cultures of *Arabidopsis thaliana* was promoted by irradiation with blue light (400–500 nm) while red light (600–700 nm) was ineffective. In *Cymbidium* orchid, we found that compared to blue LEDs, blue LEDs with a small amount of red LED light in 25% red LEDs + 75% blue LEDs was the most suitable for PLB regeneration from callus.

Some authors have suggested that photoreceptors have evolved in response to the effects of different light conditions on the growth and development of plant tissue (Bonnett, 1972; Seibert et al., 1975; Kaldenhoff et al., 1994; Burritt and Leung, 2003). Our results suggest that under different light conditions, two photoreceptors, phytochrome and blue absorbing photoreceptor, might influence the induction and proliferation of callus, as well as the formation of PLBs from callus of *Cymbidium* orchid. In callus cultures, phytochrome appears to play a more dominant role in the interaction between phytochrome and blue absorbing photoreceptor. In the PLB formation from callus, it appears that blue absorbing photoreceptor has a positive and phytochrome has a negative effect. However, further experiments are necessary to clarify these mechanisms.

The results of this study and the findings of other reports discussed in this paper suggest that the response of cultured tissues to different light conditions might be dependent on species or even clonal specificities as well as specific light conditions. In conclusion, our results show that LEDs can be used to improve callus cultures and PLB formation from callus in *Cymbidium* orchid. With further improvements, it is reasonable to expect that the LED irradiation systems could be used for the micropropagation of *Cymbidium* in the future.

REFERENCES


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<和文抄録>
シンビジウムのカルス誘導、カルス増殖およびカルスからのプロトコーム状球体形成に及ぼす赤色および青色発光ダイオードの影響

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シンビジウム（Cymbidium Twilight Moon 'Day Light')のプロトコーム状球体（PLB）切片からのカルス誘導、カルス増殖およびカルスからのPLB形成に及ぼす赤色および青色発光ダイオード（LED）光源の影響について調査した。まず、カルス誘導に及ぼす光源の種類および赤色/青色LED混合比の影響を調査するため、100ml容三角フラスコ内の修正Vacin・Went寒天培地（0.1 mg l⁻¹ NAAおよび0.01 mg l⁻¹ TDZ添加）にPLB切片を置床し、種々の混合比（赤色100%、赤色75%+青色25%，赤色50%+青色50%，赤色25%+青色75%，および青色100%）のLED光源下および植物育成用蛍光灯（PGF）下で培養した（いずれも16時間照耀、45 μmol m⁻² s⁻¹ PPFD）。その結果、PLB切片からのカルス誘導には赤色100%LED光源が最も有効であることが明らかとなった。また、カルス増殖は赤色75%+青色25%LED光源下で促進されたが、PGF光源下での反応と比べ有意差はなかった。これに対し、カルスからのPLB形成は赤色25%+青色75%LED光源下で最も促進された。以上のことから、LED光源はシンビジウムPLB切片からのカルス誘導、カルス増殖およびカルスからのPLB形成に対して有効であることが明らかになった。