Original article

In vitro propagation of *Lobelia chinensis* Lour. under different LED lights

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**Abstract**

The current study was performed to verify the effect of light quality especially of light-emitting diodes (LEDs) on the *in vitro* regeneration of *Lobelia chinensis*. Nodal explants were cultured on MS medium supplemented with 30 g L\(^{-1}\) sucrose and treated with red (R), blue (B), white (W) combination red/blue (1:1; RB), and red/blue/green (1:1:1; RGB) LED sources. After 4 weeks of culture regeneration capabilities, growth characteristics, and bioactive compounds were evaluated. RB treatments were found suitable for shoot regeneration, shoot growth, and root regeneration from shoots. Photosynthetic pigments, total phenolics, flavonoid content, and DPPH, ABTS, and FRAP antioxidant activities were highest with plants regenerated under RB LED. HPLC analysis of plants revealed the highest accumulation of catechin and myricetin in the plants regenerated under RB light sources.

**Keywords**

Antioxidant activities, *In vitro* regeneration, Light quality, Light emitting diodes

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Introduction

*Lobelia chinensis* Lour. is an important medicinal plant that is used in traditional Chinese medicine and it possesses varied pharmacological activities such as anticancer, anti-inflammatory, antioxidant, antipyretic, detumescent, diuretic, and, immunomodulatory effects [1]. The major phytochemicals are phenolics, flavonoids, alkaloids, and terpenes [1]. There is a huge demand for this plant for its utilization in traditional Chinese and oriental medicine. The natural resource of this plant has dwindled and there are also instances of adulterants in *L. chinensis* herbal products [2]. Therefore, in the current study, we applied plant tissue culture methods for the production of *L. chinensis* biomass and utilization of biomass for pharmaceutical and cosmeceutical uses.

Light is one of the prominent environmental factors that affect the plant’s growth, development, and physiological processes. Light intensity, quality, and photoperiod all are reported to influence the growth and physiological characteristics of the plants. In research and commercial laboratories, light emitting diodes (LED) are taking the place of conventional light sources like fluorescent, metal halide, high-pressure solid, and incandescent lights due to their distinctive qualities such as long life, low energy consumption, small mass and volume, low radiant heat output, and their capacity to emit a narrow spectrum of light [3].

The influence of monochromatic LED light sources such as red, blue, and green and their combination have been experimented on varied plants [3]. However, there hasn’t been enough focus on the application of wavelength-specific LEDs to influence morphogenesis, micropropagation, and the accumulation of bioactive compounds in regenerated plants. Additionally, because plant responses to certain environments are extremely genotype-dependent, each species, variety, growth stage, and tissue type must be evaluated separately [3]. To the best of our knowledge, there have been no studies on the micropropagation of *L. chinensis* and the effect of light quality on *in vitro* morphogenesis, and growth of plantlets. In the current study, we assessed the micropropagation potential, growth of plantlets, and accumulation of antioxidants and bioactive compounds in regenerated plants of *L. chinensis* grown *in vitro* under different LED treatments.

Materials and Methods

1. Plant material

*Lobelia chinensis* Lour. stem segments containing one or two nodes and alternate leaves without roots (1.5 cm in length) were collected from stock plants of *Lobelia chinensis* which were maintained in the greenhouse at Chungbuk National University, Korea. The explants were surface disinfected with 1% (w/v) bavistin and then washed for five minutes in 10% (v/v) Tween-20. Following surface sterilization with 0.1% (w/v) aqueous mercuric chloride (HgCl₂) for a period of 2-3 minutes, the explants were placed in 95% alcohol for 20-30 seconds. The explants were then cultured on MS medium [4] supplemented with 30 g L⁻¹ sucrose. Prior to autoclaving 2.4 g L⁻¹ gelrite was added and the pH of the medium was set at 5.7 with 1N HCl or NaOH and then autoclaved for 15 minutes at 15 psi and 121°C. All of the chemicals were of an analytical grade (Duschefa, Harlem, The Netherlands). 15 mL of medium was distributed to 60 mm x 15 mm Petri dishes. Explants were cultivated horizontally on the medium surface, dishes were sealed with parafilm, and cultures were incubated in a tissue culture room at a temperature of 25°C, with a 16 light/8 dark photoperiod that provided 40 µmol m⁻² s⁻¹ of irradiance, cool fluorescent lamps, and 60% relative humidity. Explants were subcultured once in four weeks.

2. Light Quality

To investigate the effect of light quality on the regeneration of plants, we used light sources (GF-320s; Good Felling Co. Ltd., Seong-nam, Korea). The cultures were placed under four different light sources: red (R), blue (B), red1/blue1 (RB), and red1/green1/blue1 (RGB), and white (W) LED was used as control. The cultures were maintained under 60 µmol m⁻² s⁻¹ and as spectral energy sources, the emission of blue LED was 440 nm, red was at 650 nm, and green was at 550 nm. The red or blue LED was provided with a 60 stick/400 tip (GFLE-102R) respectively, tip size: 3 mm, stick width: 100 mm, stick length: 290 mm, tip interval: 9.9 mm; red + blue LED was provided with a red 30 stick/400 tips and blue 30 stick/400 tip; red + blue + green LED provided with a red 20 stick/400 tip, blue 20 stick/400 tips, and a green 20 stick/400 tips used to arrange the panel. The light intensity and spectral parameters were adjusted using an LI-250A light meter with a Q50604 (LI-COR, USA).

3. Data collection

All the cultures were maintained for four weeks and data on the number of shoots per explant, shoot length (cm), leaf area (mm²), number of roots (per plant), root length (cm), and fresh weight (mg/explant) were documented.

4. Estimation of chlorophyll and carotenoid content

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid of *in vitro* regenerated plants were assessed by using 200 mg fresh weight of tissue samples from the third leaf from the top of plantlets and were subjected to extraction using 80% acetone. The absorbance was measured using a
spectrophotometer (Libra S22, Biochrome Ltd., Cambridge, UK) at the following wavelengths maxima (Amx): Chlorophyll a at 663 nm, chlorophyll b at 645 nm, and total carotenoids at 470 nm.

5. Preparation of plant extract

The dried samples were refluxed (LS-2050-S10, LS-TECH, Korea) with 30 mL 80% ethanol at 80 °C for 1 h and passed through filter paper (Advantec 110 mm, Toyo Roshi Kaisha Ltd., Japan). The final volume of the solution was set at 30 mL using 80% ethanol.

6. Estimation of total phenolic content

Total phenolic content (TPC) was estimated by using the Folin Ciocaltu reagent method [5]. Briefly, a known amount of sample was taken and made up to 3 mL with distilled water, and 0.1 mL of 2 N Folin Ciocaltu reagent was added, followed by incubation for 6 minutes, and then 0.5 mL of 20% Na₂CO₃ was added to each tube. Tubes were kept in warm water for 30 minutes and the absorbance was read at 760 nm using a UV-visible spectrophotometer. Gallic acid was used as the standard compound.

7. Estimation of total flavonoid content

The flavonoid content of extracts was analyzed as per the method of HARBORNE [5]. To brief, 0.1 mL of extract was taken and made up to the volume to 3 mL with distilled water followed by the addition of 0.15 mL of 10% AlCl₃ and 2 mL of 1 M NaOH after 5 min of incubation at room temperature. Solutions were vortexed and absorbance was measured at 510 nm. Catechin was used as standard.

8. Analysis of antioxidant activities

8.1. 2,2 Diphenyl 1 picrylhydrazyl (DPPH) radical scavenging assay

Extract (0.1 mL) was added with 1.9 mL of 0.1 mM DPPH solution prepared in ethanol. The tubes were vortexed and incubated in the dark for 15 min. The discoloration of the DPPH solution was measured at 517 nm against ethanol as blank using a UV-visible spectrophotometer. Gallic acid was used as standard and the activity of the extracts was expressed as mg gallic acid equivalent (GAE)/g extract [6].

8.2. 2,2’-azino-bis (3-ethylbenothiazoline-6-sulphonic acid (ABTS) assay

The ABTS solution was prepared by mixing 7 mM of ABTS and 2.45 mM potassium persulfate in a ratio of 1:1 and stored in the dark for 24 h. At the time of analysis, the ABTS solution was diluted with phosphate buffer (pH 7.3) to obtain the value of 0.70 at 732 nm. Fifty microliters of the extract were added to 950 microliters of diluted ABTS solution and the mixture was allowed to stay in the dark for 10 min then absorbance was measured at 732 nm using UV-visible spectrophotometry. Antioxidant activity was expressed in percentage i.e., ABTS radical scavenging activity = absorbance of control solution-absorbance of sample solution/absorbance of control solution x 100 [6].

8.3. Ferric reducing antioxidant power (FRAP) assay

FRAP reagent was prepared by mixing 300 mM acetate buffer of pH 3.6, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM FeCl₂.6H₂O in the ratio 10:1:1. 0.2 mL of extract was added with 3 mL of FRAP reagent, tubes were vortexed and incubated for 6 min at room temperature, and absorbance was measured at 593 nm using a UV-visible spectrophotometer. Ascorbic acid was used as standard and activity is expressed as mg ascorbic acid equivalent (AAE)/g extract [6].

9. Quantification of phenolic compounds using high-performance liquid chromatography (HPLC)

Extraction and analysis of phenolic compounds were carried out by the procedure of BURIN et al. [7]. The powdered sample (0.1 g) was mixed with 10 mL of 80% ethanol and the extract was obtained by ultrasonication as explained above. The extract was concentrated using nitrogen gas dissolved in 0.5 mL 80% ethanol and used for analysis. The extract was filtered through a membrane filter (0.45 µm) and used for analysis. HPLC equipment (2690 Separation Module, Waters Chromatography, Milford, USA) included a photodiode array detector (PDA), and compound separation was performed using a Fortis C18 column (5 µm, 150 x 4.6 mm). The mobile phase consists of acetic acid and water (1:99 v/v) (solvent A) and acetic acid and acetonitrile (1:99 v/v) (solvent B) and was filtered using Whatman Glass microfiber filters before use. The flow rate was 1.0 mL.min⁻¹ and the column temperature was 25°C. The peaks were detected at 280 nm and compounds were identified and quantified based on the retention time of standards and peak areas.

10. Statistical analysis

All experiments were conducted in a completely randomized design. Five replicate cultures were maintained for each treatment and mean values of replicates were presented as results. Three different readings were taken with spectrophotometric analysis. HPLC analysis was carried out with three replicate samples. The experimental data were subjected to analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMR) at p ≤ 0.05. All data analysis was carried out using IBM SPSS version 22.0.
Results

Regeneration of plants with different LED light sources and growth characteristics

Shoot regeneration of *L. chinensis*, the growth of shoots, and the rooting of shoots were significantly influenced by the types of LED light conditions to which the culture was exposed (*Table 1 and Figure 1*). The number of shoots regenerated from nodal explants was 4.1 and 4.8 per node plant with the cultures exposed to R and B LED, whereas the optimum shoots of 5.8 per explant were recorded with RB LED treatments. Similarly, a maximum of 19.1 roots was regenerated per shoot with RB treatments. The fresh weight of plantlets was also the highest (862.0 mg/plant) with cultures exposed under RB LEDs. Leaf area was highest with *L. chinensis* plants which are regenerated under B LEDs (*Table 1 and Figure 2*).

Analysis of metabolites accumulation and antioxidant activities

Analysis of photosynthetic pigments from *L. chinensis* leaves showed that plants regenerated under B LED possessed 19.8 mg g⁻¹ fresh weight (FW) of chlorophyll a, 6.6 mg g⁻¹ FW of chlorophyll b, 26.4 mg g⁻¹ FW of total chlorophyll, and 5.3 mg g⁻¹ FW of carotenoids. The plants that were grown in R, RB, RGB, and W LED sources also possessed similar levels of chlorophyll and carotenoid contents (*Figure 3*).

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**Table 1.** Effect of light quality on regeneration of plants of *Lobelia chinensis* after 4 weeks of culture

<table>
<thead>
<tr>
<th>Light quality</th>
<th>No. of shoots/node</th>
<th>Shoot length (cm)</th>
<th>Leaf area (mm²)</th>
<th>No. of roots/plant</th>
<th>Root length (cm)</th>
<th>FW (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
<td>4.1b</td>
<td>2.3bc</td>
<td>14.5c</td>
<td>18.0ab</td>
<td>1.9c</td>
<td>590.0c</td>
</tr>
<tr>
<td>R</td>
<td>4.1b</td>
<td>3.1a</td>
<td>23.5b</td>
<td>17.1b</td>
<td>2.0bc</td>
<td>788.0ab</td>
</tr>
<tr>
<td>B</td>
<td>4.8ab</td>
<td>2.0c</td>
<td>36.2a</td>
<td>10.9c</td>
<td>2.0bc</td>
<td>755.0ab</td>
</tr>
<tr>
<td>RB</td>
<td>5.8a</td>
<td>2.4bc</td>
<td>35.5a</td>
<td>19.9a</td>
<td>2.3ab</td>
<td>862.0a</td>
</tr>
<tr>
<td>RGB</td>
<td>5.2ab</td>
<td>2.5b</td>
<td>30.6a</td>
<td>15.1b</td>
<td>2.4a</td>
<td>658.0bc</td>
</tr>
</tbody>
</table>

Nodal segments were cultured on Murashige and Skoog (MS) medium supplemented with 30 g L⁻¹ sucrose.

W: White; RGB: Red1: Green1: Blue1; RB: Red1: Blue1; R: Red; B: Blue. Data represent mean values (n=10).

Mean different letters differ significantly from each other per DMRT (*p* ≤ 0.05).
The total phenolic and flavonoid content of the plants grown under different LEDs was investigated and results are presented in Figure 4. We found that the total phenolic contents were highest in the plants that were grown under RB mixed light treatments (23.75 mg GAE g\(^{-1}\) DW) followed by R LED (20.10 75 mg GAE g\(^{-1}\) DW) and RGB (17.53 75 mg GAE g\(^{-1}\) DW) treatments. The total flavonoid content was highest in plants cultivated under RB LED (3.96 mg CE g\(^{-1}\) DW) followed by RGB treatment (2.76 mg CE g\(^{-1}\) DW).

We have recorded the potent antioxidant activity of plant extracts that were cultivated under RB LEDs as depicted by ABTS, DPPH, and FRAP assays (Figure 5). The antioxidant assay showed the highest scavenging ABTS (99.17%), and DPPH (31.24%) radicles by the plant extracts grown under RB treatments. Similarly, plants grown under RB LED exhibited the highest FRAP activity (19.95 mg/g) compared to the plants grown under other light sources.
Analysis of phenolic compounds using HPLC

We evaluated the phenolic compounds present in the which were regenerated under different LED treatments through HPLC analysis and the results are presented in Figure 6. The phenolic compounds such as protocatechuic acid, catechin hydrate, phloretic acid, rutin, ferulic acid, salicylic acid, naringin, myricetin, luteolin, quercetin, apigenin, kaempferol, and biochanin were detected in the extracts of plants grown under different LEDs. Only myricetin and catechin were in higher concentrations and again plants that were regenerated under RB LED had 4.30 µg⁻¹ g DW and 6.55 µg⁻¹ g DW respectively (Figure 6).

Discussion

In the current study, the in vitro regeneration potential of L. chinensis and growth characteristics were optimum with the plants regenerated under RB light when compared to monochromatic R or B or RGB combination (Table 1 and Figures 1 and 2). It was reported that the different spectral qualities of light stimulated plant morphogenesis in vitro,
Figure 6. Amount of myricetin and catechin hydrate obtained from *Lobelia chinensis* plants grown under different LED treatments. W: white, R: red, B: blue, RB: red1: blue1, RGB: red1: blue1: green1. Data are means ± standard deviation (n = 5). Data are means ± standard deviation (n = 5).

and also demonstrated varied physiological and morphological responses [3]. Previous studies have revealed enhanced shoot multiplication *in vitro* and shoot growth in *Gerbera jamesonii* cv. Dura [8] under RB treatments compared to monochromatic R or B blue light sources. However, better shoot regeneration was reported under R LED treatments in *Stevia rebaudiana* [9]. These results demonstrate that the morphogenetic response differs in different species and this might be due to variation in endogenous growth regulator levels on exposure to varied light treatments. The results of our experiments showed that RB treatment resulted in the highest induction of roots from shoots (Table 1). These results are concurrent with *Gerbera jamesonii* cv. Shy Pink [10]—wherein RB light sources induced the highest root induction.

Our results demonstrated that blue light is responsible for better leaf growth and the highest leaf area was recorded with plants grown under blue light sources (Table 1). Similar to the current results B LEDs favored the growth of leaves regenerated *in vitro* in *Gerbera jamesonii* cv. Shy Pink [10]. The results of our study have demonstrated the highest biomass accumulation (862.0 mg/plant) was with the plants regenerated under RB LEDs. Earlier reports have shown that the biomass of plants cultivated under different light sources varied considerably. For example, while fluorescent light treatments were good enough for biomass accumulation in *Gerbera jamesonii* cv. Dura plants [8]. The results of the study revealed that optimum chlorophyll and carotenoid contents were with the plants regenerated under B LED light (Figure 3). It was reported that the positive effect of chlorophyll synthesis in *Rehmannia glutinosa* [11] plants grown under R light, while in *Lectuca sativa* plants blue light influenced the accumulation of carotenoids [12].

*L. chinensis* plants that were regenerated under RB LED possessed the highest amount of phenolics, and flavonoids (Figure 4), and extracts from these plants showed higher levels of antioxidant activities (Figure 5). TAULAVUORI et al. [13] suggested that both B and R light may be needed to regulate the accumulation of phenolics in *Ocimum basilicium*. Therefore, we believe that both B and R light may be needed for the regulation biosynthesis of phenolics and flavonoids in *L. chinensis*.

HPLC analysis of plants regenerated under RB LED showed the presence of varied phenolic compounds including protocatechuic acid, catechin hydrate, phloretic acid, rutin, ferulic acid, salicylic acid, naringin, myricetin, luteolin, quercetin, apigenin, kaempferol, and biochanin, whereas concentrations of myricetin and catechin were highest among them (Figure 6). Myricetin and catechin are the major flavonoids found in several species and are known for varied biological activities [14,15]. Therefore, the *L. chinensis* plant biomass produced *in vitro* could be used for varied therapeutic uses.

Conclusion

In this study, we investigated the effect of light quality on *in vitro* regeneration, growth, and accumulation of bioactive compounds in *Lobelia chinensis*. The results showed that RB LED treatment is superior for plant regeneration as the plants regenerated under this light displayed enhanced vegetative growth and also possessed higher concentrations of phenolics and flavonoids. These findings suggest the efficacy of LED light sources in plant tissue culture laboratories in place of traditional fluorescent lamps.

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LED effects on micropropagation of L. chinensis

Conflicts of Interest

The authors declare no conflict of interest.

References