Blue and Red Light-Emitting Diodes with or without Sucrose and Ventilation Affect in Vitro Growth of *Rehmannia glutinosa* Plantlets

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Single-node, in vitro cuttings of *Rehmannia glutinosa* were transplanted to MS basal media and grown for 30 d. Plantlets were grown under various culture conditions: four different light qualities (red LEDs, blue LEDs, mixed LEDs, and fluorescent); with sucrose (30 mg·L^{-1}) or without (0 mg·L^{-1}); with air exchanges (3.5 h^{-1}) or without (0.1 h^{-1}). Highest dry weights were obtained from plantlets under blue LEDs with 3.5·h^{-1} air exchanges. Light source did not affect shoot elongation in ventilated conditions, but without ventilation, the shoots of plantlets under red LEDs were twice as long as for plantlets growing under other types of lighting. Plantlets grown without sucrose showed little difference in photosynthesis under any of the tested light qualities. In contrast, the photosynthetic rate of those in the sucrose-containing media varied according to light source.

Keywords: Air exchanges, Environmental condition, Growth response, Light source, Photosynthetic characteristics

The photosynthetic ability of in vitro plantlets (Fujiwara et al., 1987) can be improved by increasing the light intensity and CO_2 concentration in the growth environment (Kozai et al., 1990). Light quality also plays an important role in photosynthesis, influencing the way in which light is absorbed by the chlorophyll. The light sources generally used for plant growth are fluorescent, metal halide, high-pressure sodium, and incandescent lamps. However, these sources contain unnecessary wavelengths that are of low quality for promoting growth.

Recently, light-emitting diodes (LED) have been developed for accelerating plantlet growth. Their effects on chlorophyll synthesis (Tripathy and Brown, 1995), photosynthesis (Tennessen et al., 1994), and morphogenesis (Hoenecke et al., 1992) have been studied in a variety of plants. Compared with traditional lamps, the improved features of the LED include smaller mass and volume, as well as a longer life (Bula et al., 1991; Brown et al., 1995).

Light intensity (PPF) greatly affects photosynthesis, respiration, and morphology of *Rehmannia glutinosa* plantlets (Cui et al., 2000; Seon et al., 2000). However, no reports are available about the effect of light quality on growth of this species. The objective of our study was to determine how growth parameters of *R. glutinosa* plantlets were affected by light source (LEDs vs. fluorescent lamps), in combination with supplemental sucrose and ventilation.

Shoot tips of *R. glutinosa* Libosch were cultured on agar media. The basal media consisted of MS major salts and Fe-EDTA (Murashige and Skoog, 1962), Ringe and Nitsch microelements with vitamins (Ringe and Nitsch, 1968), supplemented with 1.0 mg·L^{-1} BA and 0.3 mg·L^{-1} IAA to induce shoots. The pH was adjusted to 5.8 with 0.1 N NaOH before the media were solidified with 0.8% (m/v) bacto agar. Regenerated shoots were propagated on MS basal media at four-week intervals, then cut into 2 cm, single-nodes. Each sample was placed in a 130 mL glass test tube containing 20 mL of MS basal medium with either 30 g·L^{-1} sucrose or no sucrose.

The single node cuttings were cultured in a growth chamber. Environmental conditions were maintained at 25°C, 70% relative humidity, 2000 μmol·mol^{-1} CO_2, and 3.5 air exchanges per hour. To provide for air exchange, 0.2 μm microfilters were attached to polypropylene film as stoppers. One week after culture, the single-node stems were exposed to one of four light source treatments (blue LED; red LED; mix of half blue and half red LED; or fluorescent lamps) with a 16-h photoperiod and grown under these test conditions for three weeks. PPF was maintained at 100 μmol·m^{-2}·s^{-1} by adjusting the distance between the test tubes and the lamps. Environmental conditions inside the chamber were monitored daily with a data logger Model LI-1000 (LI-COR, Lincoln, NE,

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USA).

A total of 768 blue LEDs and 768 red LEDs were arranged and mounted on the ceiling of a 280 mm × 310 mm panel as the separate blue and red light sources. The mixed LEDs comprised 384 each of the blue and red LEDs, which were arranged and mounted as described above. As the fourth treatment, two cool-white fluorescent lamps were placed 15 cm above the test tubes. Net photosynthetic rates, chlorophyll content, shoot lengths, as well as shoot fresh and dry weights were recorded after 30 days of culture. To determine dry weight, the leaves and stems were oven-dried at 70°C until they reached constant mass. CO_2 concentrations inside and outside the tubes were measured after 30 d of culture, using a gas chromatograph Model HP 6890 (Hewlett Packard, Los Altos, CA, USA). Net photosynthetic rate per plantlet was calculated according to the method of Fujiwara et al. (1987).

The spectral energy distribution of the blue and red LEDs, and of the fluorescent lamps is shown in Figure 1; blue LED had a peak emission at 466 nm, red LED at 665 nm. These wavelengths agree with the known maximum absorptions for chlorophyll *a* and *b*, thereby indicating optimum photosynthetic efficiency (McCree, 1972). Tanaka et al. (1998) reported similar distributions for the spectral energy of blue (450 nm) and red (660 nm) LEDs. Therefore, the LEDs used in our experiment were considered efficient light sources, having narrow wavelength bands.

Shoot length was greatly affected by the number of air exchanges. No matter which light source was used, significantly longer shoots were produced when the number of exchanges was 3.5 h^{-1}, compared to 0.1 h^{-1} (Fig. 2). The presence of sucrose in the media

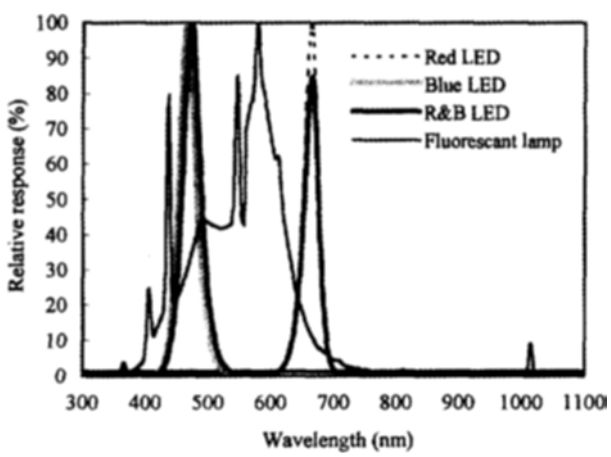

Figure 1. The spectral energy distribution of red and blue light-emitting diodes, and fluorescent lamps.

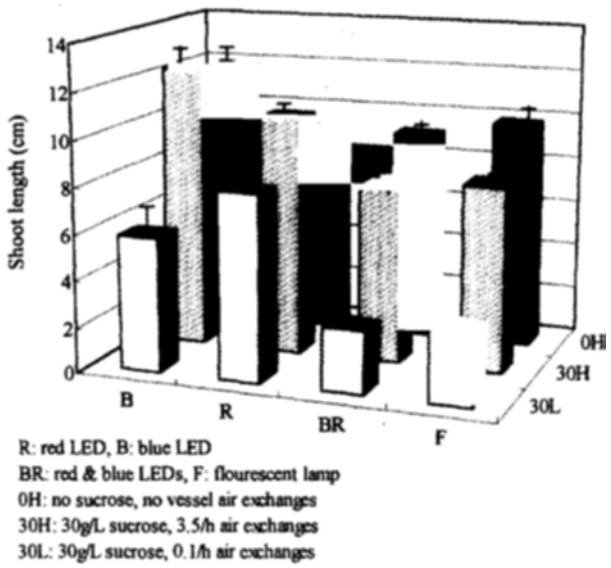

Figure 2. Effect of light source, sucrose concentration, and air exchanges on shoot length of plantlets after 30 d of culture.

did not affect shoot lengths for plantlets experiencing 3.5 air exchanges.

Although blue LED at 3.5 h^{-1} air exchanges was the most effective in promoting shoot elongation, regardless of whether sucrose was supplemented, the full effect of blue LED on shoot growth has not been determined. Blue light has been shown to promote axillary shoot growth (Chee, 1986; Chee and Pool, 1989), but Mortensen and Stromme (1987) reported less internodal elongation under blue light at high PPFs. Dougher and Bugbee (1998) suggested that plant response to blue light was dependent on PPF level and species. In future studies, species and environmental conditions should be carefully considered when determining the effect of blue and red LEDs on plant development, including shoot elongation.

Dry weights were not affected by light source, but were greatly influenced by the number of air exchanges. For all light sources, plantlets grown in culture media containing 30 $g \cdot L^{-1}$ sucrose, and with 3.5 air exchanges increased their dry weights by more than three-fold compared with those at air exchanges of 0.1 h^{-1} (Fig. 3). Plantlet growth was severely suppressed at 0.1 h^{-1} air exchanges, regardless of light source. When plantlets were grown with no supplemental sucrose and with 3.5 h^{-1} air exchanges, higher dry weights were obtained with treatments of either red LED or fluorescent lamps. Sager et al. (1982) also reported the positive effect of increased dry weights with red LED. In contrast, Sung et al. (1998) found that dry weights

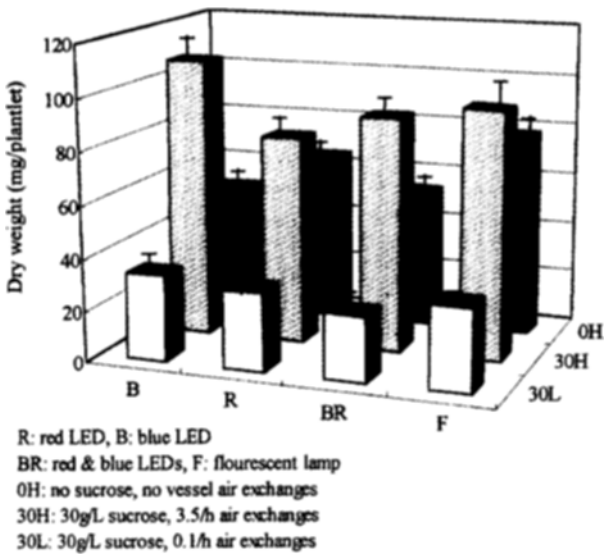

Figure 3. Effect of light source, sucrose concentration, and air exchanges on dry weights of plantlets after 30 d of culture.

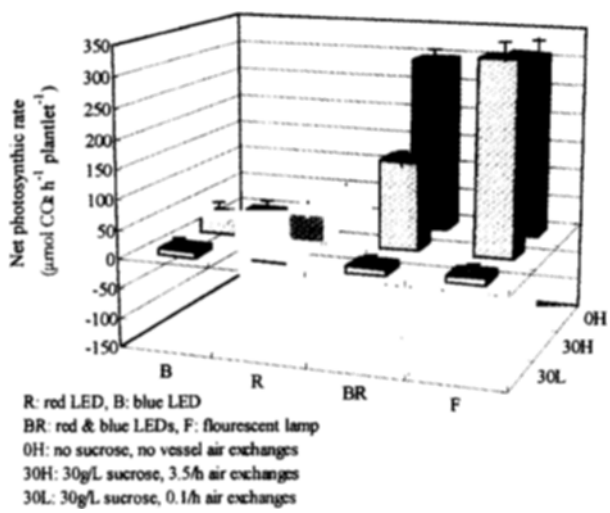

Figure 4. Effect of light source, sucrose concentration, and air exchanges on net photosynthetic rates of plantlets after 30 d of culture.

and stem lengths increased significantly under blue light at low PPF (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

Our net photosynthetic rates also were affected more by the number of air exchanges rather than by the type of light source. A remarkably higher net photosynthetic rate was observed with an air exchange of 3.5 h^{-1} for all light sources, whereas an air exchange of 0.1 h^{-1} caused diminished photosynthetic activity (Fig. 4). The net rate for plantlets grown without sucrose and with a 3.5 h^{-1} air exchange was more than six-fold higher compared with that of plantlets grown with sucrose and with an air exchange of 0.1 h^{-1}. In contrast to the high net photosynthetic rates resulting from mixed LED or fluorescent lights at 3.5-h^{-1} air exchanges, plantlets under either blue or red LEDs, at 3.5-h^{-1} air exchanges, had extremely low net photosynthetic rates. This conclusion was inconsistent with that based on shoot lengths and dry weights of the plantlets grown under the same air exchanges, and cannot be properly explained. Because errors could have been made during the measuring procedure, the testing should be repeated under the same experimental conditions.

In summary, shoot lengths, dry weights, and net photosynthetic rates of *R. glutinosa* plantlets increased two to three fold with an air exchange of 3.5 h^{-1}. At the higher number of exchanges, growth was stimulated on both the sucrose-containing and the sucrose-absent media. Therefore, sucrose supplements are not necessarily required if the number of air exchanges is increased to promote photosynthetic activity.

The pattern was the same for plantlet growth under different types of light sources. Under the 3.5-h^{-1} air exchanges, growth increased regardless of light source or presence of the sucrose supplement. Although shoot lengths under either blue or red LED were greater than under mixed LED or fluorescent lamps, the plantlets overgrew and appeared fragile (data not shown). Plantlets under mixed LED or fluorescent lamps were healthy, with normal shoot lengths.

Further experiments should be carried out to determine the physiological and morphological differences in plantlets grown under various types of LEDs.

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