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Light Quality Affects *in Vitro* Growth of Grape 'Teleki 5BB'

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We investigated the effects of various qualities of light-emitting diode (LED) light sources on the growth and carbohydrate accumulation of grape rootstock 'Teleki 5BB' cultured *in vitro*. Shoot fresh and dry weights and net photosynthetic rates were increased when plants were exposed to fluorescent lighting (control), red light, or a mixture of blue plus red, but were unaffected by blue-only radiation. Shoot elongation was significantly stimulated by red light whereas the combination of blue and red light was associated with the shortest shoots. However, the number of nodes did not differ among these treatments. Under monochromic blue or red light, sugar content and starch accumulation increased under the mixed-radiation treatment.

Keywords: blue light, light-emitting diode, mixture radiation, red light, *Vitis berlandieri* × *Vitis riparia*

Since the middle of the 19th century, high-quality rootstocks have been selected for grape production because of their compact growth habit, improved fruit pigmentation, earlier harvesting time, and proven resistance to phyloxera (Howell, 1987). As with other plant species, the vitrification of grape plus the addition of growth regulators such as cytokinin or auxin to the culture medium has led to their mass propagation *in vitro* (von Arnold and Eriksson, 1984; Densco, 1987; Gaspar et al., 1987). Grape plantlets have commonly been cultured under low light intensity and high relative humidity, and with sucrose or growth regulators supplemented in the culture medium. Thus, poor *in vitro* environments can limit their photosynthesis and growth (van Huylenbroeck et al., 2000; Shim et al., 2001).

When hairy roots of red beet (*Beta vulgaris* L.) are cultured under bioreactors, blue or far-red light qualities are more effective than conventional fluorescent lamps in enhancing not only carbohydrate accumulation but also betaxanthin and betacyanin contents (Boo et al., 2002). Shin et al. (2003) have reported that betalain synthesis can be improved if one utilizes either 1:1 blue:far-red light (B/Fr) or a higher ratio. Other researchers have also suggested that plant growth and morphogenesis are affected not only by light quality but also by phytohormone content (Palmer and Smith, 1970; Nauk and Langille, 1978).

Light is the energy source for photosynthesis and plant development. Traditionally, fluorescent, metal halide, or inflorescent lamps have been used for *in vitro* plant production. Recently, artificial lighting sources, e.g., light-emitting diodes (LEDs), with monochromic spectral regions also have been integrated into experimental *in vitro* studies of growth and morphogenesis (Kim et al., 2004; Moon et al., 2006).

An LED lighting system has several benefits including long life, no heat byproduct, small mass, and monochromic spectral quality, and thus can be easily used to promote stem elongation, lateral branching, flower stalk elongation, or variations in leaf pigment content (Bula et al., 1991; Brown et al., 1995; Miyashita et al., 1997; Tanaka et al., 1998). Light quality affects chlorophyll synthesis in wheat, as well as disease occurrence in pepper and cucumber, and photosynthesis in kudzu-vine (Schuerger and Brown, 1994; Tripathy and Brown, 1995). However, few reports have described the effect of light quality on growth, morphogenesis, or carbohydrate accumulation. Therefore, our objective here was to examine the effects of LED-provided monochromic blue and red lights, as well as their combination, on the carbohydrate content and performance of grape rootstock 'Teleki 5BB' (*Vitis berlandieri* × *Vitis riparia*) when plants were cultured without supplemental growth regulators in the medium.

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MATERIALS AND METHODS

Plant Material and Culture Conditions

Leafy single-node cuttings (10 mm long) of grape (*V. berlandieri* × *V. riparia*) 'Teleki 5BB' rootstock were cultured photomixotrophically in an MS (Murashige and Skoog, 1962) medium (70 mL per flask) supplemented with 30 g L^{-1} sucrose (pH adjusted to 5.8 before autoclaving). The explants were cultured without additional growth regulators in 500-mL (air volume) glass flasks that were capped with transparent polypropylene film and a microporous polypropylene membrane. These culturing vessels had 2.8 air exchanges per hour. Figure 1 shows our four different light treatments: 1) the control, cool-shine fluorescent lamp (FL; OSRAM 1528; OSRAM, Korea); 2) monochromic blue light (B; peak at 450 nm); 3) monochromic red light (R; peak 660 nm); and 4) a 1:1 mixture of blue and red light (BR). The B, R, and BR sources were provided by LEDs (GF-series; Goodfeeling, Korea). Photosynthetic photon flux (PPF), as determined on an empty culture shelf, was maintained at 50 ± 10 μmol m^{-2} s^{-1} for all treatments throughout the culture period. All explants were kept for 28 d in a controlled environment growth chamber at 25°C air temperature, 70% relative humidity, and 400 ± 100 μmol mol^{-1} CO_2.

Estimation of Net Photosynthetic Rate

Time courses for net photosynthetic rates (NPR) per plantlet were estimated by measuring the differences in CO_2 concentrations between the inside and outside of the cul-

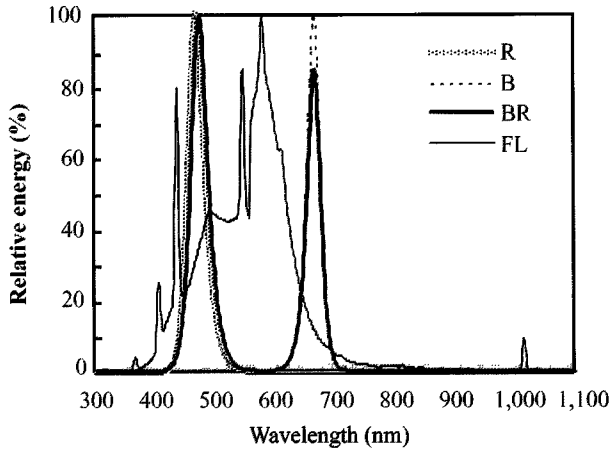

Figure 1. Spectral distributions for fluorescent lamp (FL), blue light (B), red light (R), and blue plus red light (BR) treatments.

ture vessel, taking into account the number of air exchanges and vessel air volume (Fujiwara et al., 1987). Two vessels, each containing four plantlets, were randomly selected for these determinations. CO_2 concentrations were assessed weekly with a gas chromatograph (HP 6890 Series; Hewlett Packard, USA), an HP PoraPlot Q capillary column (25 m × 0.53 mm × 30 m), and a thermal conductivity detector. Gas samples (3 mL per measurement) were taken with a syringe, and a 50:1 split ratio was maintained during the sampling period.

Carbohydrate Analysis

On Day 28, all treatment plants were harvested, and eight leaf samples were stored in liquid nitrogen. One g (fresh weight) of leaf tissue for each treatment was homogenized in 20 mL of 80% ethanol. The homogenates were centrifuged at 10,000 × g for 3 min, and the supernatant was used to analyze the soluble sugar content. The pellet was further prepared for starch analysis, and the residue was washed three times with 20 mL of 40% ethanol. Starch was then solubilized using an HCl-DMSO pretreatment (Boehringer Mannheim, 1989), and was hydrolyzed by incubation with amyloglucosidase at 60°C for 2 h. The starch concentration was quantified as the amount of glucose formed, based on a dinitrosalicylic acid assay. Formation of reducing sugars was measured spectrophotometrically at 570 nm according to the dinitrosalicylic acid method of Chaplin (1986).

Statistical Analysis

Statistical analysis was performed using the SAS System (Version 6.21; SAS, USA). Mean and standard errors served to describe differences among treatments for the parameters

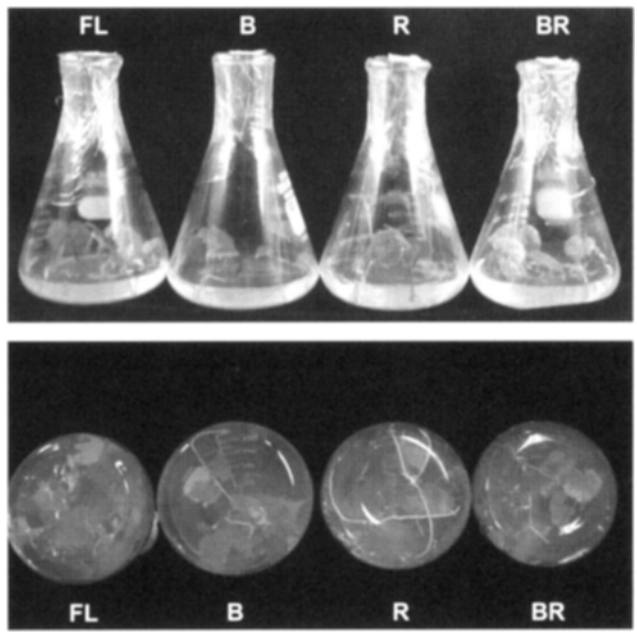

Figure 2. Grape rootstock 'Teleki 5BB' cultured under different light qualities on Day 0 (up) and Day 28 (down).

of fresh and dry weights, number of nodes, shoot lengths, net photosynthetic rates, and starch/sugar contents. Statistical significances among the mean values were assessed using ANOVA or a Duncan's multiple range test. A probability of $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

As assessed after 28 d, shoot fresh and dry weights of our grape plantlets were increased by exposure to monochromic red light (R), a mixture of blue and red radiation (BR), or conventional fluorescent lamps (FL), while plants grown under monochromic blue light (B) showed significantly lower weight increments, suggesting that this treatment was inhibitory (Fig. 2; Table 1). No significant effects on fresh weight were observed among the R, BR, and FL treatments. Ouyang et al. (2003) have demonstrated that, when *in vitro*-cultured calli of *Cistanche deserticola* are illuminated with monochromic blue light, the plants show significant increases in their fresh and dry weights. Likewise, their phenylethanoid glycoside production is better than that attained when fluorescent lamps or monochromic green, red, or yellow lights are used. Miyashita et al. (1997) have reported that the growth of single-node potato explants is affected by monochromic red light of 11, 15, 28, 47 or 64 μmol m^{-2} s^{-1}. In the current study, blue-light treatment did not promote

Table 1. Fresh and dry weights per plantlet of grape rootstock 'Teleki 5BB' cultured under different light qualities for 28 d.

Reatment	Fresh weight (mg)		Dry weight (mg)	
	Shoot	Total	Shoot	Total
Fluorescent lamp (FL; control)	370.8a*	608.5a	54.9a	76.6a
Monochromic blue light (B)	260.5b	457.8b	40.7b	54.5b
Monochromic red light (R)	412.5a	597.5a	57.2a	66.7b
1:1 Blue + red light (BR)	297.8a	499.0a	46.2a	64.2b

*Mean values followed by different letters within a column are significantly different at the 5% level, by Duncan's multiple range test (n = 8).

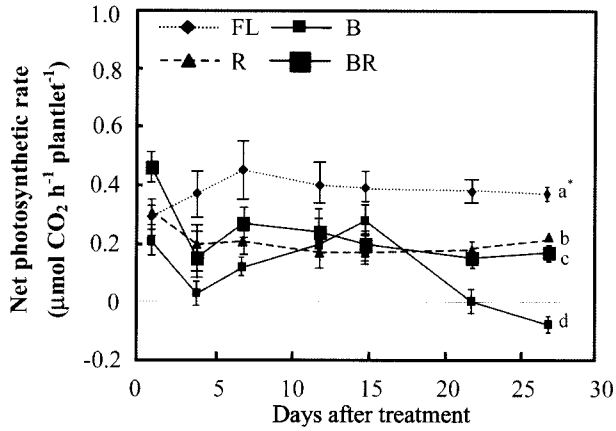

Figure 3. Time courses for net photosynthetic rate per plantlet cultured under different light qualities for 28 d. Treatment codes are described in Table 1. Mean values followed by different letters within a column are significantly different at 5% level, by Duncan's multiple range test.

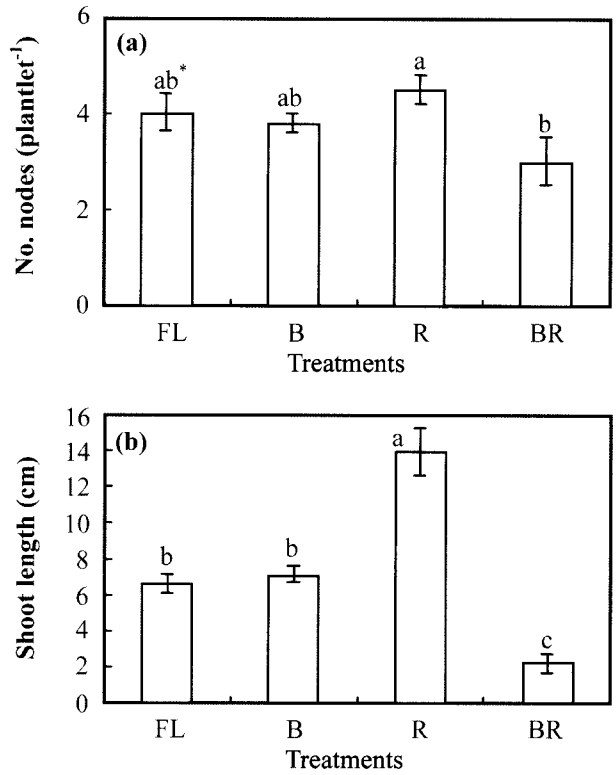

Figure 4. Number of nodes (**a**) and shoot length (**b**) per plantlet of grape rootstock 'Teleki 5BB' cultured under different light qualities for 28 d. Treatment codes are described in Table 1. Mean values followed by different letters within a column are significantly different at 5% level, by Duncan's multiple range test (n = 8).

any improvement in fresh or dry weights. However, the latter did increase when plants were exposed to continuous blue light during the culture period, suggesting that this particular parameter might be influenced not by light quality but by intensity. Kim et al. (2005) also have observed a decrease in fresh or dry weight when *Chrysanthemum* plantlets are cultured under monochromic blue light at 90 μmol m^{-2} s^{-1}. By Day 3 of our experiments, the net photosynthetic rate (NPR) per plantlet had been significantly reduced for all light treatments except FL (Fig. 3). NPR was the highest for B-treated plants within the first 15 d, but then gradually declined. Although the monochromic blue light apparently inhibited a rise in fresh and dry weights, as suggested by low measured values, the positive effect of blue light on photosynthetic rates observed here has also been described by Drozdova et al. (2001), who showed that the photosynthetic activity of radish plants is significantly increased by monochromic blue light compared with levels measured under red-light treatment. For our plants, FL treatment conferred the highest NPR on Day 28, while negative NPR values were calculated for plantlets grown under blue light. This suggests that monochromic blue exposure enhances respiratory rates while inhibiting photosynthesis during the photoperiod.

Although hypocotyl elongation was not affected by the different light qualities during the culture period, lengths of the first and second nodes were significantly increased by red light (data not shown). In fact, the second nodes of plants exposed to R were more than twice as long as those treated with FL. Mixed BR radiation was associated with shorter nodes. Red light affects internode elongation not only in grape explants but also in several flowering plants, and is a regulator of flower stalk elongation in potted *Cyclamen* (Heo et al., 2002, 2003). However, Tanaka et al. (1998) also have reported that monochromic red light promotes leaf lengthening rather than internodal growth in cymbidium plantlets. The distance between internodes on our grape plantlets tended to be shorter under BR treatment than under either B or R light. Mixed radiation as well as exposure to the monochromic blue and red lights did not increase the number of nodes (Fig. 4). Shoot-length growth was significantly inhibited by BR treatment, whereas stems under red light were more than twice as long as those under FL. A similar red-light effect on stem elongation has been described with *in vitro Pelargonium* plantlets (Appelgren, 1991). In contrast, we found no significant difference in lengths among stems treated with B, BR, or FL. We conclude that stem development in our grape plantlets under the various light qualities probably was due to individual internode elongations rather than an increase in node numbers.

On Day 15, sugar content was higher in our BR-treated plants than in those exposed to monochromic blue light (Fig. 5). Likewise, the reducing sugar content in BR plants was more than three times greater than with B or FL. However, after Day 15, these starch and reducing sugar contents markedly declined in the BR leaves. After 28 d, the monochromic blue and red lights, as well as FL, had no effect on sugar content. The level of sucrose was significantly increased under BR treatment during the culture period, but decreased with blue light. Starch accumulation in the grape plantlets was stimulated by BR and FL treatments but diminished by monochromic B or R. On Day 15, the amount of starch was more than three times greater under BR compared with the B treatment. Changes in carbohydrate contents under different light qualities are controlled by key enzyme activities related to sugar and starch metabolism in several species (van Huylenbroeck and Riek, 1995; Ouyang et al., 2003). Keiller and Smith (1989) have reported that, compared with white light alone, the supplementation of far-red light increases the soluble sugar content and decreases starch levels in radish plants. Here, we observed significantly higher carbohydrate contents on Day 15 that coincided with accelerated leaf and explant growth that required a

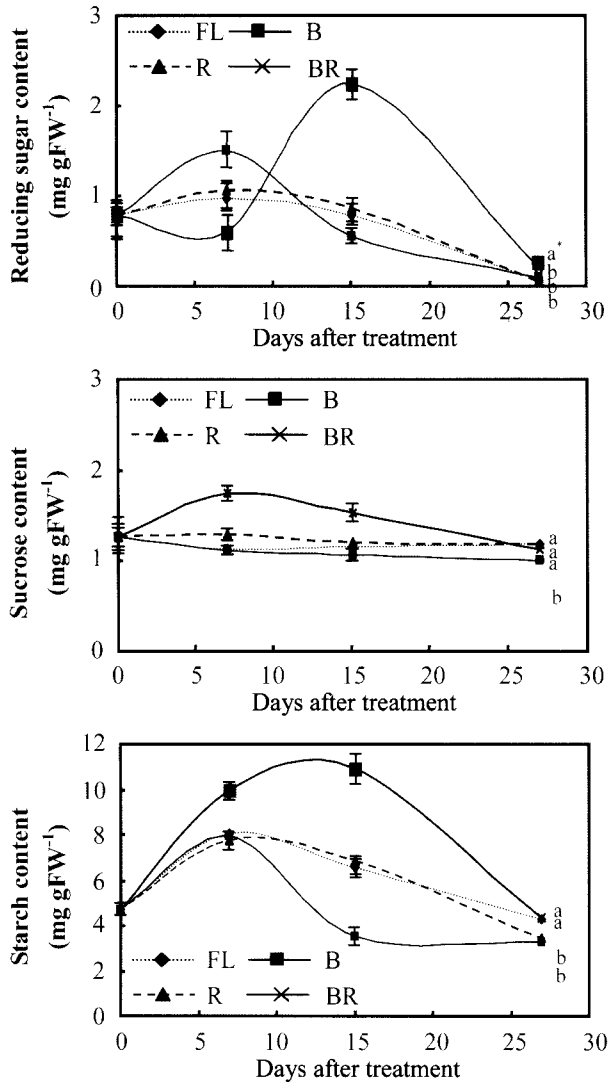

Figure 5. Carbohydrate status of grape rootstock 'Teleki 5BB' cultured under different light qualities for 28 d. Treatment codes are described in Table 1. Mean values followed by different letters within a column are significantly different at 5% level, by Duncan's multiple range test (n = 8).

greater sink for their development. Even though we did not measure the enzyme activity related to sugar metabolism that of some key enzymes, such as sucrose-*p*-synthase or adenosine-diphosphoglucose pyrophosphorylase, increased in the first 15 d concurrent with vigorous plantlet growth. Lain et al. (2002) also have shown that an LED mixture of blue and red radiation enhances the bulblet dry weights of 'Pesaro' lilies compared with their response to monochromic red or blue light (Lian et al., 2002). All of these results suggest that the significantly higher carbohydrate contents measured under BR treatment were induced by either synergistic or singular action of the photoreceptors.

When mediated by a phytochrome photoreceptor, red light increases dry weights in several plant species (Heo et al., 2002, 2003). This has also been proven in physiology and molecular biology studies. Lercari et al. (1999) have reported that, under continuous red and blue light, tomato hypocotyl cultures regenerate more shoots due to phytochrome activity. In contrast, Chee (1986) has indicated that grape shoot growth, as manifested by increases in node numbers or dry weights, can be better promoted by blue light than by red, which implies that the former inhibits the unfolding of leaves. Moreover, this blue- or red-light influence on growth can vary according to growth stage or species. Lin (2000) has noted that blue light deters *Arabidopsis* development, probably because of a cryptochrome photoreceptor. However, the action mechanism for members of the cryptochrome group, including cryptochromes 1 and 2, is not yet clearly understood.

In conclusion, the photosynthetic ability of our grape plantlets was significantly reduced by monochromic blue light compared with the red or red plus blue mixture light qualities. Moreover, red light promoted shoot elongation. Sugar and starch contents were higher under blue light in the first 15 d, as manifested by the plants' more vigorous shoot growth and higher net photosynthetic rates. In future work, researchers should not only consider the activity of key enzymes associated with sugar and starch metabolism, but also investigate the relationship between this metabolism and the action of photoreceptors such as phytochromes and cryptochromes.

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