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Effect of light emitted by diodes on growth and pigment content of black currant plantlets *in vitro*

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Summary

The effects of cool white, natural white, and warm white lights, which have a continuous spectrum throughout the region of surfactant, and blue-red light spectrum on *in vitro* growth and development of black currant (*Ribes nigrum* L.) was studied. It was demonstrated that the spectral composition of light affected length and fresh mass of shoots and roots as well as concentrations of chlorophylls, carotenoids, and anthocyanins. The plants grown under warm white light had the longest shoots $(2.5 \pm 0.2 \text{ cm})$ and fresh mass of shoots $(166 \pm 12 \text{ mg})$ and roots $(80 \pm 16 \text{ mg})$ relatively to one's grown under other light types. Under blue-red and warm white lights black currant leaves possessed the highest concentrations of chlorophyll *a* $(2.66 \pm 0.31 \text{ and } 2.17 \pm 0.14 \text{ µmol·gwm}^{-1}$, respectively), chlorophyll *b* $(1.15 \pm 0.15 \text{ and } 0.87 \pm 0.05 \text{ µmol·gwm}^{-1})$, carotenoids $(0.89 \pm 0.09 \text{ and } 0.78 \pm 0.05 \text{ µmol·gwm}^{-1})$, respectively) and anthocyanins $(1.37 \pm 0.20 \text{ and } 1.09 \pm 0.05 \text{ µmol·gwm}^{-1})$, respectively). Thus, blue-red (B:R = 1:4) and warm white lights may be used as an alternative light source for upland black currant culture systems.

Key words

anthocyanins, carotenoids, chlorophylls, LED-lighting, micropropagation

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Introduction

Light in the form of solar energy is used by green plants to assimilate carbon from carbon dioxide. Green leaves use only photosynthetically active radiation (PAR) with wavelengths of 380 - 710 nm for photosynthesis. Red (663 and 642 nm) and blue (430 nm and 453 nm) lights are the main ones affecting plant growth because they are predominantly absorbed by chlorophylls (Lefsrud et al., 2008).

Pigment composition of the photosynthetic apparatus depends on the genotype, environmental conditions and the stage of development of the plant. Plants perceive red light via phytochrome receptors (PhyA, PhyB, etc.), represented by the forms absorbing red (Pr) and far red light (Pfr). Both receptors form trigger plant responses associated with germination, elongation of the stem, leaf extension and induction of flowering (Pinho, 2008). Blue light is sensed by cryptochromes and phototropins and regulates de-etiolation, phototropism, movement of chloroplasts, endogenous rhythms, root growth, light induced stomatoplasty, redox equilibrium and levels of cyclic nucleotides (Kritsky, 1984; Macedo et al., 2011). Up to now, there are many studies on the dependence of plant growth on illumination conditions (Duong et al., 2003; Navvab, 2009; Li et al., 2010, 2013), but studies regarding the effects of different types of light on the concentrations of pigments are scarce.

Optimization of the light regime of cultured plants is progressed in parallel with the technical improvement of lighting systems. In the past, addressing the issue of artificial lighting radiation, scientists used to a great extent an empirical approach that was associated with relatively limited knowledge about the needs of plants in lighting type (Craford, 2002). Nowadays, using of light-emitting diodes (LEDs) is the most promising way to get a sample light spectrum. These diodes are cconsidered to be ideal light sources for in vitro plant cultures due to their benefits, including controlled wavelengths and intensity of lighting, spatial light energy distribution and polarization (Choi et al., 2002). These light sources are also valuable economically due to low power consumption, high efficiency of electric energy usage for light transformation, long operation life and ease of maintenance. LED-based lighting systems have successfully replaced traditional lighting providing better plant growth in several in vitro plant cultures, including cotton, bananas, grapes, strawberries, potatoes and corn (Nhut et al., 2003; Poudel et al., 2008; Li et al., 2010).

Black currant (*Ribes nigrum* L.) is propagated usually vegetatively by hardwood cuttings. This type of propagation of planting material is simple but not always optimal to get high quality planting material (Ružić and Lazić, 2006). At present, micropropagation is used to produce and multiply the planting material *in vitro*. It has a number of advantages over traditional methods of plant propagation. Micropropagation of black currant allows obtaining large quantities of healthy plants for relatively short time period. Application of *in vitro* tissue culture resulted in a generation of number black currant cultivars (Orlikowska, 1984; Wainwright and Flegmann, 1984; Vujović et al., 2012). However, there are no studies on the influence of light with different spectra on growth characteristics of black currant, pigment levels and chemical composition of *in vitro* grown plants. Therefore, present study aimed to investigate the effects of LED light on morphometric

characteristics and concentrations of photosynthetic pigments in *R. nigrum* plantlets *in vitro*.

Material and methods

Plant material and growth conditions

Black currant plantlets (cultivar 'Raduzhna'), previously established in our laboratory, were transferred to glass jars with 25 mL of Murashige and Skoog medium supplemented with 3% sucrose, 100 mg·L⁻¹ myo-inositol, 6.0 g·L⁻¹ agar and 0.1 mg·L⁻¹ indole-3-butyric acid (IBA) (Murashige and Skoog, 1962). The pH of the medium was adjusted to 5.8 prior to sterilization and cultures were maintained during 20 days in a growth room at 25 \pm 2°C, under photoperiod of 16 h of light and 8 h of darkness (50 µmol·m⁻²·s⁻¹) and one of the four lighting types described below. Each treatment was performed in two independent replications with six samples in each (n =12).

Lighting types

Ten-millimeter-long nodal shoots were excised from the proliferated shoots and cultured under different light treatments. The LED light system was obtained from Shenzhen Sanxin Lighting Co., Ltd. (Guangdong, China). Light-emitting diode (LED) array types were as follows (Figure 1):

- CW: cool white light (6000K) as control light (similar to fluorescent light);
- (2) NW: natural white light (4000K);
- (3) BR = 1:4: 20% blue light with a wavelength of 460 nm and 80% red light with a wavelength of 660 nm;
- (4) WW: warm white light (2700K).



Figure 1. Photographs of the plantlets illuminated by the LED bars: 1 – cool white light (6000K); 2 – natural white light (4000K); 3 – 20% blue light with a wavelength of 460 nm and 80% red light at a wavelength of 660 nm; 4 – warm white light (2700K)

Morphometric plant characteristics

Six plantlets of each treatment were randomly selected for the evaluation of growth characteristics after 20 days of cultivation. The fresh mass of shoots and roots was measured by a precision balance. The length of the aerial plant part was measured using a ruler from the base of stalk to the last expanded leaf.

Concentrations of chlorophyll and carotenoids

For pigment extraction, leaves were homogenized with icecold 96% ethanol (1:10, w:v) in the presence of 10 mg mL⁻¹ CaCO, to prevent pheophytinization. The homogenates were centrifuged at 8000×g for 10 min (4°C). Supernatants were collected and the pigments were repeatedly extracted twice from pellets with 1 mL ice-cold 96% ethanol and extracts were pooled. Concentrations of pigments were measured spectrophotometrically. Specific absorption coefficients for chlorophyll a, chlorophyll b, and total carotenoids were used (Lichtenthaler, 1987). Carotenoid concentrations were calculated as described previously (Gitelson et al., 2001). For anthocyanin determination, the extract was acidified with concentrated HCl to its final concentration of 1%. The anthocyanin content was measured spectrophotometrically at wavelength 530 nm and an absorption coefficient of 30 mM⁻¹·cm⁻¹ was used for calculations (Gitelson et al., 2001; Semchuk et al., 2009).

Statistical analysis

Data are presented as means \pm S.E.M. Statistical analysis was performed using Mynova computer program (version 1.3) with ANOVA followed by a Student–Newman–Keuls (SNK test). The probability value of P < 0.05 was considered to be statistically significant.

Results and Discussion

Effects of LED lighting on plant growth parameters

Different LED lighting had diverse effects on the length and fresh mass of shoots (Fig. 2, 3a) and fresh mass of roots (Fig. 3b) in black currant plantlets. The longest stems were observed in the black currant plants kept under WW light $(2.5 \pm 0.2 \text{ cm})$, while CW and NW lights provided intermediate stem length (1.9 \pm 0.2 cm and 2.0 \pm 0.2 cm, respectively), and the shortest plants were found under BR light $(1.2 \pm 0.1 \text{ cm})$ (Fig. 2). An inhibitory effect of BR LEDs on stem elongation of cymbidium, banana and zantedeschia has been demonstrated previously (Tanaka et al., 1998; Nhut et al., 2003; Jao et al., 2005). Blue light inhibited stem elongation in chrysanthemum plants (Shimizu et al., 2006). The greatest stem length of chrysanthemum plants was observed under red LEDs and far-red LEDs among six different light qualities used: fluorescent, blue LEDs, red LEDs, red and blue LEDs, red and farred LEDs, and blue and far-red LEDs (Kim et al., 2004). Warm white light contains a wide range of red and far-red light that may contribute to the intense growth of plantlets. Among the mixed light treatments, biomass of black currant shoots was significantly higher when they were grown under warm white light than under other ones used here. Thus, the WW light produced a significant increase in fresh masses of shoots (Fig. 3a) and roots (Fig. 3b) of





BR

ww

CW

NW

Note: CW – cool white light (6000K); NW – natural white light (4000K); BR – 20% blue light at a wavelength of 460 nm and 80% red light at a wavelength of 660 nm; WW – warm white light (2700K). Data are presented as means \pm S.E.M, n = 9-12.

*Significantly different from the cool white light treated group of plants (P < 0.05) using ANOVA followed by a SNK test

black currant. In this study, the shoots had fresh masses of 75 \pm 11, 112 \pm 9, 166 \pm 12 and 64 \pm 10 mg under CW, NW, WW and BR lights, respectively (Fig. 3a). Exposure of plants to CW, NW and BR lights led to 55%, 33%, and 62% lower fresh masses of shoots as compared to WW light group (Fig. 3a). Fresh masses of the shoots were also lower by 33 and 43% with CW and BR lights, respectively, as compared to NW light (Fig. 3a). The stimulating effect of WW light on the fresh mass of black currant platelets is likely due to its broad spectrum, which might penetrate better through the canopy than red-blue LED light and force photosynthesis. Similar results were reported by Lin et al. (2013) where biomass of lettuce was greater under red-blue-white light than under red-blue one. This indicated that red or blue light responses might also depend on the minor components of spectrum. By contrast, Silva et al. (2014) showed that the sugarcane plantlets showed better performance in fresh biomass of plants when they were cultivated under higher intensity blue light and equal proportion of blue and red LED light. Li et al. (2010) reported that fresh mass and dry mass of upland cotton plantlets in vitro had the greatest mass when cultured under blue-red (1:1) LED light, and the lowest one under fluorescent lamp. The best growth of the stem under blue-red light was obtained in other plant species such as lily (B:R = 1:1), banana (B:R = 4:1), strawberry (B:R = 3:7), and chrysanthemum (B:R = 3:7)1:1) (Lian et al., 2002; Duong et al., 2003; Nhut et al., 2003; Kim et al., 2004).



Figure 3. The effects LED lighting on fresh masses of shoots (A) and roots (B) of black currant

Note: CW – cool white light (6000K); NW – natural white light (4000K); BR – 20% blue light at a wavelength of 460 nm and 80% red light at a wavelength of 660 nm; WW – warm white light (2700K). Data are presented as means \pm S.E.M, n = 9-12.

*Significantly different from the cool white light treated group of plants (P < 0.05) using ANOVA followed by a SNK test

Roots play an important role in the plant, providing other plant parts with water and minerals. Therefore, well-developed roots can enhance normal plant development. In our experiments, the roots had the highest fresh mass when plants were rown under WW light (80 \pm 16 mg). Plants with intermediate root masses were grown under NW light (15 \pm 6 mg). The lowest fresh mass of roots had the plantlets grown under CW and BR lights (4 \pm 1 and 2 \pm 1 mg, respectively) (Fig. 3b). Li et al. (2010) showed that red LED seemed to be the most suitable for the root growth of upland cotton plantlets. Red light induced the root formation of in vitro cultured flamingo plantlets (Budiarto, 2010). However, root growth of in vitro cultured Doritaenopsis plants was optimal under blue LEDs in comparison with red light (Shin et al., 2008). Warm white light contains an extended range of red light that could positively affect growth and development of roots. Light may influence root mass through photomorphogenic action, i.e., root elongation can be phytochrome controlled (Vinterhalter et al., 1990).

Therefore, according to our data on the growth of shoots and roots, one can conclude that WW LED light is the most favorable for the development of black currant in comparison with lights with other spectra. This may be due to the fact that this light has an optimal ratio between blue and red spectra. However, this effect also might depend on the plant species or even cultivars (Li et al., 2010, 2013).

Effects of LED lighting on chlorophyll content

The content of chlorophylls in plants depends on many factors, including light wavelength and intensity (Brant et al., 2011). In the current study, the plantlets illuminated with blue-red light (1:4) had higher content of chlorophylls as compared to ones under CW, NW or BR light illumination conditions (Fig. 4). Fig. 4 shows influence of light type on concentrations of chlorophylls a and b. The concentration of chlorophyll a was the highest in the plants grown under BR (2.66 \pm 0.31 µmol·gwm⁻¹) and WW lights $(2.17 \pm 0.14 \,\mu\text{mol}\cdot\text{gwm}^{-1})$ compared to the values under other light types (Fig. 4a). The concentration of chlorophyll a was lower by 30% and 35% in the black currant plantlets grown under CW and NW lights as compared to those kept under BR light (Fig. 4a). The plantlets cultivated under BR LED treatment showed the highest concentration of chlorophyll b (1.15 \pm 0.15 µmol·gwm⁻¹) as compared to those cultivated under other LED light systems studied (Fig. 4b). It is known that red light can promote photosynthesis (Saebo et al., 1995; Moon et al., 2006; Lin et al., 2011). By contrast, Li et al. (2010) showed that upland cotton plantlets had the highest chlorophyll content under blue LED, and this light source might be beneficial to provide high chlorophyll level in upland cotton plantlets. Plantlets of Saccharum officinarum L. showed higher total chlorophyll content under white LED light than under blue-red LED light (Silva et al., 2014).

Effects of LED light on carotenoid and anthocyanin concentrations

Carotenoids are well-known as light-harvesting pigments, which absorb light in the blue-green region of the solar spectrum and transfer the absorbed energy to chlorophylls. They also play a vital role in protection of photosystem II (PSII) from deactivation by triplet chlorophyll and singlet oxygen (Kim et al., 2005). The highest concentrations of carotenoids were revealed in plantlets reared under BR and WW LEDs ($0.89 \pm 0.09 \ \mu\text{mol}\cdot\text{g}\text{wm}^{-1}$ and $0.78 \pm 0.05 \ \mu\text{mol}\cdot\text{g}\text{wm}^{-1}$, respectively), intermediate one – in plantlets under CW LED ($0.66 \pm 0.06 \ \mu\text{mol}\cdot\text{g}\text{wm}^{-1}$) and the lowest one – under NW LED lights ($0.59 \pm 0.04 \ \mu\text{mol}\cdot\text{g}\text{wm}^{-1}$) (Fig. 5a). Our findings are consistent with the data of Johkan et al. (2010) who found that carotenoid concentration in the leaves of lettuce seedlings treated with blue-red LED lights increased from 263 $\ \mu\text{g}$ mg⁻¹ under white fluorescent lamp to a greater than 304 $\ \mu\text{g}$ mg⁻¹ dry mass during 17 days of growth (Johkan et al., 2010).

In our experiments, the concentration of anthocyanins reached the highest values in plantlets kept under WW and BR lights (1.09 \pm 0.05 and 1.37 \pm 0.20 µmol·gwm⁻¹, respectively). Exposure to CW light resulted in 19% and 36% lower concentrations of anthocyanins as compared to WW and BR light systems, respectively (Fig. 5b). We observed by 17% and 34% reduced concentrations of anthocyanins in plantlets cultivated under NW light as compared to WW and BR lights (Fig. 5b). Choi et al. (2015) also showed that BR light was effective in the enhancement of anthocyanin levels in the fruits of strawberry plants (Choi et al., 2015). Red LED light (660 nm) can stimulate accumulation of anthocyanins in red leaf cabbages in contrast to blue or green LED wavelengths (Mizuno et al., 2011). The content of anthocyanins was also increased in salad seedlings under BR light (Johkan et al., 2010).



Figure 4. Concentrations of chlorophyll *a* (A) and chlorophyll *b* (B) in black currant plantlets cultivated in MS medium supplemented with 0.1 mg·L⁻¹ IBA during 20 days under different LED lights

Note: CW – cool white light (6000K); NW – natural white light (4000K); BR – 20% blue light at a wavelength of 460 nm and 80% red light at a wavelength of 660 nm; WW – warm white light (2700K). Data are presented as means \pm S.E.M, n = 9-12

*Significantly different from the cool white light treated group of plants (P < 0.05) using ANOVA followed by a SNK test

Potential mechanisms responsible for the effects of different light treatments on plant growth and level of pigments

In the current study, WW light influenced the growth period, accelerating plant development. Morphological parameters indicate that plants subjected to WW light grew faster than those under CW, NW or BR light illumination conditions (Figures 2, 3). Warm white light (WW) light had a positive effect on the stem length, masses of shoots and roots. These effects could be related to the stimulation of phytochrome signaling by WW. The latter light contains a large amount of red and far red components, which could activate phytochromes. The phytochromes are photoreceptors existing in two states, one sensitive to light with wavelength of 660 nm [red light absorbing (Pr) state] and another sensitive to 730 nm [far red light (Pfr) state]. Phytochrome in the Pfr state triggers the phosphorylation and subsequent proteolytic degradation of phytochrome interacting factors (PIFs) that controls directly or indirectly expression of genes responsible for light-dependent morphogenesis (Ni et al., 2014; Shin et al., 2016) (Fig. 6).

Phytochromes are inactivated by red light, which decreases PIF degradation and start of stem elongation (Leivar and Monte, 2014). Low red : far red ratio activates PIF4, PIF, and PIF7 factors that promote shoot elongation (Li et al., 2012; de Wit et al., 2016b),



Figure 5. Concentrations of carotenoids (A) and anthocyanins (B) in black currant plantlets cultivated in MS medium supplemented with $0.1 \text{ mg} \cdot \text{L}^{-1}$ IBA during 20 days under different LED lights

Note: CW – cool white light (6000K); NW – natural white light (4000K); BR – 20% blue light at a wavelength of 460 nm and 80% red light at a wavelength of 660 nm; WW – warm white light (2700K). Data are presented as means \pm S.E.M, n = 9-12.

*Significantly different from the cool white light treated group of plants (P < 0.05) using ANOVA followed by a SNK test

in particular through regulation of biosynthesis and transport of auxins (de Wit et al., 2016a). In turn, auxins play an important role in the elongation responses during shade avoidance (Kohnen et al., 2016; Müller-Moulé et al., 2016).

The induction of stem elongation by far red light is confirmed by data of Brown et al. (1995), which showed that this light conditions led to taller stems with a greater mass in sweet pepper (*Capsicum annuum* L.) in comparison with red light treatment. We also observed an increase in the length of the black currant under the WW light compared with BR light. The phenomenon could also be due to the presence of higher amount of yellow wavelengths in WW compared to the CW and NW. Pinho et al. (2007) also showed an increase of the leaves number of lettuce after the treatment with yellow light.

Our results indicate that the highest concentrations of chlorophylls, carotenoids and anthocyanins were provided in black currant leaves under WW and BR lights (Fig. 3 - 5).

This effect could be related to the spectral composition of light emitted by these LEDs. These results are consistent with previous studies that showed that leaves contain more chlorophyll and carotenoids under BR LEDs than under other treatments (Shin et al., 2008; Hogewoning et al., 2010; Wojciechowska et al., 2013).



Inhibition of elongationgrowth responses PIF-mediated elongation growth responses

Figure 6. Scheme of the phytochrome-mediated light signaling pathways

Note: CW – cool white light (6000K); NW – natural white light (4000K); BR – 20% blue light at a wavelength of 460 nm and 80% red light at a wavelength of 660 nm; WW – warm white light (2700K). Data are presented as means \pm S.E.M, n = 9-12.

*Significantly different from the cool white light treated group of plants (P < 0.05) using ANOVA followed by a SNK test

Warm white light (WW) LED emits higher percentage of red light with long wavelength and less of blue light with short wavelength and, hence, can stimulate the photosynthetic photoreceptors. The correct B:R ratio in the LED lighting can be used for obtaining the best response of photosynthetic and physiological plant systems (Hogewoning et al., 2010; Wojciechowska et al., 2015). It is also important to highlight that despite WW light stimulated growth of black currant plants, BR light promoted higher levels of the plant pigments that could be relevant to the production quality.

Conclusions

This study showed that warm white LED light is optimal for acceleration of growth of black currant plantlets *in vitro*. Warm white light is also effective for promoting root growth. Blue and red LED light is important for biosynthesis of chlorophylls and carotenoids. This is the first report on measurement of the effects of different LED lights on black currant *in vitro* to determine which types are important for black currant growth.

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