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INFLUENCE OF BLUE AND RED LEDs ON DEVELOPMENT AND NUTRITIVE VALUE OF *PERILLA FRUTESCENS* (L.) CULTIVATED IN CLIMATE CHAMBERS

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ABSTRACT

In this study influence of supplemental blue and red LED lighting on growth, nutrient solution uptake and concentration of secondary metabolites was investigated. *Perilla* plants were cultivated in climate chambers, where main light was provided by fluorescent tubes with PPFD between 123-177 $\mu molm^{-2}s^{-1}$. Additionally, blue and red LEDs were used, providing 11 $\mu molm^{-2}s^{-1}$ or 12 $\mu molm^{-2}s^{-1}$, respectively. Temperature was set on 24/19°C (day/night), relative air humidity on 64/56% (day/night), and day length was 16 hours. Results showed that small portion of supplemental blue light (~9%) increased fresh mass (FM), dry mass (DM), and nutrient solution uptake (NSU) up to 50.33%, 10.85% or 31.80%, respectively. Supplemental red light (~6%) increased nutrient solution uptake up to 23.56%, while fresh and dry mass were higher than control, but without statistical significance. Regarding nutritive value of *Perilla,* supplemental blue light significantly increased carotenoid concentration (Car) (+13.37%), but polyphenols (PP), anthocyanins (Anth) and flavonoid (Fl) concentrations did not differ from the control. In treatment with supplemental red light, only flavonoid concentration was significantly increased (+14.34%). Use of supplemental blue or red LEDs in closed systems with controlled conditions increase or tends to increase plants fresh mass, dry mass, nutrient solution uptake rate, as well as concentration of some secondary metabolites.

Keywords: *Perilla, blue and red LEDs, climate chambers, growth and nutritive value.*

INTRODUCTION

Perilla frutescens (L.) Britt. (Lamiaceae), known as Beefsteak plant, Shiso in Japan, Tía tô in Vietnam, is an Asian herbaceous plant native to mountainous areas from India to China, but mainly cultivated and consumed in Korea, Japan, Thailand and Vietnam. *Perilla* is an annual plant adopted to warm, humid climates and grows well on semi-shade or sun. Except for culinary use, its fresh leaves and seeds are well-known for treatments of various diseases like tumor, heart diseases,

diabetes, anxiety, depression, infections and intestinal disorders. The health promoting effects of *Perilla* have been attributed to its high content of secondary metabolites such as polyphenols, flavonoids and anthocyanins (Grbic et al., 2016a). Sato et al. (2002) found a total phenolic content of 727 mg/ 100 g FW. Depending on the cultivar, between 700 and 1200 mg polyphenol content/100 g FW were detected by Müller-Waldeck et al., 2010. Hong and Kim (2010), detected total flavonoid content in *Perilla* of 7.23 mg/ g DW.

Secondary metabolite synthesis can be significantly affected by light intensity and temperature, causing morphological and physiological changes in plants (Hwang et al., 2014). Some observations were already made in *Perilla* plants regarding influence of light on ingredient contents. Depending on light conditions, Park et al. (2013) found an anthocyanin content of 100-400 mg/100 g FW. Nishimura et. al (2009) investigated influence of different light conditions on growth and content of secondary metabolites of red-leafed *Perilla* cultivated in climate chamber. According to their study, dry weight and growth were positively affected by red-enriched light treatments (red alone, blue and red, green and red), while anthocyanin content was 1.3-1.7 times higher in red light treatment than in any other treatments. Importance of light spectra on plant development and concentration of secondary metabolites was also shown in the study of Grbic et al. (2016a), where *Perilla* plants were grown under greenhouse light conditions manipulated by means of colored plastic films. Light intensity can also affect *Perilla* leaf color, which vary from green to purple.

As the blue and red spectral ranges are supposed to be primary energy sources for photosynthesis and mainly absorbed by important photoreceptors as phytochromes, cryptochrome and phototropin, aim of this study was to investigate influence of these specific light spectra on plant growth, nutrient solution uptake rate, and content of secondary metabolites of *Perilla frutescens* cultivated under controlled growing conditions.

MATERIAL AND METHODS

Plant material and cultivation

In both experiments *Perilla frutescens* var. *crispa* (Thunb.) H. Deane was investigated. Bronze-leafed "H&V" *Perilla* seed from Vietnam was used. The main leaf colour is green, later partly turning bronze or purple.

Seeds were sown into plug trays and after the young plants had 4-6 fully developed leaves, they were transplanted into 18 Mitscherlich pots (Bergmann, 1958) filled with a substrate mixture (6 l). Continuous supply with nutrient solution was ensured by textile wicks, placed in the substrate of these pots so that its lower end was immersed in the pot saucer with nutrient solution, while upper end was in the plants root zone. In each pot four plants were cultivated.

Then all plants were acclimatized for one week under artificial light provided by fluorescent tubes. After acclimatisation pots were placed in two climate chambers. One was proposed as control (CC2), where plants grew under fluorescent tubes; in another one (CC1) growth conditions were the same as in CC2, but beside

fluorescent tubes as a main light source, four LED bars (length 120 cm) were horizontally placed 20 cm bellow fluorescent tubes in order to influence the light spectrum. There were 16 plants per treatment. Experiment with supplemental blue LEDs took four and experiment with red LEDs took three weeks.

Growth conditions

Climate parameters in chambers were constant and controlled by computer. Temperature was set to 24/19°C (day/night), relative air humidity to 64/56% (day/night), and day length was 16 hours. As main light source in climate chambers 14 fluorescent tubes F58W/827 (Sylvania, Luxline Plus, Germany) were used, placed 1 m above the bottom of the chamber. Light emitting diodes used in climate chamber experiments were monochromatic blue (443 nm) and red light (629 nm), providing 11 $\mu molm^{-2} s^{-1}$ or 12 $\mu molm^{-2} s^{-1}$, respectively. Wavelengths were measured with the Avantes Ava Spec NIR 256 spectrometer.

Photosynthetically active radiation (PPFD) was measured with the micro quantum/temperature sensor 2060-M of PAM-2000 portable chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) at height of 40 cm from the chambers bottom. Average PPFD values during experiments are given in Table 1. As it is shown, there are some differences in value between CC2 and CC1 during one experiment, what was result of unequal aging of fluorescent tubes.

Table 1. Average PPFD in $\mu mol\ m^{-2}\ s^{-1}$ measured in climate chamber CC1 (Control) where plants grew under fluorescent tubes (FT), and in CC2 where plants grew under fluorescent tubes (FT) and supplemental blue (first Experiment) and red (second Experiment) LEDs

Climate chambers	First Experiment	Second Experiment
CC1 (FT-Control)	123.29	177.33
CC2 (FT+supplemental LEDs)	125.62	197.31

Growing place, media and nutrients

Experiment was carried out from April to July 2013 in climate chambers of the Humboldt University of Berlin, Research station Berlin-Dahlem.

Growing substrate used in the experiments was consisting of white peat (65%), black peat (20%) and perlite (0.2-6 mm) (15%), produced by the company Gramoflor GmbH & Co. KG (Vechta, Germany).

The basic composition of nutrient solution used during experiment was: N (110 ppm), P (50 ppm), K (225 ppm), Ca (120 ppm), Mg (80 ppm), HCO_3 (90 ppm), SO_4 (60 ppm) and microelements, of which Fe (10 ppm). The EC value equalled 1.5 mS cm^{-1} and pH value 5.8, whereas pH value was adjusted with 85% H_3PO_4. The HYDROFER computer programme compatible with an Excel programme (Böhme, 1993) was used to calculate the required amounts of fertilizers, salts and acids.

Plant growth determination

Plant height was measured once a week, beginning after acclimatization and from then on in seven-day-intervals till harvest.

Fresh and dry mass were determined at the end of experiment. All leaves were removed from stems and weighed without petioles, which was figured as fresh mass (FM). After that these leaves were dried at 60°C to constant weight and figured as dry mass (DM) in %.

Nutrient solution uptake rate

Immediately after potting, two litres of nutrient solution were poured in the Mitscherlich pot saucers. From then on, every second day the nutrient solution left in saucer was recorded, poured away and new two litres were added. At the end of experiment all differences recorded between nutrient uptake and solution left in saucers were summed and figured as nutrient solution uptake rate (NSU).

Determination of secondary metabolites

Secondary metabolites were determined once a week. For the analyses, first fully developed leaves from the top of the plants were taken and whole leaf area without main leaf vain was used. Photosynthetically active pigment carotenoid was determined using spectrophotometer (Unicam UV/Vis Spectrometer UV2) according to Lichtenthaler and Buschmann (2001).

Total polyphenols were determined spectrophotometrically with Folin-Ciocalteu reagent as described previously (Zheng and Wang, 2001). After the extraction with 80% methanol and 5% HCl, 100 µl of each sample was filled into test cuvettes. 1 ml of Folin-Ciocalteu‘s reagent was added and after five minutes 1 ml Na_2CO_3. The absorbance was measured after one hour at 760 nm. For the calibration gallic acid has been used. Contents of anthocyanins were measured using pH-differential method as described by Wrolstad et al. (2005) and expressed as shisonin equivalent calculated using the formula according to Meng et al. (2006). Extraction was as same as for phenols. 2x250 µl of each sample was filled into test cuvettes and mixed with potassium chloride (pH 1.0) or sodium acetate (pH 4.5), respectively. Both mixtures were measured at 520 and 720 nm. Total flavonoids were also determined spectrophotometrically according to method of Bahorun et al. (2004) using quercetin as a standard solution. After extraction with 80% methanol, 500 µl of each sample was filled into test cuvettes. 2 ml of 2% aluminium chloride was added and after 10 minutes, absorbance was measured at 367 nm.

Data evaluation

All data were evaluated with statistical software SAS. Mean values and standard deviations were calculated and analysed using ANOVA (Tukey test, significance level $P \leq 0.05$). Before analysis of variance, data were tested for normality with Shapiro-Wilk test.

RESULTS AND DISCUSSION

In climate chambers influence of blue and red LED light could not be investigated at same time, hence both blue and red light treatment cold only be compared with respective control.

Plants cultivated under additional blue and red LED lighting were significantly lower than control (Tables 2 and 3). FM and DM were significantly higher in FT+B treatment, while FT+R treatment did not differ from the control (Tables 2 and 3). Positive influence of additional blue light on FM of *Perilla* cultivated in greenhouse was already reported by Grbic et al. (2016b). Noguchi and Amaki (2016) showed similar effects of blue light on growth and FM of Mexican mint (Lamiaceae). In their study elongation of main and lateral shoots was reduced, but FM was increased under blue light. Positive effect of supplemental blue light might be explained by its enhancing impact on amount of guard cells and stomata opening (Frecilla et al., 2000; Kang et al, 2009). Therefore, a higher CO_2- uptake can be achieved. In combination with more photosynthetic usefully radiation, higher biomass accumulation might be a result.

Results from the Tables 2 and 3 show also some tendencies regarding light intensity and cultivation period - smaller light intensity and longer cultivation period could increase plant height, FM, DM, and NSU.

Table 2. Plant height, fresh and dry mass, and nutrient solution uptake rate (NSU) of the *Perilla* plants after four weeks cultivation in climate chambers under fluorescent tubes (FT) and additional blue LED lighting (FT+B). Control plants were grown under PPFD of 123.29 µmol m^{-2} s^{-1} and plants with additional blue LEDs under PPFD of 125.62 µmol m^{-2} s^{-1}. Values in brackets relate to respective control (100%).

Treatment	Plant height (cm)	Fresh mass (g/plant)	Dry mass (g/plant)	NSU (l/plant)
Control (FT)	51.53±1.43 a	24.74±1.14 b	3.91±0.25 b	3.71±0.12 b
FT+B	40.28±1.72 b (-21.82%)	37.19±1.29 a (+50.33%)	4.34±0.21 a (+10.85%)	4.90±0.03 a (+31.80%)

Different letters indicate significant difference among treatments (Tukey-test, $P \leq 0.05$, n=16)

Nutrient solution uptake rate was significantly affected by both additional lighting (Tables 2 and 3). Blue light increased it up to 31.80%, red light up to 23.56%. Sams et al. (2016) investigated impacts of light quality on nutrient uptake. In their study, additional blue and red light increased nutrient uptake in marigold plants grown in greenhouse. Higher nutrient uptake can be explained by higher needs due to the enhanced biomass formation, especially for plants cultivated under blue light. Hwang et al. (2014) investigated influence of different light intensities and cultivation periods on Perilla plants and found out that increased light intensity or longer cultivation period under increased light intensity decrease plant height.

Table 3. Plant height, fresh and dry mass, and nutrient solution uptake rate of the *Perilla* plants after three weeks cultivation in climate chambers under fluorescent tubes (FT) and additional red LED lighting (FT+R). Control plants were grown under PPFD of 177.33 µmol m^{-2} s^{-1} and plants with additional red LEDs under PPFD of 197.31 µmol m^{-2} s^{-1}. Values in brackets relate to respective control (100%).

Treatment	Plant height (cm)	Fresh mass (g/plant)	Dry mass (g/plant)	NSU (l/plant)
Control (FT)	27.61±0.94 a	15.80±1.81	2.32±0.26	1.76±0.17 b
FT+R	23.00±1.12 b (-16.72%)	18.08±2.52 (+14.46%)	2.86±0.39 (+23.57%)	2.18±0.23 a (+23.56)

Different letters indicate significant difference among treatments (Tukey-test, P≤0.05, n=16)

Additional blue light showed positive influence on carotenoid concentration, but FT+R did not differ from the control (Tables 4 and 5). Sams et al. (2016) showed that even low PPFD of supplemental blue light may increase total carotenoid concentration. Key enzyme of carotenoid biosynthesis pathway is phytoene synthase. Previous studies demonstrated an enhancing effect of blue light on activity of phytoene synthase (Spurgeon, 1979; Li, 2008).

Table 4. Concentration of carotenoids (Car), polyphenols (PP), anthocyanins (Anth), and flavonoids (Fl) in fresh *Perilla* leaves after four weeks cultivation in climate chambers under fluorescent tubes (FT) and additional blue LED lighting (FT+B). Control plants were grown under PPFD of 123.29 µmol m^{-2} s^{-1} and plants with additional blue LEDs under PPFD of 125.31 µmol m^{-2} s^{-1}.

Treatment	Car (mg/100 g FM)	PP (mg GAE/100 g FM)	Anth (mg SHE/100 g FM)	Fl (mg QE/100 g FM)
Control	8.78±0.40 b	958.75±70.70	286.51±46.68	1124.12±168.11
FT+B	9.95±0.62 a (+13.37%)	864.03±84.05 (-9.88%)	326.97±97.02 (+14.12%)	1057.55±27.81 (-5.92%)

Different letters indicate significant difference among treatments (Tukey-test, P≤0.05, n=4)

Concentrations of polyphenols and anthocyanins were not influenced by additional blue and red light (Tables 4 and 5), but their absolute concentrations still correspond to those found by Müller-Waldeck et al. (2010) and Park et al. (2013).

Table 5. Concentration of carotenoids (Car), polyphenols (PP), anthocyanins (Anth), and flavonoids (Fl) in fresh *Perilla* leaves after three weeks cultivation in climate chambers under fluorescent tubes (FT) and red LED lighting (FT+R). Control plants were grown under PPFD of 177.33 $\mu molm^{-2}$ s^{-1} and plants with additional red LEDs under PPFD of 197.85 $\mu molm^{-2}$ s^{-1}.

Treatment	Car (mg/100 g FM)	PP (mg GAE/100 g FM)	Anth (mg SHE/100 g FM)	Fl (mg QE/100 g FM)
Control	9.72±0.95	1027.17±182.15	439.38±126.61	902.29±47.42 b
FT+R	10.01±0.35 (+2.88%)	988.06±175.04 (-3.80%)	435.62±121.73 (-0.86%)	1031.65±34.21 a (+14.34%)

Different letters indicate significant difference among treatments (Tukey-test, $P \leq 0.05$, n=4)

These concentrations were also higher than concentrations obtained in greenhouse conditions investigated by Grbic et al. (2016b). It can be assumed that controlled environmental condition lead to higher concentration of polyphenols and anthocyanins. But like anthocyanin content is also affected by temperature (Azuma et al., 2012), all growing conditions must be considered. Polyphenols and especially anthocyanins are part of a protection system to avoid oxidative damages. Supplemental light intensity provided by LEDs was moderate. Therefore, higher contents of antioxidants seem not to be necessary.

Regarding flavonoid concentration, it was significantly higher in FT+R treatment, FT+B did not show significant difference (Tables 4 and 5).

CONCLUSION

Even though additional blue light increased total light intensity only about ~9%, FM, DM and NSU were increased up to 50.33%, 10.85% or 31.80%, respectively. These experiments showed that blue light was more suitable for obtaining more fresh and dry mass than red light. Small portion of blue light was also enough to increase carotenoid concentration, while additional red light (~6%) influenced flavonoid concentration in a positive manner. Therefore, low-energy treatments can be used to increase yields without negative impact on nutritional quality.

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