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The Nutritional Treasure of Leafy Vegetables-*Perilla frutescens*

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Abstract

Most of the Asian leafy vegetables and herbs have culinary, nutritional and medicinal importance. However, intensive cultivation systems, necessary for rising market demand, are rarely investigated. *Perilla frutescens* (L.) Britt. (Lamiaceae) is an Asian herbaceous plant native to mountainous areas from India to China but mainly cultivated and consumed in Korea, Japan, Thailand, and Vietnam. Except for culinary use, its fresh leaves are well known for a range of beneficial medicinal properties. The health promoting effects of *Perilla* have been attributed to its high content of secondary metabolites such as polyphenols, flavonoids and anthocyanins. The aim of the experiments was to investigate the influence of different growing conditions, in particular light spectra on plant growth. Furthermore, the influence of the light conditions on the content of secondary metabolites as polyphenols, flavonoids, and anthocyanins has been examined. In this study, effects of natural light supplemented by monochromatic light emitted by blue, green and red light emitting diodes (LEDs) in greenhouse conditions have been investigated. Supplemental LEDs were providing additional 7-12 µmol $m^{-2}s^{-1}$ of photosynthetically active radiation (PAR). Results showed that use of additional LED lighting had a significantly promoting effect on plant parameters as height and fresh matter of *Perilla*. The different LED light spectra did not influence synthesis of anthocyanins and polyphenols. However, flavonoid concentration in green LED treatment was 74.26% higher than those found in control. The concentration of investigated secondary metabolites found in control was comparable to other studies with *Perilla*, thus its cultivation in temperate region could be possible without negative impact on bioactive compounds.

Keywords: Beefsteak plant, secondary metabolites, LEDs, light spectra

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Introduction

Perilla frutescens (L.) Britt. (Lamiaceae), also known as Shiso, is an Asian herbaceous plant native to mountainous areas from India to China but mainly cultivated and consumed in Korea, Japan, Thailand, and Vietnam. In their cuisine leaves, inflorescences, fruits, seed, and sprouts of *Perilla* are used raw or cooked. In addition, essential oil distilled from the leaves is used for preparation of meals, as well as sweetening agent (Guzman and Siemonsma, 1994).
According to scientific nomenclature of *Perilla* two varieties are described: variety *frutescens* - mainly used in Korea as fresh vegetable or for making pickles, and variety *crispa* - a strongly branching crop mainly used in Japan and Vietnam, with smaller curly leaves rich in anthocyanins (Fig. 1). These varieties are cross-fertile (Nitta et al., 2003).

Figure 1: Three types of Perilla (a) Korean (var. *frutescens*), (b) Vietnamese (var. *crispa*), (c) Japanese (var. *crispa*) with different content of
Polyphenols: (a) 127-180 mg/100g FM; (b) 250-320 mg/100g FM; (c) 180-280 mg/100g FM and Anthocyanins: (a) 20 mg quercetin/100g FM; (b) 31-202 mg quercetin/100g FM; (c) 17-36 mg quercetin/100g FM

Because of high presence of anthocyanin *Perilla frutescens* var. *crispa* is used in food colouring and in the production of pickled plums (Brenner, 1993). Zhang et al. (2009) isolated essential oils of fresh leaves of different *Perilla* accessions and identified eight chemotypes: PK (perillaketone), DLP (D-limonene and piperitone), PT (piperitenone), MS (myristicin), AL (apiol), EM (elemicin), DEK (dehydroelsholtzia ketone) and PA (perilla aldehyde), the last type is the only one with culinary use. Except for culinary use, fresh leaves are well-known for a range of beneficial medicinal properties and therefore used in traditional medicine for treatments of various diseases like cancers, heart disease, diabetes, anxiety, depressions, 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., 2016). Their content is highly dependent on environmental factors and cultivars. Light and temperature can significantly affect not only plant growth and development, but also the secondary metabolite synthesis (Hwang et al., 2014). The possibility to cultivate *Perilla* in greenhouse in temperate regions was already shown in previous studies (Grbic et al., 2016). The aim of this study was to investigate the influence of different blue, green and red spectra on plant growth by means of supplemental LED lighting. Furthermore, the influence of the light spectra on the content of secondary metabolites as polyphenols, flavonoids, and anthocyanins has been examined.

Material and Methods

In this study, *Perilla frutescens* var. crispa Vietnamese type was investigated. Experiment was carried out from 12th of May to 23rd of June 2014 in research greenhouse of the Humboldt University of Berlin, Research station Berlin-Dahlem. Plants were cultivated in Mitscherlich pots filled with a substrate mixture (6 L) consisted of white peat (65%), black peat (20%) and perlite (0.2-6 mm) (15%). There were four treatments: control - cultivated under natural light, and three LED treatments - blue, green and red. In each treatment 24 plants were cultivated and supplied with nutrient solution whose EC value equalled 1.5 mS cm^{-1} and pH value 5.8. They were growing under 16 hours photoperiod, at a mean temperature of 27.4 ± 3.4°C and a mean relative air humidity of 48.6 ± 12.2%. Photosynthetically active radiation (PAR) was recorded on the top of the canopy three times a day: at 08.30 am, 12.30 pm and 04.30 pm; average value calculated for the control (natural light, NL) was 154 ± 57 µmol m^{-2}s^{-1}. Additionally, three LED lightings were used: blue (bLED, peak at 443 nm) providing 11 µmol m^{-2}s^{-1}, green (gLED, peak at 515 nm) 7 µmolm^{-2}s^{-1} and red (rLED, peak at 629 nm) 12 µmol m^{-2}s^{-1}. They provided light 16 hours/day.
For weekly determinations of secondary metabolites, first fully developed leaves were taken. Total phenol content was determined spectrophotometrically with Folin-Ciocalteu reagent as described

previously (Zheng and Wang, 2001, Grbic et al., 2016). Total flavonoids were also determined spectrophotometrically according to the method of Bahorun et al. (2004) using quercetin as a standard solution. For anthocyanin content pH-differential method of Wrolstad et al. (2005) was used and expressed as shisonin equivalent, calculated with the formula from Meng et al. (2006).
Plant height was measured once a week starting after acclimatization until harvest. Fresh matter was determined at the end of experiment, four weeks after transplanting. All leaves were removed from stems and weighed without petioles, as marketable part of this plant, which was figured as fresh matter (FM).
For statistical evaluation of the data, statistical software SAS was applied. Mean values and standard deviations were calculated and analysed using ANOVA (Tukey test, significance level P ≤ 0.05). Before analysis of variance, data were tested for normality with Shapiro-Wilk test.

Results and Discussion

Additional LED lighting showed positive influence on plant height but without significant differences between LED treatments. Compared to control blue and green light increased plant height up to 30%, while additional red light increased it up to 24% (Table 1). In the study of Noguchi and Amaki (2016), Mexican mint (Lamiaceae) responded similar to red and green light, which promoted the elongation of main and lateral shoots. Mizuno et al. (2011) showed positive influences of monochromatic blue LED light on the elongation of main stem and petioles of two cabbage cultivars. Regarding yield indicated as fresh matter, supplemental blue light increased it up to 34% and red light up to 33%, while the increase in green light treatment did not significantly differ from any of treatments (Table 1). Other authors have also reported positive effects of supplemental LED light. Additional blue light applied alone or in combination with red light increased plant biomass in tomato (Ouzounis et al., 2016), stimulated biomass accumulation in lettuce (Johkan et al. 2010) and Chinese cabbage (Li et al., 2012). Wollaeger and Runkle (2014) showed positive influence of monochromatic red light on shoots fresh mass of salvia (Lamiaceae), tomato and petunia.

Table 1: Influence of natural light (NL) and additional blue (bLED), green (gLED) and red (rLED) LED lighting on plant height, fresh matter, and polyphenol (PP), anthocyanin (Anth) and flavonoid (FL) content of *Perilla frutescens*. Tukey-test $p \leq 0.05$, n=24 for plant height and fresh matter; n=4 for PP, Anth and FL.

Light treatment	Plant height (cm)	Fresh matter (g/plant)	PP (mg/100 g FM)	FL (mg quercetin/ 100 g FM)	Anth (mg shisonin/ 100 g FM)
Control (NL)	33.88 ± 3.25 b	14.91±2.11 b	880.20	412.85 b	201.34 a
NL+bLED	44.03±3.20 a	17.82±1.66 a	420.33	500.23 b	44.45 b
NL+gLED	44.32±3.20 a	17.28±1.41 ab	322.18	718.84 a	23.31 c
NL+rLED	42.05±3.10 a	18.15± 1.56a	353.61	458.19 b	26.36 bc

As it shown in Table 1, LED light differently affected secondary metabolites. Polyphenol content did not significantly differ among the treatments; moreover, it was remarkably reduced in LED treatments. In contrary, flavonoid content was increased by LED light with the highest content obtained under supplemental green light. Additional LED lighting, with the lowest value in the green light treatment, significantly reduced the anthocyanin content.

Conclusions and Outlook

These results indicate that the plant height and fresh matter of *Perilla* with high nutritional value could be enhanced with even small intensities of supplemental LED lighting. Since the plant response to different wavelengths is species-specific, their influence on the concentration of secondary metabolites should be investigated more in detail, whether they are applied alone or as a mixture.

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