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Full Length Article

Shorter Wavelength Blue Light Promotes Growth of Green Perilla (*Perilla frutescens*)

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Abstract

Light-emitting diodes have been tested as alternative light sources in plant growth facilities. It was discovered that growth of green perilla is specifically promoted by blue light. In this study, we investigated whether growth promotion of blue light differed at different bandwidths of 5 nm intervals in the 430–470 nm spectra. Cool white fluorescent lamps were used as the control. Leaf area, leaf fresh weight, and leaf dry weight increased more than 30% at bandwidths shorter than 450 nm. Similarly, plant height, stem fresh weight, stem dry weight, and stem diameter increased over two- to four-fold at bandwidths shorter than 445 nm. Anthocyanin content also increased at wavelengths shorter than 445 nm. These findings indicate that blue light shorter than 450 nm promotes highly leaf and stem growth of green perilla than that longer than 451 nm does. Thus, employing blue light shorter than 450 nm in plant growth facility lighting systems could provide a practical advantage for producing quality perilla leaves for fresh vegetables.

Keywords: Artificial light source; Fresh vegetable; Light-emitting diode (LED); Differential growth response; Plant growth facility; Wavelength interval

Introduction

Green perilla is an annual plant species native to Southeast Asia, particularly the regions from Northern India to Southern China (Brenner, 1993; Nitta *et al.*, 2005). Green perilla is also adapted to Korea and other warm and humid Asian countries, including Japan and Thailand. In Korea, green perilla has mostly been grown for seed oil production and, collaterally, some leaves were used for potherb. However, there has been a recent, prominent new trend in green perilla production in Korea. Production of leaves for a fresher vegetable has become a majority in green perilla cultivation, and its cultivation area exceeds about 1,000 ha (Choung, 2005).

One of the major reasons for the increase in consumption of green perilla leaves originates from consumer interest in functionality of food products (Hong and Kim, 2010). In fact, green perilla has traditionally been used as a medicinal herb for its known beneficial effects on human health. In numerous studies, seeds and leaves of green perilla have been reported to have antibacterial, anticancer, antidote, antiproliferant, antiseptic, antiulcer, hypocholesterolemic, immunostimulant, and sedative activities (Duke, 2002). Its characteristic flavor together with multiple beneficial effects of perilla leaves attracted its use as a major fresh and seasoned vegetable in the Korean diet.

Consumption of perilla leaves as a vegetable has entailed changes in its cultivation system to meet the high demand and hygiene standard. Perilla leaves are currently produced in greenhouses with lighting systems to ensure longer vegetative growth and preventing the transition to flowering for increased leaf yield. Furthermore, attempts have been made to produce quality perilla leaves in plant factory systems with artificial illumination systems. Thus, it is necessary to establish the optimal lighting condition for producing perilla leaves in artificial facilities based on the response of perilla plants to artificial lights.

Most perilla leaf production facilities equipped with artificial light systems utilize conventional incandescent light bulbs and fluorescent tubes. Recent commercialization of light-emitting diode (LED) lighting provides an alternative illumination technology for plant cultivation facilities (Yeh and Chung, 2009). LEDs have been tested for practical utility in cultivation of many crop species, particularly for their high energy efficiency, long lifetime, and spectrum specificity (Massa *et al.*, 2008). Results from previous studies generally indicate that LED light is equally efficient for plant photosynthesis as conventional lighting sources and LEDs provide economic benefits through

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higher efficiency of energy, space, and other input resources for plant cultivation (Massa *et al.*, 2008; Yeh and Chung, 2009; Olvera-Gonzalez *et al.,* 2013). Therefore, LED illumination has been tested for growth of green perilla plants in a growth chamber, a plastic greenhouse, and a glasshouse facility (Choi, 2011). The results showed that growth of perilla plants was stimulated under blue light. Leaf fresh weight (FW) and dry weight (DW) of young perilla plants grown for 5 weeks at a light intensity of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increased over 15- and 20-fold, respectively under blue light than those under white light illumination (Choi, 2011). Similar growth promotion was observed in two varieties during the vegetative growth period up to 8 weeks after planting (Choi, 2011). Therefore, in this study, we further examined whether the growth promoting effects of blue light is different in a specific bandwidth of blue light spectrum spanning from 430 to 470 nm at higher light intensity.

Materials and Methods

Plant Materials and Growth Conditions

Seedlings were prepared and light treatments were conducted in a closed growth chamber system at Chonbuk National University from September to December 2012. Green perilla (cv. Soim) seeds were sown at medium density in seedling trays filled with a mixture of peat moss, vermiculite, and pearlite (BM2, Berger Peat Moss, QC, Quebec, CAN). Seedlings were grown to the four-leaf stage in a chamber under cool white fluorescent lamps [blue (400–500 nm): green (500–600 nm): red (600–700 nm) = 26:41:33; DULUX L55W/840, OSRAM, Munich, Germany)] at 150 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux (PPF). Uniform seedlings at the four-leaf stage were transplanted to plastic pots (diameter, 200 mm; depth 150 mm) filled with the same artificial soil mixture used for planting. A single seedling was transplanted in each pot, and the seedlings were subjected to light treatments in a closed growth chamber system compartmented with rooms equipped with LED at 5 nm wavelength intervals in the range of 430 to 470 nm (Luxpia Co. Ltd., Seoul, Korea) (Table 1). Cool white fluorescent lamps were used as the control and their spectral characteristics are shown in Fig. 1. Average PPF was adjusted to 150 $\mu mol\ m^{-2}\ s^{-1}$ for all treatments with a 16/8 h day/night cycle. Twenty-one plants were placed in each wavelength treatment with three replications per treatment. The chamber system had day and night temperature at 22/18°C, relative humidity of 70%, and CO_2 concentration at 400 $\mu mol\ m^{-2}$, respectively. A nutrient solution was formulated at N-P-K-Ca-Mg = 0.8-2.0-6.0-3.0-2.0 mg L^{-1} [pH = 5.5–6.0, electrical conductivity (EC) = 1.5 mS cm^{-1}] and replenished to maintain the pH and EC at constant levels. Forty mL of nutrient solution was supplied to each pot three times per day via an automatic root dripping hydroponic system.

Plant Growth Measurements and Statistical Analysis

Eleven uniform plants were sampled per replication 41 days after the treatments, and plant growth was analyzed by measuring leaf number, leaf length, leaf width, leaf area, leaf FW, leaf DW, leaf color, plant height, stem FW, stem DW, and stem diameter according to the guidelines established by Rural Development Administration (RDA, 2006), Republic of Korea. Chlorophyll content was measured by a nondestructive method using a SPAD meter (SPAD 502, Minolta, Tokyo, Japan). Experiments were conducted in a completely randomized design, and data were analyzed using the SAS V9.1.3 software (SAS Institute, Cary, NC, USA).

Results

Leaf Growth

Leaf area increased about 33% at the 430–434 nm interval but did not change significantly at the other wavelength treatments. Increase in leaf area was accompanied by a significant increase in FW and DW, but only in the 430–434 nm interval. Leaf FW and DW increased 33% and 31%, respectively, at the 430–434 nm interval. This increase in leaf area, leaf FW, and leaf DW paralleled the increase in leaf number. Leaf number increased 13–19% at all wavelength intervals (Fig. 2).

Leaf shape changed from cordate to a slightly elongated cordate by the increase in length, particularly at wavelengths shorter than 449 nm, and the general decrease in width at all treatments except the shortest wavelength interval. Leaf length increased 8–16% at a wavelength shorter than 449 nm. Leaf width did not change significantly at any wavelength interval (Fig. 3).

Stem Growth

Stem growth was drastically enhanced by all blue light wavelengths, but the enhancement was much higher at the shorter wavelength intervals (Fig. 4). For example, stem FW and DW increased about 400% and 266%, respectively, at wavelength intervals shorter than 449 nm, whereas they increased less than 300% and 200%, respectively at wavelength intervals longer than 450 nm. Similarly, stem diameter increased over 150% at wavelength intervals shorter than 449 nm, whereas it increased less than 140% at the wavelength intervals longer than 450 nm. Plant height also increased over 200% at all wavelength intervals but the increase was higher at wavelength intervals shorter than 449 nm (Fig. 4).

Leaf Color

Chlorophyll content of leaves responded substantially differently from other traits to wavelength in that it increased at the wide wavelength spectrum (Table 2).

Table 1: Blue LED lamps with each peak wavelength at a 5 nm bandwidth interval in the spectrum of 430 to 470 nm (B1–B8 treatment, respectively) were used to investigate the effect of each 5 nm bandwidth on green perilla seedling growth

Treatment	Lighting source	Wavelength range (nm)	Peak wavelength (nm)
B1	Blue LED	430-434	432
B2		435-439	436
B3		440-444	440
B4		445-449	446
B5		450-454	450
B6		455-459	456
B7		460-464	460
B8		465-470	466
Control	Cool-white fluorescent lamps	400-500 nm: 500-600 nm: - 600-700 nm = 26:41:33	

*Average photosynthetic photon flux (PPF) was adjusted to 150 μmol m^{-2} s^{-1} for all treatments

Table 2: Chlorophyll content and color of green perilla leaves 41 days after treatments under different wavebands of blue light at 5 nm intervals (B1–B8) in the range of 430 to 470 nm

Treatment[x] (nm)	Chlorophyll Content[y]	Leaf color			
		Front		Back	
		Hue	Saturation	Hue	Saturation
B1	33.9cd	125.8a	23.9a	119.5a	15.5a
B2	34.4abc	125.7a	23.3a	117.0a	14.3ab
B3	34.2bcd	125.7a	22.4a	112.5ab	13.2b
B4	35.0abc	126.5a	19.5b	99.4c	9.6c
B5	35.0abc	123.7b	19.4b	87.3d	8.4cd
B6	35.5abc	125.8a	20.2b	104.3bc	9.1c
B7	35.9ab	126.5a	19.1b	102.4bc	8.9c
B8	36.1a	125.8a	18.8b	96.2cd	7.9cd
Control	32.6d	126.3a	18.6b	64.0e	7.1d
$LSD_{0.05}$[z]	1.7	1.5	1.6	12.0	1.8

[x]Different wavelengths of blue light in the range of 430 to 470 nm were treated at 5 nn intervals with LED lamps emitting each specific wavelength of light. Cool white fluorescent lamps were used as the control
[y]Values indicate optical density difference measured by a chlorophyll meter (SPAD 502, MINOLTA, Tokyo, Japan)
[z]Least significant difference at P = 0.05

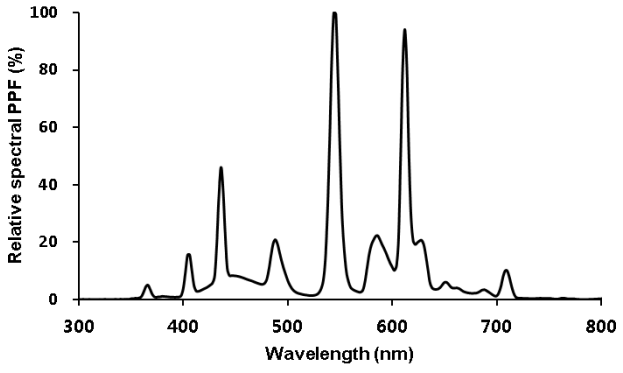

Fig. 1: Spectral characteristics of the white fluorescent lamps used as control light. Blue (400–500 nm), green (500–600 nm) and red (600–700 nm) fractions are at a ratio of 26:41:33

The color of both the front and back sides of leaves became significantly darker, but the change was more prominent on the back side at shorter wavelengths. For example, the hue of the back side became darker at all wavelength intervals, whereas saturation was deeper at wavelength intervals shorter than 449 nm (Table 2).

Discussion

The growth promoting effect of blue LED on green perilla was reproduced in this experiment under PPF of 150 μmol m^{-2} s^{-1}, which was about three times higher intensity than that used in a previous report (Choi, 2011). In fact, the promotion of plant growth by blue LED alone compared to that by control white light is a rare observation in crop plants. Similar increase in FW and DW of shoot and root have been reported in lettuce plants grown under blue LED than those grown under broad spectrum white light or red LED at PPF of 200 μmol m^{-2} s^{-1} (Kook *et al.*, 2013). Blue light alone is sufficient to develop leaves plants, leaves with normal photosynthetic function (Hogewoning *et al.*, 2010). More frequently, supplementation with blue LED to red LED by up to 20% of total intensity promotes normal growth and development in many plant species such as spinach, radish, wheat, rice and Arabidopsis (Goins *et al.*, 1998; Yorio *et al.*, 1998; Matsuda *et al.*, 2004; Massa *et al.*, 2008). However, the growth response of plants to light quality and strength is often species dependent and may be genotype specific. Supplemented blue light fractions significantly affect leaf area and chlorophyll content in lettuce, but show no substantial effect on soybean or wheat (Dougher and Bugbee, 2001). Different genotypes of potato show different blue light requirements for stem elongation (Yorio *et al.*, 2001). In contrast to our results here, growth of barley seedlings was significantly reduced under blue LED than under red, blue, green, or yellow LEDs compared to that under fluorescence light (Lee *et al.*, 2010).

Growth of green perilla leaves and stems, particularly that of the stem, was stimulated strikingly at a wavelength shorter than 450 nm. This new finding that shorter wavebands of blue light showed higher growth stimulation in green perilla is significant, as all previous reports on blue light effects on plant growth were based on observations using peak wavebands, mostly of 460 nm (Yorio *et al.*, 2001; Matsuda *et al.*, 2004; Fan *et al.*, 2013; Kook *et al.,* 2013) and 470–475 nm (Avercheva *et al.*, 2009; Lee *et al.,* 2010), or rarely at 450 nm (Choi, 2011) and 445 nm (Hogewoning *et al.*, 2012).

Though the mechanisms for this unique growth stimulation by shorter wavebands of blue light are not clearly understood, the nature of light as a signal as well as an energy source provides clues. Light provides plants with energy for growth. It is widely known that the efficiency of blue light for CO_2 fixation of crop plants is about 10–20% lower than that of white light (McCree, 1971). However, no difference was observed in quantum yield for CO_2 fixation between cucumber plants grown under red or blue LED at 100 μmol m^{-2} s^{-1} irradiation (Hogewoning *et al.*, 2010).

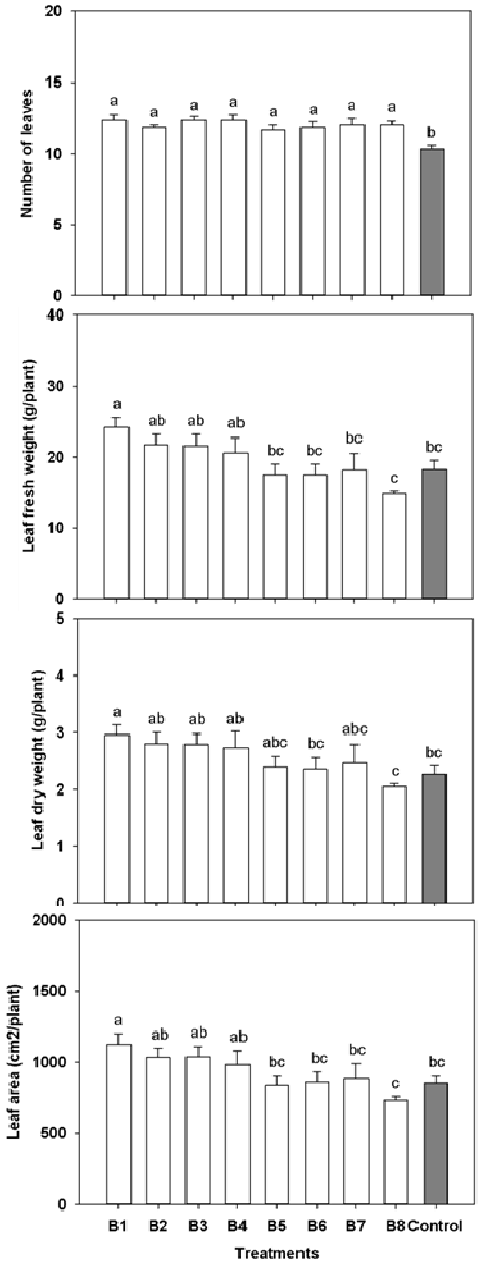

Fig. 2: Effects of different wavebands of blue light at 5 nm intervals (B1– B8) in the range of 430 to 470 nm on leaf growth of green perilla after 41 days of treatment. Cool white fluorescent lamps were used as the control. Means indicated by the same letter are not significantly different at P = 0.05. Least significant difference values for leaf number, leaf fresh weight (FW), leaf dry weight (DW), and leaf area are 0.9, 4.54, 0.60, and 208.70, respectively

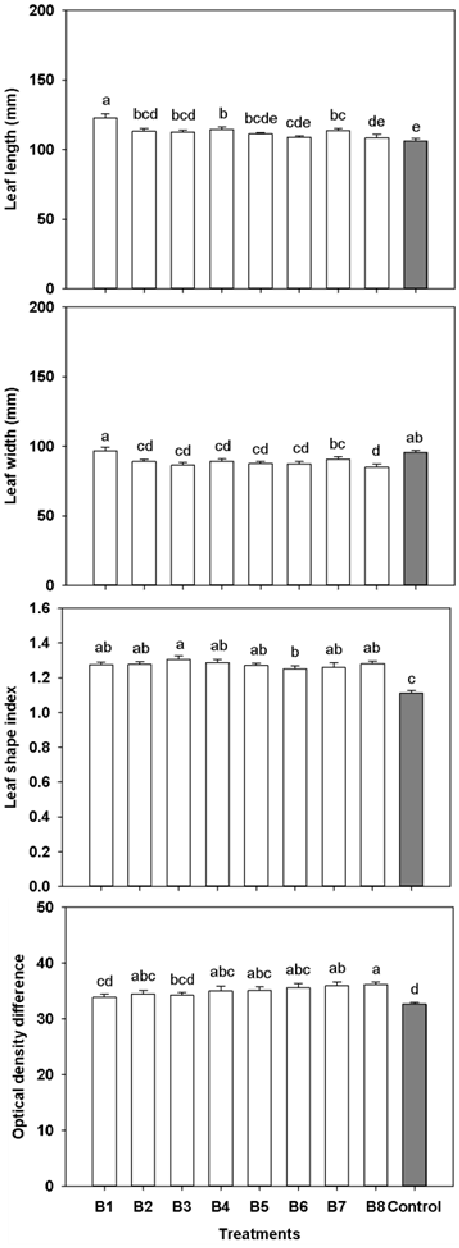

Fig. 3: Effects of different wavebands of blue light at 5 nm intervals (B1–B8) in the range of 430 to 470 nm on leaf shape of green perilla after 41 days of treatment. Cool white fluorescent lamps were used as the control. Means indicated by the same letter are not significantly different at P = 0.05. Least significant difference values for leaf length, leaf width, and leaf shape index are 5.10, 5.30, and 0.05, respectively

Moreover, the quantum yield is highest at 430 nm (McCree, 1971). These observations indicate that the efficiency of light use is also affected by light intensity and plant species, suggesting the importance of understanding characteristic responses of individual plant species to specific light conditions to set optimal light conditions for production in growth facilities.

Photosynthetic rate is also affected by gas exchange activity through stomata. Stomatal density and pore area per leaf area increases about 30 and 50%, respectively in cucumber leaves grown under blue LED than in those grown under red LED, and contribute to the increased net photosynthesis in leaves grown under blue LED (Savvides

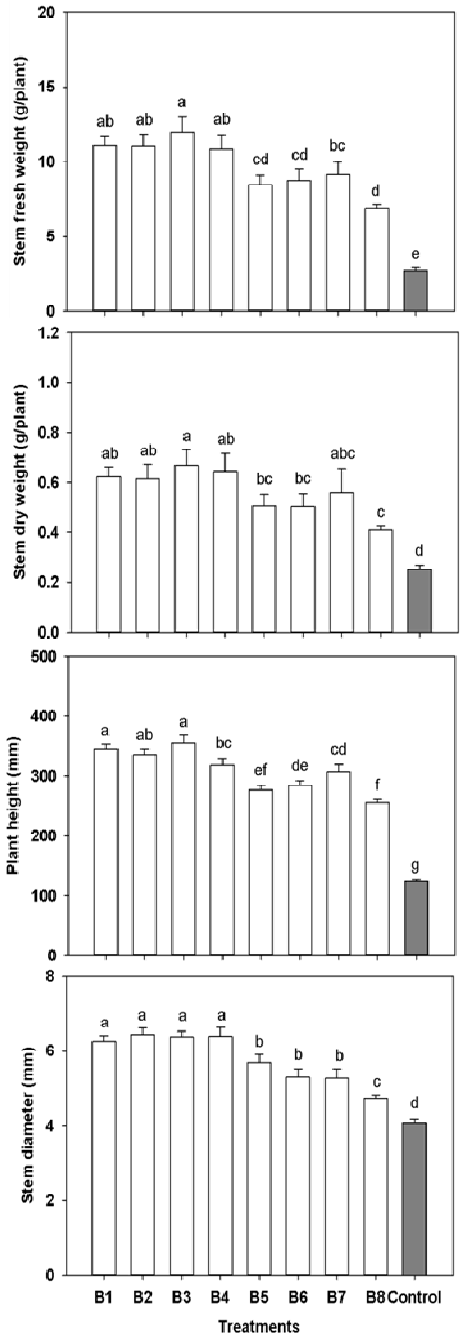

Fig. 4: Effects of different wavebands of blue light at 5 nn intervals (B1–B8) in the range of 430 to 470 nm on stem growth of green perilla after 41 days of treatment. Cool white fluorescent lamps were used as the control. Means indicated by the same letter are not significantly different at P = 0.05. Least significant difference values for stem fresh weight (FW), stem dry weight (DW), plant height, and stem diameter are 2.04, 0.16, 25.9, and 0.52, respectively

et al., 2012). Stimulation of stomatal opening is an old and proven effect of blue light. Blue light induces changes in the concentrations of potassium and sucrose as major osmotic effectors in guard cells and the changes act as signals to open stomata (Kim and Lee, 2007).

Blue light-induced morphological adjustments are often mediated by blue light receptors such as cryptochrome (Taiz and Zeiger, 2010). As increases in stem and leaf FW was accompanied by increases in stem and leaf DW (Figs. 2 and 4), these increases were obviously related to changes in primary and secondary growth processes. In other words, the blue light signal mediated by cryptochrome might affect activities associated with primary and secondary growth. Primary growth is characterized by cell division and expansion, which requires cell wall acidification and synthesis of cell wall materials such as cellulose, hemicelluloses, and pectin (Szymanski and Cosgrove, 2009; Sanchez-Rodrguez *et al.*, 2010). According to the acid-growth hypothesis, activation of the proton pump by indole acetic acid is required followed by increased turgor pressure and synthesis of cell wall materials (Kutschera, 1994). Thus, growth promotion by shorter bandwidths of blue light might require division and expansion of cells mediated by blue light receptors such as cryptochrome (Liu *et al.*, 2011). Thus, further investigations on the role of blue light receptors are required to elucidate the mechanisms of higher stimulatory effects of shorter bandwidths of blue light.

Chlorophyll, carotenoids and picoerythrovillin in mesophyll cells are the major pigments harvesting light energy. Chlorophyll biosynthesis is promoted under red and blue light. Our results indicate that a wide spectrum of blue light is equally effective for chlorophyll biosynthesis. This result is in agreement with previous findings in other plants species (Kim *et al.*, 2004; Kook *et al.*, 2013). However, in Chinese cabbage, a combination of red and blue light at a 6:1 ratio increases chlorophyll accumulation by about 25% compared to that of blue or red light alone (Fan *et al.*, 2013).

Leaf color of green perilla is also affected by anthocyanin, which is a secondary metabolite possessing antioxidant activity (Saito and Yamazaki, 2002). Blue light is required for anthocyanin accumulation in Arabidopsis (Chen *et al.,* 2006) or is equally effective as a red and blue combination in lettuce (Shoji *et al.,* 2010). Anthocyanin content increases by mediation of a blue light receptor in *Artemisia annua* (Hong *et al.*, 2009). In contrast, UV-B (Arakawa *et al.*, 1985) or red light (Zhou and Singh, 2002) is most effective for anthocyanin accumulation in apple or cranberry fruits, respectively, suggesting that there is species and tissue specificity in the effect of light on anthocyanin biosynthesis. The stimulatory effect on anthocyanin content was significantly higher at wavebands shorter than 445 nm in green perilla. This result also has a practical significance in conjunction with consistent consumer preference for functional vegetables. Anthocyanin, a traditional food colorant, has important therapeutic value on disorders of the cardiovascular and urinary systems, liver, and intestines, as well as the common cold (Konczak and Zhang, 2004; Mazza, 2007). Thus, green perilla containing higher anthocyanin could add market value and provide an additional quality

improvement benefit on top of yield increase to producers.

Taken together, blue light shorter than 445 nm had effects to promote higher leaf and stem growth and also anthocyanin synthesis in green perilla. In Korea, green perilla leaves for human consumption as a fresh vegetable are mostly produced in growth facilities. Therefore, employing shorter wavebands of blue light in the lighting system could provide a practical advantage to increase production of quality vegetable green perilla leaves.

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