Hortic. Environ. Biotechnol. 56(5):639-653. 2015.
DOI 10.1007/s13580-015-1064-3

ISSN (print) : 2211-3452
ISSN (online) : 2211-3460

Research Report

Growth, Photosynthetic and Antioxidant Parameters of Two Lettuce Cultivars as Affected by Red, Green, and Blue Light-emitting Diodes

Ki-Ho Son[1,2] and Myung-Min Oh[1,2*]

[1]*Division of Animal, Horticultural and Food Sciences, Chungbuk National University, Cheongju 28644, Korea*
[2]*Brain Korea 21 Center for Bio-Resource Development, Chungbuk National University, Cheongju 28644, Korea*

*Corresponding author: moh@cbnu.ac.kr

Received May 11, 2015 / Revised June 28, 2015 / Accepted October 1, 2015

Abstract. The addition of green light-emitting diodes (LEDs) to a combination of red and blue LEDs, which promote photosynthesis and growth in plants, is known to enhance plant growth in closed-type plant production systems. However, there is limited information on the effects of supplementary green light. This study aimed to determine the effect of red (R), green (G), and blue (B) LED ratios on the growth, photosynthetic, and antioxidant parameters in two lettuce (*Lactuca sativa*) cultivars, red leaf 'Sunmang' and green leaf 'Grand Rapid TBR'. The seedlings were grown for 18 days and then cultivated in growth chambers equipped with LED lighting systems for 4 weeks. Combinations of six LED lighting sources (R:B = 9:1, 8:2, 7:3; R:G:B = 9:1:0, 8:1:1, 7:1:2) were manufactured to emit red (655 nm), blue (456 nm), or green (518 nm) lights under photosynthetic photon flux density of 173 ± 3 μmol·m^{-2}·s^{-1}. Red LEDs were found to improve growth characteristics such as fresh and dry weights of shoots and roots, and leaf area in combination with blue LEDs. The substitution of blue with green LEDs in the presence of a fixed proportion of red LEDs enhanced the growth of lettuce. In particular, the fresh weights of red leaf lettuce shoots under R8G1B1 were about 61% higher than those under R8B2. Furthermore, analysis of leaf morphology, transmittance, cell division rate, and leaf anatomy under treatments with green LEDs supported the enhanced growth of the two lettuce cultivars tested. Meanwhile, growth under blue LEDs led to the accumulation of antioxidant parameters in 'Sunmang'. Thus, the results of this study suggest that the percentage of red, green, and blue LEDs is an important factor for the growth, development, and biosynthesis of secondary metabolites in plants and especially the supplemental irradiation of green LEDs based on the combination of red and blue LEDs can improve lettuce growth.

***Additional key words*:** cell division, *Lactuca sativa*, leaf area, supplementary green light, transmittance

Introduction

Among the various environmental factors that affect plant growth and development, light is a main impetus for the plant life cycle. It provides energy for photosynthesis and acts as a signal that induces various physiological responses in plants, which are affected by light intensity, light quality, and photoperiod (Jiao et al., 2007). Plants transfer the energy required for photosynthesis using chlorophylls that absorb visible light. In addition, plants recognize light in the visible spectrum as well as neighboring wavelengths through the stimulation of photoreceptors such as phytochromes (red and near infrared wavelengths), and cryptochromes or phototropins (blue and ultraviolet-A wavelengths). This leads to photomorphogenesis, which is associated with growth and development as a result of signal transduction (Carvalho et al., 2011). Thus, the selection of an optimal light source is an essential task in closed-type plant production systems, which are fully reliant on artificial light sources since wavelength characteristics of irradiated light influence yield and quality of crops.

Compared to conventional lighting sources such as fluorescent, metal halide, and high-pressure sodium lamps that are used as the main or supplementary light source for plant cultivation, light-emitting diodes (LEDs) have various advantages. In particular, a unique advantage is the precise control of light quality due to their narrow range of wavelengths. Previous studies using these conventional lighting sources were limited in their ability to control the light quality since the light could only be controlled by films that convert or block a broad range of wavelengths (Matsuda et al., 2008; Ohashi-Kaneko et al., 2007). However, the development of LEDs made it possible to develop light irradiation technology to improve the productivity and quality of crops and to conduct plant physiological studies that are related to light quality (Massa et al., 2008). Finally, the application of LEDs for the purpose of crop cultivation has been recently tested in closed-type plant production systems (Bian et al., 2014).

Red and blue lights have an essential role in plant growth

and development because chlorophyll a and b in leaf cells effectively absorb both red (600-700 nm) and blue (400-500 nm) wavelengths of light (Hopkins and Huner, 2004). Red LEDs were effective at promoting an increase in plant biomass such as fresh and dry weights, height, and leaf area (Johkan et al., 2010), while blue LEDs were found to simulate photosynthetic function and induce the formation and development of chlorophylls, rather than having a direct effect on plant growth (Wu et al., 2007; Wang et al., 2009; Heo et al., 2012; Savvides et al., 2012; Son et al., 2012). Moreover, it was reported that a combination of red and blue LEDs promoted the photosynthetic rate compared with the effect of monochromatic red or blue LEDs (Goins et al., 1997; Matsuda et al., 2007; Samuolienė et al., 2011; Savvides et al., 2012). In our previous study, the effects of various ratios of red and blue light on lettuce growth were determined (Son and Oh, 2013). Based on the findings from these studies, red and blue LEDs have often been used in closed-type plant production systems.

Green light (500-600 nm) was considered to be an ineffective signal or energy source for photomorphogenesis or photosynthesis in plants due to it having higher reflectiveness and a lower absorption rate than red and blue light in lettuce leaves (Johkan et al., 2012). Indeed, many previous studies have reported that green light has negative effects on plants, including stem elongation (Folta, 2004), decreased chlorophyll content, inhibited stomatal opening (Son et al., 2012; Talbott et al., 2002), and growth inhibition (Terashima et al., 2009). However, several studies have shown positive effects of green light. Folta (2004) reported that green light influenced the physiological and morphological responses of plants including photosynthesis and photomorphogenesis in addition to red and blue lights. Moreover, it was reported that green light with a relatively lower rate of absorption compared to red and blue light (about 90% absorption) in the upper plant leaf could stimulate photosynthesis in the lower canopy due to high transmittance passing light energy (Nishio, 2000; Sun et al., 1998; Terashima et al., 2009). Additionally, mixed red and blue LEDs supplemented with green LEDs promoted lettuce growth (Kim et al., 2004), and the manipulation of wavelengths and intensity of light within the green spectrum had positive effects on the growth and pigmentation of lettuce (Johkan et al., 2012). However, evidence to support the role of green light as an essential light source for plant growth and development is not sufficient. Thus, the objective of this study was to determine the effect of supplementary green LEDs in combination with various proportions of red and blue LEDs on lettuce growth and nutritional quality related to antioxidant parameters. Our findings provide important basic information for the design of light sources for use in a closed-type crop production system.

Materials and Methods

Plant growth conditions

Seeds of red leaf lettuce (*Lactuca sativa* L. 'Sunmang'; Nongwoo Bio, Suwon, Korea) and green leaf lettuce (*Lactuca sativa* L. 'Grand Rapid TBR'; Asia Seed, Seoul, Korea) were sown in a plug tray (32 mL per cell, two seeds per cell), containing growing medium (Myung–Moon; Dongbu Hannong, Seoul, Korea). The seedlings were then grown in a growth chamber (DS-96S; Dasol Scientific, Hwaseong, Korea) under normal growing conditions [20°C, fluorescent lamps + high-pressure sodium lamps, photosynthetic photon flux density (PPFD) 170 ± 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 12-h photoperiod] for 18 days. Sixteen seedlings per treatment (one seedling per pot; 10.6 × 10.6 × 11.5 cm, L × W × H) were transferred to another chamber (DS-50CPH; Dasol Scientific, Hwaseong, Korea) equipped with six different LED treatments. Distilled water (2 L) subirrigated the plug tray at intervals of 2-3 days for 18 days and nutrient solution for lettuce (N:P:K = 17.3:4.0:8.0, pH 5.5, EC 1.16 $dS \cdot m^{-1}$) was subirrigated to a tray (45 × 45 cm) containing 16 pots once a week for the rest of the cultivation period. All plants were grown at 20°C, PPFD of 173 ± 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and a 12-h photoperiod for 4 weeks. The pots were systematically rearranged to minimize disproportionate light distribution at the same time each day.

Light treatments

To evaluate the effect of substituting green LEDs for red or blue LEDs, six lighting treatments were designed (Table 1), which were based on the result from our previous study related to the ratios of red to blue LEDs (Son and Oh, 2013). The six plate-type lighting sources (48 × 48 cm, L × W) were manufactured to emit specific light quality using red (655 nm) (Bright LED Electronics, Seoul, Korea), blue (456 nm), and green (518 nm) (Itswell, Incheon, Korea) LEDs as follows: R9B1 (R:G:B = 9:0:1; based on chip number), R9G1 (R:G:B = 9:1:0), R8B2 (R:G:B = 8:0:2), R8G1B1 (R:G:B = 8:1:1), R7B3 (R:G:B = 7:0:3), and R7G1B2 (R:G:B = 7:1:2). Fluorescent and high-pressure sodium lamps were used as the control lighting source. The spectral distribution of each lighting source was measured at a height of 25 cm from the lighting source to the bottom and at five points (center and four edges of a tray) using a spectroradiometer (LI-1800; Li-Cor, Lincoln, NE, USA) (Fig. 1). From the spectral data, the photon flux and fractions in far-red (700-800 nm), red (600-700 nm), green (500-600 nm), and blue (400-500 nm) light of each treatment were determined from bandwidth integration (Table 1).

Growth characteristics

The fresh and dry weights of shoots and roots, total leaf area, and specific leaf weight (SLW) were measured 4 weeks

Table 1. Spectral data for various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs). Data were recorded at the plant canopy (25 cm from LED lighting sources) with a spectroradiometer. The spectral data were acquired from five points (a center and four edges of each tray of pots) and the means are shown (n = 5)

Parameter	Treatment						
	R9B1[z]	R9G1	R8B2	R8G1B1	R7B3	R7G1B2	Control[x]
Photon flux (μmol·m^{-2}·s^{-1})							
PPFD (400-700 nm)	174	175	172	176	174	174	170
Far-red (700-800 nm)	0	0	0	0	0	0	8
Red (600-700 nm)	152	161	130	136	114	118	30
Green (500-600 nm)	0	14	0	14	0	14	85
Blue (400-500 nm)	22	0	42	26	60	42	47
Fraction (%)[y]							
PPFD	100	100	100	100	100	100	100
Far-red	0	0	0	0	0	0	4
Red	87	91	76	77	66	68	18
Green	0	9	0	8	0	8	50
Blue	13	0	24	15	34	24	28

[z]Ratios of red, green, and blue LEDs based on the number of LED chips.
[y]Fraction of far-red, red, green, and blue wavelengths in terms of photosynthetic photon flux density (PPFD).
[x]Control: fluorescent lamp + high-pressure sodium lamp.

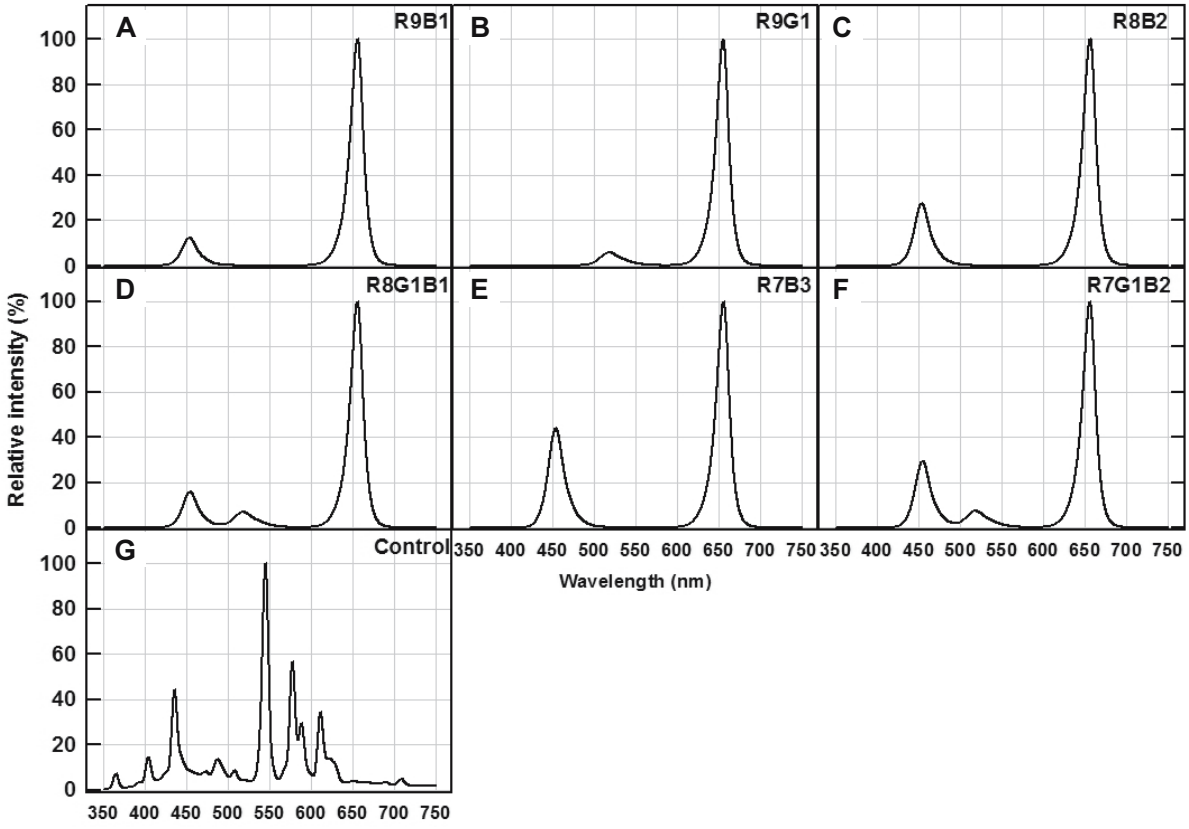

Fig. 1. Relative spectral distribution of various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) used in this study. (A) red:green:blue = 9:0:1, (B) red:green:blue = 8:0:2, (C) red:green:blue = 7:0:3, (D) red:green:blue = 9:1:0, (E) red:green:blue = 8:1:1, (F) red:green:blue = 7:1:2, (G) control (fluorescent lamp + high pressure sodium lamp). Photosynthetic photon flux density (PPFD) of all treatments was 173 ± 3 μmol·m^{-2}·s^{-1} in each treatment. Spectral scans were measured at 25 cm from the lighting sources and at five points (a center and four edges of each tray of pots).

after the onset of LED treatment. The fresh weights of shoots and roots were measured by an electronic scale (Si-234; Denver Instrument, NY, USA), and were then dried at 70°C in an oven (VS-120203; Vision Scientific, Daejeon, Korea) for 3 days to determine the dry weight. The total leaf area was measured using a leaf area meter (LI-3000A; Li-Cor, Lincoln, NE, USA). The SLW was calculated by dividing the dry weight of shoots by the leaf area.

Chlorophyll content

To analyze the chlorophyll (Chl) content of leaves, samples were collected 4 weeks after the onset of LED treatment. The Chl content of lettuce was determined by a modified version of the method described by Arnon (1949). Each sample (0.2 g) was macerated with a mortar and pestle and extracted with 5 mL of 80% (v/v) acetone. The extract (1.5 mL) was placed in a micro-tube, centrifuged at 905 ×*g* for 5 min, and the supernatant was then used to measure the Chl content. The absorbance of samples was read at both 663 nm (Chl a) and 645 nm (Chl b) using a spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). Total Chl, Chl a, and Chl b content of lettuce leaves were calculated using the following formula:

$$\text{Total Chl content } [\mu g \cdot mL^{-1}] = (20.3 \times A_{645}) + (7.22 \times A_{663})$$

$$\text{Chl a } [\mu g \cdot mL^{-1}] = (12.72 \times A_{663}) - (2.58 \times A_{645})$$

$$\text{Chl b } [\mu g \cdot mL^{-1}] = (22.88 \times A_{645}) - (5.50 \times A_{663})$$

where A_{645} and A_{663} is the absorbance of each sample. The Chl content is expressed as μg Chl per 0.2 g of fresh weight of lettuce leaves.

Photosynthetic rate and leaf transmittance

At 3 weeks after the onset of LED treatment, the photosynthetic rate (Pn) of the fully expanded fourth leaf from the top was measured using a portable photosynthesis system (LI-6400; Li-Cor, Lincoln, NE, USA). Considering diurnal variation in the Pn, the measurement was performed from 11 a.m. to 1 p.m. and the flow rate, CO_2 levels, PPF, and leaf temperature within a leaf cuvette was maintained at 350 $\mu mol \cdot s^{-1}$, 400 $\mu mol \cdot mol^{-1}$, 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 20°C, respectively.

Leaf transmittance was measured using a portable UV/VIS spectroradiometer (JAZ-EL200; Ocean Optics, Dunedin, FL, USA) with a 400-μm premium fiber (QP400-2-SR) and cosine corrector diffuser (CC-3-UV-S). At 4 weeks after the onset of LED treatment, the transmittance rate of each LED treatment was obtained by scanning the light spectrum from 400 to 700 nm at an interval of 0.38 at 1 cm below a fully expanded leaf that was parallel to the LED panels. The value was calculated with spectrometer operating software (SpectraSuite; Ocean Optics, Dunedin, FL, USA).

Cell division analysis

About 100 mg of each sample (completely unfolded young leaves) was collected at 4, 11, and 17 days after the onset of LED treatment. A high resolution DNA staining kit (CyStain UV Precise P; Partec, Münser, Germany) was used for this analysis, according to the manufacturer's instructions, and the sample was chopped with a sharp razor blade in 0.4 mL of nuclei extraction buffer (solution A), filtered through a 30-μm nylon sieve, and then 1.6 mL of staining buffer (solution B) was added. In each sample, analyses involved a minimum of 3,000 particles (total count) using Ploidy Analyzer (Partec, Münster, Germany). Data are presented as the percentage of the total number of nuclei in G1, S, and G2M phase.

Leaf cell density

Sampling and analysis of the epidermal cell density was performed using the method described by Ceulemans et al. (1995). At 3 weeks after the onset of each LED treatment, epidermal tissue near the central vein of the leaf was collected from fully expanded leaves that were the fourth leaf from the bottom using colorless nail polish and adhesive cellophane tape. Stomatal and epidermal cells were observed by fluorescence microscopy (JSB-F40; Samwon, Goyang, Korea) and each density (cell number per unit leaf area) was calculated as followed:

$$\text{Stomatal density} = s/(e + s)$$

$$\text{Epidermal cell density} = e/(e + s)$$

where s and e are the total numbers of stomatal and epidermal cells per unit area of leaf, respectively.

Antioxidant parameters; total phenolic concentration and antioxidant capacity

The total phenolic concentration and antioxidant capacity of lettuce was determined using the modified Folin-Ciocalteu reagent method (Ainsworth and Gillespie, 2007) and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) method (Miller and Rice-Evans, 1996), respectively. About 0.2 g of each sample was collected at 4 weeks after the onset of LED treatment and stored in a deep freezer at -70°C (DF8524; Il-ShinBioBase, Dongducheon, Korea) until analysis. The methods used for the extraction and analysis of total phenolic concentration and antioxidant capacity followed those described previously (Son and Oh, 2013). The absorbance of samples was read at 765 nm for total phenolic concentration and 730 nm for antioxidant capacity using a spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan). The total phenolic concentration and antioxidant capacity of lettuce are expressed as mg gallic acid equivalents (GAE) per g of fresh weight and mM trolox equivalents antioxidant capacity (TEAC) per g of fresh weight of lettuce leaves, respectively.

Statistical analysis

Four plants per treatment were used to measure all parameters. The experiment was repeated twice to verify reproducibility. Data were analyzed using the statistical analysis system (SAS 9.2; SAS Institute, USA) program. Two-way ANOVAs were performed using Fisher's LSD test to assess the interaction effects of cultivar and light source on growth characteristics. ANOVA was performed and Duncan's multiple range test was used to compare the means for the other parameters.

Results

Light spectrum

The light spectrum of each LED treatment was clearly distinct because the blue, green, and red LEDs that were used in this study had short wavelength ranges (± 25 nm from the peak wavelength) (Fig. 1). Fluorescent and high pressure sodium lamps that were used as the control treatment showed a multiple light spectrum, as expected. Based on the quantum from 400 nm to 700 nm, blue (400-500 nm), green (500-600 nm), and red (600-700 nm) lights were measured and the percentage of the three visible light sections was calculated for each LED treatment (Table 1). The average PPFD of all the LED treatments was 173 ± 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The difference in the PPFD of red or blue lights was between 4 and 9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared with the two LED treatments that had the same ratio of red and blue LEDs. The PPFD of green light irradiated to lettuce plants was 14 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in all of the LED treatments containing green LEDs.

Growth characteristics

Both lettuce cultivars grown under each treatment for 4 weeks showed significant differences according to the ratios of red, green, or blue light in terms of fresh and dry weights of shoot and roots, leaf area, and SLW (Table 2). For the red leaf lettuce 'Sunmang', the fresh and dry weights of shoots and roots, and leaf area increased as the proportion of red LEDs increased within the combination of red and blue LEDs (except for treatments with green LEDs). The fresh weight under R9B1 was about 1.8 and 1.4 times higher than that of R7B3 and the control, respectively. In contrast, increasing the proportion of blue LEDs had a negative effect on the fresh and dry weights of shoots and roots, and leaf area whereas SLW representing leaf thickness increased as the proportion of blue LEDs increased resulting in the highest

Table 2. Growth characteristics of lettuce plants grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 4 weeks after the onset of LED treatment (n = 4)

Cultivar	Light source	Fresh weight (g/plant)		Dry weight (g/plant)		Leaf area (cm^2/plant)	Specific leaf weight ($DW \cdot mg \cdot cm^{-2}$)
		Shoot	Root	Shoot	Root		
'Sunmang' (red leaf)	R9B1	42.97 a[z]	2.92 def	1.86 a	0.12 b	1238.19 b	1.46 g
	R9G1	47.14 a	2.51 efg	1.93 a	0.10 b	1506.16 a	1.25 h
	R8B2	26.70 c	1.94 g	1.35 c	0.09 b	587.62 de	2.23 ab
	R8G1B1	43.01 a	3.14 cde	1.84 a	0.12 b	1137.68 b	1.57 efg
	R7B3	23.87 c	1.82 g	1.25 c	0.10 b	517.80 e	2.34 a
	R7G1B2	32.18 b	2.27 fg	1.59 b	0.11 b	713.02 d	2.16 b
	Controly	31.53 b	2.43 efg	1.43 bc	0.11 b	925.81 c	1.49 fg
'Grand Rapid TBR' (green leaf)	R9B1	53.93 a	3.72 abcd	2.28 a	0.16 a	1430.22 ab	1.54 efg
	R9G1	50.18 ab	2.01 g	1.81 bc	0.11 a	1587.91 a	1.08 h
	R8B2	38.27 bc	4.04 ab	1.67 bc	0.17 a	808.88 c	1.97 c
	R8G1B1	55.02 a	3.94 abc	2.44 a	0.16 a	1357.58 ab	1.73 de
	R7B3	39.34 bc	4.14 ab	1.75 bc	0.17 a	869.53 c	1.96 c
	R7G1B2	50.34 ab	4.37 a	2.06 ab	0.18 a	1122.98 bc	1.78 d
	Control	35.64 c	3.42 bcd	1.49 c	0.17 a	865.14 c	1.67 def
Significance[x]							
Cultivar (C)		***	***	***	***	***	NS
Light source (L)		***	***	***	**	***	***
C × L		NS	***	NS	*	NS	***

[z]Different letters within columns indicate significant differences by Duncan's multiple range test. Significant at $p \leq 0.05$.
[y]Control: fluorescent lamp + high-pressure sodium lamp.
[x]NS,*,**,***indicates non-significant, significant at p = 0.05, 0.01 and 0.001, respectively.

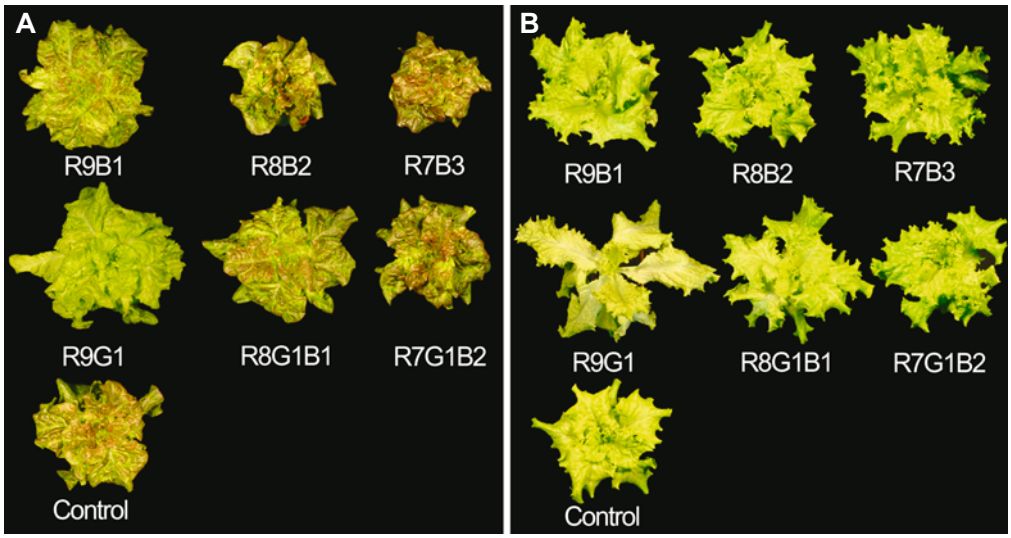

Fig. 2. Lettuce plants grown under various combinations of red (R) and blue (B), and RB with green (G) LEDs at 4 weeks after the onset of LED treatment in both 'Sunmang' (A) and 'Grand Rapid TBR' (B). Control represents fluorescent lamp and high-pressure sodium lamp.

value under R7B3. The replacement of some blue LEDs with green LEDs under the same red LED ratios increased the fresh and dry weights of shoots and roots, and leaf area (Table 2). Shoot fresh weights of R8G1B1 and R7G1B2 were about 61% and 35% higher than those of R8B2 and R7B3, respectively, which were significant. However, SLW was significantly decreased under treatments containing green LEDs. The replacement of red LEDs with green LEDs under about 10% blue LEDs (R9B1→R8G1B1) had no significant effect on any growth parameters, whereas the replacement of red LEDs with green LEDs under about 20% blue LEDs (R8B2 →R7G1B2) induced a significant increase in all growth parameters except for SLW.

For the green leaf lettuce 'Grand Rapid TBR', the growth responses were similar with the red leaf lettuce 'Sunmang' according to the ratio of red to blue LEDs (except for treatments under green LEDs). The fresh weight under R9B1 was about 1.4 and 1.5 times higher than that under R7B3 and the control treatment, respectively. As the level of blue LED light increased, the SLW also increased, which was similar to the results observed for 'Sunmang'. The effect of green LEDs on lettuce growth was observed in the same way as 'Sunmang'. Replacing blue LEDs with green LEDs under about 80% of red LED light (R8B2→R8G1B1) increased shoot growth parameters such as fresh and dry weights, and leaf area, whereas the SLW was significantly decreased. The use of green LEDs instead of blue LEDs under about 70% of red LEDs (R7B3→R7G1B2) also appeared to increase shoot growth, although there was no significant difference between the two treatments. However, the complete replacement of blue LEDs by green LEDs (R9B1→R9G1) did not affect the shoot growth and decreased SLW. The replacement of red LEDs by green LEDs, regardless of the blue LED ratio, had no clear effect on lettuce growth in 'Grand Rapid TBR'.

The light quality generated by red, green, and blue LEDs altered leaf shape and pigment in both lettuce cultivars at 4 weeks after the onset of LED treatment (Fig. 2). Considering the ratios of red to blue LEDs, the leaf length of 'Sunmang' decreased with the increasing proportion of blue LEDs. The leaf color became reddish under the treatments with a high proportion of blue LEDs. The addition of green LEDs, particularly in place of some of the blue LEDs, generally induced leaf expansion. The leaf shape for the green leaf lettuce 'Grand Rapid TBR' grown under treatments that did not contain blue LEDs (R9G1) was more elongated than that grown under other treatments, and the difference in leaf pigment was not clearly observed.

Table 3 shows the chlorophyll content of lettuce grown under various LED treatments for 4 weeks. The total chlorophyll content for 'Sunmang' tended to increase as the proportion of blue LEDs in the LED treatments composed of red and blue LEDs increased. The contents of total chlorophyll, Chl a, and Chl b of R8B2 and R7B3 were significantly higher than those under R9B1 and the control treatment. The replacement of blue LEDs with green LEDs under about 90% or 80% red LEDs (R9B1→R9G1, R8B2→R8G1B1) led to a significant inhibition of chlorophyll biosynthesis, whereas below 70% red LEDs (R7B3→R7G1B2) there was no significant difference. Under 10% or 20% blue LED light, irradiation with green LEDs instead of red LEDs (R9B1→ R8G1B1, R8B2→R7G1B2) did not affect the change in chlorophyll content. The total chlorophyll content under LED treatments with 20% more blue LEDs, such as R8B2,

Table 3. Chlorophyll (Chl) contents of lettuce plants grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 4 weeks after the onset of LED treatment (n = 4)

Cultivar	Light source	Total Chl ($\mu g \cdot mL^{-1}$)	Chl a ($\mu g \cdot mL^{-1}$)	Chl b ($\mu g \cdot mL^{-1}$)
'Sunmang' (red leaf)	R9B1	87.01 b[z]	67.16 b	19.85 c
	R9G1	59.85 c	45.57 c	14.28 d
	R8B2	132.10 a	102.74 a	29.37 b
	R8G1B1	92.93 b	71.93 b	21.00 c
	R7B3	143.84 a	108.17 a	35.67 a
	R7G1B2	130.36 a	99.18 a	31.18 ab
	Control[y]	101.48 b	80.26 b	21.22 c
'Grand Rapid TBR' (green leaf)	R9B1	105.91 b	85.44 b	20.48 b
	R9G1	64.80 d	48.88 e	15.92 c
	R8B2	84.88 c	68.81 c	16.07 c
	R8G1B1	117.17 a	94.16 a	23.01 a
	R7B3	85.82 c	69.33 c	16.49 c
	R7G1B2	90.57 c	73.03 c	17.54 c
	Control	66.55 d	56.02 d	10.53 d

[z]Different letters within columns indicate significant differences by Duncan's multiple range test. Significant at p = 0.001.
[y]Control: fluorescent lamp + high-pressure sodium lamp.

R7G1B2, and R7B3 was significantly higher than that of the plants grown under the control (fluorescent + high-pressure sodium lamps).

In green leaf lettuce 'Grand Rapid TBR', the chlorophyll content under different ratios of red to blue LEDs showed a somewhat different trend, and a high content was observed under treatments consisting of about 10% blue LEDs such as R9B1 and R8G1B1. The substitution of blue LEDs by green LEDs under about 80% red LEDs (R8B2→R8G1B1) induced a significant increase in chlorophyll contents in 'Grand Rapid TBR', which was different from the results observed with 'Sunmang'. The total chlorophyll content for 'Grand Rapid TBR' under all LED treatments except for R9G1 without blue LEDs, was significantly higher than that under the control.

Photosynthetic rate and leaf transmittance

Changing the ratio of red, green, and/or blue LEDs led to a significant difference in the photosynthetic rate of two leaf lettuce cultivars following 3 weeks of LED treatment (Fig. 3). The photosynthetic rate per unit area in the same leaf order and position showed an opposite tendency from the result of growth in both cultivars: the treatment without blue light (R9G1) led to the lowest photosynthetic rate and the treatment

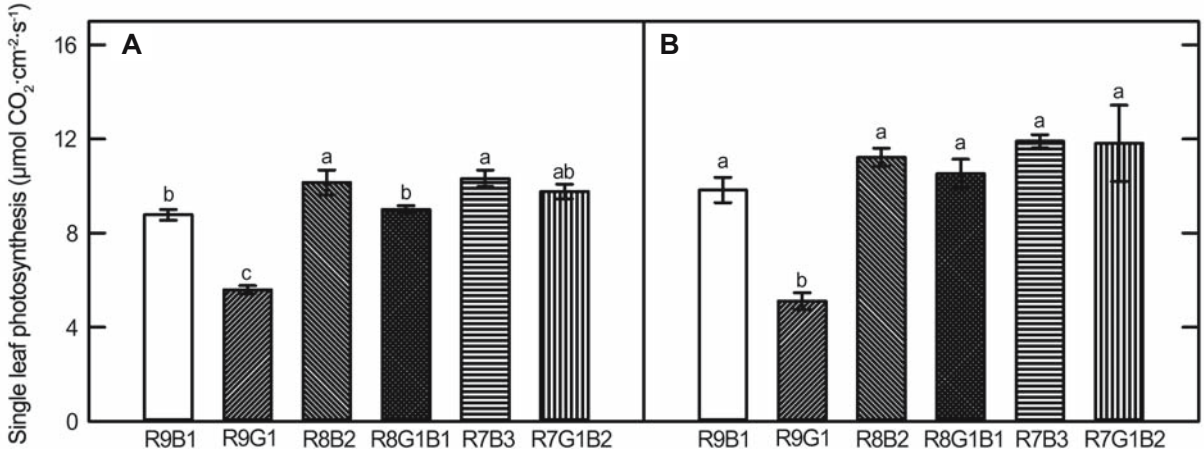

Fig. 3. Single leaf photosynthesis of both 'Sunmang' (A) and 'Grand Rapid TBR' (B) grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 3 weeks after the onset of LED treatment. The data indicate the means ± SE (n = 4). Different letters above bars indicate significant differences at p = 0.001.

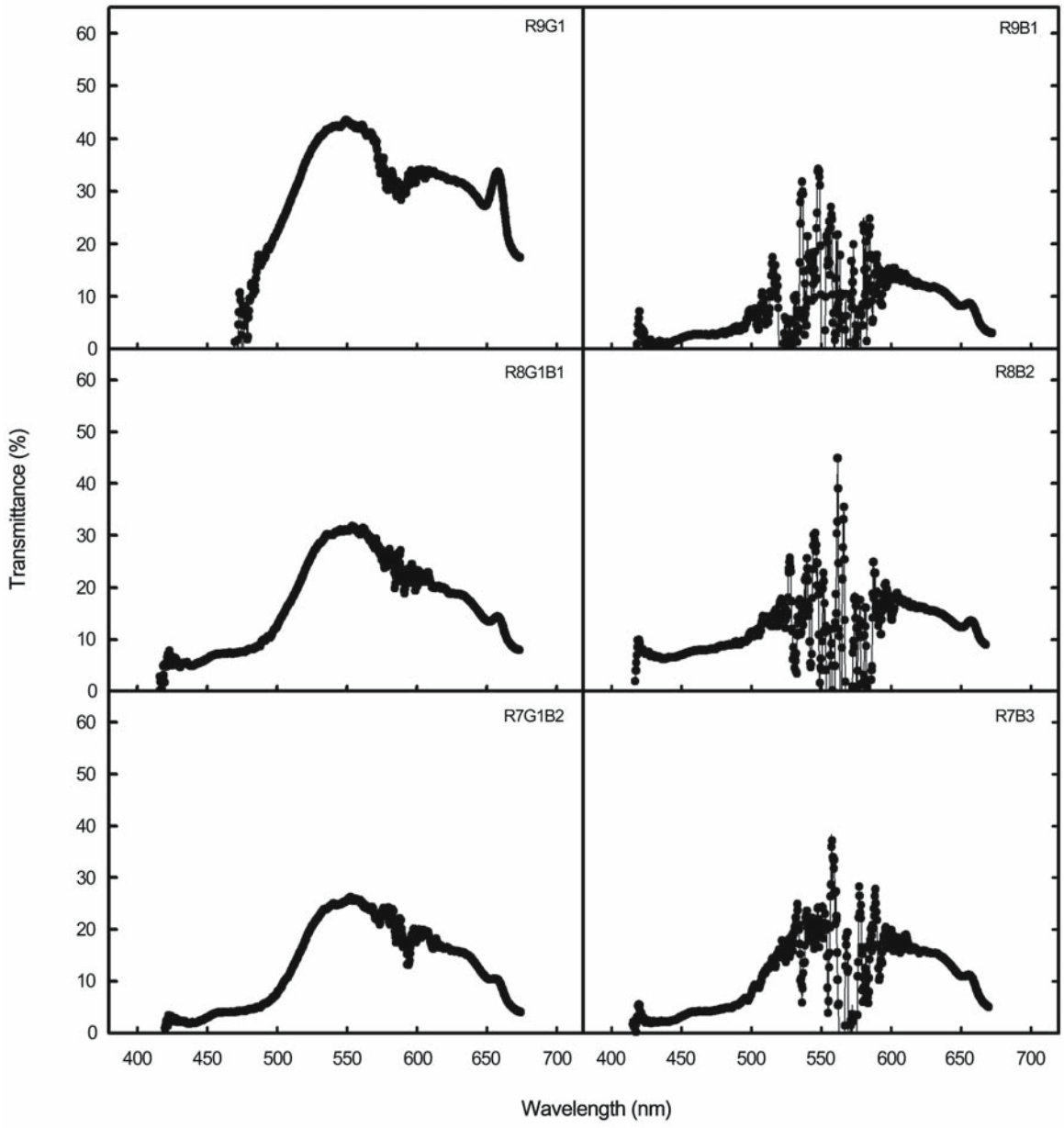

Fig. 4. Spectral distribution of light transmitted by red leaf lettuce ('Sunmang') grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 4 weeks after the onset of LED treatment. The transmittance was detected by a spectroradiometer with software at each treatment. Data are the percentages of transmitted light from lettuce leaves.

with over 10% of blue LEDs led to a high photosynthetic rate. When the proportion of red light was about 90% or 80%, the replacement of blue LEDs with green LEDs (R9B1 → R9G1, R8B2→R8G1B1) led to a significant reduction in the photosynthetic rate, whereas in the case of about 70% red LEDs (R7B3→R7G1B2), there was no significant difference, although a numerical reduction was observed in red leaf lettuce. On the other hand, the replacement of red LEDs with green LEDs at the same ratio of blue LEDs (R9B1→ R8G1B1, R8B2→R7G1B2) did not lead to a significant difference in the photosynthetic rate. For green leaf lettuce, there was no difference in the photosynthetic under the different treatments, except under the treatment without blue LEDs (R9G1).

The transmittance of two leaf lettuce cultivars, which were grown under each LED treatment was analyzed under each light source (Figs. 4 and 5). The quality of light penetrating the lettuce leaves following 4-weeks of growth under the treatments containing green LEDs was analyzed and the light transmittance (15-36%) within the range of green light (500-600 nm) was higher than that of blue and red. The transmittance each of red and blue light was 10-30% and 1-11%, respectively. In other words, the fraction of incident light within the visible light spectrum that passed through a

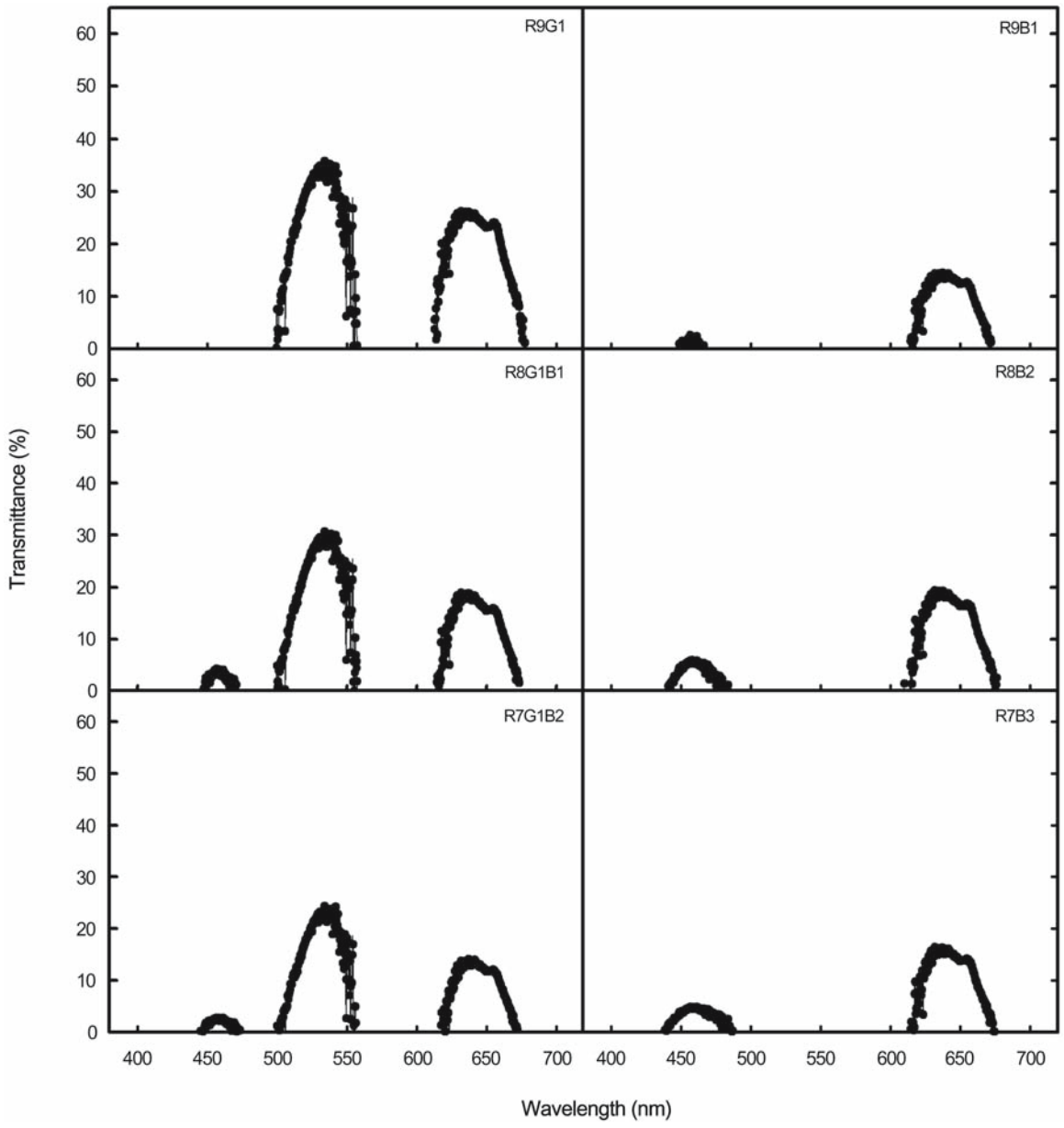

Fig. 5. Spectral distribution of light transmitted by green leaf lettuce ('Grand Rapid TBR') grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 4 weeks after the onset of LED treatment. The transmittance was detected by a spectroradiometer with software at each treatment. Data are the percentages of transmitted light from lettuce leaves.

leaf was green, red, and blue in descending order. Green leaf lettuce showed a similar tendency as red leaf lettuce. Under treatments containing green LEDs, the green wavelength exhibited a higher transmittance than blue or red wavelength.

Cell division and leaf anatomy

By analyzing the rate of cell division in red leaf lettuce, the influence of different light sources was observed (Fig. 6). The percentage of cells in the G2M phase under the treatments containing green LEDs was increased, while treatments without green LEDs such as R8B2 and R7B3 led to relatively lower percentages of cells in the G2M phase following 4 days of treatment. After 11 and 17 days of treatment, the results seemed similar to those observed after 4 days. In the graph showing the rate of cell division, the area indicating the G2M phase increased under treatments that included green LEDs (Figs. 6D and 6E). Such a trend was also observed in green leaf lettuce (data not shown).

The effect of green light on the epidermal cell density was also observed following 3 weeks of treatment (Fig. 7). In the case of red leaf lettuce, the density of epidermal cells and stomata showed an increasing tendency as blue LEDs were replaced by green LEDs under the specific ratio of red light. In particular, when the ratio of red light was about 80%, the

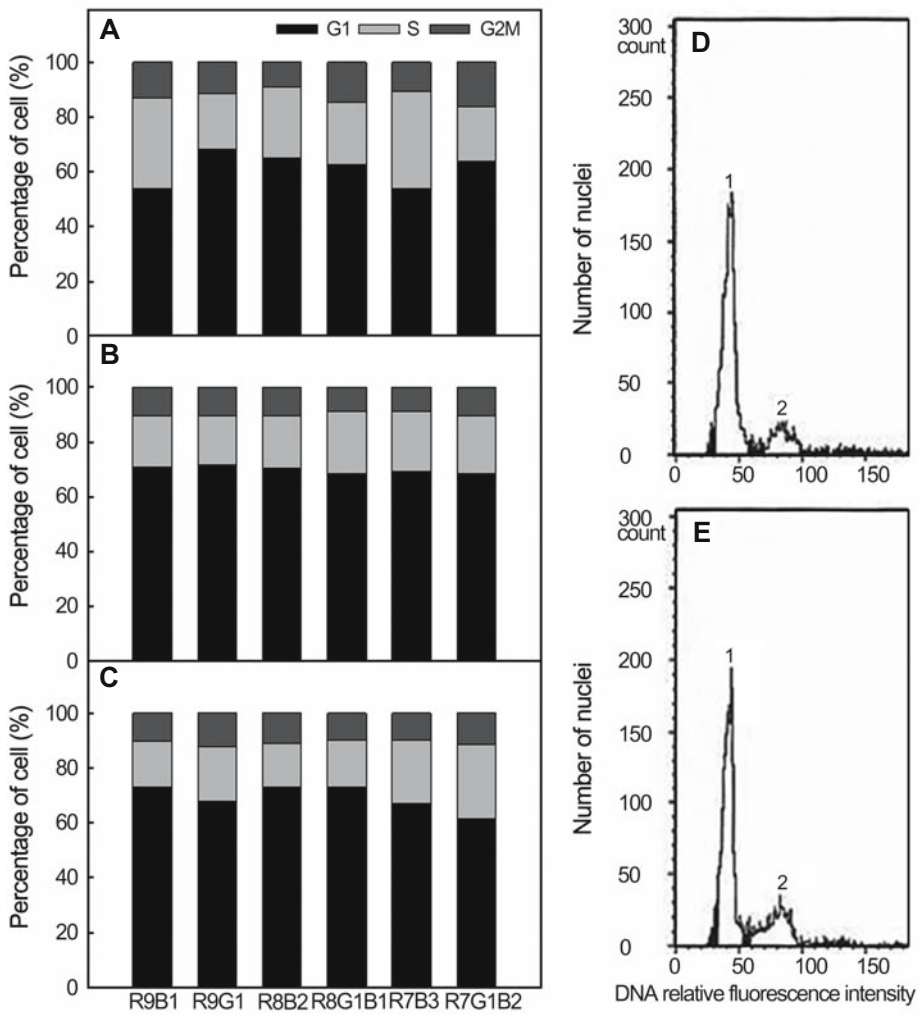

Fig. 6. Cell division of red leaf lettuce 'Sunmang' grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs). Cell division rates are shown at 4 (A), 11 (B) and 17 (C) days after the onset of LED treatment. (D-E) The picture represents endoreduplication levels in red leaf lettuce 'Sunmang' grown under R9B1 (D) and R9G1 (E) LED treatments. The data indicate the means (n = 4).

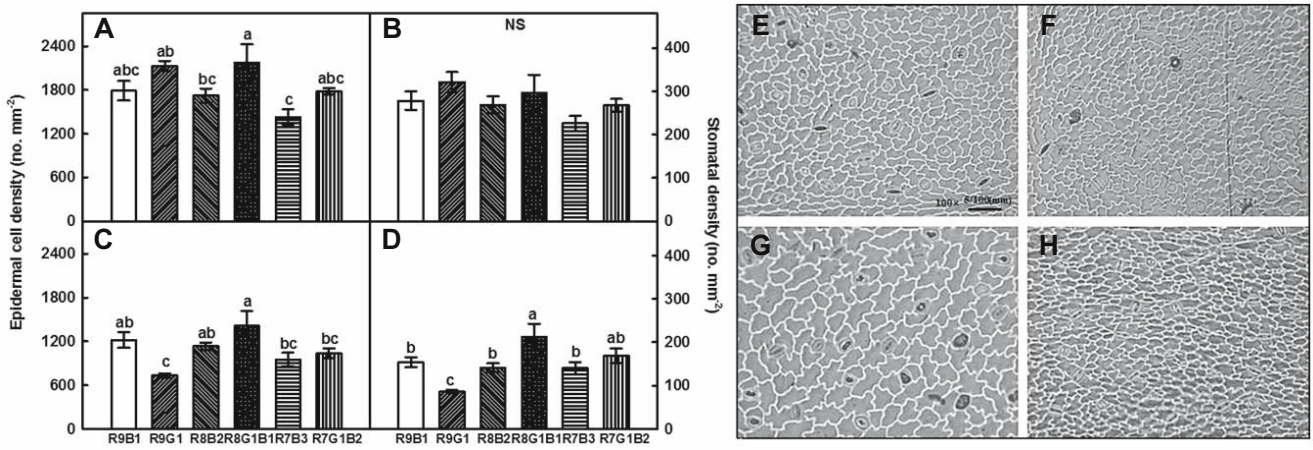

Fig. 7. Epidermal cell density (A, C) and stomatal density (B, D) in leaves of 'Sunmang' (A, B) and 'Grand Rapid TBR' (C, D) grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 3 weeks after the onset of LED treatment. (E-H) Epidermal and stomatal traits in 'Sunmang' (E, F) and 'Grand Rapid TBR' (G, H) grown under R8B2 (E, G) and R8G1B1 (F, H) LED treatments. The data indicate the means ± SE (n = 12). Different letters above bars indicate significant differences at p = 0.01.

replacement of some of the blue LEDs with green LEDs (R8B2→R8G1B1) induced a significant increase in the density of epidermal cells. Substitution of some of the blue LEDs with green LEDs (R8B2→R8G1B1) also significantly increased the stomatal density in green leaf lettuce, but the treatment without blue LEDs showed the lowest value, which was inconsistent with the result observed in red leaf lettuce. This trend can be observed by microphotography (Figs. 7E-7H).

Total phenolic concentration and antioxidant capacity

The total phenolic concentration and antioxidant capacity of red leaf lettuce following 4 weeks of treatment increased as the ratio of red LEDs decreased and the ratio of blue LEDs increased (Table 4). The total phenolic concentration and antioxidant capacity under R7B3 were significantly higher at 53-56% and 61-67%, compared with those under R9B1 and the control, respectively. In terms of the effect of substitution, replacement of blue LEDs with green LEDs led to a decrease in total phenolic concentration and antioxidant capacity. In particular, R9B1→R9G1 and R7B3→R7G1B2 led to a significant decrease, whereas when the ratio of red light was about 80%, there was a non-significant numerical decrease. On the other hand, when the proportion of blue light was constant, replacing red LEDs with green LEDs (R9B1→R8G1B1, R8B2→R7G1B2) did not lead to a significant change in the levels of both total phenolic concentration and antioxidant capacity. Light treatments that did not contain blue LEDs, except for R9G1, did not lead to a significant decrease compared to the control treatment. Total phenolic concentration and antioxidant capacity showed a similar tendency.

Table 4. Total phenolic concentration and antioxidant capacity of red leaf lettuce ('Sunmang') grown under various combinations of red (R) and blue (B), and RB with green (G) light-emitting diodes (LEDs) at 4 weeks after the onset of LED treatment (n = 4)

Light source	Total phenolic concentration (mg GAE·g^{-1} FW)	Antioxidant capacity (mM TEAC·g^{-1} FW)
R9B1	0.53 b[z]	2.42 b
R9G1	0.31 c	1.26 c
R8B2	0.64 b	2.95 b
R8G1B1	0.53 b	2.33 b
R7B3	0.83 a	3.90 a
R7G1B2	0.53 b	2.64 b
Control[y]	0.54 b	2.33 b

[z]Different letters within columns indicate significant differences by Duncan's multiple range test. Significant at p = 0.001.
[y]Control: fluorescent lamp + high-pressure sodium lamp.

Discussion

Growth characteristics

Out of red, green, and blue LEDs, red LEDs provide the major source of light for the growth of two leaf lettuce cultivars. As the ratio (or PPFD) of red LEDs increased, the fresh and dry weights of shoots and roots, and leaf area increased in both leaf lettuce cultivars. The positive effect of red light on the growth of lettuce has been reported in previous studies (Heo et al., 2012; Johkan et al., 2010), and our previous studies that compared monochromatic LEDs and the ratio of red and blue LEDs also suggested that red light was the most effective at improving the biomass of lettuce (Son et al., 2012; Son and Oh, 2013). Red light is absorbed by phytochromes, which are known to have an important role in the growth and development of plants, and have been used as an important energy source for the development of photosynthetic apparatus and the accumulation of starch (Saebo et al., 1995). Red light is therefore known to represent the effective light spectrum for promoting crop growth (Folta and Childers, 2008). The leaf shape was mainly influenced by the ratio of red to blue LEDs, and increasing the ratio of blue light suppressed leaf expansion in both cultivars (Fig. 2). This result was consistent with that of our previous experiment, which compared the effect of red to blue LED ratios (Son and Oh, 2013). On the other hand, blue light had a positive influence on the increase of SLW, which indicates leaf thickness (Table 2). This was consistent with results from the previous experiment, in which fluorescent lamps supplemented with blue light promoted increased thickness of lettuce leaf compared to treatment without blue light (Ohashi-Kaneko et al., 2007). Since blue light stimulates the formation of mesophyll tissue in leaves (Liu et al., 2014; Xiao et al., 2011), it is thought that the increased quantum of blue light increased the thickness of lettuce leaves in the present study. Furthermore, R8G1B1, R9B1, and R9G1 treatments led to a significant increase in shoot growth compared to that observed under the control treatment in both cultivars. This suggests that a specific combination of LEDs would be more effective in terms of crop production compared to a conventional lighting source.

Green light had been thought to provide an ineffective range of wavelengths for the growth and development or photosynthesis in plants (Folta and Maruhnich, 2007). Monochromatic green light (518 nm), however, was used for photosynthesis so that it led to an accumulation of biomass in lettuce, although the chlorophyll content and the growth were significantly reduced compared to those under other monochromatic wavelengths such as red and blue (Son et al., 2012). Kim et al. (2004) reported that the addition of 24% green light to a combination of red and blue LEDs led to the

enhancement of lettuce growth. Johkan et al. (2012) also demonstrated that high intensity radiation at 300 μmol·m^{-2}·s^{-1} of monochromatic green light (510 nm) improved anthocyanin biosynthesis as well as the growth and development of lettuce suggesting the availability of green light. In this study, the overall growth including fresh weight was improved when some of the blue LEDs were replaced with green LEDs to the same level as the red LEDs. In particular, shoot biomass under the treatment R8G1B1 significantly increased by about 1.6 and 1.4 times in both 'Sunmang' and 'Grand Rapid TBR' cultivars, respectively, compared to that under R8B2. However, complete replacement of blue light with green light (R9B1→R9G1) had no effect on lettuce growth. This result is consistent with that of Kim et al. (2004), who compared the combination of red and blue LEDs with and those supplemented with green LEDs. Poor lettuce growth under the R9G1 treatment without blue LEDs could be explained by the fact that blue light is an absolute wavelength range for photosynthesis along with red light, which plays an important role in the formation of chlorophyll (Banaś et al., 2012).

Meanwhile, growth under the R9G1 treatment was similar to that under R9B1 because irradiation with green LEDs caused pronounced leaf expansion. Thus, replacing some of the blue LEDs with green LEDs led to expanded leaf area and maintained leaf thickness to some extent, and could also improve overall shoot growth and development in lettuce plants. In contrast, when some of the red light was replaced with green light without any change being made to the blue light source, there was no significant increase in lettuce growth. This is not consistent with the results of Kim et al. (2004) who reported that the growth of lettuce was improved following the addition of green light under the same ratio as blue light. The proportion of green light used in this study was 8%, whereas in their study, the light treatment that improved the growth was composed of 24% green light. Therefore, green light is thought to have quantitative effects as well as qualitative effects.

Chlorophyll content

In this study, the chlorophyll content of lettuce leaves was closely associated with the ratio of blue LEDs. In our previous study, which examined the combined effects of red and blue LEDs, the SPAD value, which indicates chlorophyll content, gradually increased as the proportion of blue LEDs increased up to about 50%, and the lowest level was recorded in the treatment without blue LEDs (Son and Oh, 2013). In this study, the red leaf lettuce grown under the R9G1 treatment without blue LEDs had the lowest chlorophyll content although there was somewhat of a difference in the green leaf lettuce. Hogewoning et al. (2010) reported that the role of blue light in the biosynthesis of chlorophyll is important for the qualitative as well as quantitative aspects. In general, green wavelength is absorbed less efficiently than red and blue wavelengths (Carvalho et al., 2011). However, green light was not thought to have a negative impact on the formation of chlorophyll compared with red because there was no significant difference in the total chlorophyll content when red LEDs were replaced with green LEDs at the same level of blue LEDs (R9B1→R8G1B1, R8B2→R7G1B2). Kim et al. (2004) also demonstrated that supplementing combined blue and red LEDs with green LEDs did not change the chlorophyll content of lettuce leaves. These authors suggested that an increased leaf area by green light would be improved the chlorophyll content of the whole plant ability to absorb light although green light reduced the formation of chlorophyll and the carbon assimilation rate per unit area. Consistent with this, in the present study, replacing blue LEDs with green LEDs led to reduced chlorophyll content per unit fresh weight, but the relatively high leaf area appeared to be because of enhanced growth. Meanwhile, chlorophyll content is an important factor for photosynthesis, growth, and development of plants. In particular, chlorophyll a has a direct impact on the activation of photosynthesis through energy transfer, and has a much wider spectrum of activity than chlorophyll b (Calatayud and Barreno, 2004; Šesták, 1966). The content of chlorophyll a relied largely on the presence of blue light in red leaf lettuce because it was similar to the total chlorophyll content. Such a tendency was also seen in green leaf lettuce. The difference in chlorophyll content between the two cultivars was a result of the different composition of various pigments including anthocyanin in leaf tissues (Calatayud and Barreno, 2004). Son and Oh (2013) reported that the chlorophyll content and growth differed between cultivars. In conclusion, the chlorophyll content was mainly dependent on blue light (Banaś et al., 2012), an effect that may vary with cultivars.

Photosynthesis and transmittance

The increased ratio of red LEDs and the replacement of blue LEDs with green LEDs led to improved lettuce growth, whereas the photosynthetic rate did not support the growth results. The increased photosynthetic rate did not have a direct impact on growth (Kim et al., 2004), and measurement of the limited leaf area and the usage of particular lighting source equipped with the equipment for photosynthesis would be considered as factors that may reduce the objectivity of the result (Chow et al., 1990). However, when the photosynthetic rate per plant canopy was expressed based on the data obtained per unit area, it showed a similar pattern to that of the growth result (data not shown). Therefore, in an experiment of light quality, it seems necessary to measure

photosynthesis of the plant canopy under similar light conditions to the growth environment to obtain further accurate results. On the other hand, when the level of blue light was the same, no reduction in photosynthetic rate was observed. A significant reduction in photosynthesis under treatments without blue LEDs occurred because blue light has an essential role in photosynthesis. This can be explained by the reported effect of blue light on stomata and CO_2 fixation (Hogewoning et al., 2010).

The results of transmittance in two leaf lettuce cultivars supported growth improvement by green LEDs (Figs. 4 and 5). When the lettuce canopy begins to form a colony and leaves overlap, most of the blue and red light is absorbed and used for photosynthesis on the top of the leaves (Kim et al., 2004). However, green light is transmitted to the lower part of the lettuce canopy and would therefore have some positive effect on photosynthesis (Klein, 1992). Moreover, green light has been shown to be effective at fixing CO_2 in thick leaves (Sun et al., 1998). The red and blue wavelengths of light that are absorbed by chloroplasts in thick leaf tissue are relatively reduced compared to green light as the leaf increases in thickness (Nishio, 2000). The difference in transmittance of visible light depending on lettuce cultivar is thought to have resulted from differences in leaf shape and structure, and the content of pigments including chlorophylls.

Finally, given the growth stage and leaf thickness, supplementary irradiation with green light in combination with red and blue light might enhance growth by improving the photosynthesis rate of the whole plant canopy.

Cell division and leaf anatomy

The rate of cell division was determined by endopolyploidy analysis, which observed development of an organ and the size of plant cells. The percentage of cells in the G2M phase, which represents the rate of cell division, was higher under treatments with green LEDs (R9G1, R8G1B1, R7G1B2) than those under treatments with the same ratio of red LEDs but in the absence of green LEDs (Fig. 6), and this was consistent with the growth results. Thus, green light would be increased the rate of cell division at each growth stage. This directly involves the cell cycle, which is related to cell expansion and size and suggests the possibility of improved growth and development through the conversion of M, a mitotic period (Park et al., 2010; Vázquez-Romos and de la Paz Sánchez, 2003).

The results from the analysis of epidermal cell density and stomatal density of leaves also support a positive effect of green light on lettuce growth. It was thought that activation of cell division under treatments including green LEDs increased the density of epidermal cells and that this leads to expansion of the leaf area and improved growth and development. Stomatal density is also thought to have a positive impact on growth (Downton et al., 1985) since it increased when blue LEDs were replaced with green LEDs (R8B2→R8G1B1, R7B3→R7G1B2) in green leaf lettuce.

Total phenolic concentration and antioxidant capacity

The difference in the expression of pigments such as anthocyanin depending on light qualities in red leaf lettuce is closely related to the difference in the level of secondary metabolites produced through the phenylpropanoid biosynthetic pathway. A considerable amount of secondary metabolites is phenolic compounds, and in the case of lettuce, about 70% of phenolic compounds have antioxidant properties (Llorach et al., 2008). In this study, an increase in blue light intensity increased the total phenolic concentration and the antioxidant capacity of red leaf lettuce (Table 4). This was consistent with the amount of pigment expressed in the image of lettuce leaves (Fig. 2) and was consistent with the results of previous studies that reported an increase in antioxidant phenolic compounds following the addition of blue light to red light (Johkan et al., 2010; Stutte et al., 2009). In our previous study, blue light activated expression of the PAL gene, which leads to the synthesis of PAL, a key factor in the biosynthetic pathway of secondary metabolites (Son et al., 2012). Replacing blue LEDs with green LEDs led to a reduction in the total phenolic concentration and the antioxidant capacity, while the replacement of red LEDs with green LEDs did not have an impact on total phenolic concentration and the antioxidant capacity. However, as we reported in Son and Oh (2013), green leaf lettuce did not show a clear tendency in total phenolic concentration and antioxidant capacity according to the change of blue LED ratio (data not shown). Most of the existing LED studies relating to plants have focused on growth; however, this study analyzed changes in antioxidant phenolic compounds, which are important for determining crop quality.

Conclusion

In our study, we demonstrated that red and blue lights have a large influence on improving biomass of lettuce and accumulating secondary metabolites, respectively. In addition, the supplementation with green LEDs was found to enhance leaf expansion and cell division of lettuce, and subsequently enhance growth and development under appropriate proportions of red and blue LEDs. In visual aspects, the supplementation with green light also reduces the burden, enabling easy observation of the growth status of crops (Kim et al., 2004). Thus, the results of this study suggest that the proportion of red, green, and blue LEDs is an important factor in designing

artificial lighting sources because of its effects on growth, development, morphology, and secondary metabolite biosynthesis, and thereby the application of cultivation techniques by adjusting the light quality should be considered for the production of goal-oriented crops in closed-type plant production systems.

Acknowledgement: This work was supported by the research grant of the Chungbuk National University in 2012.

Literature Cited

Ainsworth, E.A. and K.M. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protoc. 2:875-877.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Banaś, A.K., C. Aggarwal, J. Łabuz, O. Sztatelman, H. Gabryś. 2012. Blue light signaling in chloroplast movements. J. Exp. Bot. 63:1559-1574.

Bian, Z.H., Q.C. Yang, and W.K. Liu. 2015. Effects of light quality on the accumulation of phytochemicals in vegetables produced in controlled environments: a review. J. Sci. Food Agric. 95:869-877.

Calatayud, A. and E. Barreno. 2004. Response to ozone in two lettuce varieties on chlorophyll a fluorescence, photosynthetic pigments and lipid peroxidation. Plant Physiol. Biochem. 42:549-555.

Carvalho, R.F., M. Takaki, and R.A. Azevedo. 2011. Plant pigments: the many face of light perception. Acta Physiol. Plant. 33:241-248.

Ceulemans, R., L.Van Praet, and X.N. Jiang. 1995. Effects of CO_2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (Populus). New Phytol. 131:99-107.

Chow, W.S., A. Melis, and J.M. Anderson. 1990. Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Pro. Natl. Acad. Sci. 87:7502-7506.

Downton, W.J.S., W.J.R. Grant, and S.P. Robinson. 1985. Photosynthetic and stomatal responses of spinach leaves to salt stress. Plant Physiol. 78:85-88.

Folta, K.M. 2004. Green light stimulates early stem elongation antagonizing light-mediated growth inhibition. Plant Physiol. 135: 1407-1416.

Folta, K.M. and S.A. Maruhnich. 2007. Green light: a signal to slow down or stop. J. Exp. Bot. 58:3099-3111.

Folta, K.M. and K.S. Childers. 2008. Light as a growth regulator: controlling plant biology with narrow-bandwidth solid-state lighting systems. HortScience 43:1957-1964.

Goins, G.D., N.C. Yorio, M.M. Sanwo, and C.S. Brown. 1997. Photomorphogenesis, photosynthesis, and seed yield of wheat plants grown under red light-emitting diodes (LEDs) with and without supplemental blue lighting. J. Exp. Bot. 48:1407-1413.

Heo, J.W., D.H. Kang, H.S. Bang, S.G. Hong, C. Chun, and K.K. Kang. 2012. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. Kor. J. Hort. Sci. Technol. 30:6-12.

Hogewoning, S.W., G. Trouwborst, H. Maljaars, H. Poorter, W. van Ieperen, and J. Harbinson. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Exp. Bot. 61:3107-3117.

Hopkins, W.G. and N.P.A. Huner. 2004. Introduction to plant physiology. 3rd Ed. John Wiley and Sons, Hoboken, NJ, USA.

Jiao, Y., O.S. Lau, and X.W. Deng. 2007. Light-regulated transcriptional networks in higher plants. Nature Rev. Genet. 8:217-230.

Johkan, M., K. Shoji, F. Goto, S. Hahida, and T. Yoshihara. 2010. Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. HortScience 45:1809-1814.

Johkan, M., K. Shoji, F. Goto, S. Hahida, and T. Yoshihara. 2012. Effect of green light wavelength and intensity on photomorphogenesis and photosynthesis in *Lactuca sativa*. Environ. Exp. Bot. 75: 128-133.

Kim, H.-H., G.D. Goins, R.M. Wheeler, and J.C. Sager. 2004. Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. HortScience 39:1617-1622.

Klein, R.M. 1992. Effects of green light on biological systems. Biol. Rev. 67:199-284.

Liu, M., Z. Xu, S. Guo, C. Tang, X. Liu, and X. Jao. 2014. Evaluation of leaf morphology, structure and biochemical substance of balloon flower (*Platycodon grandiflorum* (Jacq.) A. DC.) plantlets in vitro under different light spectra. Sci. Hortic. 174:112-118.

Llorach, R., A. Martínez-Sánchez, F.A. Tomás-Barberán, M.I. Gil, and F. Ferreres. 2008. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. Food Chem. 108:1028-1038.

Massa, G.D., H.-H. Kim, R.M. Wheeler, and C.A. Mitchell. 2008. Plant productivity in response to LED lighting. HortScience 43: 1951-1956.

Matsuda, R., K. Ohashi-Kaneko, K. Fujiwara, and K. Kurata. 2007. Analysis of the relationship between blue-light photon flux density and the photosynthetic properties of spinach (*Spinacia oleracea* L.) leaves with regard to the acclimation of photosynthesis to growth irradiance. Soil Sci. Plant Nutr. 53:459-465.

Matsuda, R., K. Ohashi-Kaneko, K. Fujiwara, and K. Kurata. 2008. Effects of blue light deficiency on acclimation of light evergy partitioning in PSII and CO_2 assimilation capacity to high irradiance in spinach leaves. Plant Cell Physiol. 49:664-670.

Miller, N.J. and C.A. Rice-Evans. 1996. Spectrophotometric determination of antioxidant activity. Redox Rpt. 2:161-17.

Nishio, J.N. 2000. Why are higher plants green? Evolution of the higher plant photosynthetic pigment complement. Plant Cell Environ. 23:539-548.

Ohashi-Kaneko, K., M. Takase, N. Kon, K. Fujiwara, and K. Kurata. 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Control Biol. 45: 189-198.

Park, S.-Y., E.C. Yeung, and Peak, K.-Y., 2010. Endoreduplication in *Phalaenopsis* is affected by light quality from light-emitting diodes during somatic embryogenesis. Plant Biotechnol. Rep. 4: 303-309.

Saebo, A., T. Krekling, and M. Appelgren. 1995. Light quality affects photosynthesis and leaf anatomy of birch plantlets *in vitro*. Plant Cell Tissue Organ Cult. 41:177-185.

Samuolienė, G., R. Sirtautas, A. Brazaitytė, J. Sakalauskaitė, S. Sakalauskienė, and P. Duchovskis. 2011. The impact of red and blue light-emitting diode illumination on radish physiological indices. Central Eur. J. Biol. 6:821-828.

Savvides, A., D. Fanourakis, and W. van Leperen. 2012. Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves. J. Exp. Bot. 63:1135-1143.

Šesták, Z. 1966. Limitations for finding a linear relationship between chlorophyll content and photosynthetic activity. Biol. Plant. 8: 336-346.

Son, K.-H., J.-H. Park, D. Kim, and M.-M. Oh. 2012. Leaf shape, growth, and phytochemicals in two leaf lettuce cultivars grown under monochromatic light-emitting diodes. Kor. J. Hort. Sci. Technol. 30:664-672.

Son, K.-H. and M.-M. Oh. 2013. Leaf shape, growth, and antioxidant

phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting didoes. HortScience 48:988-995.

Stutte, G.W., S. Edney, and T. Skerritt. 2009. Photoregulation of bioprotectant content of red leaf lettuce with light-emitting diodes. HortScience 44:79-82.

Sun, J., J.N. Nishio, and T.C. Vogelmann. 1998. Green light drives CO_2 fixation deep within leaves. Plant Cell Physiol. 39:1020-1026.

Talbott, L.D., G. Nikolova, A. Ortiz, I. Shmayevich, and E. Zeiger. 2002. Green light reversal of blue-light-stimulated stomatal opening is found in a diversity of plant species. Am. J. Bot. 89:366-368.

Terashima, I., T. Fujita, T. Inoue, W.S. Chow, and R. Oguchi. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50:684-697.

Vázquez-Romos, J.M. and M. de la Paz Sánchez. 2003. The cell cycle and seed germination. Seed Sci. Res. 13:113-130.

Wang, H., M. Gu, J. Cui, K. Shi, T. Zhou, and J. Yu. 2009. Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. J. Photochem. Photobiol. B: Biol. 96:30-37.

Wu, M.C., C.Y. Hou, C.M. Jiang, Y.T. Wang, C.Y. Wang, H.H. Chen, and H.M. Chang. 2007. A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. Food Chem. 101:1753-1758.

XiaoYing, L., G. ShiRong, X. ZhiGang, J. XueLei, and T. Tezuka. 2011. Regulation of chloroplast ultrastructure, cross-section anatomy of leaves, and morphology of stomata of cherry tomato by different light irradiations of light-emitting diodes. HortScience 46:217-221.