

# Quantitative Evaluation of Electric Light Recipes for Red Leaf Lettuce Cultivation in Plant Factories

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ADDITIONAL INDEX WORDS. anthocyanin, energy yield, LED, light-emitting diode, photon yield, quantitative indicator

**SUMMARY.** Red leaf lettuce (*Lactuca sativa*) has high nutritional value and is frequently used in salads. In a plant factory with full electric lighting, if the spectrum is incorrect, then red leaf lettuce will have incomplete coloration. This study aimed to establish a light recipe for the mass production of red leaf lettuce using electric light sources in a plant factory by using indicators for quantitative assessment, including energy yield (EY) [grams of fresh weight (FW) harvested per kilowatt hour of electricity input for lighting], photon yield (PY) (grams of FW harvested per mole of photons delivered), anthocyanin yield per kilowatt hour (EY<sub>A</sub>), and anthocyanin yield per photon (PY<sub>A</sub>). First, the effects of four types of light quality on FW and anthocyanin content were examined. Then, two types of light quality, light-emitting diode with a red-to-blue photon ratio of 80:20 (R<sub>80</sub>:B<sub>20</sub>) and R<sub>20</sub>:B<sub>80</sub>, were selected for an experiment involving five treatments. An optimum light recipe (SR5SB1) including R<sub>80</sub>:B<sub>20</sub> treatment during the early stage of cultivation (weeks 1 through 5 after sowing) followed by R<sub>20</sub>:B<sub>80</sub> treatment during the final stage (week 6) was proposed. The SR5SB1 treatment led to FW, EY<sub>A</sub>, and PY<sub>A</sub> of 87.8 g/plant, 1.63 mg/kWh, and 0.57 mg·mol<sup>-1</sup>, respectively. This treatment resulted in the highest EY<sub>A</sub> and PY<sub>A</sub>, with 159% and 256% more anthocyanin productivity, respectively, compared with cool white treatment (with FW, EY<sub>A</sub>, and PY<sub>A</sub> of 65.8 g/plant, 0.63 mg/kWh, and 0.16 mg·mol<sup>-1</sup>, respectively). The proposed SR5SB1 light recipe enabled cultivation of red leaf lettuce with a balanced yield and anthocyanin production.

The issue of food safety has gained attention in recent years. Currently, climate change is a major concern, and it is increasingly difficult to cultivate healthy and safe agricultural products in the field (Sazvar et al., 2018). Plant factories can provide a cultivation environment that is free from disturbances by the external environment and have steady annual production; therefore, they have great potential for safe crop production (Kozai, 2013). In Taiwan, red leaf lettuce contains anthocyanin and appears red; therefore, it is used for color enhancement in salads (Gazula et al., 2007). Lee et al. (2009) found that red leaf lettuce can help reduce

the risk of cardiovascular disease in mice. Current plant factories with full electric lighting primarily use light from white light-emitting diodes (LEDs) as the light source for cultivation; however, these light sources do not emit ultraviolet light, and the spectra are considerably different from sunlight. Previous studies have mentioned that although ultraviolet light causes substantial accumulation of anthocyanin and secondary metabolites in red leaf lettuce, excessive ultraviolet light slows the growth of lettuce (Tsormpatsidis et al., 2010).

In a plant factory with electric light, both the fixed cost of acquiring electric light sources and the subsequent operating cost expended each day are remarkably high. The cost of electric energy may exceed 25% of the total production cost (Kozai, 2013); therefore, the selection of electric light sources is a crucial topic of discussion. Scholars have conducted numerous studies of photosynthetic photon flux density (PPFD), light quality, and photoperiods. A PPFD of 300 μmol·m<sup>-2</sup>·s<sup>-1</sup> can significantly increase red perilla (*Perilla frutescens*) growth and anthocyanin content compared with lower PPFD treatments (Lu et al., 2017). Furthermore, a mixture of red and blue electric light sources can increase anthocyanin content more than green light sources can (Nishimura et al., 2009). The total phenolic concentration, total flavonoid concentration, and anthocyanin content of lettuces grown under high ratios of blue light (B<sub>59</sub>, B<sub>47</sub>, and B<sub>35</sub>) were significantly higher compared those grown with B<sub>0</sub> treatment (Son and Oh, 2013). Supplemental blue light (R<sub>14</sub>:G<sub>31</sub>:B<sub>55</sub>) for baby leaf lettuce can enhance anthocyanin accumulation and increase carotenoid concentration (Li and Kubota, 2009). Compared with a 12-h photoperiod, a 16-h photoperiod can significantly increase the concentration of rosmarinic acid in spearmint [*Mentha spicata* (Fletcher et al., 2010)]. However, this series of studies lacked a quantitative assessment of the energy consumption of electric light sources used for crop production; therefore, it lacked an important metric for the selection of lamps.

The concepts of energy yield (EY) and photon yield (PY) are aimed at assessing the effectiveness of electric light sources for cultivating crops in plant factories. The unit of EY is grams per kilowatt hour and represents the grams of shoot fresh weight

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## Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
10	%	mg·g <sup>-1</sup>	0.1
29.5735	fl oz	mL	0.0338
0.0929	ft <sup>2</sup>	m <sup>2</sup>	10.7639
2.54	inch(es)	cm	0.3937
1	mmho/cm	mS·cm <sup>-1</sup>	1
28.3495	oz	g	0.0353
28,350	oz	mg	3.5274 × 10 <sup>-5</sup>
10.7639	W/ft <sup>2</sup>	W·m <sup>-2</sup>	0.0929
(°F - 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32
(°F ÷ 1.8) + 255.37	°F	K	(K - 255.37) × 1.8

(FW) that can be produced by a plant per kilowatt hour of electricity consumed during the entire crop cycle. The higher the EY value, the more efficiently a plant converts electric energy into FW (Fang, 2013). EY can also be used to improve secondary metabolite production in plants. Using anthocyanin as an example,  $EY_A$  indicates the milligrams of anthocyanin that can be produced by a plant per kilowatt hour of electricity consumed during the crop cycle. The unit of PY is grams per mole and represents the grams of FW that can be produced by a plant per mole of photosynthetically active radiation (PAR). Higher PY values indicate greater efficiency with which a plant uses each mole of photons to produce FW. The PY is affected by *PPFD* and light quality. Similarly,  $PY_A$  represents the amount (milligrams) of anthocyanin that can be produced by a plant per mole of photosynthetically active photons. Obviously, crop yields are influenced not only by light delivery but also by nutrients received, indoor temperature, humidity, ventilation, management, and when and how to apply light.

This study paired different light qualities with different illumination strategies to examine the growth and anthocyanin production of red leaf lettuce, and it used EY and PY as indicators for assessing the effects of energy consumption of an electric light source and its spectrum on crop production in a plant factory. This study evaluated light recipes, including

when light was provided, how intense the light was (*PPFD*), how long the light was on (photoperiod), and what type of spectrum was provided during specific stages of lettuce cultivation.

## Materials and methods

**EXPT. 1: EFFECTS OF DIFFERENT AMOUNTS OF RED AND BLUE LIGHT RATIOS ON THE GROWTH AND PHYSIOLOGY OF RED LEAF LETTUCE.** The red leaf lettuce cultivar Red Oak was used in this study (Suntech Seed Co., Tainan, Taiwan). Seeds were soaked in tap water for 5 h; after which, floating seeds were removed and the remaining seeds were sown in moist sponge media (Yung-Kuang Chemical Products Co., Taichung, Taiwan) and placed in a growth chamber with a temperature of 20 °C. Cool white (CW) LED lamps (Genesis Photonics, Tainan, Taiwan) were used for 24-h illumination. The *PPFD* of each treatment group was measured using a quantum sensor (LI-250A light meter, LI-190R sensor; LI-COR, Lincoln, NE) and maintained at  $100 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at 25 cm below the light source during the sowing stage. Settings for additional environmental parameters during the cultivation period are listed in Table 1.

Expt. 1 used four different light qualities, including three R-B light combinations and CW LED lamps. The R-B treatments comprised three different photon ratios:  $R_{80}:B_{20}$ ,  $R_{50}:B_{50}$ , and  $R_{20}:B_{80}$  (Test model; Everlight Electronics Co., New Taipei City, Taiwan). The fourth treatment,

which was used as the control, used CW LEDs with a color temperature of 5500 K. The *PPFD* used during each treatment was measured using a quantum sensor and maintained at  $150 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . A spectroradiometer (USB4000; Ocean Optics, Largo, FL) was used to measure the light spectrum of each treatment (Fig. 1). Energy consumption of the light source was measured using a current clamp-on meter (368FC; FLUKE, Everett, WA), and the power consumed per unit of growing area was calculated (Table 2). Table 2 shows the absolute values of the *PPFD* in the blue, green, and red wavebands. Plants were harvested 42 d after sowing.

**EXPT. 2: EFFECTS OF DIFFERENT COMBINATIONS OF RED AND BLUE LIGHT RATIOS DURING DIFFERENT GROWTH STAGES ON THE ANTHOCYANIN CONTENTS OF RED LEAF LETTUCE.** The same red leaf lettuce cultivar that was used for Expt. 1 was used for Expt. 2, and two types of R-B LEDs were used: one system with stronger red (SR) and another with stronger blue (SB). The ratios of R-B photons were  $R_{80}:B_{20}$  and  $R_{20}:B_{80}$ . The spectra and intensity values are shown in Fig. 1 and in the second and fourth columns of Table 2. Both light sources produced a *PPFD* of  $150 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . During cultivation, three out of five LED treatments were switched to different spectra at 3, 2, and 1 weeks before harvest (Table 3). The first column of Table 3 shows the treatments of each arrangement

**Table 1. Environmental parameters, including air temperature, light parameters, nutrition solution parameters, and carbon dioxide concentration, used for red lettuce cultivation.**

Environmental parameters	Units (mean $\pm$ SD) <sup>z</sup>	Sowing stage 0–6 DAS <sup>y</sup>	Seedling stage 7–13 DAS	Growing stage 14–42 DAS
Day/night temperature	°C	20 $\pm$ 2		25/18 $\pm$ 2
Light intensity <sup>x</sup>	$\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	100 $\pm$ 5		150 $\pm$ 5
Light quality <sup>w</sup>	-	CW		$R_{80}:B_{20}/R_{50}:B_{50}/R_{20}:B_{80}/\text{CW}$
Photoperiod	h	24		16
Plants density	plants/m <sup>2</sup>	667.6	140.3	30.6
Nutrition solution recipe	-	Yamazaki solution [6N–1.5P–10K (Yamazaki, 1982)]		
EC	mS·cm <sup>-1</sup>		1.2 $\pm$ 0.1	
pH	-		6.0 $\pm$ 0.1	
Hydroponic type	-	Still water culture		Deep flow technique
Carbon dioxide concentration	$\mu\text{mol}\cdot\text{mol}^{-1}$		1200 $\pm$ 100	

<sup>z</sup>(1.8  $\times$  °C) + 32 = °F, 1 plant/m<sup>2</sup> = 0.0929 plant/ft<sup>2</sup>, 1 mS·cm<sup>-1</sup> = 1 mmho/cm.

<sup>y</sup>Days after sowing.

<sup>x</sup>The photosynthetic photon flux density [*PPFD* (400–700 nm)] was measured using a quantum sensor at 25 cm (9.8 inches) below the light source.

<sup>w</sup>Lettuce was grown using different light quality treatments. The red-blue photon ratios were  $R_{80}:B_{20}$ ,  $R_{50}:B_{50}$ , and  $R_{20}:B_{80}$ , respectively, and the cool white (CW) red-green-blue photon ratio was  $R_{20}:G_{50}:B_{30}$ .

of LED lights. They were SR6 [SR (R<sub>80</sub>:B<sub>20</sub>) for 6 weeks], SR5SB1 [SR for 5 weeks followed by SB (R<sub>20</sub>:B<sub>80</sub>) for 1 week], SR4SB2 (SR for 4 weeks followed by SB for 2 weeks), SR3SB3 (SR for 3 weeks followed by SB for 3 weeks), and SB6 (SB for 6 weeks). During Expt. 2, after 6 weeks of cultivation, FW, anthocyanin content, and chromaticity coordinates of the leaves were measured and analyzed.

## Measurements

**CHROMATICITY COORDINATES OF THE LEAVES.** Fully expanded upper leaves were selected after 6 weeks of cultivation. Each leaf was measured at three different locations. Five plants were randomly selected for sampling

and examined with a colorimeter (6807 color-guide 45/0; BYK-Gardner, Wesel, Germany). The  $L^*$  value indicated the degree of lightness (black:  $L^* = 0$ ; white:  $L^* = 100$ ), the  $a^*$  value indicated the degrees of green ( $a^* = -60$ ) and red ( $a^* = +60$ ), and the  $b^*$  value indicated the degrees of blue ( $b^* = -60$ ) and yellow ( $b^* = +60$ ). The hue angle ( $h^\circ$ ) was calculated using Eq. [1]. The  $h^\circ$  close to  $0^\circ$  indicated a color close to red, and  $h^\circ$  close to  $180^\circ$  indicated a color close to green (Owen and Lopez, 2015).

$$h^\circ = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad [1]$$

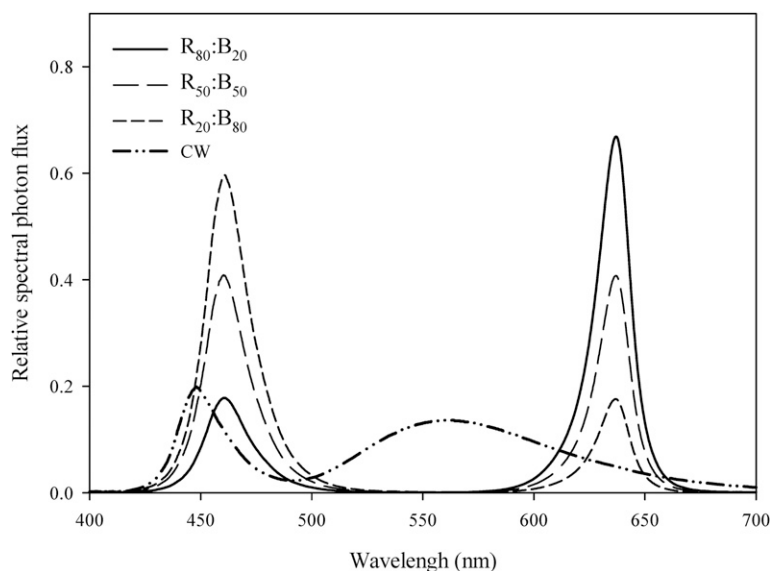
## ANALYSIS OF ANTHOCYANIN CONTENT AND SHOOT FW OF LETTUCE.

After the lettuce was harvested, the aboveground portion was removed and weighed on an electronic balance (STX2202; OHAUS, Parsippany, NJ) to obtain the shoot FW (in grams). Next, fully expanded upper leaves were selected. Five plants were randomly selected for sampling. Using the method described by Hung et al. (2008), 0.5 g of fresh lettuce leaves were randomly collected from each treatment group. After the samples had been ground with liquid nitrogen in a mortar, 2 mL of tripotassium phosphate buffer solution was added and evenly mixed. The samples were placed in a 1.5-mL centrifuge tube and centrifuged at 4 °C and 9500g<sub>n</sub> for 30 min (Z216MK; HERMLE, Wehingen, Germany). A spectrophotometer (DR2800; Hach, Loveland, CO) was used to determine the absorbance at a wavelength of 600 nm. A standard cyanidin-3-O-glucoside solution in methanol with 1% HCl (Sigma-Aldrich, St. Louis, MO) was used to establish a calibration curve to obtain the anthocyanin content [AC (milligrams per gram)] of FW.

Eq. [2] shows that the AC and the shoot FW of lettuce (grams per plant) were multiplied to obtain the total anthocyanin content [TAC (milligrams per plant)]:

$$TAC = AC \times FW \quad [2]$$

**EY AND PY.** Calculations for EY and PY were based on the formula published by Fang (2013). EY and EY<sub>A</sub> incorporated represent the FW of lettuce and the TAC that can be produced, respectively, by an electric light source per kilowatt hour of electricity consumed between sowing



**Fig. 1.** Spectral distributions of light from light-emitting diodes (LEDs) measured with a spectroradiometer. Different LED light quality treatments were used. The red-blue photon ratios were R<sub>80</sub>:B<sub>20</sub>, R<sub>50</sub>:B<sub>50</sub>, and R<sub>20</sub>:B<sub>80</sub>, respectively. The cool white (CW) red-green-blue photon ratio was R<sub>20</sub>:G<sub>50</sub>:B<sub>30</sub>. Photon flux per unit wavelength is expressed relative to the maximum.

**Table 2.** Characteristics of four light-emitting diode (LED) treatments installed 25 cm (9.8 inches) directly above the lettuce seedling. The cool white LED (CW) treatment was the control. The PPF<sub>D</sub> in the blue, green, and red wavebands for the different light treatments is shown.

Expt. 1 parameters	Treatment			
	R <sub>80</sub> :B <sub>20</sub>	R <sub>50</sub> :B <sub>50</sub>	R <sub>20</sub> :B <sub>80</sub>	CW
	Light intensity [mean ±SD (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )] <sup>z</sup>			
PPFD (400–700 nm)	150 ± 5	150 ± 5	150 ± 5	150 ± 5
Blue (400–499 nm)	30 ± 5	75 ± 5	120 ± 5	45 ± 5
Green (500–599 nm)	0	0	0	75 ± 5
Red (600–699 nm)	120 ± 5	75 ± 5	30 ± 5	30 ± 5
	Energy consumption per unit area (W·m <sup>-2</sup> ) <sup>y</sup>			
	194.0	180.0	166.6	138.8

<sup>z</sup>Photosynthetic photon flux density [PPFD (400–700 nm)] was measured using a quantum sensor at 25 cm (9.8 inches) below the light source.  
<sup>y</sup>1 W·m<sup>-2</sup> = 0.0929 W/ft<sup>2</sup>.

Table 3. Using two types of light-emitting diodes (red–blue ratios R<sub>80</sub>:B<sub>20</sub> and R<sub>20</sub>:B<sub>80</sub>) for different durations, a total of five different treatments were used for red lettuce cultivation (Expt. 2).<sup>z</sup>

Treatment	Sowing state 0–6 DAS <sup>y</sup>	Seedling state 7–20 DAS	Growing state 21–42 DAS		
	1 wk	2–3 wk	4 wk	5 wk	6 wk
	<i>PPFD</i> [mean ±SD (μmol·m <sup>-2</sup> ·s <sup>-1</sup> )] <sup>x</sup> = 150 ± 5				
SR6		R <sub>80</sub> :B <sub>20</sub>			
SR5SB1		R <sub>80</sub> :B <sub>20</sub>			R <sub>20</sub> :B <sub>80</sub>
SR4SB2		R <sub>80</sub> :B <sub>20</sub>			R <sub>20</sub> :B <sub>80</sub>
SR3SB3		R <sub>80</sub> :B <sub>20</sub>		R <sub>20</sub> :B <sub>80</sub>	
SB6			R <sub>20</sub> :B <sub>80</sub>		

<sup>z</sup>For environmental parameters other than light treatment, please refer to Table 1.

<sup>y</sup>Days after sowing.

<sup>x</sup>Photosynthetic photon flux density [*PPFD* (400–700 nm)] was measured using a quantum sensor at 25 cm (9.8 inches) below the light source.

and harvesting. The equations are as follows:

$$TPI_i = (P_i \times N_i \times L_i \div 1000) \times d_i / Pd_i \quad [3]$$

$$OTPI = \sum_{i=1}^n TPI_i \quad [4]$$

$$EY = FW / OTPI \quad [5]$$

$$EY_A = TAC / OTPI \quad [6]$$

Total power integral per plant at stage *i* (TPI<sub>*i*</sub>) is described as follows: during cultivation, stage *i* represents the electric energy consumed by the lights (in kilowatt hours per plant). The *i* represents the different stages of cultivation. Power at stage *i* (P<sub>*i*</sub>) is described as follows: during cultivation, stage *i* represents the electric power consumed per electric light source (in watts). The number of lights at cultivation stage *i* (N<sub>*i*</sub>) represents the average number of electric light sources (tubes or panels) used per square meter of growing area. Daily lighting hours at cultivation stage *i* (L<sub>*i*</sub>) represents the daily photoperiod (in hours per day). Days at cultivation stage *i* (d<sub>*i*</sub>) represents the number of days for cultivation stage *i*. Planting density at cultivation stage *i* (Pd<sub>*i*</sub>) represents the planting density (in plants per square meter). Overall total power integral (OTPI) represents the total light energy consumption during the entire cultivation period (in kilowatt hours per plant). EY is the FW per lettuce plant that can be produced by using electric lights (in grams per kilowatt hour). EY<sub>A</sub> is the TAC (milligrams) per lettuce plant that can be produced by using lights (in milligrams per kilowatt hour).

PY and PY<sub>A</sub> represent the FW and TAC, respectively, that can be

produced per mole of photons delivered from electric light sources. The equations are as follows:

$$TLI_i = (PPFD_i \times L_i \times 3.6 / 1000) \times d_i / Pd_i \quad [7]$$

$$OTLI = \sum_{i=1}^n TLI_i \quad [8]$$

$$PY = FW / OTLI \quad [9]$$

$$PY_A = TAC / OTLI \quad [10]$$

Total light integral per plant at cultivation stage *i* (TLI<sub>*i*</sub>) represents the total cumulative *PPFD* per plant (in moles per plant). Photosynthetic photon flux density at cultivation stage *i* (*PPFD*<sub>*i*</sub>) represents the average *PAR* (in micromoles per square meter per second). Overall total light integral per plant (OTLI) represents the total accumulated *PPFD* per plant during the entire cultivation period (in moles per plant). PY and PY<sub>A</sub> are measured in grams and milligrams per mole, respectively.

The quantities EY and PY can be used to calculate the overall photon energy (OPE) ratio (moles per kilowatt hour), as shown in Eq. [11].

$$OPE = EY / PY \quad [11]$$

For the *PAR* efficacy of luminaires, the unit micromoles per joule (1 μmol·J<sup>-1</sup> = 3.6 mol/kWh) is used. It represents the *PPFD* (micromoles per second per square meter) of photons generated by the light per watt (joules per second) of electric power. Although the *PPFD* per watt and OPE are expressed as the same units, they represent different concepts.

**STATISTICAL ANALYSIS.** This experiment used a completely randomized design and was replicated twice.

There were 20 samples for FW and five samples for anthocyanin content, chromaticity coordinates, EY and PY measurements, and calculations. Statistical software (SAS version 9.1; SAS Institute, Cary, NC) was used for analyses. Duncan's multiple range test was used for treatment comparisons (*P* ≤ 0.05).

## Results

**EXPT. 1: EFFECTS OF DIFFERENT AMOUNTS OF RED AND BLUE LIGHT RATIOS ON THE GROWTH AND PHYSIOLOGY OF RED LEAF LETTUCE.** Results of the experiment showed that the R<sub>80</sub>:B<sub>20</sub> treatment group produced the most FW (Table 4). Although the R<sub>20</sub>:B<sub>80</sub> treatment group did achieve color changes in the leaves, the biomass production was clearly reduced (Fig. 2). Table 4 shows the FW, AC, and TAC of the three light quality treatments and the control treatment. The R<sub>80</sub>:B<sub>20</sub> treatment significantly increased the shoot FW of lettuce, which caused the TAC to be the highest. As the proportion of blue light increased, the AC increased, but the shoot FW of lettuce significantly decreased (Table 4). The R<sub>20</sub>:B<sub>80</sub> treatment significantly increased the AC of red leaf lettuce.

In terms of TAC, R<sub>80</sub>:B<sub>20</sub> treatment increased production by 117% compared with CW treatment. Regarding the chromaticity coordinates of the leaves, R<sub>80</sub>:B<sub>20</sub> and CW treatments did not show significant differences in the four chromaticity coordinates listed in Table 4. The R<sub>80</sub>:B<sub>20</sub> and CW treatments significantly increased the values of *L*<sup>\*</sup> (lightness), *b*<sup>\*</sup> (blue and yellow), and *h*<sup>o</sup> (hue angle) compared with the R<sub>50</sub>:B<sub>50</sub> and R<sub>20</sub>:B<sub>80</sub> treatments. The R<sub>20</sub>:B<sub>80</sub> treatment significantly increased the *a*<sup>\*</sup> value (green and red) and

Table 4. Effects of different red–blue ratios [R<sub>80</sub>:B<sub>20</sub>, R<sub>50</sub>:B<sub>50</sub>, R<sub>20</sub>:B<sub>80</sub>, and cool white (CW)] on fresh weight (FW) of shoot, anthocyanin content, color coordinates, and quantitative indicators of red leaf lettuce (Expt. 1).

Treatment <sup>c</sup>	Anthocyanin content <sup>x</sup>			Color coordinates <sup>w</sup>				Quantitative indicators <sup>v</sup>				
	FW (g/plant) <sup>y</sup>	AC (mg·g <sup>-1</sup> ) <sup>y</sup>	TAC (mg/plant) <sup>y</sup>	L*	a*	b*	b <sup>o</sup>	EY (g/kWh)	EY <sub>A</sub> (mg/kWh)	PY (g·mol <sup>-1</sup> )	PY <sub>A</sub> (mg·mol <sup>-1</sup> )	OPE (mol/kWh)
R <sub>80</sub> :B <sub>20</sub>	101.2 a <sup>u</sup>	0.024 b	2.43 a	32.78 a	-6.63 c	19.96 a	108.37 a	40.52 a	0.97 a	14.69 a	0.35 a	2.75 d
R <sub>50</sub> :B <sub>50</sub>	78.6 b	0.026 b	2.02 b	24.73 b	-1.13 b	8.44 b	97.63 b	33.91 b	0.88 b	11.32 b	0.29 b	3.00 c
R <sub>20</sub> :B <sub>80</sub>	40.5 d	0.046 a	1.86 c	21.11 b	1.87 a	5.69 b	71.81 c	18.96 c	0.87 b	5.89 c	0.27 b	3.21 b
CW	65.8 c	0.017 c	1.12 d	34.00 a	-7.08 c	20.58 a	108.98 a	37.13 b	0.63 c	9.55 b	0.16 c	3.88 a

<sup>x</sup>Lettuce was grown using different light quality treatments for 42 d (different red–blue photon ratios were R<sub>80</sub>:B<sub>20</sub>, R<sub>50</sub>:B<sub>50</sub>, and R<sub>20</sub>:B<sub>80</sub>, respectively; CW red–green–blue photon ratio was R<sub>20</sub>:G<sub>50</sub>:B<sub>30</sub>).

<sup>y</sup>1 g = 0.0353 oz, 1 mg·g<sup>-1</sup> = 0.1%, 1 mg = 3.5274 × 10<sup>-5</sup> oz.

<sup>z</sup>AC = anthocyanin content, TAC = total anthocyanin content (TAC=AC × FW).

<sup>w</sup>Leaf lightness (L\*) indicates darkness and lightness (black: L\* = 0; white: L\* = 100). The color coordinates a\* value is the ratio between greenness and redness (green: a\* = -60; red: a\* = +60). The color coordinates b\* value is the ratio between blueness and yellowness (blue: b\* = -60; yellow: b\* = +60). Hue angle (b<sup>o</sup>) was calculated using Eq. [1].

<sup>v</sup>EY = energy yield (gram per kilowatt hour of energy consumption), EY<sub>A</sub> = milligram of TAC produced per kilowatt hour of energy consumption, PY = photon yield (grams per mole of photon provided), PY<sub>A</sub> = milligram of TAC produced per mole of photon provided, OPE = overall quantum energy ratio (moles of light delivered per kilowatt hour of electrical light during the full cultivation period).

<sup>u</sup>Mean separation in columns by Duncan's multiple range test at P ≤ 0.05 (only FW, n = 20; others, n = 5).

significantly decreased the b\* and b<sup>o</sup> values compared with the CW treatment, which confirmed that the leaves had turned distinctly red and dark (Table 4; Fig. 2).

As shown in Table 4, the EY, EY<sub>A</sub>, PY, and PY<sub>A</sub> after R<sub>80</sub>:B<sub>20</sub> treatment were the highest, whereas the EY<sub>A</sub> and PY<sub>A</sub> after CW treatment were the lowest. The EY and PY of the R<sub>20</sub>:B<sub>80</sub> treatment were the lowest, indicating that the electricity consumption and spectrum of the R<sub>20</sub>:B<sub>80</sub> light source benefited the accumulation of FW the least. Of the four light quality treatments, R<sub>80</sub>:B<sub>20</sub> was the most favorable and CW had the highest OPE value of all light treatments. However, regarding the EY, PY, EY<sub>A</sub>, and PY<sub>A</sub> shown in Table 4, CW treatment achieved results similar to those of R<sub>50</sub>:B<sub>50</sub> in terms of EY and PY, whereas its anthocyanin accumulation (EY<sub>A</sub> and PY<sub>A</sub>) was the least favorable.

**EXPT. 2: EFFECTS OF DIFFERENT COMBINATIONS OF RED AND BLUE RATIOS DURING DIFFERENT GROWTH STAGES ON ANTHOCYANIN CONTENTS OF RED LEAF LETTUCE.** The results showed that lettuce growth during the SB6 treatment was suppressed and that growth of the SR6 treatment group was more favorable. However, color changes were poor, and the growth and color changes of the SR5SB1 treatment group were more balanced (Fig. 3).

As shown in Table 5, although the SR6 treatment significantly increased FW, it also significantly decreased AC. The SR5SB1 treatment significantly increased the TAC of lettuce. Although the SB6 treatment significantly increased AC, the TAC was also the lowest due to its low FW. A comparison between the SR5SB1 and SB6 treatment groups (SR5SB1/SB6) showed that the ratio values for FW, AC, and TAC were 2.03, 1.00, and 2.05, respectively.

Table 5 shows that when the SR6 treatment of R<sub>80</sub>:B<sub>20</sub> was used for the entire process, the L\*, b\*, and b<sup>o</sup> values were the highest, whereas the a\* value was the lowest. Moreover, if R<sub>20</sub>:B<sub>80</sub> was used as the illumination treatment, regardless of whether it was for 6 weeks (SB6) or only 1 week (SR5SB1), then the L\*, b\*, and b<sup>o</sup> values were significantly decreased and the a\* value was significantly increased. The SR5SB1 treatment

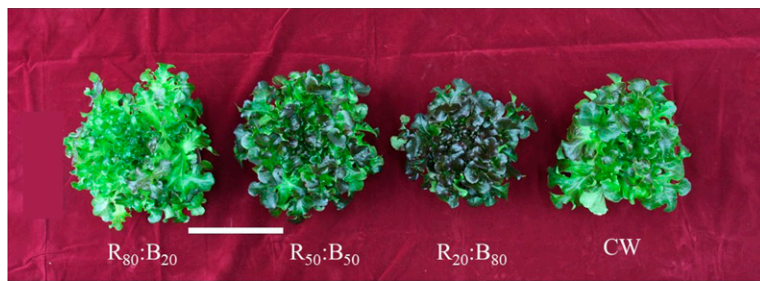


Fig. 2. Effects of different light quality treatments on the growth and development of red leaf lettuce 42 d after sowing [bar = 15.0 cm (5.91 inches)]. The different light-emitting diode (LED) light quality treatments were as follows: red-blue photon ratios of  $R_{80}:B_{20}$ ,  $R_{50}:B_{50}$ , and  $R_{20}:B_{80}$ , respectively, and the cool white (CW) red-green-blue photon ratio was  $R_{20}:G_{50}:B_{30}$ .

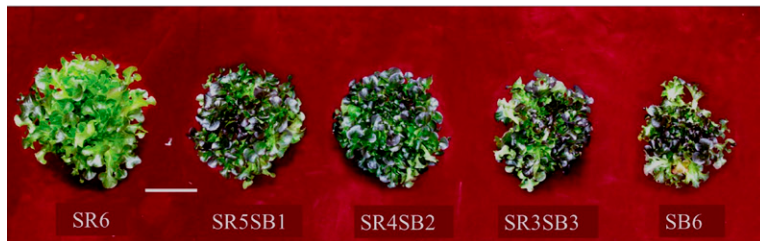


Fig. 3. Effects of different red-blue light ratios on the growth and development of red leaf lettuce 42 d after sowing [bar = 15.0 cm (5.91 inches)]. SR6 represents light quality treatment  $R_{80}:B_{20}$  for the full 6 weeks. SR5SB1 represents  $R_{80}:B_{20}$  during the first 5 weeks and  $R_{20}:B_{80}$  during the last 1 week. SR4SB2 represents  $R_{80}:B_{20}$  during the first 4 weeks and  $R_{20}:B_{80}$  during the last 2 weeks. SR3SB3 represents  $R_{80}:B_{20}$  during the first 3 weeks and  $R_{20}:B_{80}$  during the last 3 weeks. SB6 represents  $R_{20}:B_{80}$  during the full 6 weeks.

significantly increased the  $a^*$  value and decreased the  $b^{\circ}$  value.

As shown in Table 5, the SR6 treatment had the highest EY and PY, which indicated that this treatment was most beneficial for increased FW. The SR5SB1 treatment had the highest  $EY_A$  and  $PY_A$ , indicating that it was the most beneficial for anthocyanin accumulation. Interestingly, following an increase in the number of weeks of  $R_{20}:B_{80}$  usage, the OPE value also significantly increased, which indicated that this light quality treatment provided a greater number of photons per kilowatt hour of electricity than the  $R_{80}:B_{20}$  treatment.

The relative EY (rEY), relative PY (rPY), relative  $EY_A$  (r $EY_A$ ), and relative  $PY_A$  (r $PY_A$ ) represent the ratios of an electric light source to that of the control group (CW). SR6 treatment had the highest rEY and rPY. Considering the electricity consumption of photon generation, this indicated that using the SR6 light source resulted in 18% (EY) and 65% (PY) more FW, respectively, compared with using the CW light source. SR5SB1 treatment had the highest

r $EY_A$  and r $PY_A$ , which indicated that when consuming the same kilowatt hours of electricity or producing the same number of photons, this treatment resulted in 159% (EY) and 256% (PY) more anthocyanin yield, respectively, compared with CW treatment.

## Discussion

Chlorophyll a and b have absorption peaks for blue light (430–453 nm) and red light (642–663 nm) (Hopkins and Hüner, 2004). Previous studies have found that red light promoted the growth of lettuce (Johkan et al., 2010; Lee et al., 2010). Plant pigments can use red light to increase the carbon dioxide concentration in mesophyll cells, thus increasing the photosynthetic rate (Folta and Childers, 2008; Olsen et al., 2002). Research comparing the use of red-blue LEDs ( $R_{70}:B_{30}$ ) compared with white LEDs for the cultivation of lettuce found that red-blue LEDs can significantly increase the shoot dry weight, chlorophyll a and b content, and net photosynthetic rate (Amoozga et al., 2017). An analysis of a lettuce growth

model found that among various types of green light, yellow-green light (575–625 nm) resulted in the lowest growth efficiency (Dougher and Bugbee, 2001; Kong et al., 2015). The amount of green light in the CW treatment of this study reached 50%; therefore, it may have been one of the causes of inhibited red leaf lettuce growth.

Light quality ratios with high red light and low blue light can significantly increase the content of chlorophyll a and b and carotene, and the increase in carotene content provides a mechanism for the photoprotection of a plant to reduce the damage caused by free radicals (Johkan et al., 2010; Schagerl and Müller, 2006). This study also found that treatments with more than 50% blue light significantly suppressed plant growth, which is consistent with the findings of previous studies (Son and Oh, 2013).

Total phenol content is a general term for various secondary metabolites of plants, among which anthocyanin is a water-soluble antioxidant that mostly exists in vacuoles. They are primarily found in the petals, leaves, and seeds of plants as blue and purple pigments (Takeoka et al., 1997), and they protect plants and reduce the effects of light stress (Tattini et al., 2004). Son and Oh (2013) used red and blue LEDs to cultivate red leaf lettuce and found that FW and total phenol content were negatively correlated ( $r^2 = 0.7219$ ). However, treatment with more blue than red light ( $R_{41}:B_{59}$ ) significantly increased the total phenol content compared with  $R_{87}:B_{13}$  treatment, but it decreased the FW of lettuce. This result was consistent with the findings of the present study.

Phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) are key enzymes in the biosynthetic pathway of anthocyanin, and high blue light quality treatments (at least 50% of total PPF) can increase PAL activity (Heo et al., 2012). Moreover, when red leaf lettuce was cultivated for 1 week, first with red light and then with blue light, substantial accumulations of PAL and CHS were found (Son et al., 2017). This indicated that blue light can stimulate the biosynthesis of these two enzymes to increase the AC; however, the FW of lettuce tended to be decreased.

Table 5. Effects of different light recipes (SR6, SR5SB1, SR4SB2, SR3SB3, and SB6) on fresh weight (FW) of shoots, anthocyanin content, color coordinates, color coordinates, and quantitative indicators of red leaf lettuce (Expt. 2).

Treatment <sup>z</sup>	Anthocyanin content <sup>x</sup>			Color coordinates <sup>w</sup>				Quantitative indicator <sup>v</sup>				
	FW (g/plant) <sup>y</sup>	AC (mg·g <sup>-1</sup> ) <sup>y</sup>	TAC (mg/plant) <sup>y</sup>	L*	a*	b*	h°	EY (g/kWh)	EYA (mg/kWh)	PY (g·mol <sup>-1</sup> )	PYA (mg·mol <sup>-1</sup> )	OPE (mol/kWh)
SR6	108.3 a <sup>u</sup>	0.024 c	2.56 b	34.67 a	-6.64 d	19.27 a	109.01 a	43.65 a	1.00 b	15.72 a	0.36 c	2.76 c
SR5SB1	87.8 b	0.045 a	3.95 a	25.13 b	3.50 a	2.50 c	35.54 c	36.63 b	1.63 a	12.73 b	0.57 a	2.88 d
SR4SB2	88.7 b	0.038 b	3.34 b	27.54 b	1.35 b	4.19 b	72.14 b	38.67 b	1.45 b	12.87 b	0.48 b	3.00 c
SR3SB3	70.1 c	0.036 ab	2.54 c	25.85 b	0.83 c	4.32 b	79.12 b	31.98 c	1.16 c	10.17 c	0.37 c	3.14 b
SB6	43.2 d	0.045 ab	1.93 d	29.08 b	1.63 b	4.66 b	70.72 b	20.20 d	0.90 d	6.27 d	0.28 d	3.22 a

<sup>z</sup>Lettuce was grown using different light recipes. SR6 represents light treatment R<sub>80</sub>:B<sub>20</sub> for the full 6 weeks. SR5SB1 represents R<sub>80</sub>:B<sub>20</sub> during the first 5 weeks and R<sub>20</sub>:B<sub>80</sub> during the last 1 week. SR4SB2 represents R<sub>80</sub>:B<sub>20</sub> during the first 4 weeks and R<sub>20</sub>:B<sub>80</sub> during the last 2 weeks. SR3SB3 represents R<sub>80</sub>:B<sub>20</sub> during the first 3 weeks and R<sub>20</sub>:B<sub>80</sub> during the last 3 weeks. SB6 represents R<sub>20</sub>:B<sub>80</sub> during the full 6 weeks.

<sup>y</sup>1 g = 0.0353 oz, 1 mg·g<sup>-1</sup> = 0.1%, 1 mg = 3.5274 × 10<sup>-5</sup> oz.

<sup>x</sup>AC = anthocyanin content, TAC = total anthocyanin content (TAC = AC × FW).

<sup>w</sup>Leaf lightness (L\*) indicates darkness and lightness (black: L\* = 0; white: L\* = 100). The color coordinates a\* value is the ratio between greenness and redness (green: a\* = -60; red: a\* = +60). The color coordinates b\* value is the ratio between blueness and yellowness (blue: b\* = -60; yellow: b\* = +60). Hue angle (h°) was calculated using Eq. [1].

<sup>v</sup>EY = energy yield (gram of lettuce produced per kilowatt hour of energy consumption), EYA = milligram of TAC produced per kilowatt hour of energy consumption, PY = photon yield (grams of lettuce produced per mole of photon provided), PYA = milligram of TAC produced per mole of photon provided, OPE = overall quantum energy ratio (mole of light delivered per kilowatt hour of electrical light during the full cultivation period).

<sup>u</sup>Mean separation in columns by Duncan's multiple range test at P ≤ 0.05 (only FW, n = 20; others, n = 5).

This study also found that increasing the proportion of blue light significantly increased the AC in red leaf lettuce (Table 4); however, incomplete color changes were found in leaves underneath areas of leaf overlap, possibly because the blue light was absorbed by the leaves above them; therefore, the lower leaves were not illuminated sufficiently by blue light.

Red leaf lettuce must turn red to have acceptable market value (Owen and Lopez, 2015). A color scale (h°) indicates redness (0°), yellowness (90°), greenness (180°), or blueness (270°) of the lettuce. Owen and Lopez (2015) cultivated red leaf lettuce in greenhouses and found that using red and blue LEDs (R<sub>50</sub>:B<sub>50</sub>) for 1 week before harvesting significantly decreased the hue angle value (h° = 30.7–40.6), indicating successful color change from green (h° = 107.3–114.8) to red. In their control group (supplemental white LED lamps in a greenhouse), the leaves tended to turn green. This result was similar to that of the present study (SR5SB1 and SR6 hue angle values were 35.54 and 109.01, respectively) (Table 5). The present study used treatments with light changes during the cultivation process to reach a balance between yield and quality. An effective technique is promoting the accumulation of primary metabolites during an early stage of cultivation to cause plant growth, followed by short-term use of high levels of blue light to stimulate the plant to biosynthesize anthocyanin during the final stage of cultivation. Prolonging the period of high levels of blue light during quality treatment did not have a significant positive effect on the accumulation of TAC, possibly because the FW decreased due to the prolonged period of treatment with the high blue light (Table 5). During the last week of cultivation, the SR5SB1 treatment group received R<sub>20</sub>:B<sub>80</sub> treatment. These results were compared with the results of R<sub>80</sub>:B<sub>20</sub> use during the entire growing period. Although SR5SB1 treatment decreased FW by ≈19%, AC was increased by ≈90% and TAC of the entire plant was, in comparison with the plants in the R<sub>80</sub>:B<sub>20</sub> treatment group during the entire growing period, increased by 54% (Table 5).

Kozai (2013) proposed an indicator to calculate the efficiency of the use of electric lighting in plant factories and the efficiency of the use of light energy by plants [ $LUE_p$  (in grams dry weight per mole)], which was defined as the ratio of the accumulated dry weight and the light received by the plant. The EY and PY used in the present study were different from the concept of  $LUE_p$ . Equations for EY and PY both use the FW of a plant in the numerator, and EY uses the electrical energy consumed by lighting in the denominator, whereas PY uses the number of photons provided to the cultivation area as the denominator, which includes the entire growing cycle from sowing to harvesting. Commercially sold vegetables are valued according to FW, and the costs of light sources are typically calculated using the electricity consumed by the lighting system. Therefore, EY and PY may have more practical value than  $LUE_p$ . The  $LUE$  values for tomato (*Solanum lycopersicum*) seedlings are  $\approx 0.017$  in greenhouses and  $\approx 0.038$ – $0.042$  in plant factories. Plant factories have been reported to have 2- to 2.5-times more light use efficiency than greenhouses (Shibuya and Kozai, 2001; Yokoi et al., 2003). As shown in Table 5, EY was 20–45 g/kWh and PY was  $\approx 6$ – $16$  g·mol<sup>-1</sup>. When using these parameters to compare different cultivation methods, their respective definitions should be considered.

Previous studies have primarily focused on seedlings; however, the present study calculated the EY and PY of red leaf lettuce cultivated using different electric light sources in plant factories during the entire growing period (Shibuya and Kozai, 2001; Yokoi et al., 2003). The photosynthesis of red leaf lettuce is usually lower than that of green leaf lettuce (Lee et al., 2017). Additionally, the EY of red leaf lettuce is also lower than that of green leaf lettuce [the research team of the present study used CW to cultivate Boston lettuce with an EY of  $\approx 80$ – $100$  g/kWh (unpublished data)]. The increased EY and PY values were due to the enhanced effectiveness of the light supply. Moreover, the increased efficiency of the lighting itself or improvements in cultivation methods can be used to achieve increased crop FW. Changing from fluorescent lamps to LEDs can result

in an increase in lighting efficiency of  $\approx 60\%$  (Kozai, 2013). Among the LED systems used, the different spectra also resulted in changes of 54% (EY ration, SB6/CW) to 118% (EY ration, SR4SB2/CW) and of 100% (EY<sub>A</sub> ration, CW/CW) to 259% (EY<sub>A</sub> ration, SR5SB1/CW) for rEY and rEY<sub>A</sub> performance, respectively. Different electric light sources and cultivation processes also affect the energy consumed by each plant. In terms of the OPE (moles per kilowatt hour), CW was the most efficient electric light source (Table 4). However, for cultivation of red leaf lettuce, considering TAC and color change, CW was not the most efficient (Table 4). Our experiments used EY and PY to represent the red leaf lettuce FW and TAC that could be produced per kilowatt hour of electricity consumed or per mole of photons (400–700 nm) delivered, respectively. These two values could be used to determine better strategies for the production of red leaf lettuce.

During Expt. 1, R<sub>80</sub>:B<sub>20</sub> treatment was used to cultivate red leaf lettuce, resulting in significantly increased values of EY, EY<sub>A</sub>, PY, and PY<sub>A</sub> (Table 4). Figure 2 shows that the color change in lettuce was incomplete: the high  $h^p$  values in Table 4 indicate that the red leaf lettuce had leaves close to green, which is not desirable by the market. Furthermore, R<sub>80</sub>:B<sub>20</sub> treatment significantly decreased AC (Table 4). The R<sub>80</sub>:B<sub>20</sub> spectrum increased the EY<sub>A</sub> and PY<sub>A</sub> values of red leaf lettuce because the overall increase in FW caused the total TAC of the entire plant to increase. R<sub>20</sub>:B<sub>80</sub> treatment significantly increased AC and significantly decreased the  $h^p$  value, which indicated that this treatment was beneficial for leaf color change.

The results of Expt. 1 showed that R<sub>80</sub>:B<sub>20</sub> treatment was beneficial for yield increase, whereas R<sub>20</sub>:B<sub>80</sub> treatment was beneficial for color change and increased anthocyanin content. Expt. 2 attempted to combine the advantages of both treatments. R<sub>80</sub>:B<sub>20</sub> treatment was first used to produce primary metabolites in red leaf lettuce (i.e., biomass), and R<sub>20</sub>:B<sub>80</sub> treatment was used during the end of production to stimulate and induce anthocyanin production. SR5SB1 treatment significantly increased the PY<sub>A</sub> of red leaf lettuce, and the change to R<sub>20</sub>:B<sub>80</sub> treatment during the last week of the cultivation period, in comparison with SR6

treatment (i.e., R<sub>80</sub>:B<sub>20</sub> light for the entire 6-week growing period), caused the efficiency of photon use in anthocyanin production in lettuce to increase by 58%. During red leaf lettuce production, the FW of each plant and the secondary metabolite content must be considered. Using the control group (CW) as a standard to evaluate the differences among the electric light sources, a comprehensive evaluation (FW accumulation and anthocyanin content) indicated that 1 week of blue light at the end of cultivation (light recipe: SR5SB1) is recommended for commercial use.

## Conclusions

Among the four types of light quality treatments used in this study, those with high amounts of blue light were able to effectively increase the anthocyanin content of the leaves; however, they inhibited growth. With regard to total anthocyanin content per plant, if using the same light quality treatment throughout the growing period is required and the primary consideration is yield, then R<sub>80</sub>:B<sub>20</sub> treatment is recommended. To maximize the red color, R<sub>50</sub>:B<sub>50</sub> treatment can be used for the entire growing period. However, a more suitable cultivation method is R<sub>80</sub>:B<sub>20</sub> treatment during the first 5 weeks and then changing the treatment to R<sub>20</sub>:B<sub>80</sub> 1 week before harvest. This lighting strategy can balance both yield and anthocyanin content.

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