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Effect of LED Light Combinations on Photosynthetic Efficiency and Growth of Lettuce

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This study aimed to investigate the influence of red, blue, green, and yellow light-emitting diode (LED) lights with varying intensities and ratios on the photosynthetic efficiency of Boston lettuce and Ziyan lettuce; treatments were conducted using four LED lights, namely red (R), blue (B), green (G), and yellow (Y) LED lights; a light intensity of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm were applied. With respect to triple light combinations, the highest photosynthetic efficiency was observed in Boston lettuce under RBG treatment (R, 32%; B, 48%; G, 20%) and in Ziyan lettuce under RBY treatment (R, 36%; B, 54%; Y, 10%), indicating that different light combinations are required for the two types of lettuce. The plant growth, leaf pattern, nitrate level, and electric power consumption of Boston lettuce (under G, RB, and RBG treatments), Ziyan lettuce (under R, RB, and RBY treatments), and a control group (under white LED light [W]) were compared. The results indicated that Boston lettuce under RBG treatment produced a denser leaf pattern and deeper colors; its growth was inferior, but its nitrate level and electric power consumption were lower relative to those under G and W treatments. Ziyan lettuce under RBY treatment produced deeper leaf colors, but its growth did not differ significantly from that of Ziyan lettuce under R treatment; its nitrate level and electric power consumption were lower. The two aforementioned types of lettuce were suitable for consumption. The findings indicated that optimal photosynthetic efficiency improved the nitrate metabolism in plants and reduced power consumption. The growth pattern achieved with a lighting mode that promotes optimal photosynthetic efficiency has greater commercial value and can be applied in plant factories for mass-producing lettuce that meets consumption standards, thereby providing better food safety for consumers.

Key words: nitrate content, photosynthetic efficiency, spectral quality

INTRODUCTION

Lettuce is currently a main vegetable crop that is widely planted in greenhouses. It contains rich nutrients including protein, vitamins, and fiber, which are essential nutrients for humans (Connor *et al.*, 2005; Samuolienė *et al.*, 2009). Multiple types of lettuce are cultivated, and the predominant leaf color of lettuce is green followed by purple and red. Light is the energy source for plant growth; red and blue light are the main types of light absorbed by plants, and they play a key role in the morphological and physiological changes of plants, including hypocotyl elongation, leaf area growth, and metabolism (Ouzounis *et al.*, 2015; Kitazaki *et al.*, 2018). Under a natural light source, a plant is exposed to the full spectrum of light. Light quality requirements vary depending on the leaf colors of various varieties of plants, giving rise to the diverse morphologies exhibited by plants, which involve the growth speed and color depth of leaves. Briggs *et al.* (2001) believed that duration and intensity of the irradiation of red light, far red light, blue

light, and ultraviolet light trigger physiological changes in plants and regulate their growth and development. Conventionally, studies on plant growth regulation have adopted electro-illumination with a lamp spectrum greater than 400–500 nm or 600–700 nm. However, few studies have examined the optimal light quality required for plant growth (Hogewoning *et al.*, 2010). In this regard, the precise spectral range of light-emitting diode (LED) allows for researchers to examine the influence of light quality on plant growth (Tenessen *et al.*, 1994).

Stutte and Edney (2009) subjected red leaf lettuce to treatments using red LED light (R); red and blue LED light (RB); and red, blue, and green LED light (RBG); they reported that a lower dry weight was achieved with the R treatment; Kim *et al.* (2005) held that the optimal photosynthetic efficiency for plant growth can be achieved with red (R) and blue (B) LED lights, and that the incorporation of green LED light (G) enables better penetration through plant canopies; R and B can increase the growth rate of lettuce and make its leaf colors more distinct; they also allow for plant health to be assessed visually. The aforementioned findings indicate that light combinations can increase plant growth. Thus, the present study examined the optimal ratio of LEDs for increasing the yield and quality of lettuce through treatments involving various light combinations.

Dong *et al.* (2014) held that light density, light quality, and direction of photoreception influence plants' photoreceptors, which contain cryptochrome and phytochrome, leading to various physiological responses in

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plants. Dim lighting may promote leaf area growth and growth of plants, thereby optimizing photosynthetic efficiency (Steinger *et al.*, 2003), which involves photoabsorption, quenching, chlorophyll and carotenoids synthesis, responses regarding plant color, and the provision of essential nutrients for humans, (e.g., vitamins and antioxidants). This explains how various lighting conditions in the natural environment increase the levels of chemical components in plants (Naznin *et al.*, 2019).

The influence of light quality on plant growth varies depending on the plant species. For example, the stem growth in tomato, salvia, and petunia under R treatment is greater than that achieved with RB (R, 75%; B, 25%) treatment (Wollaeger and Runkle, 2014). For treatments performed in a given cultivation environment with varying light quality, a higher growth level was achieved in lettuce with R than with B (Son *et al.*, 2013). In addition, Snowden *et al.* (2016) suggested that the ratios of R and B in RB treatments influence the dry weight of lettuce, and a higher dry weight was observed under RB treatments with R:B ratios ranging from 4:1 to 7:1.

Food safety control is becoming increasingly essential because of consumers' increasing demand for food safety. In 2004, European Union (EU) announced that the nitrate concentration of facility-cultivated lettuce should be reduced to between 2500 and 4500 ppm. In a normal diet, 87% of nitrate ions come from vegetables (Nigel, 2000). The metabolites produced by the nitrate ions that are absorbed into the human body are associated with health concerns such as stomach cancer and cyanosis (Hill, 1999; Wang *et al.*, 1998). The nitrate level of vegetables varies depending on environmental factors such as plant species, harvest time, and nitrogen fertilizer use. Among vegetable species, the highest nitrate levels are found in leaf vegetables followed by root and stem vegetables; by contrast, flower vegetables and fruits have the lowest levels of nitrate (Chien-Chang and Fung, 1995). The light density during cultivation is a major influencing factor for the level of nitrate ions in vegetables (Bottex *et al.*, 2008). In an environment with insufficient light density, nitrate reductases and photosynthetic efficiency decrease, causing nitrate ions to accumulate (Huang and Sung, 2013). Nitrate metabolism in plants is based on photosynthesis, and it involves light, nitrogen sources, and increased CO₂; such metabolism can also increase photosynthetic efficiency and reduce nitrate content.

To examine the effect of efficient photosynthetic photon flux density (i.e., PPFD) required by crop growth while considering the electric energy consumption of light (Mitchell *et al.*, 2012), the present study further explored the results of another study (Tsai *et al.*, 2021), which investigated the influence of monochromatic LEDs on the photosynthetic efficiency of Boston lettuce and Ziyan lettuce and reported that a higher photosynthetic efficiency was detected in Boston lettuce under G treatment and in Ziyan lettuce under R treatment. Moreover, to investigate the influence of light quality combinations on the growth of the two types of lettuce, the photosynthetic efficiency levels achieved with vari-

ous combinations of R, B, G, and Y were assessed. Light quality combinations were examined at a light density of 120 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO₂ concentration of 1000 ppm. In addition, the influence of light quality combinations on the growth, growth pattern, nitrate level, and electric power consumption of lettuce were investigated by subjecting Boston lettuce to G, RB (R, 40%; B, 60%), and RBG (R, 32%; B, 48%; G, 20%) treatments; subjecting Ziyan lettuce to R, RB (R, 40%; B, 60%), and RBY (R, 36%; B, 54%; Y, 10%) treatments; and comparing the results of the aforementioned treatments with those of a control group under W treatment. These experiments were conducted to identify the optimal light combination for the two types of lettuce for reducing production costs and nitrate content, thereby allowing for the mass-production of high-quality vegetables in plant factories and the enhancement of food safety.

MATERIALS AND METHODS

Plant materials and nursery environment

This experiment selected two cultivars of lettuce (*Lactuca sativa*). These were green leaf Boston lettuce purchased from Formosa Farming Material Co., Ltd and red leaf Ziyan lettuce from Known-You Seed Co., Ltd.

Prior to seedling cultivation, seeds were soaked in water for 2 hours, and sowed on rock wool in a closed plastic container for 1 day to hasten germination. Sprouting seeds were selected and placed in a plant factory (length of 6.07 m, width of 2.47 m, and height of 2.4 m) where the temperature and CO₂ concentration could be regulated. The R:B ratio was 2:1, with a light density of 120 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, CO₂ concentration of 1000 ppm, and cycled gully nutrient solution controlled through the nutrient film technique. The nutrient solutions comprised Solution A and Solution B. One liter of Solution A contained 34 g of KH₂PO₄, 39.8 g of KNO₃, 97.28 g of MgSO₄, and 52.48 g of K₂SO₄; it also contained microelements, namely 22 g of MnSO₄, 29 g of HBO₃, 14 g of ZnSO₄, 1.8 g of CuSO₄, 1.2 g of Na₂MoO₄. One liter of Solution B contained 144.7 g of Ca (NO₃)₂, 16.54 g of NH₄NO₃, 39.8 g of KNO₃, and 1.86 g of ferric ethylenediaminetetraacetic acid. The nutrient solutions were prepared by diluting 600-mL Solution A and 600-mL Solution B in 120 L of water, and the electrical conductivity level was adjusted to a range of 230–250 $\mu\text{S}/\text{cm}$ with a pH value of 5.8–6.5. Indoor daytime temperature was set to 25°C (for 18 h), and nighttime temperature was set to 18°C (for 6 h); CO₂ concentration was set to 1000 ppm. The seedlings were grown for 7 days as experiment materials.

Experiment methods

The seedling phase of this experiment was conducted with various light quality combinations while other conditions were identical. A spectral quantum spectrophotometer (LM801S, LeBio Corporation, Taiwan) was used to measure the spectral energy distribution of four types of LED light quality, namely R (620–635 nm), B (460–475 nm), G (505–535 nm), and Y (585–

Table 1. The spectral energy distribution of monochromatic LED light

Spectrum ^z	Spectral distribution ratio (%)			
	Red (620–635 nm)	Blue (460–475 nm)	Green (505–535 nm)	Yellow (585–595 nm)
Red	57.52	0.02	0.11	1.66
Blue	0.01	29.30	0.71	0.02
Green	0.08	0.77	64.44	0.43
Yellow	2.96	0.01	0.07	25.00
48 R+72 B	21.00	23.85	0.61	0.57
38.4 R+57.6 B+24 G	17.85	14.29	13.77	0.54
43.2 R+64.8 B+12 Y	17.26	20.87	0.59	5.16
Red:Blue=2:1	60.88	38.54	0.41	0.08
White	4.93	4.16	12.49	5.39

^z Red: 120- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Red Light, Blue: 120- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Blue Light, Green: 120- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Green Light, Yellow: 120- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Yellow Light, 48R+72B: 48- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Red Light+72- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Blue Light, 38.4R+57.6B+24G: 38.4- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Red Light+57.6- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Blue Light+24- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Green Light, 43.2R+64.8B+12Y: 43.2- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Red Light+64.8- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Blue Light+12- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ Yellow Light, White: 120- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ White Light.

595 nm). Boston lettuce were subjected to treatments of G, RB, and RBG; Ziyan lettuce were subjected to treatments of R, RB, and RBY; and the control group was subjected to W treatment (Table 1).

Plant sampling analysis

The first sampling was conducted on the 6th day of the experiment, after which sampling was conducted every 3 days for a total of four samplings, and each sampling's leaf length and width, fresh weight, and appearance were observed. Subsequently, the sampled plants were wrapped in aluminum foil and placed in an oven at 105°C to dry for 1 day before being taken out to measure its dry weight. For each of the four plants, measurements of leaf length and width and fresh and dry weight were taken to obtain the average values for the four items.

Plant photosynthetic efficiency analysis

On the 15th day of the experiment, the plant leaf area was approximately 6 cm². A photosynthesis system (LI-6400XT, LI-COR Corporation, USA) was used; analytic clamps were used to clamp the leaves, whose photosynthetic efficiency was examined at a light density of 120 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO₂ concentration of 1000 ppm. For each of the three plants, measurements of leaf length and width and fresh and dry weight were taken to obtain the average values for the three items.

Nitrate level analysis

A light density of 120 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO₂ concentration of 1000 ppm were applied. Lettuce leaves were grown under monochromatic LEDs, double and triple LED combinations, and W LED (for the control group) for 15 days before being ground in a mortar. Sap was extracted, and 100 μL of it was collected with a pipette and placed in a microcentrifuge tube for centrifugation; 10 μL of the supernatant solution was extracted and

diluted 100 times, after which the solution was transferred through a pipette to a LAQUA Nitrate Meter (HORIBA, Japan) for testing. For each of the three plants, measurements of leaf length and width and fresh and dry weight were taken to obtain the average values for the three items.

Electric power consumption analysis

Treatments were conducted using a lighting board (length of 60 cm and width of 30 cm) to examine the electric power consumption of monochromatic LEDs and double and triple LED combinations; comparisons with the control group (under W) were conducted at a light density of 120 $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO₂ concentration of 1000 ppm. Sixteen hours of lighting was provided per day, and an electricity meter (PRODIGIT, Taiwan) was used to record the cumulative electric power (unit: w) of monochromatic LEDs and LED combinations during the 15-day experiment.

Statistic data analysis

Data were compiled using Microsoft Excel. The Scheffe's method was applied using SAS software to identify significant differences ($p \leq 0.05$) among treatments. Graphs were plotted using Sigmaplot 10 (Systat Software, USA) statistical software.

RESULTS

Analysis of photosynthetic efficiency under double light combinations

The photosynthetic efficiency of Boston lettuce and Ziyan lettuce under double light combinations are present in Fig. 1; higher efficiency was detected under RB combinations, whereas lower efficiency was detected under GY combinations. Among the RB combinations, the highest photosynthetic efficiency was achieved with 40% R and 60% B (48- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ R+72- $\mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$

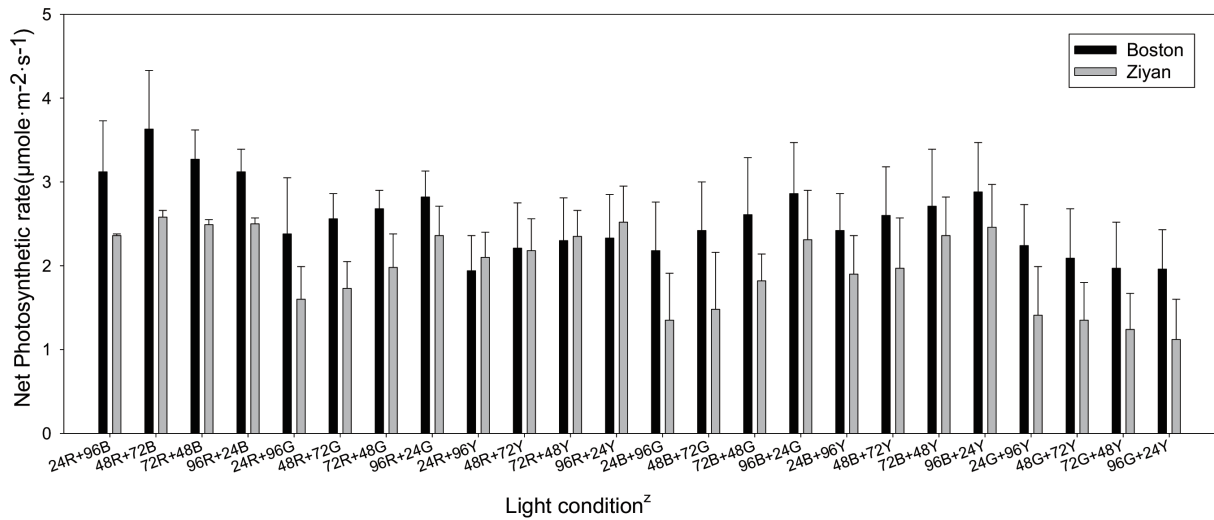


Fig. 1. Photosynthetic efficiency of lettuce (*Lactuca sativa* L. cv. Boston and cv. Ziyan) treated with double light combinations at a light density of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm.

^z The same with Table 1.

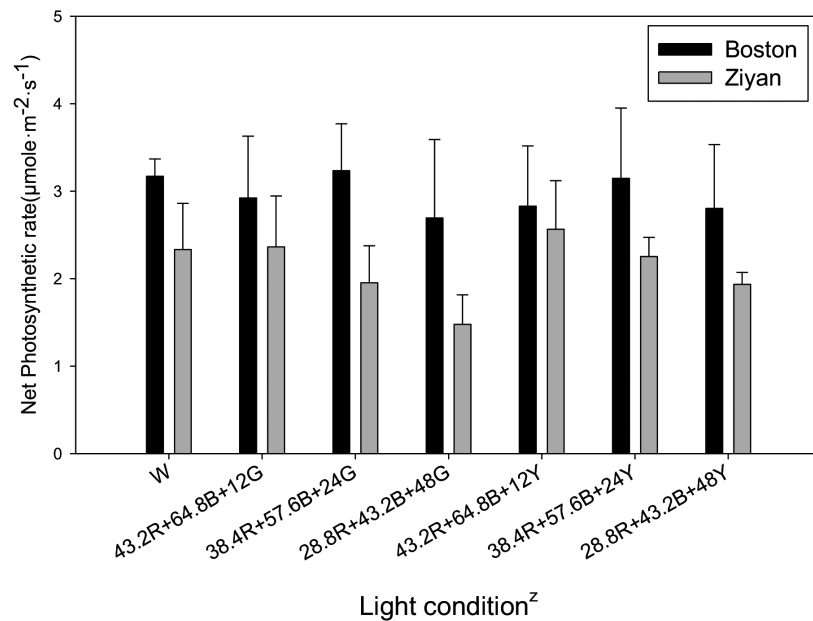


Fig. 2. Photosynthetic efficiency of lettuce (*Lactuca sativa* L. cv. Boston and cv. Ziyan) treated with triple light combinations at a light density of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm.

^z The same with Table 1.

B; optimal RB). For RG or RY light combinations, the findings indicated that photosynthetic efficiency increased with an increase in the proportion of B. For BR or BY light combinations, the findings indicated that photosynthetic efficiency increased with an increase in the proportion of B. For YG light combinations, photosynthetic efficiency decreased with an increase in the proportion of G.

Analysis of photosynthetic efficiency under triple light combinations

Boston lettuce and Ziyan lettuce were selected and subjected to the optimal RB (i.e., optimal double light combination for achieving photosynthetic efficiency) and

supplemental G and Y; comparisons with the control group under W treatment were conducted (Fig. 2). For Boston lettuce, the highest photosynthetic efficiency ($3.2 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was detected with the treatment with 32% R, 48% B, and 20% G (i.e., optimal RBG); in Ziyan lettuce, the highest photosynthetic efficiency ($2.6 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was achieved with the treatment with 36% R, 54% B, and 10% Y (i.e., optimal RBY). Under W treatment, the highest photosynthetic efficiency achieved was $3.17 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Boston lettuce and $2.33 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for Ziyan lettuce. The findings indicated that for the two types of lettuce, treatments with light combinations yielded higher levels of photosynthetic efficiency relative to W treatments.

Plant sampling analysis

Boston lettuce was subjected to treatments with W, G, optimal RB, and optimal RBG (Table 2). On the 6th day of the experiment, the greatest fresh weight was achieved under G, but no significant difference was observed among these treatments. On the 9th day of the experiment, fresh weight and dry weight of the Boston lettuce were 809.8 and 59.8 mg, respectively, under G treatment and 419.5 mg and 33.5 mg, respectively, under optimal RB treatment; no significant difference was observed between the two treatments. Furthermore, no significant difference was observed between the optimal RBG and W treatments. On the 6th day of the experiment, a higher fresh weight (2044.3 mg) was observed under G treatment, but no significant difference was observed among the four treatments; on the 15th day, a greater fresh weight (5965.8 mg) was similarly observed under G treatment, and a significant difference between the fresh weight achieved under optimal RB (3307.0 mg) and that achieved under optimal RBG (4104.0 mg) was observed. With respect to leaf length, on the 6th day of the experiment, a longer length (5.8 cm) was achieved under G treatment, and this length was significantly different from those achieved under the other three treatments; on the 9th day, a longer length (8.0 cm) was similarly achieved under G treatment, and this length was significantly different from that achieved under W treatment (6.0 cm); on the 12th day and 15th day, a longer length was similarly achieved under G treatment (9.6 and 12.4 cm on the 12th and 15th day, respectively), and this length was significantly different from those achieved

under the other three treatments. With respect to leaf width, on the 6th day, a longer width (2.4 cm) was achieved under RB treatment, but no significant difference among the four treatments was observed; on the 9th day, a longer width (3.1 cm) was achieved under W treatment, but the difference among the four treatments was not significant. On the 12th and the 15th day, a longer width was observed under G treatment (4.1 and 5.1 cm on the 12th and 15th day, respectively), but the difference between the four treatments was not significant.

Ziyan lettuce was subjected to treatments of W, R, optimal RB, and optimal RBY (Table 3). On the 6th day of the experiment, a greater fresh weight (260.8 mg) was observed under R treatment, and this weight was significantly different from those achieved under W (120.5 mg) and optimal RB (101.3 mg) treatments; on the 9th day, a greater fresh weight (445.3 mg) was similarly observed under R treatment, and this weight was significantly different from those achieved under the other three treatments; on the 12th day, a greater fresh weight (831.3 mg) was similarly observed under R treatment, and the lightest fresh weight (538.5 mg) was achieved under optimal RB treatment, but the difference among the treatments was not significant; on the 15th day, a greater fresh weight (2703.8 mg) was achieved under R treatment, and this weight was not significantly different from those achieved under optimal RB (1689.8 mg) and optimal RBY (2211.5 mg) treatments; however, it was significantly different from that achieved under W (1393.8 mg) treatment. With respect to dry weight, on the 6th day of the experiment, a greater fresh weight (24.0 mg) was

Table 2. Growth performance of lettuce (*Lactuca sativa* L. cv. Boston) under a light intensity of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm measured by day after planting and monochromatic light-emitting diode light

Days after planting	Spectrum ^c	Fresh weight (mg/plant)	Dry weight (mg/plant)	Leaf length (cm)	Leaf width (cm)
6	White	285.0 a ^y	24.5 a ^y	4.5 b ^y	2.3 a ^y
	120G	329.8 a	24.5 a	5.8 a	2.2 a
	48R+72B	284.0 a	25.8 a	3.6 c	2.4 a
	38.4R+57.6B+24G	315.0 a	27.5 a	4.1 bc	2.3 a
9	White	694.0 ab	58.0 ab	6.0 b	3.1 a
	120G	809.8 a	59.8 a	8.0 a	3.0 a
	48R+72B	419.5 b	33.5 b	5.0 bc	2.7 a
	38.4R+57.6B+24G	637.3 ab	54.0 ab	4.8 c	2.8 a
12	White	1613.3 a	130.8 a	7.5 b	3.9 a
	120G	2044.3 a	143.5 a	9.6 a	4.1 a
	48R+72B	1194.3 a	94.8 a	5.9 c	4.0 a
	38.4R+57.6B+24G	1306.3 a	110.0 a	5.9 c	3.8 a
15	White	5025.5 ab	379.0 a	9.7 b	4.7 a
	120G	5965.8 a	394.5 a	12.4 a	5.1 a
	48R+72B	3307.0 c	256.5 a	7.5 c	4.7 a
	38.4R+57.6B+24G	4104.0 bc	299.5 a	7.9 c	5.0 a

^a The same with Table 1.

^y Mean separation within the same column followed by different lowercase letters were significantly different ($p < 0.05$), as determined through Scheffe's multiple range test.

Table 3. Growth performance of lettuce (*Lactuca sativa* L. cv. Ziyan) under a light intensity of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm measured by day after planting and monochromatic light-emitting diode light

Days after planting	Spectrum ^z	Fresh weight (mg/plant)	Dry weight (mg/plant)	Leaf length (cm)	Leaf width (cm)
6	White	120.5 bc ^y	11.0 bc ^y	3.4 b ^y	1.7 c ^y
	120R	260.8 a	24.0 a	5.5 a	2.5 a
	48R+72B	101.3 c	9.8 c	2.9 b	1.8 bc
	43.2R+64.8B+12Y	229.3 ab	22.5 ab	3.7 b	2.3 ab
9	White	258.0 b	24.0 b	4.6 b	2.6 b
	120R	445.3 a	46.0 a	6.8 a	3.3 a
	48R+72B	248.3 b	22.3 b	4.2 b	2.4 b
	43.2R+64.8B+12Y	261.8 b	26.8 b	4.2 b	2.3 b
12	White	533.0 a	76.8 a	5.6 b	3.1 a
	120R	831.3 a	76.5 a	8.9 a	3.5 a
	48R+72B	538.5 a	49.5 a	5.1 b	3.0 a
	43.2R+64.8B+12Y	698.8 a	63.0 a	5.4 b	3.3 a
15	White	1393.8 b	114.8 b	8.1 b	4.6 b
	120R	2703.8 a	231.3 a	11.7 a	5.7 a
	48R+72B	1689.8 ab	141.5 b	7.2 b	4.7 ab
	43.2R+64.8B+12Y	2211.5 ab	179.0 ab	7.7 b	5.3 ab

^z The same with Table 1.^y Mean separation within the same column followed by different lowercase letters were significantly different ($p < 0.05$), as determined through Scheffe's multiple range test.

observed under R treatment, and this weight was significantly different from those achieved under optimal RB (9.8 mg) and W (11.0 mg) treatments; on the 9th day, a greater dry weight (46.0 mg) was similarly observed under R, but no significant difference was observed among treatments; on the 12th day of the experiment, a greater dry weight was observed under W (76.8 mg) treatment, but the difference among treatments was not significant; on the 15th day of the experiment, a greater dry weight (231.3 mg) was achieved under R treatment, and this weight was significantly different from those achieved under W (114.8 mg) and optimal RB (141.5 mg) treatments but not from that achieved under optimal RBY treatment. With respect to leaf length, during the experiment (the 6th–15th day), longer lengths were achieved under R treatment (5.5, 6.8, 8.9, and 11.7 cm), and a significant difference with those achieved by other treatments was observed. With respect to leaf width, on the 6th day, a longer width (2.5 cm) was achieved under R treatment, and this width was significantly different from those achieved under W (1.7 cm) and optimal RB (1.8 cm) treatments; on the 9th day of the experiment, a longer width (3.3 cm) was similarly achieved under R treatment, and this width was significantly different from those achieved under W (2.6 cm), RB (2.4 cm), and optimal RBY (2.3 cm) treatments; on the 12th day of the experiment, a longer width (3.5 cm) was still achieved under R treatment, but the difference among the treatments was not significant; on the 15th day of the experiment, a longer width was observed under R (5.7 cm), and this width was significantly different from that

achieved under W (4.6 cm) treatment.

Nitrate level analysis

On the 15th day of this experiment, the nitrate level in Boston lettuce was the highest (4440 ppm), and this nitrate level was not significantly different from that achieved under the G (3540 ppm), optimal RB (3520 ppm), and optimal RBG (3440 ppm) treatments (Fig. 3). For Ziyan lettuce, a higher nitrate level was achieved under R (2740 ppm), but this nitrate level was not significantly different from that achieved under the W (2460 ppm), optimal RB (2400 ppm), and optimal RBY (2230 ppm) treatments (Fig. 4).

Analysis of electric power consumption during lettuce cultivation

For Boston lettuce, the highest power consumption was observed for under the G treatment (12.9 kw), followed by the W (9.6 kw) and optimal RBG (8.8 kw) treatments; the lowest power consumption was observed under the optimal RB (7.3 kw) treatment. For Ziyan lettuce, the highest power consumption was observed under the R treatment (10.0 kw), followed by the W (9.6 kw) and optimal RBY (8.5 kw) treatments; the lowest power consumption was observed under the optimal RB (7.3 kw) treatment.

DISCUSSION

Regarding the influence of various types of light quality on lettuce, researchers have indicated that red and blue lights are the primary light sources that pro-

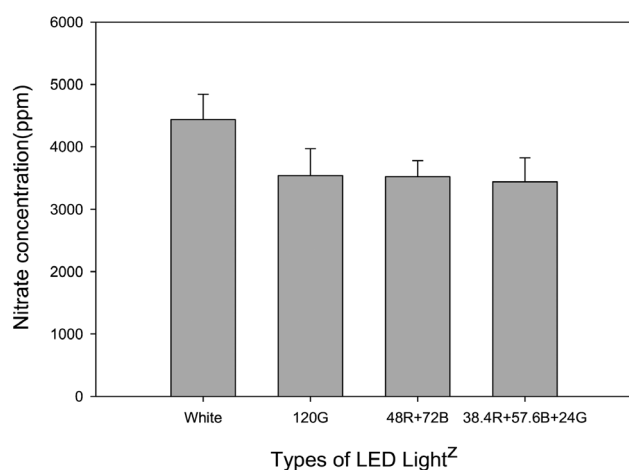


Fig. 3. Nitrate content level in lettuce (*Lactuca sativa* L. cv. Boston) leaf during 15-day cultivation period under monochromatic light-emitting diodes (LEDs) and LED combinations at a light density of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm.

^z The same with Table 1.

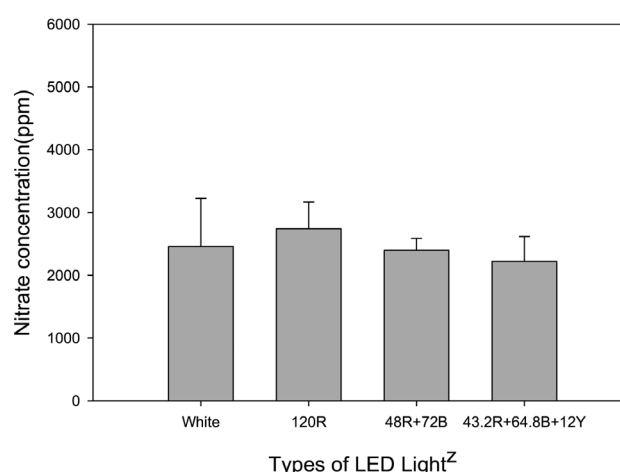


Fig. 4. Nitrate content level in lettuce (*Lactuca sativa* L. cv. Ziyen) leaf during 15-day cultivation period under monochromatic light-emitting diodes (LEDs) and LED combinations at a light density of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm.

^z The same with Table 1.

mote lettuce growth (Johkan *et al.*, 2010; Lichtenthaler *et al.*, 1980; Lin *et al.*, 2013; Hogewoning *et al.*, 2010). In addition, red and blue lights enable the photosynthetic pigments in leaves to absorb light energy, which is then converted into chemical energy. They can also enhance carotenoid and chlorophyll biosynthesis (Fan *et al.*, 2013), which promote leaf closure and increase biomass. Lettuce at the seedling stage that was subjected to RB treatment (R:B=6:1) exhibited higher growth; the primary red and blue lights can promote seedling development and increase photosynthetic efficiency, with blue light promoting chlorophyll formation in leaves, which, in turn, promotes leaf growth and enhances photosynthetic efficiency (Li *et al.*, 2010; Banaś *et al.*, 2012; Johkan *et al.*, 2010). Moreover, red and blue lights can increase the number of stomata in leaves, which is relevant to the structure of stomata and their opening, closing, and development (Muneer *et al.*, 2014). In the present study, the analysis of the photosynthetic efficiency achieved under double light combinations revealed that a higher photosynthetic efficiency was attained under RB combinations (Fig. 1); this finding corresponds to those reported by other studies.

Light quality was used to control the stem growth and flowering time of plants as well as the opening and closing of stomas in leaves; the phytochromes in the photoreceptors of plants trigger various growth patterns (Martínez-García *et al.*, 2014). Red light induces hypocotyl growth and cotyledon expansion in lettuce seedlings, and it also increases the growth rate and leaf area of these seedlings (Lee *et al.*, 2014). Blue light inhibits hypocotyl growth and reduces the growth rate of the cell wall on a leaf surface, resulting in dense leaves (McNellis *et al.*, 1995; Li *et al.*, 2010; Sergejeva *et al.*, 2018). In the present study, the optimal RB for photosynthetic efficiency had a greater proportion of B than of R; the leaf length and width of Boston lettuce and Ziyen lettuce were inferior, and its leaves were dense (Fig. 5 and Fig.

6); these findings are similar to those of other studies.

With respect to growth patterns achieved under treatments with varying light quality during cultivation, leaf color was an item that was examined and used as an indicator for assessing vegetable growth (Barrett *et al.*, 2010). Kitazaki *et al.* (2018) indicated that applying a suitable light quality contributes to the accumulation of leaf pigments or the regulation of gene expression in lettuce. Chung (2019) applied light quality adjustments and a nutrient solution formula to three types of sprouts, namely radish, broccoli, and red cabbage sprouts; in the experiment, pure red light (R100) significantly increased the fresh weight of these sprouts, whereas pure blue light (B100) significantly increased the anthocyanin concentration levels in radish and red cabbage sprouts. In addition, red oak lettuce was cultivated for 6 weeks; RB (R:B=4:1) treatment was conducted during the first 5 weeks; in the 6th week, another RB (R:B=1:4) treatment was conducted; anthocyanin content and production capacity were increased compared with the levels observed for white light treatment. Stutte and Edney (2009) subjected red leaf lettuce to RGB treatment; stimulation by B increased the anthocyanin content in leaves, which was more than twice that achieved through R treatment, but no significant difference was observed when green light was added, indicating that B was the main factor that contributed to the increase in anthocyanin content.

Kurilcik *et al.* (2008) combined blue light (455 nm), light red light (640 nm), deep red light (660 nm) and red light (735 nm) of varying density levels to cultivate chrysanthemum seedlings; their results revealed that blue light of a higher density decelerated the seedling growth, which increases the survival rate of seedlings after plant transplantation (Glowacka, 2002); the amount of photosynthetic pigments increased when the proportion of light red light was increased while the proportions of other light qualities remain unchanged. Kamiya *et al.*

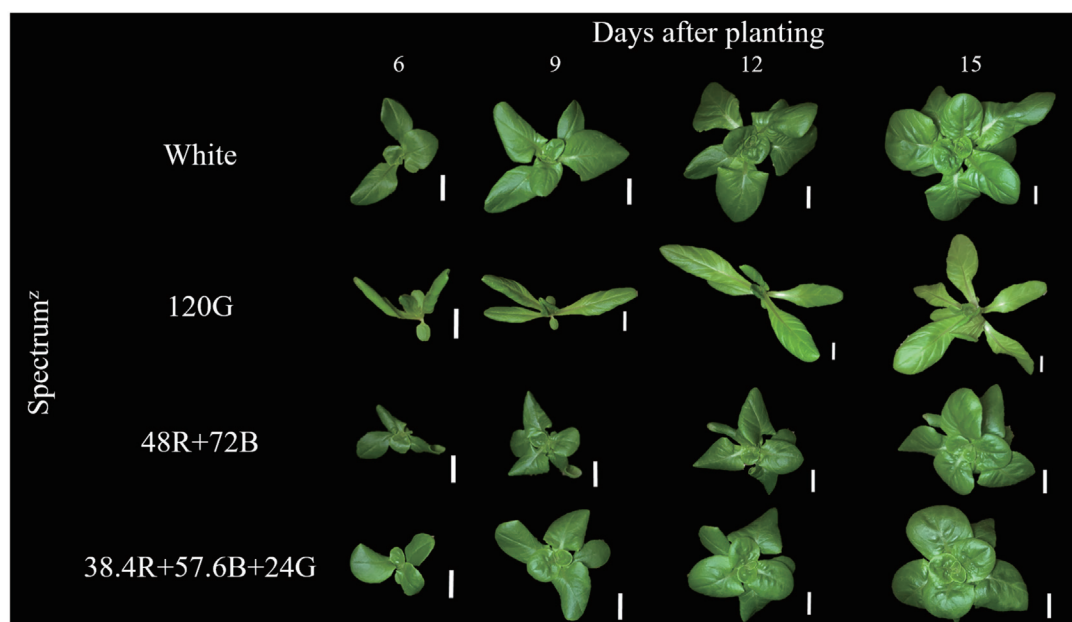


Fig. 5. Morphology of lettuce (*Lactuca sativa* L. cv. Boston) over time when cultivated under monochromatic light-emitting diodes (LEDs) and various LED combinations.

^z The same with Table 1. White bars indicate 2 cm.

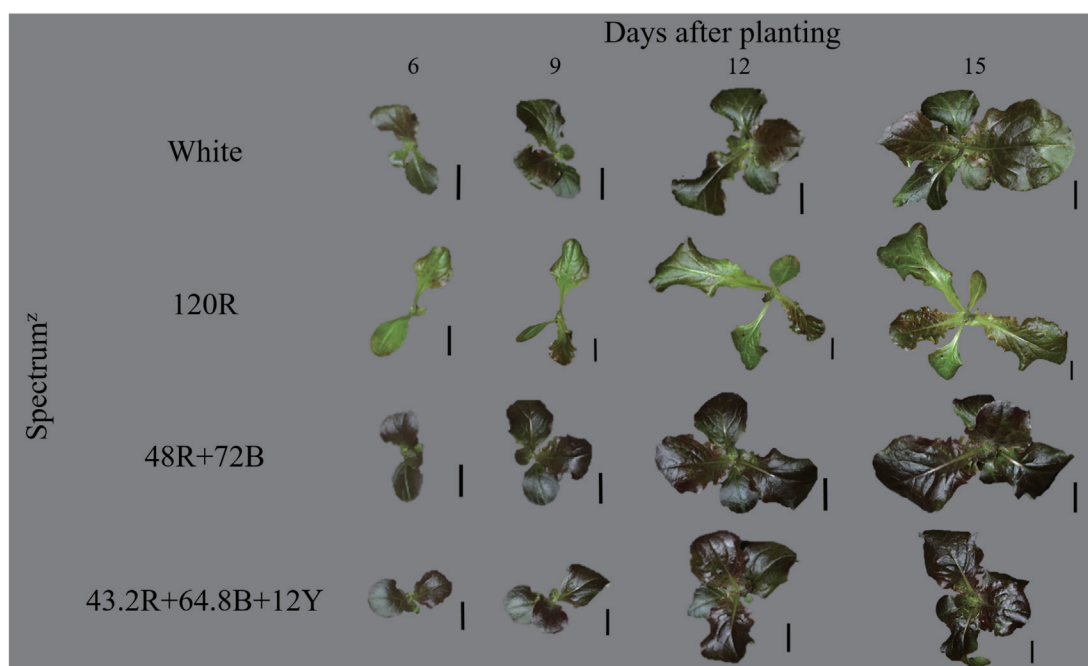


Fig. 6. Morphology of lettuce (*Lactuca sativa* L. cv. Ziyan) over time when cultivated under monochromatic light-emitting diodes (LEDs) and LED combinations.

^z The same with Table 1. Black bars indicate 2 cm.

(1981) applied blue light treatment to tobacco cells in a dark environment, and they discovered that blue light could induce the formation of 5-aminolevulinic acid synthase and increase its activity, thereby promoting chlorophyll biosynthesis; however, under green light treatment, chlorophyll synthesis and ALA decreased probably because of lower protein levels, which reduced the activity of enzymes responsible for chlorophyll esterification and metabolism. In the present study, the implementation of optimal RB and optimal RGB treatments for

Boston lettuce revealed that when the proportion of B was higher, the observed leaf length and leaf colors were shorter and deeper, respectively; when the G treatment was applied, leaves grew excessively and lighter leaf colors were observed (Fig. 5); these findings are similar to those of the aforementioned studies.

Kim *et al.* (2004a) planted lettuce that was subjected to treatments involving various light combinations; their results indicated that light combinations influenced the stomatal conductance in leaves. Because

the spectrum of white light is relatively complete, it allows for the absorption of a fuller range of light qualities; the stomatal conductance attained under white light was higher than that attained under RBG light, but the highest dry weight was observed under RBG light, indicating that leaves exhibit better stomatal conductance under white light but also that white light is not directly associated with plant growth. In another study, lettuce was cultivated under RBG, RB, cold white light (cool-white fluorescent lamps), and green cold white light (green fluorescent lamps); the results of the study indicated that a larger leaf area, higher growth, and higher stomatal conductance were attained under RBG treatment (Kim *et al.*, 2004b). Folta and Maruhnich (2007) suggested that green light treatment promotes the growth rate of *Arabidopsis* seedlings in a dark environment because the main cryptochromes exist as flavin semiquinones that absorb green light, thereby stimulating photoreceptors and promoting growth. Klein (1992) used G and B to stimulate biological systems; a larger number of stomata were observed on the leaf surface subjected to G treatment because G could penetrate plant canopies and increase the photosynthetic efficiency of leaves, thereby promoting plant growth. In the present study, Boston lettuce achieved greater growth under optimal RBG treatment than under optimal RB treatment (Table 2), suggesting that the addition of G enhanced plant growth; the finding is similar to those of the aforementioned studies.

Son *et al.* (2013) treated red leaf lettuce (*Lactuca sativa* L. 'Sunmang') and green leaf lettuce (*Lactuca sativa* L. 'Grand Rapid TBR') with RB combinations with varying ratios of R and B to investigate lettuce growth; they reported that the highest growth was uniformly observed under R treatment, suggesting that R promotes growth, whereas the lowest growth was observed when the R:B ratio was 53:47. Saito *et al.* (2010) subjected lettuce to RB, B, and R treatments; they reported that the greatest fresh weight was achieved under R treatment followed by B treatment. Glick *et al.* (1986) treated pea plants (*Pisum sativum* L. cv Alaska) with cold white light combined with red and yellow light to investigate concentrations of photosystem I (PSI) and photosystem II (PSII) reaction centers and the level of chloroplast reaction center gene transcripts; under R treatment, the proportion of PsbA transcripts in PSII was higher than that of PsbA transcripts in PSI; this demonstrated the difference between the genes in the reaction-centers of the two photosystems and indicated that the synthesis of chloroplast protein and assembly of photosystems were regulated by light quality at the post-transcriptional level. This suggests that red light promotes PSI activity in chloroplasts and increases plant growth rate. On the 15th day of the experiment conducted in the present study, the greatest fresh weight for Ziyun lettuce was achieved under R treatment (Table 3), which could be attributed to the increased chloroplast activity caused by red light.

Few studies have explored the influence of Y on plant growth, and studies have suggested that Y deceler-

ates growth rate (Glick *et al.*, 1986; Folta and Maruhnich *et al.*, 2007); however, the addition of G and Y to light combinations probably contributes to plant growth (Folta and Childers, 2008). In the present study, an analysis was performed to examine photosynthetic efficiency under treatments involving double and triple light combinations; treatments with light combinations that had higher proportions of R and B and lower proportions of G and Y were conducted; the growth results for Boston lettuce under optimal RB and RBG treatments and those for Ziyun lettuce under optimal RBY treatment were compared (Tables 2 and 3); the results suggested that these light qualities promoted the growth of the two lettuce cultivars, and this finding is consistent with those of the aforementioned studies.

Liu *et al.* (2011) subjected cherry tomato seedlings (*Solanum lycopersicum* var. *cerasiforme*) to R, B, Y, G, RB, RBG, and W treatments to examine the influence on chloroplast structure and stomata on leaves. They reported that the chloroplast shape was probably associated with amyloplast content; when amyloplast content was lower, a slenderer chloroplast shape was observed, and when Y treatment was applied, the leaves contained a small amount of amyloid and exhibited inferior development; under a microscope, the boundary between mitochondrion and plastid was undefined. Because of the lower stroma content in chloroplasts, carbohydrate synthesis declined, and the lowest level of soluble protein was observed. Under Y treatment, the area of stomas expanded, but the fewest number of stomas and lowest leaf photosynthetic efficiency was observed. Thus, the number of stomas probably affected photosynthetic efficiency. Tsai *et al.* (2021) used monochromatic lights to examine their influence on the growth of lettuce, thereby clarifying the yield of lettuce and obtaining basic information about various light qualities, which contributed to the development of subsequent light combination treatments for increasing yield. Tao *et al.* (2017) subjected lettuce to treatments with B, broad R and B (BRB), RYB, broad W (BW), sharp R (SR), sharp R and B (SRB), and narrow W (NW), and they examined the influence of light quality on leaf area and growth; the results indicated that the greatest leaf area was achieved under RBY treatment, suggesting that RBY promotes plant growth. In the present study, the highest photosynthetic efficiency and a higher growth were observed in Ziyun lettuce under optimal RBY treatment (Fig. 2), verifying that supplementary Y in light combinations could enhance plant growth (Table 3).

Chao (2015) subjected red leaf and green leaf lettuce to treatments of red, blue, and green lights to examine the nitrate levels in the two lettuce cultivars; they reported that the nitrate level under red light treatment was the highest, and that the lowest nitrate levels were observed in green leaf lettuce under blue light treatment and in red leaf lettuce under green light treatment. This suggests that the nitrate content level in green leaf lettuce was lower under B treatment because B treatment could promote the conversion of nitrogen fertilizers into nutrients for crop growth and reduce

nitrate accumulation. In red leaf lettuce, the lowest nitrate level and fresh weight were both observed under green light treatment, indicating that the low level of nitrate that metabolized under green light was related to growth, and that green light was not suitable for promoting the growth of red leaf lettuce. With respect to the nitrate level observed in Ziyan lettuce in the present study, a higher nitrate level was similarly observed under R treatment; this finding is similar to those of the aforementioned studies; the optimal RB treatment results indicated that the nitrate level was lower when the proportion of B was higher than the proportion of R, which is related to the results of the aforementioned study. On the 15th day, the effects of the optimal RBG and W treatments on Boston lettuce were compared, and the results indicated that a lower fresh weight and substantially lower nitrate level were attained under optimal RBG treatment (Fig. 3). The effects of the optimal RBY and W treatments on Ziyan lettuce were compared, and the results indicated a greater fresh weight and lower nitrate level were attained under optimal RBY treatment (Fig. 4); compared with W treatment, the two lettuce cultivars yielded higher growth under their respective optimal treatments, but the difference relative to W treatment was nonsignificant, and the growth pattern was similar.

Chen *et al.* (2021) treated lettuce with light combinations with varying proportions of R and B to examine energy efficiency, growth pattern, and chlorophyll and carotenoid levels; their results indicated that the highest energy efficiency was achieved with a R:B ratio of 9:1. This indicates that energy efficiency increases when the proportion of red light is higher; regarding the growth pattern under RB treatment, when the proportion of R was greater than 70%, the twisted pattern of the petiole became more pronounced, the leaf color was lighter, and the number of leaves increased when the proportion of R increased; the highest chlorophyll and carotenoid levels were observed under pure blue light treatment. Tao *et al.* (2017) explored chromaticity coordinates and

reported that white light is similar to the ideal light source for plant growth, and a higher growth rate was achieved under white light treatment. The growth pattern, growth, and amount of lettuce pigments required to meet producers' expectations can be achieved by identifying the optimal light combination according to the requirements of cultivation. In the present study, the optimal photosynthetic efficiency of Boston lettuce and Ziyan lettuce was achieved under optimal RBG and RBY treatments, respectively. No significant difference was observed between the two lettuce cultivars in terms of morphology and growth. In terms of electric power consumption, the consumption recorded under optimal RBG and RBY treatments was 8.3% and 11.5%, respectively; these consumption levels were lower than that recorded under W treatment (Table 4), indicating that the cultivation of various varieties of lettuce using suitable light combinations can increase yield and reduce electric power consumption, thereby decreasing production costs.

The influence of light combinations on lettuce growth and morphology was studied on the basis of basic monochromatic LED lights (Tsai *et al.*, 2021); multiple light combinations were examined, and Boston lettuce and Ziyan lettuce were planted and treated with suitable light combinations, leading to dense plant patterns, deep leaf colors, increased yields, and low nitrate levels, thereby increasing consumers' purchase intentions. Moreover, the expansion of production scale allows for electric power consumption to be reduced, which, in turn, reduces production costs, enhances market competitiveness, and enables the nitrate level requirements stipulated by EU to be met; these beneficial developments can help improve the lettuce industry and promote lettuce exportation to the international market.

Therefore, in terms of morphology, growth, and nitrate level, Boston lettuce cultivated under RBG treatment and Ziyan lettuce cultivated under RBY treatment are ideal for consumption by consumers.

Table 4. LED power consumption during 15-day cultivation of lettuce (*Lactuca sativa* L. cv. Boston and cv. Ziyan) at a light density of $120 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and CO_2 concentration of 1000 ppm

Type of Lettuce	Spectrum ^a	Electric energy consumption(kw) Electricity charge of LED light (15 days)
Boston lettuce	White	9.6
	Green	12.9
	48 R+72 B	7.3
	38.4 R+57.6 B+24 G	8.8
Ziyan lettuce	White	9.6
	Red	10.0
	48 R+72 B	7.3
	43.2 R+64.8 B+12 Y	8.5

^a The same with Table 1.

AUTHOR CONTRIBUTIONS

1. Chun-Yu Tsai, verified that the lettuce cultivars had different appearances under different light combinations quality treatments. Fresh weight, dry weight, photosynthetic efficiency, nitrate level and electric power consumption were analyzed to reveal the influence of light combinations qualities on lettuce yield. This was conducive to understanding the influence and requirements of the growth and photosynthetic efficiency of the lettuce cultivars.
2. Yung-Fu Yen, designed the analytic method for the effects of light combinations qualities on lettuce growth by using the concept of lettuce cultivars and provided advice.
3. Chyung Ay, designed the suitable lettuce growing space from the environment control basis of plant factories and provided advice on environment usage control.
4. Ikuo Miyajima, provided practical research advice on spectral qualities and lettuce cultivars and revised the study content and experiment data.
5. Kuang-Liang Huang, integrated the test programs of light combinations quality treatments for lettuce cultivars from former studies on lettuce cultivar types and managed related studies in the laboratory.

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