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Lettuce Growth, Nutritional Quality, and Energy Use Efficiency as Affected by Red–Blue Light Combined with Different Monochromatic Wavelengths

Lie Li, Yu-xin Tong, Jun-ling Lu, Yang-mei Li, and Qi-chang Yang
Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing 100089, China; and Key Laboratory of Energy Conservation and Waste Management of Agricultural Structures, Ministry of Agriculture, Beijing 100089, China

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Abstract. **Light, as the energy and signal sources for plant growth and development, is one of the most important environment factors in recently developed plant factories with artificial light (PFALs). To find the optimal combination of light wavelengths for lettuce (*Lactuca sativa* cv. 'Tiberius') plant growth in a PFAL, four treatments, each using red (R; 662 nm) and blue light (B; 447 nm) with a ratio of 4:1 and photon flux density (PFD) of 150 μmol·m^{-2}·s^{-1}, and mixing, respectively, with 50 μmol·m^{-2}·s^{-1} of green light (G; 525 nm; RBG), yellow light (Y; 592 nm; RBY), orange light (O; 605 nm; RBO) and far-red light (FR; 742 nm; RBFR), were set up during this experiment. A combination of R and B with a ratio of 4:1 and PFD of 200 μmol·m^{-2}·s^{-1} was set as the control (RB). The responses of lettuce growth, morphology, anatomical structure of the lettuce leaf, photosynthetic performance, lettuce nutritional quality, and energy use efficiency were investigated. The results showed that RBG, RBO, and RBFR increased the shoot fresh weight of lettuce by 20.5%, 19.6%, and 40.4%, and they increased the shoot dry weight of lettuce by 24.2%, 13.4%, and 45.2%, respectively, compared with those under RB. The P_n under RBY was significantly lower than that under RB, although no significant differences in chlorophyll or carotenoid content were found between RBY and RB. RBG increased the lettuce leaf area, the thickness of the leaf palisade tissue, P_n, and light use efficiency compared with those under RB. Plants grown under RBO showed better photosynthetic capacity, such as higher P_n, Φ_{PSII}, and other photosynthetic parameters. RBFR caused an increase in lettuce leaf area and energy use efficiency, but a decrease in leaf thickness and P_n of the single leaf. Moreover, tipburn injury was observed under RBFR. Therefore, these results demonstrate that RBG and RBO can be considered optimal combinations of light wavelengths for lettuce growth in a PFAL in this experiment, although plant growth can also be improved by using RBFR.**

Among the various factors that affect plant growth and development, light is a vital driving force (Metallo et al., 2018; Stuefer and Huber 1998). In PFALs, selecting the most favorable light environment for plant growth is essential because the artificial light is the sole light source for plant growth. In past decades, traditional artificial light sources, such as fluorescent, high-pressure sodium, and metal halide lamps, have been used in tissue culture, growth chambers, and greenhouses to supplement natural light for vegetable production (Bantis et al., 2018). Light-emitting diodes (LEDs) can provide several unique advantages compared with the traditional light sources, such as energy-savings with less heat generation, lower degradation, and longevity. Recently, LEDs have been gradually deemed an appropriate light source in PFALs. Furthermore, LEDs can provide special and mixed wavelengths that can be set according to the photosynthetic requirements of plant (Nelson and Bugbee, 2014). Therefore, the light conditions in a PFAL can be controlled precisely to meet the needs of plants.

Compared with light quantity and light period, light quality has much more complex effects on plant morphology and physiology (Folta and Carvalho, 2015; Xu et al., 2015). Therefore, in past decades, numerous studies have focused on plant growth and development affected by light quality (Ouzounis et al., 2015). In general, R (620–700 nm) and B (400–500 nm) are often used in combination for plant development because their absorptions by photosynthetic pigments are higher and they are more effective for plant production than other wavelengths (Olle and Viršile, 2013). Hence, several previous studies focused on combinations of R and B and the effects of their different ratios on plant growth (Li et al., 2013; Wang et al., 2016).

It has been recognized that plants are capable of sensing and responding to the light spectrum with a broad range, from ultraviolet (280–400 nm) to FR (720–780 nm) regions. Different light qualities or wavelengths have distinctly different biological influences on plants, including plant growth, morphology, photochemical compounds, photosynthesis, organ growth, development, and nutritional quality. For example, G can be absorbed by cryptochrome (*cry*) and decrease the activity of chromophores on *cry*, thus suppressing the induction of the stomata opening of plant leaves by B that is absorbed by *cry* (Sellaro et al., 2010). In addition, G can supply energy for the lower canopy and deep leaves of plants, especially those with overlapping leaves, such as lettuce (Sun et al., 1998). Moreover, the nitrate concentration was decreased under short-term continuous combinations of red, blue, and green LEDs (Bian et al., 2018). Park and Runkle (2017) found that far-red light (FR) increased plant growth mainly through leaf expansion and an increase in the whole-plant net photosynthetic rate (P_n). Several studies also found that FR decreased anthocyanins, carotenoids, and chlorophyll concentrations of lettuce plants but increased soluble sugar and nitrate concentrations (Li and Kubota, 2009; Zou et al., 2019). Studies have shown that under the irradiation of ultraviolet-B, morphological changes of plants will occur, such as dwarfing and leaf area reduction, but the nutritional quality of plants will be improved (Harbaum-Piayda et al., 2016). Evidence for Y (580–600 nm) suppression of lettuce growth has been reported (Dougher and Bugbee, 2001). Fan et al. (2013) reported that the dry mass, soluble sugar, and protein concentrations were decreased in nonheading Chinese cabbage under single Y. In addition, O (600–620 nm), which is generally classified as R, is part of the photosynthetically active spectrum; however, few studies have investigated the effects of this wavelength on plant growth.

Some researchers have studied the effects of monochromatic light on plant growth; however, abnormal growth usually resulted (Wang et al., 2009). Some studies chose white light as the background light (Chen et al., 2016). However, white light, whether from fluorescent lamps or LEDs, provides a broad and complicated spectrum containing some wavelengths of little use for plant growth. Therefore, it is necessary to investigate an optimal combination of specific light wavelengths for plant growth rather than using white light with a broad spectrum or simple mixed R and B (Gupta and Jatothu,

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Y.-X.T. is the corresponding author. E-mail: tongyuxin@caas.cn9790015395.

2013). Moreover, additional research is needed to determine the appropriate and ideal combination of light wavelengths for plant production as well as to deepen our understanding of the impact of combinations of R, B, and less well-studied light wavelengths of Y, G, O, and FR on plants (Bantis et al., 2018). In addition, to improve the energy use efficiency in PFALs, it is necessary to find the optimal light environment for plant growth (Kozai et al., 2016).

Therefore, to investigate the optimal combination of light wavelengths for lettuce (*Lactuca sativa* cv. 'Tiberius') grown in a PFAL, four treatments were used by combining the R and B with each monochromatic G, Y, O, and FR and using the combination of R and B as the control in this experiment. Lettuce growth and morphology, anatomical structure, photosynthetic performance, lettuce nutritional quality, and energy use efficiency were investigated.

Materials and Methods

Plant factory

This experiment was conducted in a PFAL at the Chinese Academy of Agricultural Science (CAAS) in Beijing, China. The cultivation beds were equipped with vertically movable LED panels (Datang New Energy Technology Co., Ltd., Shenzhen, China). Plants were planted in Styrofoam that floated on the nutrient solution on each cultivation bed. An air conditioner with a cooling capacity of 14 kW (HFW-75-2; Beijing Zhongke Shiheng Technology Co., Ltd, Beijing, China) was used to control the air temperature and relative humidity inside the PFAL. CO_2 was supplied from a gas cylinder.

Plant materials and cultivation conditions

Lettuce (*Lactuca sativa* cv. 'Tiberius') seeds were sown in sponge blocks with seeding at a density of 1066 plants/m^2 in a growth chamber (GLED-250PY; Beijing Luxi Technology Co., Ltd., Beijing, China) with an air temperature of 20 °C. When the seeds geminated, the sponge blocks with seedlings were moved to the PFAL. Fluorescent lamps (TL-D56W; Osram) with a light intensity of 150 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were used as the light source with a photoperiod of 16 $h \cdot d^{-1}$. Seedlings with three true leaves after sowing were transplanted to a cultivation bed with a nutrient circulation system in which the nutrient solution depth was 0.05 m and the cultivation density was 32 plants/m^2. The Yamasaki lettuce nutrient solution was used and circulated daily for 1 hour. The electric conductivity (EC) and pH were adjusted to 1.1 to 1.2 $dS \cdot m^{-1}$ and 5.8 to 6.0, respectively. Lettuce growth conditions in this experiment were maintained at 24/20 °C (light/dark), 65% ± 10% relative humidity, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2 level, and 16 $h \cdot d^{-1}$ photoperiod.

Experimental setup

Four treatments with a mixture of R (662 ± 30 nm) and B (447 ± 30 nm) combined with either monochromatic G (525 ± 30 nm), Y (592 ± 10 nm), O (605 ± 30 nm), or FR (742 ± 30 nm) were set up, and the combination of R and Bt was set as the control (Table 1). The PFD was controlled by adjusting the DC power supply (PKU-MS605D). The distance between the cultivation bed and the LED panel was maintained at 0.3 m during this experiment. Side reflectors (width, 0.2 m; PVC) with a light reflectivity of 0.94 were used on both sides of the shelf. The PFD on the cultivation bed surface was controlled by adjusting the DC power supply (PKU-MS605D). The PDF was measured by a spectrometer (Avaspec-2048CI; Avates, Apeldoorn, the Netherlands).

Measurements of plant growth and morphology

Five lettuce samples were measured on day 21 after transplanting. Plants were dried with absorbent paper. Each plant was separated into the shoot and root using a sharp scalpel and forceps. Leaf area was measured by an area meter (LI-3100C; Lincoln, NE). The shoot fresh weight and shoot dry weight were measured using an electronic balance (Si-234; Denver Instrument, NY).

Observation of leaf anatomical structure

The fully expanded second young leaves were soaked in FAA (38% formalin/glacial acetic acid/70% ethanol; 1:1:18, v/v) for at least 24 h. After washing, alcohol dehydration, xylene transparency, wax immersion, embedding, slicing, xylene dewaxing, alcohol rehydration, saffron dying for 1 to 2 hours, fixed green dying for 30–60 s, and xylene transparency for 5 min, the neutral gum seals were examined using a microscope (Eclipse E100; Nikon, Japan) and photographed using an imaging system (DS-U3; Nikon). Micrographs were processed using image analysis software (case viewer 2.0), the thickness of leaves, palisade tissue, and sponge tissue were measured, and the proportion of palisade tissue thickness to leaf thickness was calculated. Five leaves from different plants were taken from each treatment, and every index for each micrograph was either measured or calculated 10 times.

Photosynthetic performance

Determination of the contents of chlorophyll and carotenoid. The fully expanded second young leaves were ground and mixed with 95% ethanol. The samples were kept in dark conditions until they turned white. Absorbances of the extraction at 665, 649, and 470 nm were separately measured by a spectrophotometer (ultraviolet-1800; Shimadzu, Kyoto, Japan). The contents of chlorophyll (Chl) and carotenoid (Car) were determined using the following equations derived from Wellburn (1994).

$$\mathrm{Chl\,a}\left(\mathrm{mg \cdot g^{-1}}\right) = \frac{(13.95\mathrm{A665} - 6.88\mathrm{A649})\mathrm{V}}{1000\mathrm{W}}$$

$$\mathrm{Chl\,b}\left(\mathrm{mg \cdot g^{-1}}\right) = \frac{(24.96\mathrm{A649} - 7.32\mathrm{A665})\mathrm{V}}{1000\mathrm{W}}$$

$$\mathrm{Car}\left(\mathrm{mg \cdot g^{-1}}\right) = \frac{[(1000\mathrm{A470} - 2.05\mathrm{Chl\,a} - 114.8\mathrm{Chlb})/245]\mathrm{V}}{1000\mathrm{W}}$$

Measurements of the net photosynthetic rate (P_n) and stomatal conductance (g_S). P_n and g_S were measured using a portable photosynthetic instrument (LI-6400XT; Lincoln, NE) for fully expanded second young leaves. To measure the P_n and g_S under the light source of actual plant growth, a transparent leaf chamber was installed. The environmental conditions of the leaf chamber were maintained at 24 °C, 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2 level, and 60% to 70% relative humidity. The measurement order among the treatments was arranged randomly for every repetition, and each treatment was repeated three times.

Measurements of chlorophyll fluorescence parameters. Chlorophyll fluorescence parameters were measured using LI-6400XT on the fully expanded second young leaves using the fluorescence leaf chamber. The maximum PSII quantum yield (F_v/F_m), the efficiency of excitation capture by the open PSII center (F_v'/F_m'), actual photochemical quantum efficiency (Φ_{PSII}), electron transfer rate (ETR), photochemical quenching (qP), and nonphotochemical quenching (NPQ) were measured and calculated using LI-6400XT (Hormann et al., 1994). Measurement order was arranged randomly for every repetition, and each treatment was repeated three times.

Measurements of carbon dioxide photosynthesis curves. Carbon dioxide photosynthesis curves were measured with the photosynthetic instrument LI-6400XT on fully expanded second young leaves. During the determination of the corresponding carbon dioxide photosynthesis curve, the initial CO_2 concentration was set at 400 $\mu mol \cdot mol^{-1}$, followed by 200, 150, 100, 50, 400, 600,

Table 1. Five treatments with different combinations of light wavelengths with consistent light intensities of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

	Spectral composition (%)					
Treatment	Red	Blue	Green	Yellow	Orange	Far red
RB	80	20	0	0	0	0
RBG	60	15	25	0	0	0
RBY	60	15	0	25	0	0
RBO	60	15	0	0	25	0
RBFR	60	15	0	0	0	25

800, and 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, sequentially (Wang et al., 2018). To measure the P_n and g_S under the light source of actual plant growth, a transparent leaf chamber was installed. The temperature of the leaf chamber was maintained at 24 °C, and the relative humidity was 60% to 70%. Data were recorded when the P_n and g_S were stable. Measurement order was arranged randomly for every repetition, and each treatment was repeated three times. The slope of the proportional stage of the CO_2 photosynthetic curve was fitted to Rubisco activity (carboxylation efficiency), whereas the maximum net photosynthetic rate was fitted to P_m.

Observations of stomata. To perform stomata observations, rubber elastic impression material was applied to the front and back of the fully expanded young leaves and uncovered when the rubber was dry. Glycerol was applied to a slide, and the rubber impression with the leaf imprint side was quickly covered on the slide. After the glycerol had dried, the rubber was uncovered and then covered with a piece of the slide. A microscope (XSP-13CC; Shanghai Caikon Optical Instrument Factory, China) was used to observe the aperture area and stomatal density. The values of the aperture area and stomatal density were the averages of their values on the two sides of the leaves.

Determination of soluble sugar, ascorbic acid, nitrate, and soluble protein

Soluble sugar. Soluble sugar was determined using the method of Fairbairn (1953). Fresh leaf samples of 0.2 g were cut and mixed, and 5 mL of deionized water was added to extract the soluble sugar in a boiling water bath for 30 min (repeated twice). Anthrone ethyl acetate reagent (0.5 mL) and concentrated sulfuric acid (5 mL) were added to 0.5 mL of the extract. The absorbance was measured at a wavelength of 630 nm after natural cooling with the spectrophotometer. The content of soluble sugar was calculated based on the measured absorbance and standard curve.

Ascorbic acid. Ascorbic acid was determined by the 2,6-dichloroindophenol sodium method (Kumar et al., 1992). Fresh leaf samples of 0.2 g were cut and mixed well. Oxalic acid (3 mL, 2%) was added to the homogenate. Then, the homogenate was poured into a 100-mL capacity bottle. The residue was washed with 1% oxalic acid. The washing liquid was poured into the bottle. Then, 1 mL of 30% zinc sulfate was added and the bottle was shaken. Potassium ferro cyanide (1 mL, 15%) was added to remove the fat-soluble pigments, and 1% oxalic acid was used to fix the volume. Then, it was shaken and strained into a clean beaker. Then, 2 mL of 2,6-dichloroindophenol sodium solution and 5 mL of xylene were added to the aforementioned extract (4 mL). The absorbance was determined according to the standard curve method.

Nitrate. Nitrate was determined using the method of Cataldo et al. (1975). A mixture of 0.5 g fresh leaf samples and 10 mL deionized water was heated in a boiling water bath for 30 min; then, it was cooled and filtered. A 0.1-mL portion of the extract was absorbed and 0.4 mL of 5% salicylic acid–sulphuric acid solution was added. The solution was mixed and maintained at room temperature for 20 min; then, 9.5 mL of 8% NaOH solution was slowly added. The absorbance of the extract at a wavelength of 410 nm was measured with a spectrophotometer. The content of NO_3-N was calculated based on the measured absorbance and the NO_3-N standard curve. The lettuce nitrite content was calculated according to the content of NO_3-N.

Soluble protein. Soluble protein was determined using the method of Bradford (1976). Fresh leaf samples of 0.2 g were placed into 2-mL centrifuge tubes; then, 1.6 mL deionized water was added. After homogenizing with a tissue grinder, the sample was centrifuged for 10 min at 10,000 $r \cdot min^{-1}$. Then, 1 mL of supernatant was added to 5 mL Coomassie Brilliant Blue G-250 solution in the test tube. The absorbance was measured at a wavelength of 595 nm. The soluble protein content was calculated by the absorbance and a standard curve.

Electric energy use efficiency

Electric energy use efficiency (EUE) is the ratio of the chemical energy accumulated by the whole plant dry weight to the electric energy consumed by the light source.

$$\mathrm{EUE} = \frac{D \cdot S \cdot f \cdot DW}{W_e}$$

where *DW* is the average dry weight of the samples (g); *D* is the planting density (32 plants/m^2); *S* is the planting area (m^2); *f* is the conversion coefficient between the dry weight and chemical energy (20 $kJ \cdot g^{-1}$); and W_e is the electric energy consumed by the light source (J) (Kozai, 2013).

Light use efficiency

Light use efficiency (LUE) is the ratio of the chemical energy accumulated by the whole plant dry weight to the light energy received at the plant community surface.

$$\mathrm{LUE} = \frac{D \cdot S \cdot f \cdot DW}{W_l}$$

where *DW* is the average dry weight of the samples (g); *D* is the planting density (32 plants/m^2); *S* is the planting area (m^2); *f* is the conversion coefficient between dry weight and chemical energy (20 $kJ \cdot g^{-1}$); and W_l is light energy received at the plant community surface ($J \cdot m^{-2}$) (Kozai, 2013).

Statistical analysis

One-way analysis of variance (ANOVA) was performed for each treatment, and Duncan's multiple range test at $P = 0.05$ was used to make post hoc multiple comparisons among the mean values of parameters that differed significantly according to the ANOVA. This experiment was repeated three times. All statistical analyses were performed using the SPSS statistics program (SPSS Inc., Chicago, IL).

Results

Growth and morphology. The shoot fresh weights of lettuce plants under RBG, RBO, and RBFR were increased by 20.5%, 19.6%, and 40.4% respectively, and the shoot dry weights of lettuce plants were increased by 24.2%, 13.4%, and 45.2%, respectively, on day 21 after transplanting compared with those under RB (Fig. 1A and B). As shown in Fig. 1C, the leaf areas of lettuce under RBG and RBFR were increased by 10.8% and 33.2%, respectively, on day 21 after transplanting compared with that under RB.

Leaf anatomical structure. There were no significant differences in leaf thickness among RB, RBG, and RBO, whereas the leaf thickness under RBY and RBFR was decreased by 10% and 15%, respectively, compared with that under RB (Fig. 2A). Palisade tissue thickness under RBG was increased by 29.2% compared with that under RB (Fig. 2B). Sponge tissue thickness was decreased by 13.8% and 16.2% under RBG and RBFR, respectively, compared with that under RB (Fig. 2C). The largest proportion of palisade tissue thickness to leaf thickness was found under RBG (Fig. 2D).

Photosynthetic performance. Compared with that under RB, the content of Car under RBO was increased by 12% (Table 2). The lowest contents of Chl and Car were found under RBFR. As shown in Table 2, significantly higher P_n values were found under RBG and RBO compared with that under RB. P_n and g_S were decreased by 23.9% and 50.0%, respectively, under RBFR compared with those under RB.

There were no significant differences in F_v/F_m among all treatments, as shown in Table 3. Significantly higher F_v'/F_m', Φ_{PSII}, and ETR were observed under RBO, whereas significantly lower Φ_{PSII}, ETR, and qP were found under RBFR compared with those under RB. Compared with that under RB, significant lower NPQ was found under RBO (Table 3).

The differences in P_n between RB and RBY and between RBO and RBFR became increasingly greater with the increase in the CO_2 concentration (Fig. 3). For the fitting parameters of carbon dioxide photosynthesis curves (Fig. 4), no significant differences in Rubisco activity under RB, RBG, RBY, and RBO were observed. The maximum net photosynthetic rates, P_m, of lettuce grown under RBY and RBO were 1.08 and 1.06 times as much as that under RB, respectively. The lowest values of Rubisco activity and P_m were found under RBFR (Fig. 4).

No significant differences in the aperture area among RB, RBY, RBO, and RBFR were observed, whereas the aperture area was decreased by 31.7% under RBG compared with that under RB (Fig. 5A). The stomatal densities under RBG and RBO were increased by 35.1% and 41.8%, respectively, compared with that under RB (Fig. 5).

Soluble protein, ascorbic acid, soluble sugar, and nitrate. The contents of soluble sugar, nitrate, and soluble protein were greatly influenced by the different combinations of

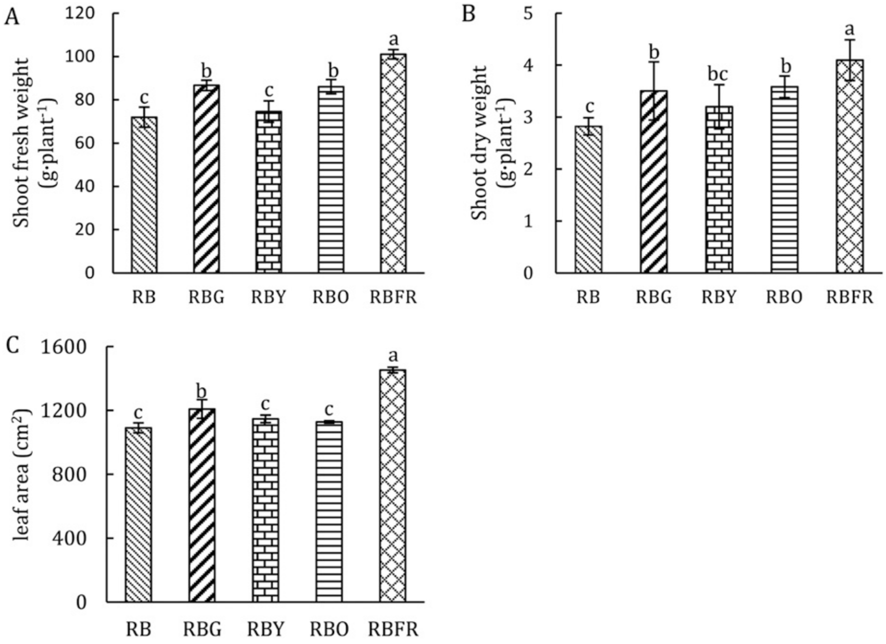

Fig. 1. Lettuce shoot fresh weight, shoot dry weight, and leaf area on day 21 after transplanting under different combinations of light wavelengths (**A–C**). Different letters in each histogram show significant differences according to Duncan's test ($P = 0.05$).

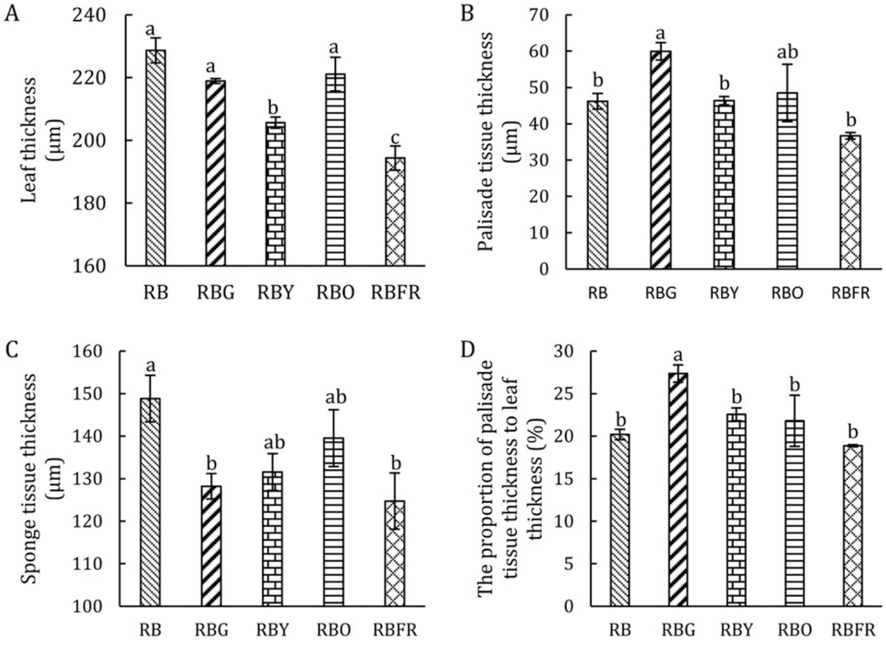

Fig. 2. Leaf anatomical structure as affected by different combinations of light wavelengths. Different letters in each histogram show significant differences according to Duncan's test ($P = 0.05$).

light wavelengths (Fig. 6), whereas no significant differences in ascorbic acid contents were observed among all treatments. The soluble sugar contents under RBY and RBFR were increased by 46.9% and 86.7%, respectively, compared with that under RB. The content of nitrate was increased by 21.9% under RBFR, whereas it was decreased by 17.3% under RBY compared with that under RB.

Energy use efficiency. The EUE under RBFR was 1.35 times as much as that under RB. The lowest EUE was found under RBY. The LUE values under RBG, RBO, and RBFR were 1.27, 1.24 and 1.28 times as much as that under RB, respectively (Fig. 7).

Discussion

Different combinations of light wavelengths were proven to have distinct effects on both shoot fresh weight/dry weight and photosynthetic capacity. In this study, increases were demonstrated in lettuce shoot fresh weight/dry weight and P_n when lettuce was grown under RBG and RBO, whereas only an increase in lettuce shoot fresh weight/dry weight was found under RBFR (Fig. 1, Table 2). RBO improved lettuce plant shoot fresh weight/dry weight, mainly because of the improved photosynthetic ability, such as the enhancements in P_n, Φ_{PSII}, ETR, and qP, and the decrease in NPQ (Table 3). Light is not only the driving force for photosynthesis but also an important signal for affecting plant morphology (Chen et al., 2016). Increases of lettuce leaf area were found under RBG and RBFR. These results agree with previous studies that found that both G and FR can act as shade signals for plant growth and cause shading avoidance symptoms in plants, including leaf enlargement, petiole elongation, and upward orientation of leaves (Zhang et al., 2011). RBG can increase lettuce plant shoot fresh weight/dry weight, probably due to an increase in lettuce leaf area and P_n. Conflicting effects on lettuce plant shoot fresh weight/dry weight and P_n were observed under RBFR. The possible explanation for this result was that P_n measured on a single leaf cannot represent P_n of the whole plant (Yorio et al., 2001). These results agree with those of previous studies showing that plant shoot fresh weight/dry weight was affected by both the photosynthetic ability and plant morphology (Hernández and Kubota, 2016; Wang et al., 2016). However, although the highest plant shoot fresh weight/dry weight was shown under RBFR, tipburn injury was often evident before lettuce harvesting, so its commercial value was markedly reduced. Although there are several causes of tipburn injury in a PFAL, tipburn occurred under RBFR in this experiment mainly because of the rapid lettuce plant growth and increasing demand of calcium in the newly and/or inner developed leaves, especially before harvesting (Florkowski et al., 2000). The large demand of calcium for new cell wall formation and fast leaf expansion cause calcium deficiency, which can easily induce tipburn injury (Borkowski et al., 2016). Zhang et al. (2018) showed that Y was not suitable as the only light source in a PFAL because it restrained the formation of chloroplasts and chlorophyll, which subsequently constrained lettuce growth. However, in this study, no significant influences on lettuce shoot fresh weight/dry weight and leaf photosynthesis were shown under RBY because no significant differences in pigment contents and chlorophyll fluorescence parameters were found between RBY and RB (Fig. 1, Table 2).

The anatomical structure and stomatal characteristics of lettuce were also dramatically influenced by different combinations of light wavelengths. Previous studies found that thick palisade tissue enhanced the CO_2 assimilation capacity of the plant leaf because most of the chloroplasts are distributed in the palisade tissue (Gotoh et al., 2018; Vogelmann, 2008). In this study, higher palisade tissue thickness and higher proportions of palisade tissue thickness to leaf thickness under RBG may have enhanced the photosynthetic efficiency of the lettuce plant (Fig. 2B and D). This may also explain the

Table 2. Pigment content, net photosynthetic rate, and stomatal conductance (g_S) as affected by different combinations of light wavelengths.

Treatment	Chlorophyll content ($mg \cdot g^{-1}$)	Carotenoids content ($mg \cdot g^{-1}$)	P_n ($\mu mol \cdot m^{-2} \cdot s^{-1}$ CO_2)	g_S ($mol \cdot m^{-2} \cdot s^{-1}$ H_2O)
RB	1.68 ab	0.25 b	6.36 b	0.22 ab
RBG	1.57 b	0.26 ab	6.70 a	0.17 abc
RBY	1.70 a	0.27 ab	6.30 b	0.14 bc
RBO	1.80 a	0.30 a	7.00 a	0.24 a
RBFR	1.14 c	0.19 c	4.84 c	0.11 c

Different letters in each column indicate significant differences according to Duncan's test ($P = 0.05$).

Table 3. Chlorophyll fluorescence parameters of lettuce plant as affected by different combinations of light wavelengths.

Treatment	F_v/F_m	F_v'/F_m'	Φ_{PSII}	ETR	qP	NPQ
RB	0.81 a	0.53 b	0.34 b	30.50 b	0.65 ab	1.54 ab
RBG	0.81 a	0.55 ab	0.32 bc	29.23 b	0.59 b	1.47 ab
RBY	0.81 a	0.57 ab	0.34 b	30.57 b	0.61 b	1.34 bc
RBO	0.81 a	0.59 a	0.43 a	38.14 a	0.72 a	1.16 c
RBFR	0.82 a	0.54 b	0.27 c	23.42 c	0.50 c	1.72 a

Different letters in each column indicate significant differences according to Duncan's test ($P = 0.05$).

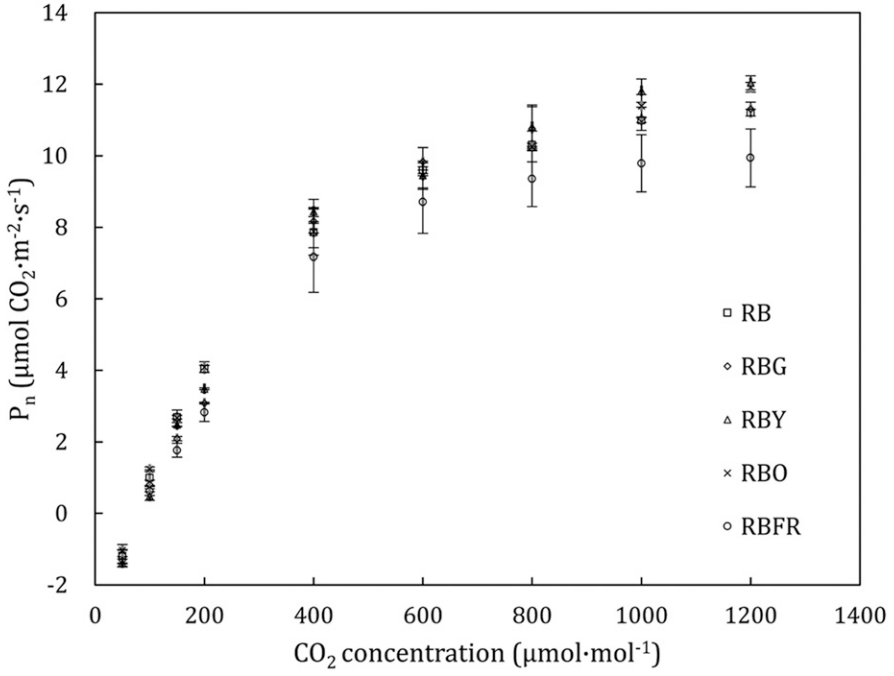

Fig. 3. Carbon dioxide photosynthetic response curves of lettuce under different combinations of light wavelengths.

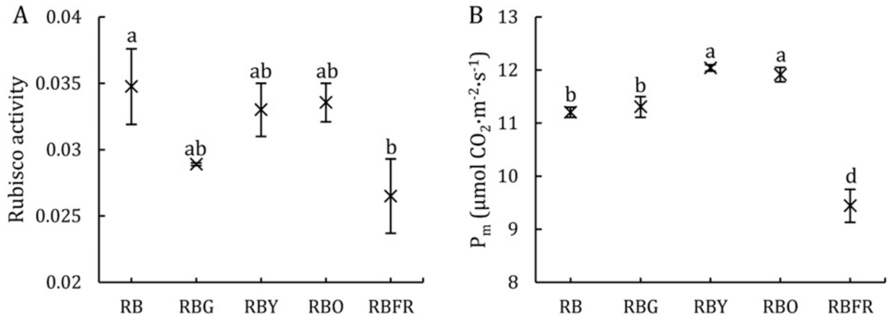

Fig. 4. Rubisco activity (**A**) and P_m (**B**) as affected by different combinations of light wavelengths. Different letters show significant differences according to Duncan's test ($P = 0.05$).

higher P_n under RBG. In addition, when light passes through the palisade tissue cells, "light channeling" will be generated, which is conducive to light entering the inside of the leaves (Vogelmann, 2008). The result is that because RBG increased the leaf palisade tissue thickness, this may promote more RBG light entering the deeper lettuce leaves. This hypothesis is consistent with the fact that G can pass deeper into the mesophyll of plant leaves (Evans and Vogelmann, 2003). When light enters spongy tissue composed of irregular cells, it will show a scattering phenomenon, which increases the probability of photon absorption. Therefore, thick sponge tissue is conducive to leaf photosynthesis (Taiz and Zeiger, 2015). In this study, thinner spongy tissue was found under RBG, but the P_n was higher than that under RB. One possible explanation is that more G could enter the spongy tissue compared with other light, because G could contribute to improving the light environment within plant leaves (Smith et al., 2017). Kozai et al. (2015) also reported that G has 30% higher transmissivity by green leaves, whereas B and R are mostly absorbed by the uppermost layer of leaves in the plant canopy. P_n of lettuce leaf under RBFR can be suppressed by the thinner palisade and sponge tissues under RBFR (Fig. 2). The stomatal aperture area under RBG was the smallest among all the treatments, and this result agrees with previous findings that G can reverse the stomatal opening that is promoted by B (Frechilla et al., 2000), but the stomatal density under RBG increased dramatically compared with that under RB (Fig. 5). This may promote leaf photosynthesis under RBG.

The photosynthetic capacity of plants can be expressed by the carbon dioxide photosynthetic response curve. At the proportion stage of the carbon dioxide photosynthetic response curve, the CO_2 concentration is the limiting factor of photosynthesis (Wang et al., 2018). The slope of the proportion stage is affected by ribulose bisphosphate carboxylase/oxygenase (Rubisco), which is the carboxylase of ribulose bisphosphate (RuBP) bound to CO_2. Therefore, the slope of the proportion stage can be fitted to Rubisco activity (carboxylation efficiency), which affects the value of P_n (Taiz and Zeiger, 2015). In this experiment, Rubisco activity of lettuce under RBFR was significantly lower compared with that under RB. This result may contribute to a lower P_n under RBFR (Table 2). At the saturation stage of the carbon dioxide photosynthetic response curve, CO_2 is no longer the limiting factor for photosynthesis, but the amount of the CO_2 receptor, RuBP, becomes the limiting factor. Moreover, the regeneration of RuBP is

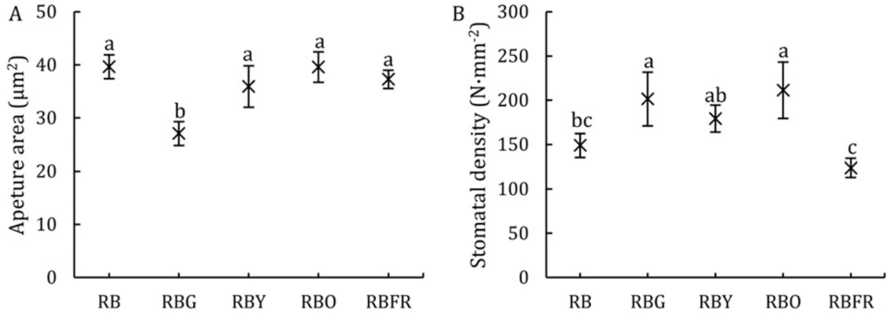

Fig. 5. The leaf stomatal aperture area and density as affected by different combinations of light wavelengths. Different letters show significant differences according to Duncan's test (*P* = 0.05).

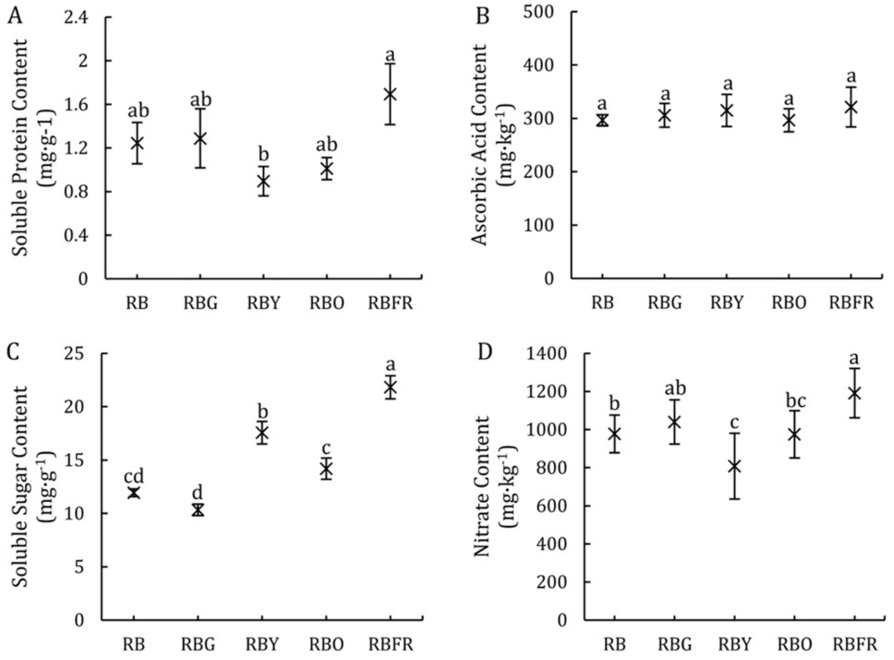

Fig. 6. The contents of lettuce soluble protein (**A**), ascorbic acid (**B**), soluble sugar (**C**), and nitrate (**D**) as affected by different combinations of light wavelengths. Different letters show significant differences according to Duncan's test (*P* = 0.05).

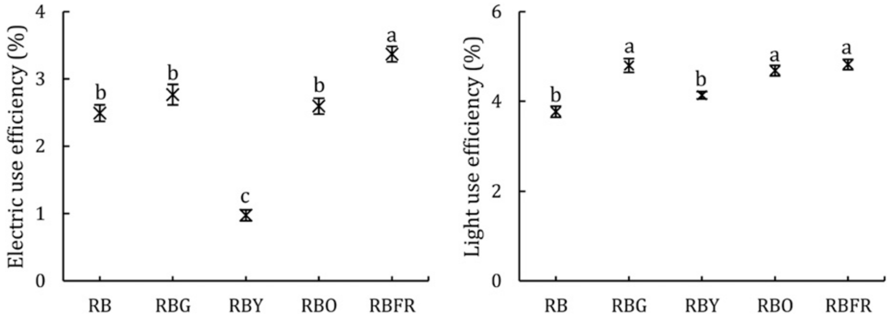

Fig. 7. Effects of different combinations of light wavelengths on electric use efficiency and light use efficiency. Different letters show significant differences according to Duncan's test (*P* = 0.05).

affected by the ATP supply. ATP synthesis is influenced by the electron transfer rate (ETR) and ATP synthase activity. Therefore, P_n at the saturation stage is ultimately affected by ETR and ATP synthase activity (Taiz and Zeiger, 2015). During this experiment, a significantly higher ETR and the maximum photosynthetic rate of the carbon dioxide photosynthetic response curve (P_m) were found under RBO compared with those under RB. This indicated that lettuce plants under RBO showed better photosynthetic capacity. On the contrary, significantly lower ETR and P_m under RBFR caused lower photosynthetic capacity. Although no significant difference in ETR was shown between RB and RBY (Table 3), significantly higher P_m under RBY was found compared with that under RB (Fig. 4). This can be attributed to the fact that the activity of the ATP synthase of lettuce under RBY may be higher than that under RB.

The values of F_v/F_m were within an allowable range for plant growth under the five treatments used during this experiment (Table 3). This indicates that the lettuce plants had no detectable damage to PSII under the five different combinations of light wavelengths during this experiment (Wang et al., 2009). Based on these results, RBO increased lettuce plant shoot fresh weight/dry weight, mainly due to its enhancement of photosynthetic capacity. An increase in the carotenoid content was found under RBO. Previous studies found that energy transfer from carotenoids to chlorophyll could be demonstrated in vitro (Cerullo et al., 2003). Therefore, the increase in carotenoid content under RBO improved the amount of light captured by the PSII reaction center. RBO dramatically increased Φ_{PSII} and F_v'/F_m', and it slightly increased qP. This result suggested that the increase in Φ_{PSII} under RBO is mostly attributed to the increase of qP because Φ_{PSII} is the product of qP and F_v'/F_m' (Wang et al., 2009). Moreover, a significant increase in ETR was found under RBO (Table 3). This increase could promote the process of photophosphorylation and provide more ATP for carbon assimilation because the electrons in the primary reaction promote electron transfer on the photosynthetic membrane (thylakoid membrane) and provide proton power for photophosphorylation to form ATP (Taiz and Zeiger, 2015). As shown in Tables 2 and 3, RBO had better performance with the g_S and stomatal characteristics compared with RB. All these changes in the plant may promote the increase of the net photosynthetic rate and the increase of shoot fresh weight/dry weight under RBO.

Many secondary metabolites are key components for defense against adverse circumstance as well as major contributors to specific odors, tastes, and colors of the plant (Theoharis et al., 2015). Therefore, soluble sugar, ascorbic acid, nitrate, and soluble protein as secondary metabolites are important components of plant nutritional quality. Generally, higher soluble sugar results in a more desirable lettuce taste (Lin et al., 2013). Ascorbic acid is a potent water-soluble antioxidant in humans that protects the body from free radicals, whereas higher nitrate contents in vegetables are harmful for human health; soluble protein is an important nutritional indicator (Chen et al., 2014; Mattila et al., 2011). During this study, no significant improvement in the edible qualities of the lettuce plants was observed under RBG or RBO. However, Chen et al. (2016) found that white light, supplemented with G, increased the soluble sugar content and decreased the nitrate content. Different combinations of light wavelengths showed no significant influences on ascorbic acid content in this experiment. RBY enhanced the content of soluble sugar and reduced the nitrate accumulation, indicating that RBY can improve the taste of the lettuce plant. This result disagrees with those of previous studies that indicated that single Y decreased soluble sugar and protein content of nonheading Chinese cabbage (Fan et al., 2013), and that combining Y with white light increased the nitrate content of green oak leaf lettuce (Chen et al., 2016). In addition, RBFR had the highest values of soluble sugar and nitrate content in this experiment. Zou et al. (2019) also found that supplementing with FR, R, and B increased the concentrations of soluble sugar and nitrate of the plant. Although the RBFR had the highest level of nitrate (1191 $mg \cdot kg^{-1}$), it was far less than China's regulatory limit for nitrate content ($\leq 3000\ mg \cdot kg^{-1}$) in leafy vegetables (Fig. 6). The most suitable light source can be selected according to the needs of production. There were some different nutritional quality results according to this research and previous studies. The possible reason is that the diverse responses of plants to light entail sophisticated sensing of quantity, wavelength, direction, and duration. The light environment among each study was not completely consistent (Theoharis et al., 2015).

EUE and LUE are important factors for determining the economic feasibility of using artificial light (Kozai, 2013). The radiation efficiencies of the experimental light source in this study were different; they were 44.1%, 12.7%, 11.2%, 21.4%, 33.4%, and 30.4%, respectively, for B, G, Y, O, R, and FR. Therefore, although higher biomass under RBG and RBO were found, the EUE showed no significant difference between RB and RBG or RBO. However, significantly higher biomass caused higher LUEs under RBG and RBO. Significantly higher EUE and LUE were found under RBFR. This result indicated that the EUE under RBFR was higher than that under RB.

Conclusions

In this study, lettuce growth, photosynthetic capacity, nutritional quality, and LUE were significantly affected by different combinations of light wavelengths. To select optimal combinations of light wavelengths for lettuce growth in a PFAL, all these indexes were analyzed. Compared with RB, higher shoot fresh weight/dry weights were observed under RBG and RBO. Lettuce plant shoot fresh weight/dry weight under RBG was increased, mainly because of the increase in lettuce leaf area and the P_n, which was caused by a thicker palisade thickness and higher stomatal density. Lettuce shoot fresh weight/dry weight under RBO was increased because of the several aspects of the photosynthetic capacity were improved, such as net photosynthetic rate and electron transport rate. No significant difference in lettuce shoot fresh weight/dry weight was found between RB and RBY; however, RBY showed better nutritional quality performance, with an increase in soluble sugar content and a decrease in nitrate accumulation. The highest lettuce plant shoot fresh weight/dry weight was obtained under RBFR, and it resulted from the highest leaf area. Furthermore, the EUE of lettuce plants can be improved by using RBFR, and the LUE can be improved by using RBG, RBO, and RBFR. However, the nutritional quality was reduced due to the higher nitrate content, and more serious tipburn injuries were seen under RBFR. Therefore, based on this analysis, RBG and RBO can be considered optimal combinations of light wavelengths for lettuce growth in a PFAL.

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