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Continuous Irradiation with Alternating Red and Blue Light Enhances Plant Growth While Keeping Nutritional Quality in Lettuce

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***Abstract*. Plant factories with artificial lighting have been developed to improve food production, functional ingredients, and profitability. Intensive research has been performed to elucidate the effects of light intensity and wavelength on plant growth and nutritional quality with the use of light-emitting diodes (LEDs). In particular, the effects of monochromatic red, blue, or simultaneous red + blue light have been studied because these wavelengths are predominantly used for photosynthesis. We examined the effects of alternating red and blue light provided by LEDs over a period of 24 hours on the growth and nutritional properties of leafy lettuce. The results clearly show that alternating red and blue light accelerated plant growth significantly compared with white fluorescent lamps or red and blue LEDs at the same daily light integral. Plants grown under alternating red/blue light had a greater net assimilation rate and total and projected leaf area (an indicator of the fraction of leaf area that absorbs more light) than other plants. Additionally, alternating red and blue light maintained high concentrations of sugars, ascorbic acid, and anthocyanins in leaves. Taken together, the results indicate that continuous irradiation with alternating red and blue light could enhance growth while maintaining the nutritional quality in lettuce.**

Plant factories with artificial lighting have been developed for efficient production of food crops and are now used for the commercial production of leafy greens and herbs in many countries (Kozai, 2013). As the demands for year-round production of lettuce continue to increase in the food service industry, including fast food restaurants, the efficient operation of plant factories with artificial lighting are expected to take advantage of their characteristics, which are unaffected by weather and are able to produce high yields with uniform quality year round.

Recently, intensive research has been performed to elucidate the effect of light intensity and wavelength on plant growth with the use of LEDs, which offer many advantages, including minimal electricity consumption, low heat generation, small size, and long life compared with conventional artificial lighting, such as high-pressure sodium lamps and fluorescent lamps (Ohtake et al., 2015; Tamulaitis et al., 2005). In particular, the effects of red and blue light on plant development and growth have been extensively studied because these wavelengths are predominantly absorbed by photosynthetic pigments and efficiently drive photosynthesis (Abidi et al., 2013; Massa et al., 2008; Pfündel and Baake, 1990; Yamori, 2016; Yamori and Shikanai, 2016). It has been reported that monochromatic red or blue light alone is not suitable for normal plant growth. For example, plants grown only under red light had decreased photosynthetic rate and showed abnormal growth, compared with the simultaneous red and blue light or white light (Goins et al., 1998; Wang et al., 2015). Moreover, blue light alone, especially at high intensity, caused chloroplast avoidance responses, which induce chloroplasts to escape from the intense light, reducing photosynthesis (Kim et al., 2004; Loreto et al., 2009; Tholen et al., 2008; Wada et al., 2003). On the other hand, simultaneous red and blue light improved photosynthesis and growth relative to red or blue light alone (Brown et al., 1995; Hogewoning et al., 2010; Li et al., 2013; Nanya et al., 2012; Ohashi-Kaneko et al., 2006; Yorio et al., 2001). Thus, it is now recognized that multiple simultaneous light sources, such as red and blue light, are more appropriate for plant growth than monochromatic sources.

Not only the spectral composition but also the mix of irradiation (simultaneous or alternate) can affect plant growth and physiology. Jao and Fang (2004) reported that alternating red and blue light reduced growth of potato plantlets relative to simultaneous red and blue light. On the other hand, Shimokawa et al. (2014) and Chen et al. (2017) reported that alternating red and blue light could affect growth of lettuce, but the authors could not conclude whether it had benefit for plant growth compared with simultaneous red and blue light because the growth period was short or the daily light integral (DLI) and day length were not comparable among treatments. Recently, Kuno et al. (2017) showed that alternating red and blue light could enhance growth in leafy lettuce compared with simultaneous red and blue light under equal DLI and day length, but Jishi et al. (2016) showed the opposite result in cos lettuce. Thus, the effect of alternating red and blue light on plant growth and the underlying mechanisms remain to be clarified. If alternating red and blue light can enhance plant growth compared with simultaneous red and blue light and/or fluorescent lamps, it can also reduce electricity costs by shortening the time to harvest (Ohtake et al., 2015).

Here, we examined the effects of alternating red and blue light by LEDs at different intervals but at the same DLI on the growth and nutritional properties of leafy lettuce. We posed three questions: 1) Does continuous irradiation with alternating red/blue light enhance plant growth? 2) Does it affect nutritional quality, including concentrations of sugar, ascorbic acid, and anthocyanins? 3) What mechanisms are involved in plant growth promotion by alternating red and blue light?

Materials and Methods

Plant material and cultivation conditions. Seeds of leafy lettuce (*Lactuca sativa* L. ‘Summer Surge’; Takii Seed Co., Kyoto, Japan) were sown in sponge blocks, and the seedlings were grown at 23 °C under a photosynthetic photon flux density (*PPFD*) of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool white fluorescent lamps (FHF32-EX-N-H; Panasonic Co., Ltd., Japan). At 10 d after sowing (DAS), the seedlings were transplanted into growth chambers equipped with similar cool white fluorescent lamps (R:G:B = 32:45:23)

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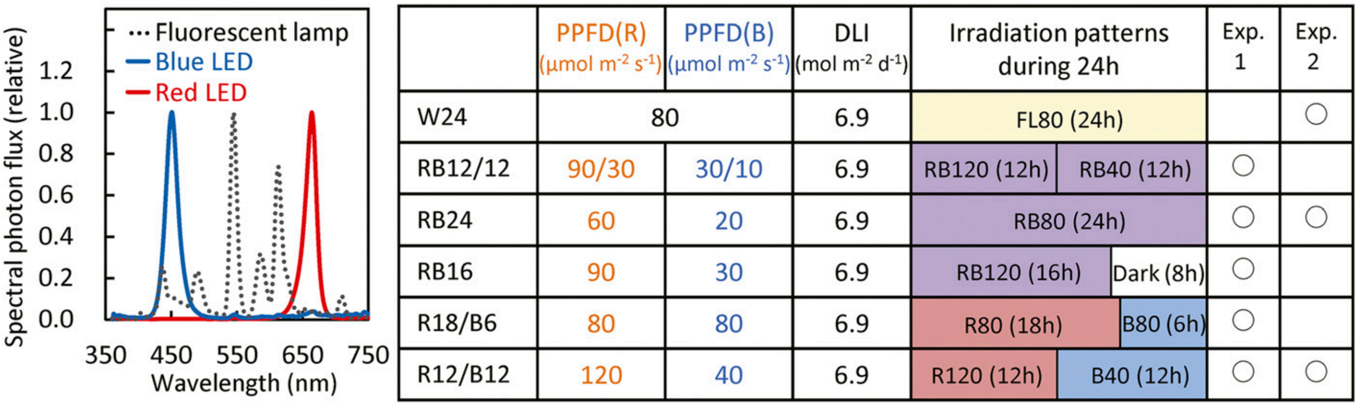

	PPFD(R) (μmol m⁻² s⁻¹)	PPFD(B) (μmol m⁻² s⁻¹)	DLI (mol m⁻² d⁻¹)	Irradiation patterns during 24h	Exp. 1	Exp. 2
W24	80		6.9	FL80 (24h)		○
RB12/12	90/30	30/10	6.9	RB120 (12h) RB40 (12h)	○	
RB24	60	20	6.9	RB80 (24h)	○	○
RB16	90	30	6.9	RB120 (16h) Dark (8h)	○	
R18/B6	80	80	6.9	R80 (18h) B80 (6h)	○	
R12/B12	120	40	6.9	R120 (12h) B40 (12h)	○	○

Fig. 1. Irradiation wavelengths and patterns. Plants were grown under cool white fluorescent lamps as well as red (with a peak at 660 nm) and blue (with a peak at 450 nm) light-emitting diodes in six irradiation patterns with equal daily light integrals: 1) constant white light at 80 μmol·m^{-2}·s^{-1} (W24); 2) constant red and blue light at 120 μmol·m^{-2}·s^{-1} for 12 h and 40 μmol·m^{-2} s^{-1} for 12 h (RB12/12); 3) constant red and blue light at 80 μmol·m^{-2}·s^{-1} (RB24); 4) red and blue light for 16 h at 120 μmol·m^{-2}·s^{-1} (RB16); 5) alternating red light at 80 μmol·m^{-2}·s^{-1} for 18 h and blue light at 80 μmol·m^{-2}·s^{-1} for 6 h (R18/B6); and 6) alternating red light at 120 μmol·m^{-2}·s^{-1} for 12 h and blue light at 40 μmol·m^{-2}·s^{-1} for 12 h (R12/B12).

as well as blue and red LEDs (Showa Denko KK, Japan) with various irradiation patterns (Fig. 1). The DLIs of all light sources were set to 6.9 mol·m^{-2}·d^{-1} in all of the following six light conditions according to Shimokawa et al. (2014) (Fig. 1): (1) constant white light at 80 μmol·m^{-2}·s^{-1} (W24); (2) constant red and blue light at 120 μmol·m^{-2}·s^{-1} for 12 h and 40 μmol·m^{-2}·s^{-1} for 12 h (RB12/12); (3) constant red and blue light at 80 μmol·m^{-2}·s^{-1} (RB24); (4) red and blue light for 16 h at 120 μmol·m^{-2}·s^{-1} (RB16); (5) alternating red light at 80 μmol·m^{-2}·s^{-1} for 18 h and blue light at 80 μmol·m^{-2}·s^{-1} for 6 h (R18/B6); and (6) alternating red light at 120 μmol·m^{-2}·s^{-1} for 12 h and blue light at 40 μmol·m^{-2}·s^{-1} for 12 h (R12/B12). The *PPFD* was measured with a light quantum meter (LI-250Q; LI-COR Inc., Lincoln, NE) on the surface of the culture panel, set at 30 cm below the LED or fluorescent lamps. Twenty plants per treatment were grown in a hydroponic culture system (TAPS-1SW, 1540 mm W × 780 mm D × 2000 mm H; Espec Mic Corp., Japan) in Otsuka House A nutrient solution (OAT Agrio Co., Ltd., Japan) with an electrical conductivity of 2.1 ± 0.1 dS·m^{-1} and a pH of 6.2 ± 0.3, which were automatically controlled by a nutrient regulator (TAPS-1W; Espec Mic Corp.). The air temperature, relative humidity, and CO$_2$ concentration were set to 23 °C, 70%, and 1000 μmol·mol^{-1}, respectively. The planting density was 37.6 m^{-2} from 10 to 16 d, 26.9 m^{-2} from 16 to 22 d, and 16.1 m^{-2} from 22 to 31 d, which was adjusted by sampling 6 plants equidistantly at 16, 22, and 31 DAS. Plants were cultivated under irradiation patterns 2 to 6 in Expt. 1 and under patterns 1, 3, and 6 in Expt. 2. Expt. 1 was used to elucidate the effects of photoperiod, light intensity, and the combination of red and blue light. On the basis of those results, we compared plant growth between alternating red and blue, simultaneous red and blue, and conventional fluorescent lamp under a 24-h photoperiod in Expt. 2. Each cultivation experiment was repeated at least twice and the representative data from the independent experiments are shown.

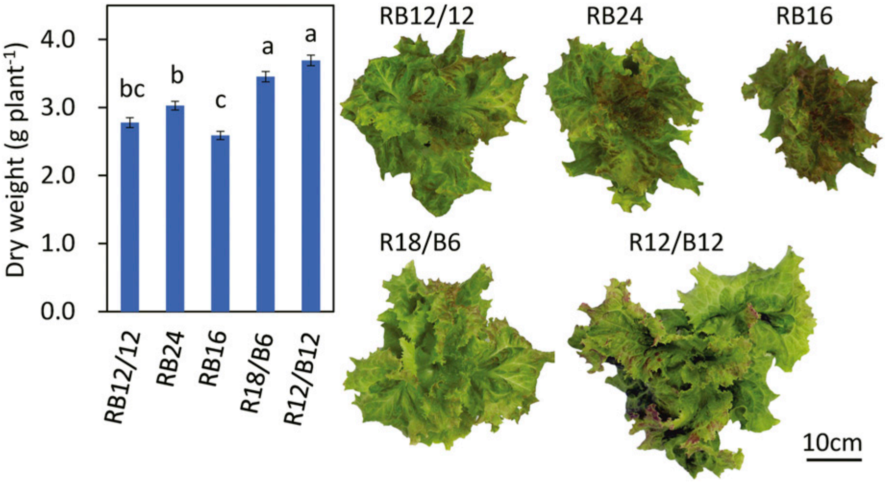

Fig. 2. Total leaf dry weight at 31 d after sowing and photos of each cultivation condition in Expt. 1. Plants were grown as in Fig. 1. Data are means ± SE (n = 6). Bars labeled with the same letter are not significantly different among treatments by Tukey's honest significant difference test at $P < 0.05$. Abbreviations are as in Fig. 1. Typical pictures of plant color and morphogenesis are shown.

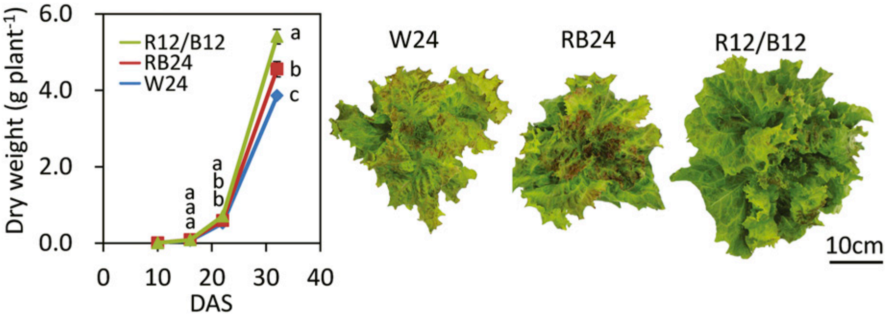

Fig. 3. Plant growth during cultivation and photos of each cultivation condition at 31 d after sowing in Expt. 2. Plants were grown as in Fig. 1 and harvested at 10, 16, 22, and 31 d after sowing. Data are means ± SE (n = 6). Points labeled with the same letter are not significantly different among treatments by Tukey's honest significant difference test at $P < 0.05$. Abbreviations are as in Fig. 1. Typical pictures of plant color and morphogenesis are shown.

Growth analysis. At 10, 16, 22, and 31 DAS, plants were harvested, and the marketable fresh weights and total dry weights, projected leaf area (PLA), total leaf area (LA), number of leaves, plant height, and maximum leaf width and length were measured. PLA and LA were measured in image analysis software (LIA32 v.0.376) from digital images taken vertically downward (Maeshiro et al., 2013). Number of leaves

Table 1. Parameters for the plant growth at 16, 22, and 31 d after sowing (n = 6).

	Marketable fresh wt (g)	Dry wt (g)	Number of leaves	Plant ht (cm)	Maximum leaf length (cm)	Maximum leaf width (cm)	Leaf length-to-width ratio	Dry matter ratio (%)	Leaf mass per area (g DW/m^2)	Total Chl content (g·m^{-2})	Chl *a*/*b*
16 DAS											
W24	0.79 ± 0.06 a	0.07 ± 0.01 a	4.0 ± 0.0 a	6.6 ± 0.2 a	6.43 ± 0.19 a	5.60 ± 0.27 a	1.16 ± 0.04 b	6.40 ± 0.14 c	8.7 ± 0.1 c	0.114 ± 0.002 b	3.80 ± 0.03 c
RB24	0.86 ± 0.08 a	0.09 ± 0.01 a	3.7 ± 0.2 a	5.7 ± 0.1 b	5.50 ± 0.13 b	5.05 ± 0.26 a	1.10 ± 0.04 b	8.17 ± 0.17 b	12.7 ± 0.3 b	0.124 ± 0.004 a	3.98 ± 0.04 b
R12/B12	0.77 ± 0.03 a	0.08 ± 0.01 a	3.5 ± 0.2 a	6.8 ± 0.2 a	6.57 ± 0.27 a	4.83 ± 0.15 a	1.36 ± 0.04 a	9.52 ± 0.59 a	14.6 ± 0.8 a	0.113 ± 0.002 b	4.09 ± 0.05 a
22 DAS											
W24	6.29 ± 0.23 b	0.52 ± 0.02 b	7.0 ± 0.0 a	10.6 ± 0.2 b	11.5 ± 0.2 b	11.4 ± 0.3 a	1.01 ± 0.02 a	6.23 ± 0.10 b	8.9 ± 0.3 b	0.132 ± 0.002 b	3.75 ± 0.03 b
RB24	7.31 ± 0.64 ab	0.59 ± 0.04 b	7.3 ± 0.2 a	9.4 ± 0.2 c	10.3 ± 0.3 c	11.7 ± 0.5 a	0.89 ± 0.02 b	6.27 ± 0.21 b	9.7 ± 0.1 b	0.155 ± 0.002 a	4.09 ± 0.02 a
R12/B12	8.28 ± 0.13 a	0.71 ± 0.02 a	7.2 ± 0.2 a	11.8 ± 0.3 a	12.4 ± 0.1 a	12.2 ± 0.1 a	1.02 ± 0.02 a	7.55 ± 0.10 a	13.5 ± 0.8 a	0.154 ± 0.003 a	4.05 ± 0.04 a
31 DAS											
W24	58.4 ± 0.9 c	3.86 ± 0.07 c	16.7 ± 0.2 a	18.7 ± 0.3 a	19.1 ± 0.4 a	23.2 ± 0.3 b	0.83 ± 0.02 a	6.53 ± 0.15 a	8.9 ± 0.4 a	0.132 ± 0.004 b	3.62 ± 0.03 b
RB24	66.7 ± 2.8 b	4.55 ± 0.20 b	17.7 ± 0.8 a	16.7 ± 0.2 b	16.5 ± 0.5 b	22.2 ± 0.4 b	0.75 ± 0.03 a	6.25 ± 0.12 a	10.0 ± 0.2 a	0.157 ± 0.006 a	3.92 ± 0.04 a
R12/B12	80.2 ± 1.7 a	5.41 ± 0.19 a	17.0 ± 0.3 a	19.3 ± 0.5 a	19.7 ± 0.6 a	25.5 ± 0.3 a	0.77 ± 0.02 a	6.36 ± 0.12 a	10.1 ± 0.5 a	0.150 ± 0.005 a	3.87 ± 0.04 a

Chl = chlorophyll; DAS = days after sowing.

was counted for the leaves greater than 1 cm. Dry matter ratio (DMR) was calculated as dry weight per fresh weight in the most newly expanded leaves without petiole. Leaf mass per area (LMA) was calculated as follows: LMA = (fresh weight of the leaf disc) × DMR / unit area. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) were estimated from total dry weight and leaf area as (Blackman, 1919; Hunt, 1990; West et al., 1920; Yamori et al., 2011): RGR = $(1/W)(\Delta W/\Delta t) = [\ln(W_2) - \ln(W_1)]/(t_2 - t_1)$, where W_1 and W_2 are total dry weights of the whole plant at times t_1 and t_2; NAR = $(1/L)(\Delta W/\Delta t) = (W_2 - W_1)/(t_2 - t_1)[\ln(L_2) - \ln(L_1)]/(L_2 - L_1)$, where L_1 and L_2 are total leaf areas of the whole plant at times t_1 and t_2; LAR = L/W = $[L_1/W_1 + L_2/W_2]/2$.

Measurements of gas exchange. Gas exchange was measured with a portable gas exchange system (LI-6400; LI-COR Inc.) as described previously (Yamori et al., 2012; Zhang et al., 2015). After 30 min of illumination to obtain steady-state photosynthesis, the net photosynthetic rate in the most newly expanded leaves of 31- to 35-d-old plants grown under W24 was measured under growth light conditions in Expt. 2: white light at 80 μmol·m^{-2}·s^{-1} (W24), red and blue light at 80 μmol·m^{-2}·s^{-1} (RB24), red light at 120 μmol·m^{-2}·s^{-1} (R12/B12), and blue light at 40 μmol·m^{-2}·s^{-1} (R12/B12). Then we estimated daily carbon gains by integration of the photosynthetic measurements as described previously (Chazdon, 1986; Ellsworth and Reich, 1992). Because it takes time to reach the maximum rate of photosynthesis following an increase in irradiation (Yamori et al., 2012), the estimated daily carbon gains may be slightly overestimated, although there should not be any significant differences among growth light conditions.

Nutritional quality of leafy lettuce. At 31 DAS, plants were harvested for nutritional analyses by the Japan Food Research Laboratories (http://www.jfrl.or.jp/e/index.html). Bulked samples of four or five plants per treatment were analyzed for plant moisture by the atmospheric pressure drying method, protein by Kjeldahl method, total fat by acid hydrolysis method, and ash by the direct ashing method. Contents of vitamin A, vitamin C (ascorbic acid), fructose, glucose, and sucrose were quantified by high-performance liquid chromatography. Contents of free amino acids were quantified by automatic amino acid analysis. Anthocyanin and chlorophyll (Chl) contents were analyzed in the most newly expanded leaves. Anthocyanins were extracted in hydrochloric acid/methanol (1:99, v/v) and quantified by spectrophotometer (V-570, Jasco, Japan) according to Ubi et al. (2006). Chl was extracted in *N,N*-dimethylformamide and quantified by spectrophotometer according to Porra et al. (1989).

Statistical analysis. Values were compared between irradiation treatments by Tukey's post hoc test in Excel software (BellCurve for Excel, v. 2.15; Social Survey Research Information Co., Ltd. Tokyo, Japan). Differences were considered significant at $P < 0.05$.

Results

Plant growth. In Expt. 1, plant growth at 31 DAS was significantly greater under RB24 than under RB16 (Fig. 2), indicating that 24 h of light enhanced plant growth relative to 16 h. Growth was significantly greater under R18/B6 and R12/B12 than under RB24 and RB12/12, indicating that alternating red/blue light enhanced plant growth. Growth was marginally greater under R12/B12 than under R18/B6. These results clearly indicate that both continuous light and alternating red and blue light enhanced lettuce growth.

In Expt. 2, we performed further analysis to elucidate how alternating red and blue light improves plant growth, analyzing growth under W24, RB24, and R12/B12 at 10, 16, 22, and 31 DAS. Plant dry weight and marketable fresh weight at 31 DAS were greatest under R12/B12, intermediate under RB24, and lowest under W24 (Fig. 3; Table 1). The number of leaves was similar among conditions. Plant height and the maximum leaf length were greater under W24

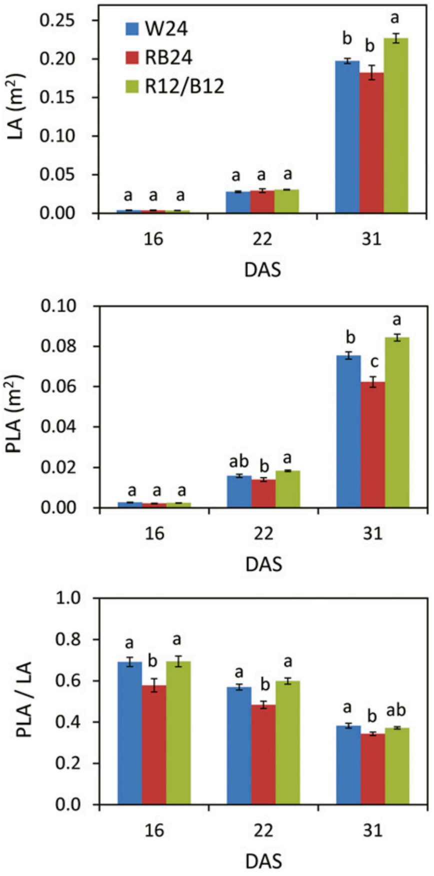

Fig. 4. Total leaf area (LA), projected leaf area (PLA) and PLA/LA in Expt. 2. Plants were grown as in Fig. 1 and measured at 16, 22, and 31 d after sowing. Data are means ± SE (n = 6). Points labeled with the same letter are not significantly different among treatments by Tukey's honest significant difference test at $P < 0.05$. Abbreviations are as in Fig. 1.

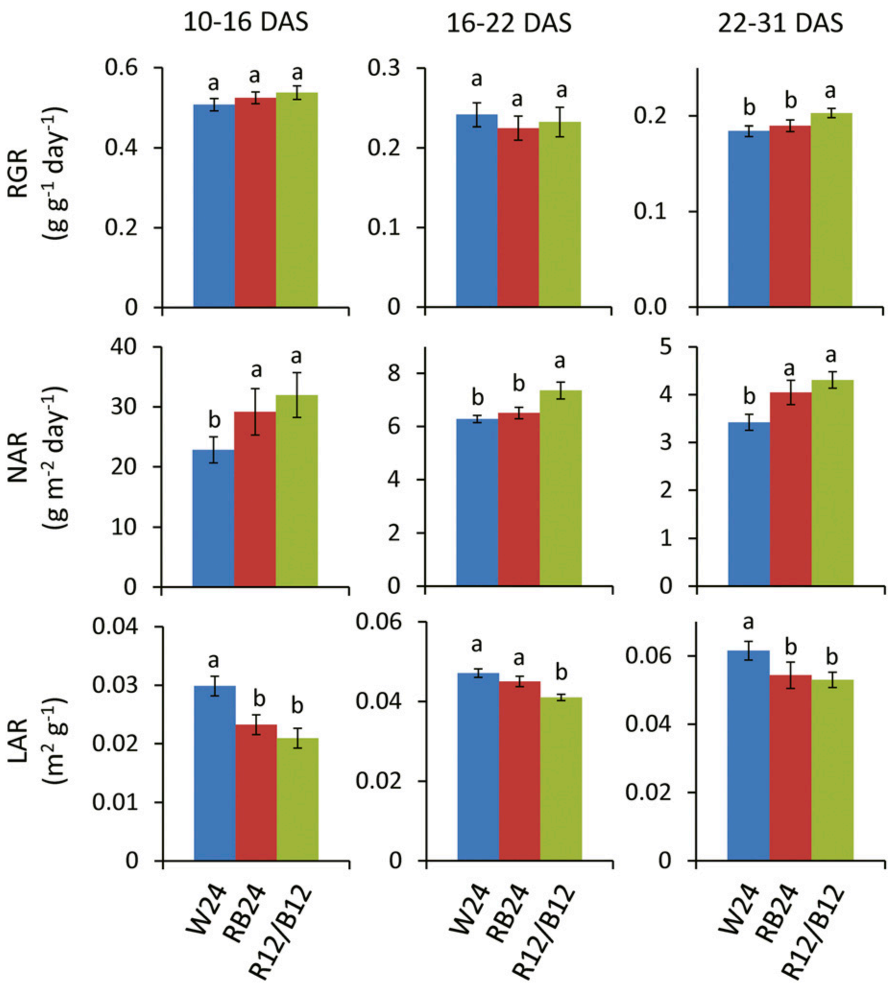

Fig. 5. Effects of irradiation patterns on plant growth in Expt. 2. Relative growth rate (RGR), net assimilation rate (NAR), and leaf area ratio (LAR) (data from Fig. 3). Cultivation experiment (n = 6) was repeated four times and all data were used for statistical analysis. Bars labeled with the same letter are not significantly different among the three light treatments by Tukey's honest significant difference test at $P < 0.05$. Abbreviations are as in Fig. 1.

Table 2. Daily carbon gain estimated from measurements of net photosynthetic rate. Net photosynthetic rate was measured in each light condition in Expt. 2 (Supplemental Fig. 2) and the daily carbon gain was estimated by the integration. Data are means ± SE (n = 6). Abbreviations as in Fig. 1.

	Daily carbon gain ($\mathrm{mmol{\cdot}m^{-2}{\cdot}d^{-1}}$)
W24	216.3 ± 2.3 b
RB24	237.6 ± 6.3 ab
R12/B12	248.6 ± 8.0 a

and R12/B12 than under RB24. On the other hand, the maximum leaf width was similar among the three conditions at 16 and 22 DAS, but it was greater under R12/B12 at 31 DAS. LA was similar irrespective of growth conditions at 16 and 22 DAS, but was greatest under R12/B12 at 31 DAS (Fig. 4). PLA was greatest under R12/B12 at 22 and 31 DAS. PLA/LA was significantly greater under W24 and R12/B12 at 16 and 22 DAS and showed a similar tendency at 31 DAS. DMR and LMA in the most newly expanded leaves were significantly greatest under R12/B12 at 16 and 22 DAS (Table 1). Although there were no significant difference at 31 DAS, LMA tended to be greater under R12/B12 than under W24. Total Chl content and Chl *a/b* were greater under RB24 and R12/B12 than under W24 at 22 and 31 DAS.

There was no significant difference in RGR at 10–16 or 16–22 DAS, but it was significantly greatest under R12/B12 at 22–31 DAS (Fig. 5). NAR tended to be greatest under R12/B12, intermediate under RB24, and lowest under W24, whereas LAR showed the opposite trend. These results indicate that the enhancement of RGR under R12/B12 is attributable to an improvement in NAR, which is determined mainly by photosynthetic rate. This is supported by the significantly greater daily carbon gain under RB12/12 than under W24 estimated from photosynthetic measurements in each growth condition (Table 2; Supplemental Fig. 2).

Nutritional quality. The contents of protein, fat, carbohydrate, β-carotene, α-tocopherol, and γ-tocopherol were similar among W24, RB24, and R12/B12. On the other hand, the contents of ascorbic acid, fructose, and anthocyanins tended to be greater in plants grown under R12/B12 than under other growth conditions, though only marginally significantly ($P < 0.1$; Supplemental Fig. 1A). The same trends were observed in 'Red Oak' lettuce (Supplemental Fig. 1B). Thus, it is fair to say that continuous irradiation with alternating red and blue light could keep nutritional quality while enhancing plant growth in lettuce.

Discussion

Although the development of LEDs has brought new opportunities for plant factories with artificial lighting, the cost of electricity for lighting is still high. Because there is great potential to reduce costs by designing more efficient lighting systems, many studies have investigated the effects of light intensity and wavelength on plant growth (Hogewoning et al., 2010; Joshi et al., 2017; Li et al., 2013; Li and Kubota, 2009; Lin et al., 2013; Zhang et al., 2015). There has been controversy in the recent literature regarding the effect of alternating red and blue lighting on the growth of lettuce: Kuno et al. (2017) showed that alternating irradiation promoted growth compared with simultaneous irradiation of red and blue light at the same DLI and day length at 30 DAS, whereas Jishi et al. (2016) showed no significant difference between the two conditions at 21 DAS. It is highly possible that differences in cultivation stage could explain this difference. This conclusion is supported by our results, which clearly show that alternating red and blue lighting accelerated plant growth significantly compared with simultaneous red and blue, especially from 22 to 31 DAS (Figs. 2–5). Thus, the effect of alternate irradiation appears to make a significant difference to plant growth at a later stage of cultivation.

We further analyzed the mechanisms of growth promotion from the viewpoints of both photosynthesis and plant morphogenesis. Plants grown under alternating red and blue light had a high NAR (Fig. 5), which is the primary indicator of the rate of photosynthesis per unit area (Potter and Jones, 1977). This is supported by the significantly greater daily carbon gain estimated from photosynthetic measurements under alternating red and blue light than under white light (Table 2). Furthermore, LMA was significantly greatest under alternating red and blue light at the early stage and tended to be greater than under white light at 31 DAS (Table 1). Because it is commonly recognized that LMA shows good correlation with photosynthesis (Poorter et al., 2014; Reich et al., 1991), the results indicate that the plants grown under alternating red and blue light had high photosynthetic activity on the basis of leaf area, especially during early growth.

Next, we focused on the effect of alternating red and blue light on plant morphogenesis. The length-to-width ratio of the maximum leaf was greater under alternating red and blue light than under simultaneous red and blue light at 16 and 22 DAS (Table 1), indicating that alternating red and blue light promoted leaf elongation, which might have an advantage in light absorption. Moreover, PLA/LA was significantly greater under alternating red and blue light than under simultaneous red and blue light at 16 and 22 DAS and was marginally greater at 31 DAS (Fig. 4). Because the higher PLA/LA value means less self-shading (Furuyama et al., 2017), the result indicates that plants grown under alternating red and blue light had a lower proportion of leaf area shaded by their own leaves and thus could absorb more light than plants grown under simultaneous red and blue light. Taken together, the results show that alternating red and blue light contributed to elongated leaves with less self-shading and high photosynthetic activity, improving plant growth relative to simultaneous red and blue light or white light.

In addition, we analyzed the effect of the alternating red and blue light on nutritional quality. Generally, sugar concentration is closely related to taste (Chadwick et al., 2016; Meyers and Brewer, 2008), ascorbic acid (i.e., vitamin C) is an essential antioxidant (Alasalvar et al., 2001; Kelebek et al., 2009), and anthocyanins are believed to have antioxidant properties (Lu et al., 2017; Wang and Lin, 2000). Our results indicate that the concentrations of all three were marginally greater under alternating red and blue light than under white light (Supplemental Fig. 1). Thus, we conclude that continuous irradiation with alternating red and blue light could maintain high concentrations of sugars, ascorbic acid, and anthocyanins, improving the nutritional properties of lettuce.

In this study, the DLIs were set to 6.9 $\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$, and the ratio of red to blue was adjusted to 3:1 using LED light. Because it has been reported that DLI as well as the R:B ratio affect both plant growth and quality (i.e., phytochemicals) (Goto, 2012), further research is needed to optimize DLI and the R:B ratio to maximize plant growth and quality in a commercial plant factory.

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