A Combination of Downward Lighting and Supplemental Upward Lighting Improves Plant Growth in a Closed Plant Factory with Artificial Lighting

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Abstract. “Plant factory with artificial lighting” (PFAL) refers to a plant production facility that can achieve mass production of vegetables year round in a controlled environment. However, the high-density planting pattern in PFALs causes low light conditions in the lower canopy, leading to leaf senescence in the outer leaves and thus to reductions in plant yields. In the present study, the effect of supplemental upward lighting underneath the plants on photosynthetic characteristics and plant yield was examined in lettuce, in comparison with supplemental downward lighting from above the plants at the same light intensity. Supplemental upward lighting increased the curvature factor of the photosynthetic response to light from above the plants. Moreover, supplemental upward lighting significantly enhanced the lettuce yield by retarding the senescence of the outer leaves. Here, we propose a novel cultivation system with a combination of downward lighting and supplemental upward lighting that can effectively increase plant growth and yield in PFALs.

“Plant factories with artificial lighting” are a new type of facility that can produce high yield with high quality all year round in

a controlled environment (e.g., lighting, temperature, CO₂ concentration, and nutrients) (Kozai, 2013a; Yamori et al., 2014). PFALs have many advantages over conventional farming and greenhouses (Merrill et al., 2016). For example, a PFAL creates a closed space that is independent from environmental, seasonal, and geographical limitations. Moreover, resources are more efficiently used, including land, water, nutrients, and labor. In addition, high-quality and safe plants can be produced without pesticides under this strict closed environment (Merrill et al., 2016). PFALs have already been used for commercial production of leafy vegetables and herbs in many countries, including Asia, Europe, and North America (Kozai, 2013b; Kozai et al., 2015).

In a PFAL, a large number of leafy vegetables (e.g., lettuce and spinach) are grown at a high planting density to achieve high yields. However, this dense cultivation causes a strong shading effect on leaves beneath the plant canopy, leading to a dramatic decrease in photosynthesis in the lower canopy (Brouwer et al., 2012; Tewolde et al., 2016). Light is the most important environmental factor affecting photosynthesis and thus yield because plant growth and yield depend on photosynthesis (Yamori, 2016; Yamori and Shikanai, 2016; Yamori et al., 2016). The light intensity in the outer leaves is usually below the photosynthetic light compensation point [i.e., the photosynthetic photon flux density (PPFD) at which photosynthetic rate is zero], meaning that the rate of photosynthesis falls below the rate of respiration (Zhang et al., 2015). The outer leaves senesce fast and appear yellow (McCabe et al., 2001), resulting in large reductions in plant yield at the time of shipment. A recent study indicated that supplemental upward lighting from underneath the plants could delay the senescence of outer leaves (Zhang et al., 2015). However, it is unclear whether supplemental upward lighting from underneath the plants could improve the yield in comparison with supplemental downward lighting from above the plants at the same light intensity because their experiments were examined under different total light intensity. Because the free energy from sunlight is completely excluded from PFALs, additional electrical energy is required to provide growth light for plant cultivation, accounting for as much as 80% of the total electricity consumption (Kozai, 2007; Kozai, 2013a). This causes a great increase in the electricity bill and reduces income substantially. To use the light source efficiently, it is important to investigate the effects of supplemental upward lighting from underneath on plant growth in comparison with supplemental downward lighting from above at the same light intensity and propose the lighting environment most suitable to the plant cultivation in PFALs.

Currently, only leafy vegetables and some herbs (shade-type plant species) are suitable for commercial production in PFALs because they can grow well even under relatively low light intensities (100–200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD; Kozai et al., 2015). It is difficult to cultivate many vegetables (especially, sun-type plant species), including tomato, paprika, and cucumber, under such low light intensities in a PFAL because they require higher light intensities for growth. The light–response curve of the photosynthetic rate differs between sun- and shade-type plant species. Both the light compensation point and light saturation point of the photosynthetic rate are low in shade species (Givnish, 1988; Lichtenthaler et al., 1981), allowing them to efficiently grow under low light intensities. In contrast, sun species demand more light to drive photosynthesis because they have a higher light compensation point and light saturation point than shade species.

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The shape of the photosynthetic light–response curve varies with the direction of irradiation (Moss, 1964; Ögren and Evans, 1993; Terashima, 1986). In general, the photosynthetic light–response curve obtained by irradiating the adaxial side of a dorsiventral leaf shows a higher curvature factor value than that obtained by irradiating the abaxial side of the same leaf (Terashima and Takenaka, 1986). The difference in the curvature factor of the adaxial and abaxial curves became smaller after 7 d of the leaf being inverted and was slightly reversed by 11 d (Terashima, 1986). However, how the photosynthetic light–response curve can be altered by combining downward lighting and supplemental upward lighting has not been examined. If supplemental upward lighting could alter the shape of the photosynthetic light–response curve and thus the light saturation point of sun-type species possible under relatively low downward lighting with supplemental upward lighting. In the present study, to develop a new cultivation system fitting for plant productions under PFALs, we studied the effects of supplemental upward or downward lighting at the same light intensity on photosynthetic characteristics and plant growth in lettuce and measured the light–response curve of photosynthetic rate to analyze whether the supplemental upward lighting could effectively alter the curvature factor of the photosynthetic response to light from above the plants.

Materials and Methods

Plant materials and growth conditions.

Romaine lettuce seeds (*Lactuca sativa* L. var. Romana; Takii Seed Co., Kyoto, Japan) were sown in urethane cubes (W 2.3 cm × D 2.3 cm × H 2.7 cm), and the seedlings were grown in an environmentally controlled growth chamber at 20/17 °C (photoperiod/dark period) under a PPFD of $350 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from cool white fluorescent lamps with a 12-h photoperiod. At 4 weeks after sowing, uniformly sized seedlings at the three-leaf stage were transplanted to a cultivation room under a 25/20 °C photoperiod/dark period temperature (Wang et al., 2016; Zhang et al., 2015) and 14-h photoperiod. The plants were grown in a deep-flow hydroponic system, supplied with Enshi formula nutrient solution (EC: 2.0 ± 0.2 and pH: 7.0 ± 0.5 ; Asao et al., 2013; Zhang et al., 2015). The cultivation system could provide light both from above (i.e., downward lighting) and underneath (i.e., upward lighting) the plants (Fig. 1). The light treatments were divided into three groups: 1) control: plants were grown solely under downward lighting at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 2) supplemental downward lighting: plants were grown under downward lighting at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with supplemental downward lighting at a PPFD of 30 or $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; and 3) supplemental upward lighting: plants were grown under downward lighting at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with supplemental upward lighting at a PPFD of 30 or $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with supplemental upward lighting at a PPFD of 30 or $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the height of the outer leaves (3.0 cm). The planting density is 33 plants/ m^2 , and the interval between plant rows and between light-emitting diode (LED) tubes is 9 cm. The LEDs for supplemental upward lighting have installed on the aluminum plates to release the heat efficiently and so there was no significant effect on the leaf temperature by the supplemental upward lighting (InfRed, Fujitsu, Japan). The downward lighting for plant cultivation was provided by cool white fluorescent lamps (Supplemental Fig. 1A), and supplemental downward or upward lighting was supplied with white LEDs (Supplemental Fig. 1B). Each treatment was replicated five times.

Gas exchange and chlorophyll fluorescence.

The plants were divided into six layers of leaves counted from the lowest leaf; the first to third layers were considered outer leaves, and the fourth to sixth layers were considered inner leaves. Photosynthetic rate and stomatal conductance (g_s) in the newest fully expanded leaves (i.e., inner leaves; in the sixth layer) and the outer leaves (in the third layer) in plants grown for 3 weeks after transplanting were measured under each growth-light condition with a portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln, NE) as described previously (Yamori et al., 2009, 2010; Zhang et al., 2015). For the measurement of photosynthetic light–response curves, the control plants which were grown solely under downward lighting have been examined. Maximum potential photochemical efficiency (i.e., the ratio of variable to maximum fluorescence, F_v/F_m) was measured using an Imaging-PAM fluorometer (Walz, Effeltrich, Germany) as described in Zhang et al. (2015).

Chlorophyll and nitrogen content. Immediately after the photosynthesis and chlorophyll fluorescence measurements, leaf discs (0.85 cm in diameter) were taken from the six layers of leaves (the same leaves used to measure F_v/F_m) of each treatment. Total chlorophyll content was quantified according to Porra et al. (1989). Leaf nitrogen content was measured with a Vario EL III elemental analyzer (Elementar, Hanau, Germany) according to Yamori et al. (2005).

Plant growth and ascorbic acid content. The plants were harvested at 3 weeks after transplanting (i.e., after 3 weeks under different light treatments), and plant growth was analyzed. The ascorbic acid content of plants in each treatment was determined using a RQFlex plus reflectometer (Merck, Darmstadt, Germany) according to Zhang et al. (2015).

Electricity consumption measurements. The electrical energy consumption of the white LEDs was measured using a multimeter and a clamp ammeter (Hioki 3169-01; Hioki E.E. Corporation, Nagano, Japan) and was used to evaluate the economics of each light treatment (Zhang et al., 2015).

Statistical analysis. Data are presented as means $\pm$ SD. Analysis of variance with a post hoc Tukey's test was performed in the SPSS statistical software (SPSS, Chicago, IL). Differences were considered significant at $P < 0.05$.

1) Control

2) Supplemental downward lighting

3) Supplemental upward lighting

Fig. 1. Schematic diagram of the experimental design of the present study. In cultivation beds, a lighting system that could provide light both from above (i.e., downward lighting) and underneath (i.e., upward lighting) the plants was installed. The supplemental light treatments were divided into three groups: (1) control: plants were grown solely under downward lighting at a photosynthetic photon flux density (PPFD) of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; (2) supplemental downward lighting: plants were grown under downward lighting at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with supplemental downward lighting at a PPFD of 30 or $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; and (3) supplemental upward lighting: plants were grown under downward lighting at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with supplemental upward lighting at a PPFD of 30 or $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the height of the outer leaves. The picture of supplemental upward lighting underneath the plants has been shown.

Results

Leaf characteristics. In plants grown without any supplemental lighting, the total chlorophyll content (Fig. 2A) and F_v/F_m (Fig. 2B) remained high in the inner leaves (fourth to sixth layer), but dramatically decreased from the third to first layer in the outer leaves. Similar results were obtained when plants were grown with supplemental downward lighting.

Supplemental upward lighting resulted in higher total chlorophyll content and F_v/F_m values in the outer leaves of plants compared with plants grown without any supplemental upward lighting (Fig. 2A and B). In addition, the treatment with supplemental upward lighting at a PPFD of $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ resulted in a higher total chlorophyll content

Fig. 2. (A) Total chlorophyll content and (B) maximum quantum yield (F_v/F_m) in lettuce leaves from the six leaf layers in plants grown under different light treatments. Data represent means $\pm$ SD ($n = 5$). “200” denotes plants grown solely under downward lighting at a 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (PPFD); “200 + 30 down” or “200 + 60 down” denotes plants grown under downward lighting at a 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD with supplemental downward lighting at a 30 or 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD; and “200 + 30 up” or “200 + 60 up” denotes plants grown under downward lighting at a 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD with supplemental upward lighting at a 30 or 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD.

in the outer leaves than the treatment with supplemental upward lighting at a PPFD of 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 2A and B).

Photosynthesis and nitrogen content. In the newest fully expanded leaves (inner leaves; sixth layer), the photosynthetic rate and g_s were highest in the treatment with supplemental downward lighting at a PPFD of 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, followed by 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD, whereas plants in the control and supplemental upward lighting treatments at a PPFD of 60 or 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ showed similar values (Fig. 3A and B). The nitrogen content in the newest fully expanded leaves (inner leaves; sixth layer) showed a similar trend to the photosynthetic rate in the inner leaves (Fig. 4A).

In the outer leaves (third layer), all the plants without supplemental upward lighting (even those with supplemental downward lighting) showed negative photosynthetic rates (Fig. 3C). However, supplemental upward lighting increased the g_s and photosynthetic rate (Fig. 3C and D). The treatment with supplemental upward lighting at a 60 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD increased the photosynthetic rate in the outer leaves to a greater extent than that at a 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD (Fig. 3C). Moreover, supplemental upward lighting increased the leaf nitrogen content in the outer leaves (third layer; Fig. 4B), which showed the same pattern to the photosynthetic rate in the outer leaves (third layer; Fig. 3C).

Fig. 3. Photosynthetic rate and stomatal conductance (g_s) of plants grown under different light treatments. Data represent means $\pm$ SD ($n = 5$). (A) Photosynthetic rate and (B) g_s of the newest fully expanded leaves (i.e., inner leaves; in the sixth layer), and (C) photosynthetic rate and (D) g_s of the outer leaves (in the third layer) were measured under each growth-light condition. Bars labeled with different letters indicate that the data are significantly different among the five light treatments (Tukey's HSD test, $P < 0.05$). Abbreviations are the same as those in Fig. 2.

Fig. 4. Nitrogen content in the (A) inner and (B) outer leaves of lettuce plants grown under different light treatments. Data represent means $\pm$ SD ($n = 5$). Bars labeled with different letters indicate that the data are significantly different among the five light treatments (Tukey's HSD test, $P < 0.05$). Abbreviations are the same as those in Fig. 2.

Photosynthetic light-response curves under different light intensities of supplemental upward lighting. The photosynthetic light-response curves in the newly fully expanded leaves (inner leaves; sixth layer) of control plants were measured to assess the leaf photosynthetic capacity under different levels of supplemental upward lighting (0, 30, 60, or 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD; Fig. 5). The maximum photosynthetic rate at the light saturation point was similar in each photosynthetic light-response curve (Fig. 5). However, supplemental upward lighting increased the photosynthetic rate at lower PPFDs compared with

the control, whereas the light compensation point was reduced by supplemental upward lighting (Fig. 5). The curvature factor, which indicates the bending rate of the photosynthetic light-response curve, increased with increasing supplemental upward light intensity (Fig. 5; Table 1).

Plant growth. Plant growth and yield were significantly enhanced by both supplemental downward lighting and supplemental upward lighting, but supplemental upward lighting was more effective, and the most remarkable increase was found in the treatment with supplemental upward lighting

at a $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD (Fig. 6A; Supplemental Table 1). Supplemental downward lighting tended to have a higher waste compared with the control (Fig. 6B). Conversely, supplemental upward lighting significantly reduced the waste (Fig. 6B). As a result, the marketable leaf fresh weight (i.e., the remaining leaves after removal of the outer senesced leaves) was much higher in the treatment with supplemental upward lighting than in the treatment with supplemental downward lighting (Fig. 6A).

Ascorbic acid content of the romaine lettuce. The ascorbic acid content in outer leaves and total leaves was unaffected by supplemental downward lighting, but was significantly increased by supplemental upward lighting (Fig. 7). The highest ascorbic acid content was obtained in the treatment with supplemental upward lighting at a $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD (Fig. 7).

Discussion

A combination of downward and upward lighting could be an economically feasible and more cost-effective alternative to traditional solely downward lighting. Our results clearly showed that supplemental upward lighting significantly retarded the senescence of outer leaves in plants, resulting in a higher F_v/F_m (Fig. 2B) and higher photosynthetic rate (Fig. 3) compared with plants grown solely under downward lighting. Because nitrogen, an essential constituent of chlorophyll and photosynthetic enzymes including Rubisco, significantly influences the rate of photosynthesis (Yamori et al., 2011), supplemental upward lighting would improve photosynthesis by the maintenance of higher nitrogen content in the outer leaves (Fig. 4B). Moreover, supplemental upward lighting significantly increased ascorbic acid content in outer leaves and thus total leaves, compared with plants grown under solely downward lighting (Fig. 7). The previous research indicated that ascorbic acid content increased with increases in growth-light intensity in lettuce (Kosma et al., 2013), but the present study clearly showed that the total amount of ascorbic acid in plants was severely affected by the direction of irradiation to plants. Furthermore, supplemental upward lighting gave a significant reduction in the waste by retarding the senescence of the outer leaves (Fig. 6B), leading to a significant increase in marketable leaf fresh weight (Fig. 6A). Considering only the cost of electricity, the increased cost of supplemental downward lighting (23.3 or 44.8 JPY/plant) resulted in a 12.7% or 27.1% increase in marketable leaf fresh weight (a profit equivalent to 12.9 or 33.1 JPY/plant) compared with control plants grown solely under downward lighting at a $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD (Supplemental Table 2). However, supplemental upward lighting more efficiently increased the marketable leaf fresh weight (35.4% or 56.3%) by reducing the waste, leading to an 80.0 or 117.1 JPY/plant

increase in profit (Supplemental Table 2). Because the electricity requirement for lighting is the largest expense to growers, the effective use of electricity through a more efficient lighting system can contribute to the profitability of a PFAL (Kozai et al., 2015). Our results clearly show that supplemental upward lighting is more effective for plant cultivation in PFALs than supplemental downward lighting. LED lighting is a revolutionary product that has allowed growers to put lights in places that were difficult or impossible to reach in the past. The unique characteristics of LEDs, such as their small size, long lifetime, reduced heat, and precise wavelength specificity (Merrill et al., 2016), make them ideal as feasible and efficient light sources for supplemental upward lighting. Future developments in lighting systems for PFALs should consider supplemental upward lighting to achieve a high yield and good quality, as well as good economic benefits.

In the present study, it took 7 weeks until harvesting lettuce after sowing seeds, because plants were grown under ambient CO_2 concentration of 400 ppm. Optimization of growth CO_2 concentration, hydroponic system, and air conditioners with air fans would be able to promote plant growth and thus shorten the cultivation period (Kozai, 2013a; Merrill et al., 2016). In addition, the present data indicated that downward lighting at a PPFD of $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with supplemental upward lighting at a PPFD of $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ increased lettuce yield compared with supplemental upward lighting at a PPFD of $30 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 6). The upper limits of light intensity for supplemental upward lighting might be greater than that of $60 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Therefore, further research is required to optimize the light

intensity of supplemental upward lighting and also the combination with the light intensity of downward lighting for maximizing lettuce yield in PFALs.

Fig. 5. Photosynthetic light-response curves of the newest fully expanded leaves (i.e., inner leaves; in the sixth layer) in control plants with different levels of supplemental upward lighting [0, 30, 60, or $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density (PPFD)]. Data represent means $\pm$ SD ($n = 5$). “200” denotes the photosynthetic light-response curve measured in leaves without supplemental upward lighting. “200 + 30 up,” “200 + 60 up,” and “200 + 90 up” denote the photosynthetic light-response curve measured in leaves with supplemental upward lighting at 30, 60, and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD, respectively.

Table 1. Parameters for the light-response curve of photosynthetic rate, derived from the nonrectangular hyperbolic response.

	Maximum rate of photosynthesis (A_{max}) ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Curvature factor (θ)
0	15.0 ± 0.8 a	0.705 ± 0.035 b
30 _{up}	14.8 ± 0.7 a	0.768 ± 0.032 ab
60 _{up}	14.6 ± 0.7 a	0.822 ± 0.041 a
90 _{up}	14.3 ± 0.7 a	0.862 ± 0.038 a

“0” denotes the photosynthetic light-response curve measured in leaves without supplemental upward lighting. “30_{up},” “60_{up},” and “90_{up}” denote the photosynthetic light-response curve measured in leaves with supplemental upward lighting at 30, 60, and $90 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density, respectively. Curves are fitted to the data shown in Fig. 5.

Fig. 6. (A) Total leaf fresh weights, (A) marketable leaf fresh weights, and (B) wastes of the outer senesced leaves of plants grown under different light treatments at 3 weeks after transplanting. Data represent means $\pm$ SD ($n = 5$). Bars labeled with different letters indicate that the data are significantly different among the five light treatments (Tukey’s HSD test, $P < 0.05$). Abbreviations are the same as those in Fig. 2.

Fig. 7. Ascorbic acid content in the outer leaves, inner leaves, and total leaves of lettuce plants grown under different light treatments. Data represent means $\pm$ SD ($n = 5$). Bars labeled with different letters indicate that the data are significantly different among the five light treatments (Tukey's HSD test, $P < 0.05$). Abbreviations are the same as those in Fig. 2.

Supplemental upward lighting makes it possible to grow plants with a high light-saturated rate of photosynthesis. At present, the number of plant species suitable for commercial production in PFALs is limited because the light intensity used is relatively low (e.g., below a $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD). Accordingly, only leafy vegetables and some herbs can be produced under such low light conditions (Kozai, 2013b). Low light intensities are insufficient for sun-type plant species, which have a higher light saturation point (Givnish, 1988; Lichtenthaler et al., 1981) and thus need more light for growth. However, our results clearly show that supplemental upward lighting altered the shape of the photosynthetic light-response curve by improving the curvature factor and had a significant effect on the treatment with supplemental upward lighting at the higher light intensity (Figs. 5 and 6; Table 1). This indicates that supplemental upward lighting of the abaxial side of a leaf can lower the light saturation point of photosynthesis even under relatively low growth light from above. Our results suggest that a new efficient lighting system with supplemental upward lighting could make it possible to realize PFAL cultivation even for sun-type plant species.

Conclusions

The aim of our research is to establish a new cultivation system in PFALs. The newly proposed system using a combination of downward lighting and supplemental upward lighting retarded the senescence of the outer leaves and improved the photosynthesis, leading to significant increase in lettuce yield. Moreover, supplemental upward lighting increased the curvature factor of the photosynthetic light-response curve, providing the feasibility to cultivate sun-type species under relatively low downward lighting with supplemental upward lighting. This novel cultivation system with supplemental upward lighting could be a widespread application in PFALs because it can maximize plant yield through a suitable light source layout and make PFALs applicable to a wide range of plant species.

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Supplemental Fig. 1. The relative spectral photon flux of (A) cool white fluorescent lamps and (B) supplemental downward or upward lighting (white LEDs). The wavelengths of light sources were recorded at 240–800 nm with a spectrometer (SR9910-v7; Irradiant Ltd., Tranent, UK).

Supplemental Table 1. Leaf number and total leaf area of lettuce under different light treatments at harvest time.

Light treatments	Leaf number	Total leaf area (m ²)
200	35.2 ± 1.5 c	0.361 ± 0.288 bc
200 + 30 _{down}	40.2 ± 3.0 bc	0.353 ± 0.130 c
200 + 60 _{down}	45.0 ± 1.0 ab	0.464 ± 0.153 a
200 + 30 _{up}	45.6 ± 1.0 ab	0.431 ± 0.970 ab
200 + 60 _{up}	47.8 ± 1.6 a	0.469 ± 0.133 a

Data represent means ± SD ($n = 5$). Values within columns followed by different letters were significantly different (Tukey's HSD test, $P < 0.05$).

Supplemental Table 2. Evaluation of the feasibility of different light treatments in real production. Based on local surveys, the retail price of lettuce was 200 JPY/100 g and the electricity bill was 17.49 JPY/KW h. (A) Electricity bill of supplemental upward lighting per plant was calculated as: electricity consumption of white LEDs $\times 14/1000/8 \times 17.49$, where 14 was the photoperiod and 8 was the number of plants illuminated by supplemental white LEDs from underneath the plants; (B) retail price per plant was calculated as: marketable leaf fresh weight/100 $\times 200$; net retail price per plant was calculated as B minus A.

PPFD ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Marketable leaf fresh weight (g)	Electric consumption of supplemental lighting (KWh plant/d)	Electricity consumption during the treatments	(A) Electricity bill of supplemental lighting (JPY plant)	(B) Retail price (JPY plant)	(B – A) Net retail price (JPY plant)
200	143.7 ± 25.2 c	0.000 ± 0.000 c	0.00 ± 0.00 d	0.0 ± 0.0 d	287.3 ± 50.5 d	287.3 ± 50.5 c
200 + 30 _{down}	161.8 ± 11.7 c	0.051 ± 0.002 b	1.33 ± 0.04 b	23.3 ± 0.8 b	323.6 ± 23.6 c	300.2 ± 23.7 c
200 + 60 _{down}	182.6 ± 3.8 b	0.098 ± 0.001 a	2.56 ± 0.03 a	44.8 ± 0.5 a	365.2 ± 7.6 b	320.4 ± 7.2 c
200 + 30 _{up}	194.5 ± 8.4 b	0.048 ± 0.002 b	1.24 ± 0.05 b	21.6 ± 0.9 b	388.9 ± 16.7 b	367.3 ± 16.6 b
200 + 60 _{up}	224.5 ± 8.7 a	0.098 ± 0.002 a	2.55 ± 0.04 a	44.6 ± 0.8 a	449.0 ± 17.4 a	404.4 ± 17.6 a

Data represent means ± SD ($n = 5$). Values within columns followed by different letters were significantly different (Tukey's HSD test, $P < 0.05$).