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Both Multi-Segment Light Intensity and Extended Photoperiod Lighting Strategies, with the Same Daily Light Integral, Promoted *Lactuca sativa* L. Growth and Photosynthesis

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Abstract: With the rise of plant factories around the world, more and more crops are cultivated under artificial light. Studies on effects of lighting strategies on plant growth, such as different light intensities, photoperiods, and their combinations, have been widely conducted. However, research on application of multi-segment light strategies and associated plant growth mechanisms is still relatively lacking. In the present study, two lighting strategies, multi-segment light intensity and extended photoperiod, were compared with a constant light intensity with a 12 h light/12 h dark cycle and the same daily light integral (DLI). Both lighting strategies promoted plant growth but acted via different mechanisms. The multi-segment light intensity lighting strategy promoted plant growth by decreasing non-photochemical quenching (NPQ) of the excited state of chlorophyll and increasing the quantum yield of PSII electron transport (PhiPSII), quantum yield of the carboxylation rate (PhiCO_2), and photochemical quenching (qP), also taking advantage of the circadian rhythm. The extended photoperiod lighting strategy promoted plant growth by compensating for weak light stress and increasing light-use efficiency by increasing chlorophyll content under weak light conditions.

Keywords: photoperiod; weak light stress; circadian rhythm; chlorophyll fluorescence; photosynthesis; *Lactuca sativa* L.

1. Introduction

Plant factories with artificial lighting (PFAL) are a relatively new method of efficient agricultural cultivation that are becoming more and more important in combatting increasingly serious global food supply problems [1]. Unusual weather, water shortages, and reduction of cultivated land area all threaten to reduce the production of the crops worldwide; however, these environmental factors do not threaten PFAL crop growth as they are thermally insulated, artificially lit, air conditioned, and have air circulation fans along with supplies of CO_2 and nutrient solution, making it so the environment can be fully controlled [2–4]. Crops in PFALs always rely on artificial lighting in that the light energy drives photosynthesis, which means that electricity accounts for 25%–30% of the total production costs and that light sources account for the majority of all energy consumption, about 60%–80% [1]. Therefore, improving the efficiency of light sources would greatly reduce the cost of PFALs, which would further encourage their sustainable development because costs and ecological impacts could be reduced.

In PFALs, light energy can be provided for crops at any time because day and night no longer determine lighting schedules. This means that any photoperiod can be chosen to optimize the growth and quality of many crops. Gaudreau [5] reported that lengthening the photoperiod resulted in substantial gains in the fresh weight of lettuce. Longer photoperiods not only increased the growth of the lettuce under low CO_2 concentrations at the same day light integral (DLI), but also compensated for weak photosynthetic photon flux density (PPFD) [6]. One study revealed that increasing photoperiod under weak light cultivation conditions compensated for weak light stress [7]. Vlahos et al. [8] reported that the growth of *Achimenes* species can be promoted under weak light intensities at the same DLI compared with those under high light intensities, as the lower light intensities combined with longer photoperiods resulted in higher light-use efficiencies. Overall, these results have suggested that under the same DLI, longer photoperiods promote the growth of lettuce.

Light is not only an energy source, but also an important environmental signal [9,10]. Morphological adaptations and light-dependent adjustments optimize plants for photosynthesis in a given environment [11,12]. One previous study has shown that the formation and accumulation of photosynthetic pigments in leaves was affected by light quality, which either increases light harvesting under low-light conditions or acts as screening pigments and free-radical scavengers under high-light conditions [13]. There has been plenty of research about the effect of different ratios of red and blue light [11,14–16], different light intensities [11,14–16], and various light intensities combined with different photoperiods on plant growth [11,14–16]. However, these studies have ignored the effect of light as an environmental signal affecting the circadian rhythm, which in turn affects the metabolism in many plants. Breathing in plants is regulated daily, alternately cycling between high activity and low activity in roughly 24 h, determined by the circadian rhythm, which also has a great influence on the growth of crops [17–19]. Dodd [20] reported that the circadian rhythm confers an advantage to plants, benefitting photosynthesis, growth, survival, and competitive advantage. Most plants use circadian oscillators to coordinate physiological and developmental processes such as photosynthesis, respiration, and cell wall synthesis. In addition, the rhythm of these processes can change periodically during a single day. Previous lighting strategies have ignored the effects of photoperiod on circadian rhythm, missing the opportunity to use the circadian rhythm to benefit the growth of crops in PFALs [21–24].

In this study, three different lighting strategies were used, with varying photoperiods while maintaining the same DLI. The goal of the study was to determine the effects of different lighting strategies on growth of lettuce and to clarify the underlying mechanisms by investigating growth parameters, chlorophyll content, photosynthesis parameters, chlorophyll fluorescence parameters, and circadian rhythm.

2. Materials and Methods

2.1. Plant Material and Cultivation Method

Romaine (*Lactuca sativa* L. var. longifolia, 'Coastal Star') lettuce seeds were sown in rockwool cubes (5 × 5 × 5 cm) and grown in a closed cultivation room with air conditioning to control the temperature and artificial lighting for illumination at Chiba University, Japan. The photosynthetic photon flux density (PPFD) was set to 150 μmol photons m^{-2} s^{-1} with a photoperiod of 14 h per day provided by cool white fluorescent lamps (FHF32 EX-N-H; Panasonic Co., Ltd., Osaka, Japan). The plants were irrigated with a nutrient solution (Otsuka hydroponic composition; Otsuka Chemical Co. Ltd., Osaka, Japan) [25]). The EC (electric conductivity) and pH of the nutrient solution were adjusted to 1.2 dS m^{-1} and 6.0 by adding new nutrient solution, respectively. Air temperature, relative humidity, and CO_2 concentration were maintained at 20 ± 5 °C, 60%–80%, and 400 ± 10 μmol mol^{-1}, respectively.

2.2. Treatments

After two weeks, the seedlings were transplanted into three environmentally controlled mini-PFALs and subjected to the three lighting strategies, all with the same DLI. Each group had nine plants. Strategy A: PPFD was set to 150 μmol photons m^{-2} s^{-1} with a photoperiod of 16 h per day. Strategy B: PPFD was set to 200 μmol photons m^{-2} s^{-1} with a photoperiod of 12 h per day. Strategy C:

PPFD was set to 100 μmol photons m^{-2} s^{-1} for the first 3 h, then 300 μmol photons m^{-2} s^{-1} for the next 6 h, and 100 μmol photons m^{-2} s^{-1} in the last 3 h (the total photoperiod was 12 h) (Figure 1). All plants were grown under red and blue LED (light emitting diode) lamps (dimmable LED panels, using the R/B (red/blue) ratio 2:1). The red LEDs had a peak wavelength of 660 nm and a wavelength range of 600 to 700 nm. The blue LEDs had a peak wavelength of 450 nm and a wavelength range of 400 to 500 nm. Prior to introducing the plants, the PPFD was measured at the surface of rockwool cubes using a light meter (LI 250A, LI-190R; Li-Cor Inc., Lincoln, NE, USA) and adjusted accordingly. The EC and pH of the nutrient solution were adjusted to 1.6 dS m^{-1} and 6.0, respectively. The air temperature was maintained at 20 ± 0.5 °C with a relative humidity of 70% ± 5% and carbon dioxide concentrations of 400 ± 10 μmol mol^{-1}.

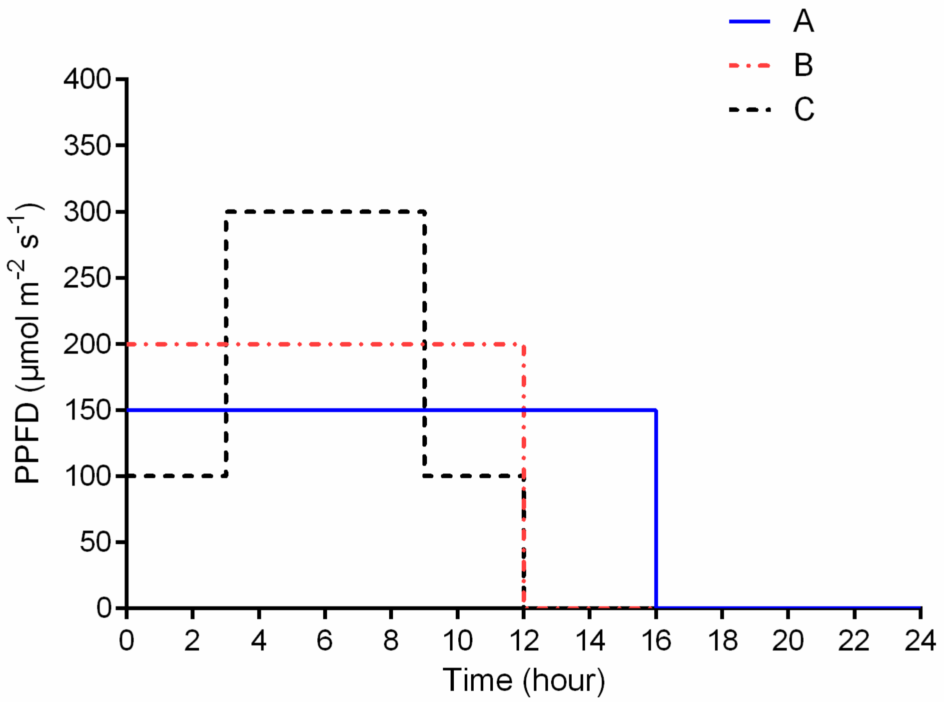


Figure 1. Lighting strategy of A, B, and C. Each strategy had the same daily light integral (DLI; 8.64 mol m^{-2} d^{-1}), and the photoperiods of A, B, and C were 16, 12, and 12 h, respectively.

In addition, nine seedlings were transplanted in a glass greenhouse in Chiba University, Japan. The EC and pH of the nutrient solution were adjusted to 1.6 dS m^{-1} and 6.0, respectively. During the cultivation period, the average air temperature was 35.3 °C/27.4 °C (day/night), average relative humidity was 62.8%, and average daily PPFD was 588 μmol m^{-2} s^{-1} inside the greenhouse.

2.3. Measurements

2.3.1. Growth Parameters and Chlorophyll Content Measurements

Six samples from lighting strategies A, B, and C were collected 4 weeks after transplant, and the fresh weight of leaves and roots were determined. For determination of dry weight of leaves and roots, samples were oven dried at 70 °C until the weight became constant, and the dry weight was recorded. The SPAD (soil and plant analyzer develotrnent) value was taken for leaf chlorophyll content using a portable chlorophyll meter (SPAD-502; KONICA MINOLTA, Osaka, Japan).

2.3.2. Gas-Exchange Parameter

In order to show that the rate of photosynthesis of lettuce grown under natural conditions changed with circadian rhythm, we continuously measured the photosynthetic rates of the lettuce, which was cultivated in the greenhouse for 60 h using a portable photosynthesis measurement system (Li–6400XT, Li-Cor Inc., Lincoln, NE, USA) on the 24th day after transplant. PPFD was 200 μmol photons m^{-2} s^{-1}, and the leaf temperature, CO_2 concentration, and relative humidity were 20 °C, 400 ± 10 μmol mol^{-1}, and 65%, respectively. Furthermore, in order to obtain a photosynthesis curve of each strategy over a continuous photosynthesis cycle, the photosynthetic rate of each strategy was continuously measured. The photosynthesis curve of strategy A was measured on the 25th, 28th, and 31st day after transplant. The photosynthesis curve of strategy B was measured on the 26th, 29th, and 32th day. The photosynthesis curve of strategy C was measured on the 27th, 30th, and 33rd day. The measurement time and PPFD were consistent with the photoperiod and light intensity under the actual cultivation conditions. The leaf temperature, CO_2 concentration, and relative humidity were 20 °C, 400 ± 10 μmol mol^{-1}, and 65%, respectively. The youngest fully expanded leaves from each treatment were used for measurements [15].

2.3.3. Light Response Curve Measurements

The P_N (net photosynthetic rate)-PPFD curve was measured on the 28th day after transplant, which was created by plotting P_N data against the corresponding light intensity. When light response curve measurements were taken, leaf temperature was set at 20 °C and PPFD settings were 1200, 1000, 800, 600, 400, 300, 200, 150, 100, 50, 10, and 0 μmol photons m^{-2} s^{-1}. In order to obtain the light-saturated maximum photosynthetic rate (P_{Nmax}) and apparent quantum yield (AQY), the P_N-PPFD curves were fitted with least squares according to previous research [26,27]. P_{Nmax} was determined as the maximum P_N at a saturated light intensity, and according to the initial slope of the P_N-PPFD curves, we determined the AQY.

2.3.4. Chlorophyll Fluorescence Parameter Measurements

Chlorophyll fluorescence parameters were measured on the 28th day to evaluate light absorption, transfer, dissipation, and distribution in the photosystem of lettuce plants grown under the different lighting strategies. The dynamics of F_o and F_v/F_m were measured on the 1st, 7th, 14th, 21st, and 28th day. Chlorophyll fluorescence parameters were measured with an open gas exchange system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, USA) and an integrated fluorescence chamber head (LI-6400-40XT, Li-Cor, Inc. Lincoln, NE, USA) after leaves were adapted to light or dark and had reached a stable state. Chlorophyll fluorescence parameters are defined as follows [28]:

F_s: the steady state chlorophyll fluorescence level.

F_0: the minimum chlorophyll fluorescence yield in the dark-adapted state.

F_0': the minimum chlorophyll fluorescence yield in the light-adapted state.

F_m: the maximum chlorophyll fluorescence yield in the dark-adapted state.

F_m': the maximum chlorophyll fluorescence yield in the light-adapted state.

F_v: the maximum variable chlorophyll fluorescence yield in the dark-adapted state.

F_v': the maximum variable chlorophyll fluorescence yield in the light-adapted state.

F_v/F_m: the maximum quantum yield of the PSII primary photochemistry was calculated as $(F_m - F_o)/F_m$.

F_v'/F_m': the efficiency of excitation energy capture by open PSII reaction centers was calculated as $(F_m' - F_o')/F_m'$.

qP: photochemical quenching of chlorophyll fluorescence was calculated as $(F_m' - F_s)/(F_m' - F_o')$.

NPQ: non-photochemical quenching of chlorophyll fluorescence was calculated as $(F_m - F_m')/F_m'$.

PhiPSII: the quantum yield of PSII electron transport was calculated as $(F_m' - F_s)/F_m'$.

PhiCO_2: The quantum yield of the carboxylation rate [29,30].

2.4. Statistical Analysis

All experiments were replicated three times for each treatment. Six plants from each replicate were used for plant growth analyses, and three plants from each replicate were used for the determination of gas-exchange parameters and chlorophyll fluorescence. All statistical analysis was conducted with GraphPad Prism Software (GraphPad Software, Inc. La Jolla, California, USA) using the Tukey's multiple range test at $p < 0.05$ level of significance

3. Results

3.1. Plant Growth and Chlorophyll Content

Multi-segment (C) and extended photoperiod (A) lighting strategies resulted in positive effects on plant growth relative to the short photoperiod (B) lighting strategy. Compared with the short photoperiod (B), multi-segment (C) lighting strategy increased total fresh weight of plants, total dry weight, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight by 10.5%, 11.5%, 11.4%, 11.3%, 12.4%, and 11.9%, respectively; however, the root/shoot ratio and chlorophyll content were not significantly affected (Figure 2).

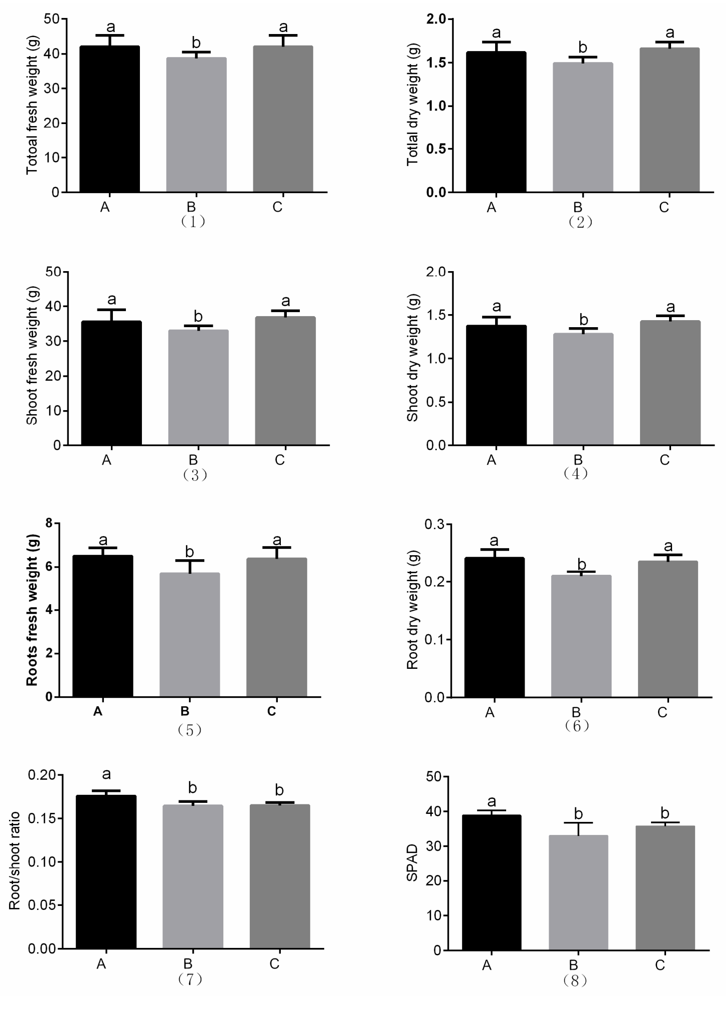


Figure 2. Total fresh weight (**1**), total dry weight (**2**), shoot fresh weight (**3**), shoot dry weight (**4**), root fresh weight (**5**), root dry weight (**6**), root/shoot ratio (**7**), and chlorophyll content (**8**) under different lighting strategies. Each value is the mean ± SE (standard error) of six replicates. Different letters indicate groups were significantly different ($p < 0.05$) according to Tukey's multiple range test.

Compared with the short photoperiod (B) lighting strategy, the extended photoperiod (A) lighting strategy increased total fresh weight, total dry weight, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, as well as both the root/shoot ratio, and SPAD by 8.6%, 8.5%, 7.6%, 7.4%, 14.5%, 6.9%, and 17.1%, respectively (Figure 2).

3.2. Mreasurements of Photosynthetic Parameters

Under 12 h of continuous lighting, the photosynthetic rate gradually increased with time, peaking at about 6 h with a maximum value of 9.2 µmol CO_2 m^{-2} s^{-1}, before it gradually decreased (Figure 3). The light source was turned off after 12 h, and the curve in the graph reflects the respiration rate of the leaves. These 24 h represent a complete photosynthesis cycle; the process was repeated three times and showed a consistent trend. However, the trend of the last two photosynthesis cycles was weaker than the first.

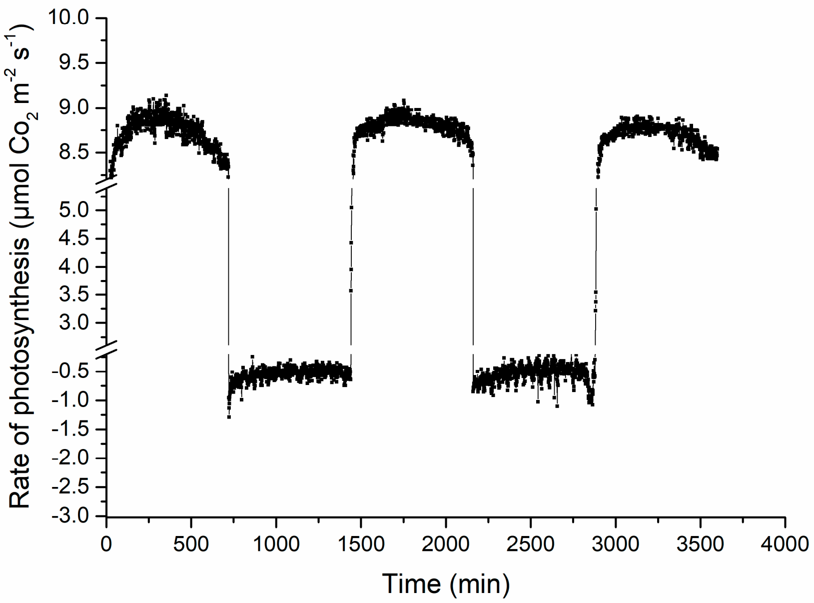


Figure 3. Photosynthetic rate curve of the lettuce plants that were cultivated in the natural light condition. The light cycle was set as 12 h lighting and 12 h dark, and in total the data over 60 h were recorded. The light intensity during the light period was 200 µmol photons m^{-2} s^{-1}.

The photosynthetic rate curves of A and B approximated straight lines, which was quite different from the photosynthetic rate curve of lettuce cultivated in the natural light environment (Figure 3), indicating that the circadian rhythm of lettuce disappeared in the constant temperature and constant light environments. The photosynthetic rate curve of C was made of three approximately straight lines (Figure 4). The photosynthetic rate curves of A, B, and C were fitted separately. The fit function (Equations (1)–(3)) was established by the photosynthetic rate curves and then the fit functions were integrated (Equations (4)–(6)). The integral value approximated the diurnal photosynthetic capacity. The integral value of the A, B, and C curves were 0.372 mol CO_2 m^{-2}, 0.366 mol CO_2 m^{-2}, and 0.386 mol CO_2 m^{-2}, respectively. The integral value of C was 3.8% higher than A, and 5.5% higher than B. However, there was no significant difference in the integral values of A and B. This means that the multi-segment lighting strategy increased the diurnal photosynthetic capacity of lettuce and increased yield.

$$\mathrm{A}:\ y = -6\times10^{-5}x^2 + 0.0496x + 383.1\left(R^2 = 0.81\right) \tag{1}$$

$$\mathrm{B}:\ y = -2\times10^{-4}x^2 + 0.1393x + 488.77\left(R^2 = 0.79\right) \tag{2}$$

$$\mathrm{C}:\ y = \begin{cases} 0.001x^2 - 0.2277x + 283.79 \quad (R^2 = 0.83) \\ -0.0009x^2 + 1.0172x + 454.19 (R^2 = 0.92) \\ 0.0002x^2 - 0.0548x + 270.18 \quad (R^2 = 0.82) \end{cases} \tag{3}$$

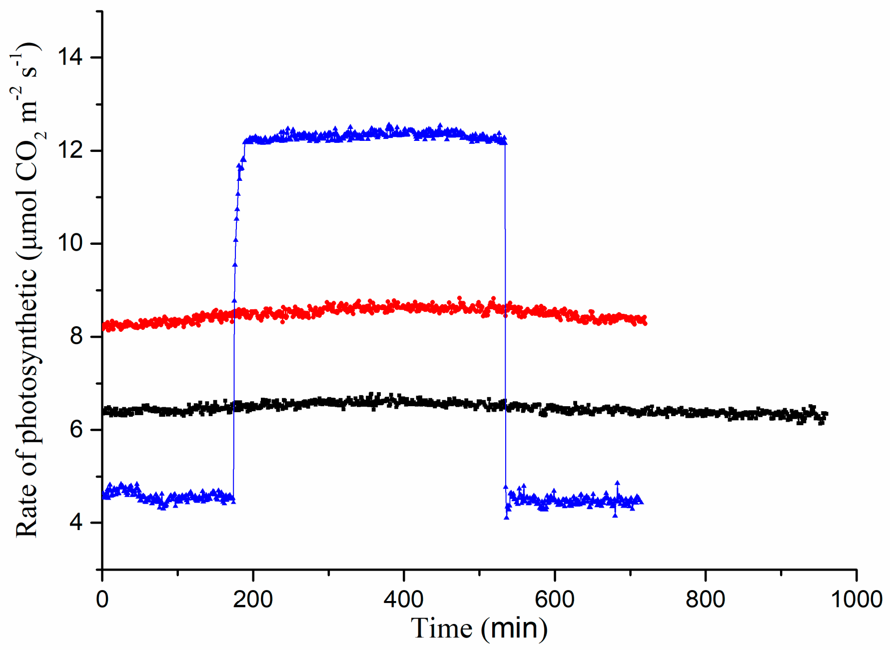


Figure 4. Photosynthetic rates of lettuce under three different lighting strategies. Photosynthetic rates were measured every minute to get the complete photosynthesis curves, and then fit using the fit function. The black, red, and blue colors represent the photosynthetic rate curve of A, B, and C, respectively.

In Equations (1)–(3), y is the net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) under different lighting strategies, and x is the time (min).

$$\mathrm{A}: \int_0^{960} \left(-6\times10^{-5}\mathrm{x}^2+0.0496\mathrm{x}+383.1\right) = 0.372\ \mathrm{mol\ CO_2\ m^{-2}} \tag{4}$$

$$\mathrm{B}: \int_0^{720} \left(-2\times10^{-4}\mathrm{x}^2+0.1393\mathrm{x}+488.77\right) = 0.366\ \mathrm{mol\ CO_2\ m^{-2}} \tag{5}$$

$$\begin{aligned}\mathrm{C}: &\left(\int_0^{180} 0.001\mathrm{x}^2-0.2277\mathrm{x}+283.79\right)+\left(\int_{180}^{540} -0.0009\mathrm{x}^2+1.0172\mathrm{x}+454.19\right)\\ &+\left(\int_{540}^{720} 0.0002\mathrm{x}^2-0.0548\mathrm{x}+270.18\right) = 0.386\ \mathrm{mol\ CO_2\ m^{-2}}\end{aligned} \tag{6}$$

In Equations (4)–(6), the upper and lower limits of the integration (min) are the start and end times of the illumination, respectively.

3.3. Light Response Curve Measurements

The photosynthetic rate increased with the increases of PPFD and grew rapidly when the light intensity was lower than 600 μmol photons m^{-2} s^{-1}. The P_N-PPFD curve of B and C became flat after light intensity reached 1000 μmol photons m^{-2} s^{-1}, whereas the P_N-PPFD curve of A became flat after light intensity had reached 800 μmol photons m^{-2} s^{-1}. This indicated that the light saturation points for the samples under lighting strategies B and C were approximately 1000 μmol photons m^{-2} s^{-1}, and A was approximately 800 μmol photons m^{-2} s^{-1} (Figure 5). The multi-segment lighting strategy (C) significantly increased P_{Nmax} by 8.79% (Figure 6, panel 1), whereas no significant effect was observed on AQY (Figure 6, panel 2) compared with the short photoperiod lighting strategy (B). The extended photoperiod lighting strategy (A) decreased P_{Nmax} by 9.3% and significantly increased AQY by 14.3%.

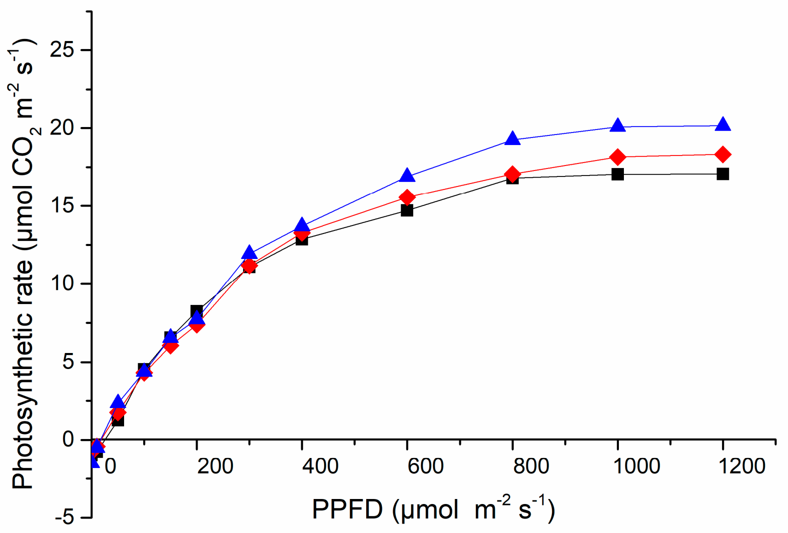
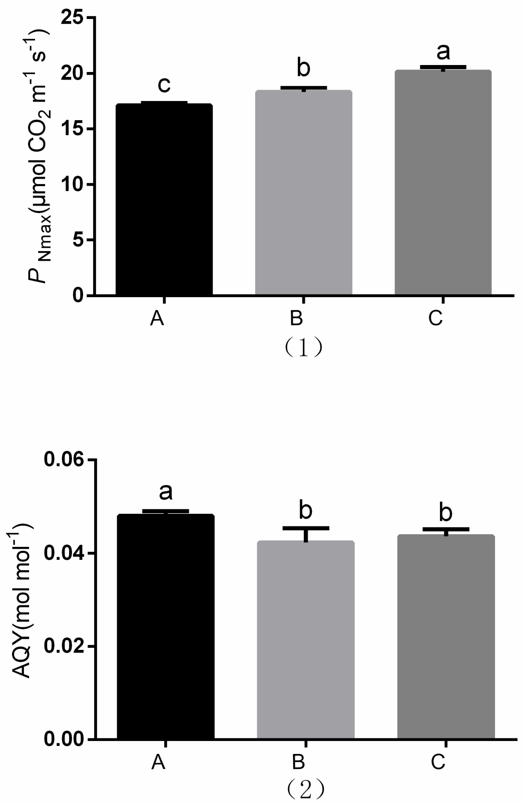


Figure 5. Photosynthetic light response curves for three different lighting strategies (P_N (net photosynthetic rate)-PPFD (photosynthetic photon flux density)). The black, red, and blue curves are the light response curves of A, B, and C, respectively.



（1）

（2）

Figure 6. Effects of different lighting strategies on light-saturated maximum photosynthetic rate (P_{Nmax}) (**1**) and apparent quantum yield (AQY) (**2**). Each value is the mean ± SE of three replicates. Different letters indicate that groups were significantly different ($p < 0.05$) according to Tukey's multiple range test.

3.4. Chlorophyll Fluorescence Parameter Measurements

Compared with strategy B, the multi-segment lighting strategy (C) increased the quantum yield of PSII electron transport (PhiPSII; Figure 7, panel 3), quantum yield of the carboxylation rate (PhiCO$_2$; Figure 7, panel 4), and photochemical quenching (qP; Figure 7, panel 5), whereas it decreased non-photochemical quenching (NPQ; Figure 7, panel 6), but had no effect on maximum quantum yield of the PSII primary photochemistry (F_v/F_m; Figure 7, panel 1) or efficiency of excitation energy capture by open PSII reaction centers (F_v'/F_m'; Figure 7, panel 2). Compared with strategy B, extended photoperiod lighting strategy (A) increased F_v/F_m but had no effect on F_v'/F_m', PhiPSII, $PhiCO_2$, qP, or NPQ.

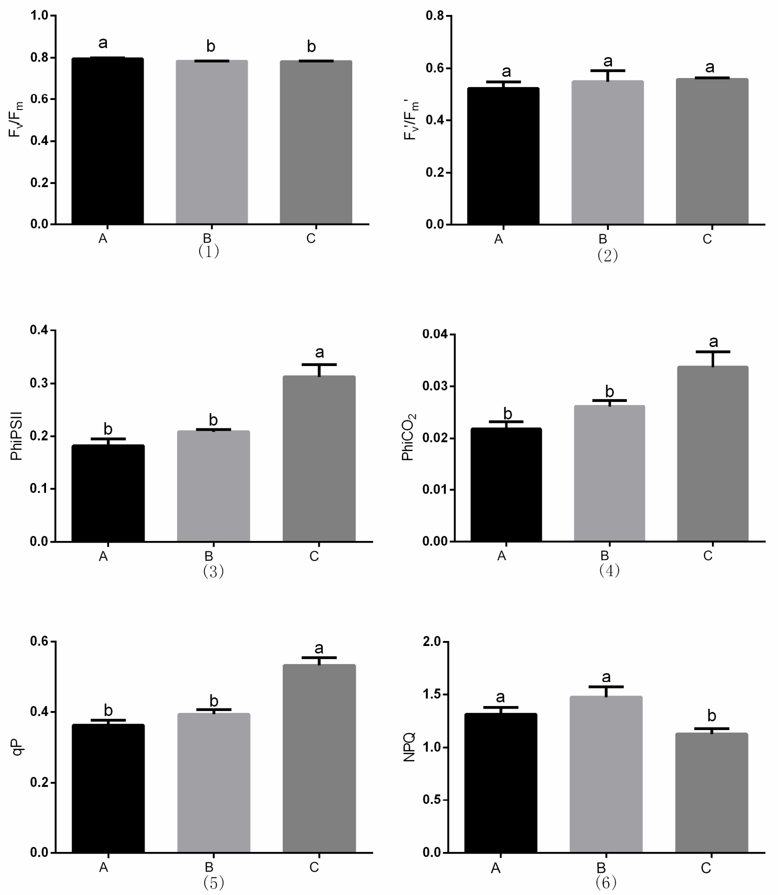


Figure 7. Effects of different lighting strategies on F_v/F_m (**1**), F_v'/F_m' (**2**), quantum yield of the PSII electron transport (PhiPSII) (**3**), quantum yield of the carboxylation rate ($PhiCO_2$) (**4**), photochemical quenching (qP) (**5**), and non-photochemical quenching (NPQ) (**6**). Each value is the mean ± SE of three replicates. Different letters indicate significant differences between groups ($p < 0.05$) according to Tukey's multiple range test.

The dynamics of F_o and F_v/F_m for lettuce grown under different lighting strategies, which were used to reflect the light conversion efficiency and the degree of environmental stress of the lettuce in different growth stages, are expressed in Figure 8. As shown in Figure 8, panel 1, the trends of F_o in the different lighting strategies were similar; the values of F_o were lowest on the first day after transplanting, and then gradually increased; however, the F_o in the A strategy decreased from the 7th day to the 14th day, then increased from the 14th day to the 21st day and had almost no change after that. Greater F_v/F_m values indicated that the environmental stress suffered by the lettuce was weaker. Furthermore, the kinetic curves of F_v/F_m under different lighting strategies were significantly different (Figure 8, panel 3); the F_v/F_m values under lighting strategy A were always greater than the other two strategies during the entire growth period, indicating that the light stress experienced by the lettuce under lighting strategy A was lower than that experienced by the other two strategies. These results showed that the lettuce suffered from varying degrees of environmental light stress under different lighting strategies, despite each strategy having the same DLI.

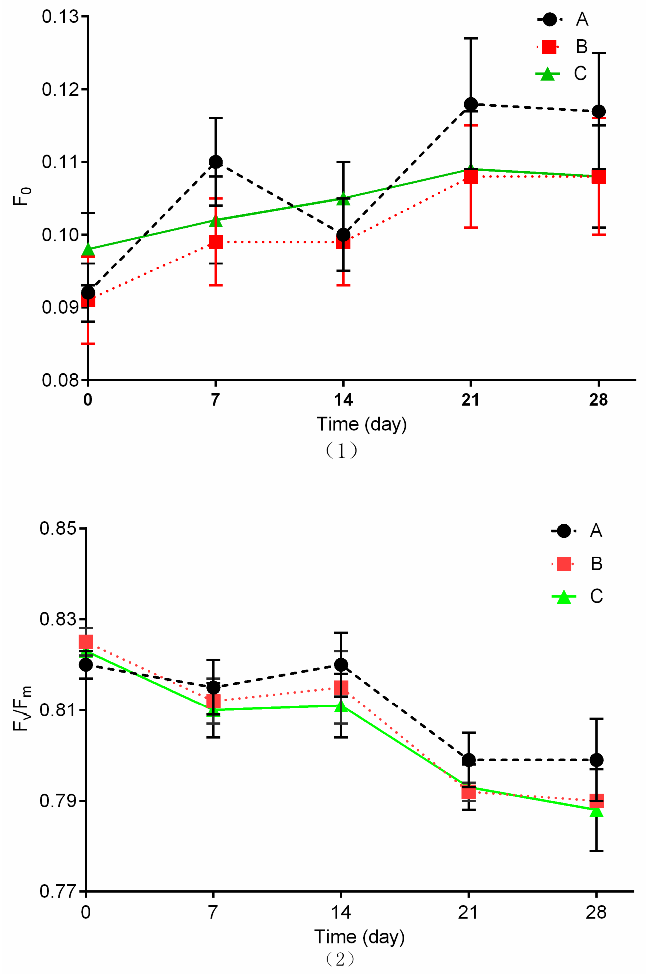


Figure 8. Dynamics of F_o and F_v/F_m in different lighting strategies. Each value is the mean ± SE of three replicates.

4. Discussion

Previous research has shown that higher light intensities promote growth and increase the production of crops [31,32], and that lettuce yields were highest under a light intensity of 600 µmol photons m^{-2} s^{-1} [24]. However, in a plant factory, higher light intensity means higher energy consumption, and thus light intensities no higher than 300 µmol photons m^{-2} s^{-1} are usually used. Previous results have also shown that the qP and PhiPSII values of lettuce were low in the 100 µmol photons m^{-2} s^{-1} treatment, which translated to low light use efficiency and plant yields [24]. Thus, the light intensity must be higher than 100 µmol photons m^{-2} s^{-1} in PFALs. To maximize the yield to cost ratio, the light intensities used in plant factories usually end up being below the optimal range, that is, natural light conditions, causing the plant to suffer from mild light stress that can limit plant production. In this study, lettuce growth was improved in both multi-segment light intensity and extended photoperiod lighting strategies, proving that both lighting strategies are effective at improving crop performance under weak light conditions in PFALs. In addition, the extended photoperiod lighting strategy improved the root/shoot ratio and chlorophyll content (Figure 2). One study reported that the root/shoot ratio decreased as PPFD increased [6], and Mozafar [33] reported that the root/shoot ratio increased along with a photoperiod increase from 12 to 18 h. This study supports the results that the root/shoot ratio under the extended photoperiod lighting strategy is promoted when light intensity is reduced with an increased photoperiod. In addition, the increase in length of photoperiod under weak light conditions compensated for the low-light stress, promoting increases in the chlorophyll content of the crop and increasing competitiveness in weak lighting conditions. Increased chlorophyll content is beneficial to plants, helping to effectively absorb sufficient light energy at weaker light intensities by maximizing photosynthetic efficiency [34]. From the results, we concluded that the extended photoperiod lighting strategy promoted the growth of roots and increased chlorophyll content, which enhanced the light-use efficiency, and thus resulted in better growth of lettuce.

According to the photosynthetic rate curve over 60 consecutive hours (Figure 3), it can be seen that after transplanting the lettuce from natural light conditions to constant artificial light and temperature conditions, the circadian rhythm was maintained for a short period of time before gradually fading. The circadian clock of the plant regulates the circadian rhythm, which synchronizes internal physiological and biochemical processes with the external day and night cycle. Circadian rhythms enable plants to maintain high photosynthetic rates during the photoperiod and maximize biomass yields, providing a competitive advantage [17,19,20]. According to the photosynthetic rates and the integral values of A, B, and C from this study, we observed that the multi-segment light intensity lighting strategy significantly increased diurnal photosynthetic capacity. This was because the multi-segment lighting strategy mimicked the changes of sunlight during a day, stimulating the crop to maintain its circadian rhythm, which maximizes photosynthetic rates despite the environmental changes. Thus, while the short photoperiod constant light intensity lighting strategy cleared the crop's circadian rhythm (Figure 4), the multi-segment lighting strategy took advantage of the circadian rhythm and increased photosynthesis when the light was strong, which enhanced the photosynthetic rate, and ultimately led to an increased yield. Therefore, this study suggested that the multi-segment lighting strategy can take advantage of circadian rhythms and increase crop yields while not increasing energy costs.

The P_{N}-PPFD curve, P_{Nmax}, and AQY of the samples were determined to investigate the photosynthetic response of lettuce plants to the aforementioned three lighting strategies. The results revealed that the light saturation point of B and C were higher than A; this was due to the adaptation and adjustment of the photosynthetic mechanism to the various light energy regimens. The weak light treatment reduced the demand for light energy by the crop, and through adaptation to the weak light environment, crops increased their ability to use weak light. Moreover, the AQY reflected the photosynthetic capacity of the leaves under weak light. Higher AQY values indicate that there are more pigment protein complexes for the plant to use to absorb and convert light energy [35–37]. Our results suggested that the lettuce plants in the extended photoperiod lighting strategy (A) had a higher ability to use weak light than the lettuce cultivated under the short photoperiod lighting strategy (B). In addition, the P_{Nmax} of A was lower than B and C, reflecting reduced leaf photosynthetic capacity in lower light treatments, which results from reduced RuBP (Ribulose-1,5-bisphosphate) carboxylase activity in leaves. These results indicated that, even though this lighting strategy reduced the photosynthetic capacity of crops, it increased the ability of crops to utilize light under weak light conditions, which reduced the impact of weak light on crop yields. Thus, overall the extended photoperiod lighting strategy (A) increased the yields of the lettuce.

Chlorophyll fluorescence parameters can be used to assess the effects of many environmental factors on photosynthesis. The value of $\mathrm{F_v/F_m}$ was shown to vary in the range of 0.8–0.84 for the majority of the C3 plants when the crops were not exposed to environmental stress. When the $F_{\mathrm{v}}/F_{\mathrm{m}}$ value was below 0.8, this indicated that the plant had been exposed to some environmental stressor, such as light stress [38–40]. All the $\mathrm{F_v/F_m}$ values were slightly below 0.8 (Figure 7, panel 3) for all three lighting strategies, which showed that lettuce plants suffered a mild light stress under these three lighting conditions. The $F_{\mathrm{v}}/F_{\mathrm{m}}$ value in the lighting strategy A was the highest, indicating that increasing the photoperiod in a weak light environment somewhat compensated for weak light stress. Also, the values of $\mathrm{F_v'/F_m'}$ were not different between the three lighting strategies, suggesting that the integrity of the photosynthetic apparatus was not affected. However, the quantum yield of PSII electron transport (Figure 7, panel 3), the quantum yield of carboxylation rate (Figure 7, panel 4), and photochemical quenching (Figure 7, panel 5) were all improved as the lettuce adapted to the multi-segment lighting strategy (C). Fu [24] reported that an appropriate increase in light intensity below the light saturation point can improve the efficiency of light energy utilization. Light energy absorbed by chlorophyll molecules goes through three consecutive processes [28,41]: first, it is used to drive photosynthesis; second, excess light energy is dissipated as heat; third, it is re-emitted as chlorophyll fluorescence [29]. It has been suggested that any increase in efficiency of one of these processes will lead to a decline in the other two, which is why the NPQ of strategy C was lower than the other two strategies [36]. In other words, the multi-segment lighting strategy exposed lettuce to a higher light intensity level for a period of time each day, which meant the lettuce was better adapted to high light intensity conditions and was able to use more of the light energy to drive photosynthesis rather than it dissipating as heat. This also explains why the P_{Nmax} of lighting strategy C was much higher than the other two strategies.

In addition, by examining the dynamics of F_o, we concluded that the F_o value in lighting strategy A was the largest, whereas F_o in the B and C strategies were not significantly different from each other. These values reflected the differences in chlorophyll content. This is supported by Figure 2, panel 8, where we can see that the chlorophyll content of lettuce in lighting strategy A was significantly higher than that of the other two strategies. The F_o in all of the three different lighting strategies increased significantly after transplanting; there are two potential explanations for this. First, the white fluorescent lamps were used during the nursery period, and the red and blue LED light sources were used after transplanting. We checked the ratios of blue light in the two light sources and there was higher ratio of blue light in the red and blue LED lamp (33%) than the fluorescent lamp (21%). It has been shown that increasing the ratio of blue light can significantly increase the chlorophyll content of lettuce and increase the ratio of chlorophyll a/b [42]. Secondly, because the lettuce was in the seedling stage, the plants had lower demand for light energy, and the lower chlorophyll content was able to meet the photosynthesis demands. After transplanting, however, with the change of light environment the lettuce was able to adapt by increasing chlorophyll content for better photosynthesis. Following the adaptation to the new light environment and the associated increase in growth, the demand for light energy also began to gradually increase. The lettuce increased the efficiency of light energy utilization by regulating the chlorophyll content, and thus F_o also gradually increased. However, F_o in strategy A decreased between the 7th and 14th days; this was likely due to the longer photoperiod of strategy A, as the longer photoperiod can help the lettuce partially compensate for the weak light. The lettuce in strategy A was better able to adapt to its light environment, therefore, it did not need to stimulate photosynthesis by increasing chlorophyll content. However, as the plants grew, the bigger leaves created more and more shading, resulting in even more severe weak light stress. Therefore, the lettuce in our experiment increased its chlorophyll content to cope with the weak light stress.

The dynamics of F_v/F_m for lettuce in all three lighting strategies were synchronous, with the maximum value appearing on the 14th day, and the minimum value appearing on the 28th day. However, the values of F_v/F_m in the different lighting strategies were significantly different, especially on the 28th day when the F_v/F_m value in strategy A was greater than the other two strategies, which was consistent with the previous conclusion that the extended photoperiod somewhat compensated for the effects of weak light stress. We also found that the F_v/F_m of lettuce in all three strategies was higher than 0.8 before the 14th day, before gradually decreasing until they were slightly lower than 0.8 on the 28th day. This showed that the lettuce in all treatment groups grew better before the 14th day, when they were not stressed by the external environment (mainly weak light stress in this study). After 14 days, however, the environmental stress gradually increased as the lettuce grew and the light intensity was not sufficient to meet the growth requirements. This trend was further exacerbated by the rapid expansion of leaf area, resulting in more and more self-shading as the number of leaves increased. Shading further reduced the light that is intercepted by the lettuce, whereas at the same time the demand for light energy rapidly increased, leading to the increased occurrence and severity of weak light stress. In addition, the values of F_v/F_m varied with the growth stage, indicating that the lettuce had different requirements for light energy at different growth stages.

5. Conclusions

Both multi-segment light intensity and extended photoperiod lighting strategies can increase the production of hydroponic lettuce crops in PFALs; however, the mechanisms by which crop production was improved were different. The former increased the production by taking the advantage of the circadian rhythm. It also increased the quantum yield of PSII electron transport, quantum yield of the carboxylation rate, and photochemical quenching, while decreasing non-photochemical quenching, thereby promoting the photosynthesis of the lettuce. The latter increased production by increasing the chlorophyll content, which increased the ability to absorb light energy in weak light intensity conditions. In addition, extending the photoperiod in a weak light environment had a slight compensatory effect, which reduced the negative effects of the weak light stress.

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