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Photosynthetic characteristics and growth performance of lettuce (*Lactuca sativa* L.) under different light/dark cycles in mini plant factories

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Abstract

We investigated the photosynthesis and growth of lettuce (*Lactuca sativa* L.) grown under three light/dark cycles in a mini plant factory with artificial illumination. A relative longer light cycle [12/12 h (light/dark)] increased not only light-response curve parameters, such as light-saturated net photosynthetic rate, light-saturation point, light-compensation point, dark respiration rate, but also upregulated CO_2-response curves parameters, such as CO_2-saturated net photosynthetic rate, initial carboxylation efficiency, and photorespiration rate, compared to those of the shorter light cycles [6/6 h and 3/3 h (light/dark)]. A longer light cycle enhanced electron transfer potential, increased the chlorophyll amount, leaf area, and biomass and reduced the root/shoot ratio and the specific leaf area. Our results imply that the prolonged light cycle led to the increase in photosynthetic capacity and significantly enhanced the growth rate of lettuce.

Additional key words: biomass accumulation; chlorophyll fluorescence; leaf gas exchange; morphogenesis; photoperiod.

Introduction

As one of the most important environmental factors for plant production, light provides the energy to induce various physiological responses of plants (Abidi *et al.* 2012). Lettuce (*Lactuca sativa* L.) is often used as a model crop in studying plant responses to the light environment due to its rapid growth, short growth cycle, low energy demands, high nutritional value, and stable yields (Křístková *et al.* 2008, Li and Kubota 2009, Lin *et al.* 2013). The light/dark period is one important component of light conditions. The light/dark cycle refers to the alternation of light and dark periods within a circadian cycle. Many studies have shown that light/dark periods play an important role in regulating lettuce photosynthetic characteristics and growth performance (Koontz and Prince 1986, Gaudreau *et al.* 1994, Kitaya *et al.* 1998, Park *et al.* 2012, Zhang *et al.* 2018, Yan *et al.* 2019). Contents of some secondary metabolites also appear to be influenced by the light/dark periods. A previous study showed that the leaves of vegetable plants contained the highest content of betacyanin, chlorophyll (Chl), total polyphenol, and antioxidant activity under a 12/12-h photoperiod compared to 6/18, 8/16, and 24/0 h (Ali *et al*. 2009).

A plant factory with artificial light (PFAL) is regarded as the best model of modern protected horticulture because of its precise management of most environmental factors (*i.e*., temperature, humidity, light intensity and quality, and duration of photoperiod) (Miyagi *et al.* 2017). A mini plant factory with artificial illumination (mini-PFAL) is a household PFAL system, which further concentrates the plant factory technology into a closed-loop refrigerator-sized environment (Takagaki *et al.* 2016). A PFAL for plant production has several potential benefits, such as higher quality pesticide-free plants, a shorter production period, and higher light energy-use efficiency compared to traditional greenhouse and field cultivation (Kozai 2013a,b). The light/dark period in the PFAL is not limited by the traditional concept of a circadian cycle and can be segmented into several short light/dark cycles based on a certain ratio. These short light/dark cycles consume the same amount of electricity per day as a light/dark period.

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Abbreviations: A_{Nmax} – CO_2-saturated net photosynthetic rate; AQY – apparent quantum efficiency; CE – initial carboxylation efficiency; C_i – intercellular CO_2 concentration; C_{isat} – CO_2-saturation point; DM – dry mass; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; LA – leaf area; LCP – light-compensation point; LED – light-emitting diode; LSD – least significant difference; LSP – light-saturation point; PFAL – plant factory with artificial light; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; R^2 – coefficient of determination; R_D – dark respiration rate; R_P – photorespiration rate; SLA – specific leaf area; SPAD – unitless value obtained with the *SPAD-502* chlorophyll meter; Γ* – CO_2-compensation point.
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In areas where electricity costs vary widely at different times of the day, the irradiance mode of short light/dark cycles can be used to provide light to plants when the price of electricity is low (Hang *et al.* 2019).

However, a short light/dark cycle breaks the traditional day-night pattern and is inevitably different from a light/dark period in its effects on the physiological and growth characteristics of plants. Different studies have described the growth of lettuce under varying light/dark cycle conditions. Ishii *et al.* (1995) reported the growth characteristics and nutrient uptake of lettuce plants grown under three light/dark cycles for 2.67/5.33 h, 4/8 h, and 8/16 h (light/dark). Their results demonstrated that lettuce plants grown under 2.67/5.33 h and 4/8 h had fewer leaves, lower fresh and dry masses, smaller leaf area (LA), and lower water and mineral uptakes compared to plants grown under 8/16 h. Hang *et al.* (2019) reported the dynamic changes in lettuce LA under three light/dark cycles of 3/3 h, 6/6 h, and 12/12 h (light/dark). Their results showed that under a light/dark period of 12/12 h, lettuce leaves were more slender and the leaf angle was steeper compared to plants grown under 6/6 h and 3/3 h. In addition, a relatively longer light cycle of 6/6 h increased leaf stomatal conductance (g_s), net photosynthetic rate (P_N), and biomass compared to plants grown under a shorter light cycle of 3/3 h.

These studies indicate that short light/dark cycles alter the morphogenesis, nutrient uptake, and biomass accumulation of lettuce compared to a light/dark period. However, relevant research on the parameterization of the response of gas exchange to different light/dark cycles has not been conducted. This lack of data has limited the development and application of predictive models of lettuce productivity in PFAL production systems. The light- and CO_2-response curves describe the relationship between P_N and PPFD; P_N and CO_2 as analytical tools widely used for the ecophysiological characterization of gas exchange at the leaf level may represent useful criteria for controlling the environment and are required tools for simulation models designed to predict potential plant behavior in response to environmental conditions (Noda *et al.* 2004, Wang *et al.* 2006, Avola *et al.* 2008, Xu *et al.* 2013). Chl fluorescence continues to be a primary means for studying photosynthetic regulation and plant-environment responses due to its sensitivity, convenience, and nondestructive properties (Rascher *et al.* 2000, Dai *et al.* 2009). Hence, the objectives of this research were to calculate and compare the coefficients of the light- and CO_2-response curves and Chl fluorescence parameters under different light/dark cycles and to identify the optimal light/dark cycle for growth to achieve the biomass accumulation and distribution required for selected lettuce plants. Two hypotheses were tested: (*1*) Based on the fact that photosynthesis is hampered under short light cycle conditions, light-saturated net photosynthetic rate (P_{Nmax}) and CO_2-saturated net photosynthetic rate (A_{Nmax}) would decrease as the light cycle duration decreases. (*2*) Lettuce plants under different light/dark cycles would exhibit different above- and belowground biomass accumulations.

Materials and methods

Plant materials and growth conditions: All the experiments were conducted at Jiangsu University, Zhenjiang, China. Romaine lettuce (*Lactuca sativa* L.) was selected as the experimental plant. Lettuce seeds were sown in a seeding tray (59 × 30 × 4.5 cm; 118-plug tray filled with sponge blocks) and germinated in a nursery greenhouse maintained at 20 ± 5°C under natural day length on 19 March, 2019. After 20 d, the seedlings were transplanted into three environmentally controlled mini-PFALs (*SRG-Y*, *Lianshuo Instrument Inc*., China) for treatment. The mini-PFALs were outfitted with a light-emitting diode (LED) light source, light timers, a sterilizer, and a systematized heat sink device. When they had three true leaves, the seedlings were planted in plastic basins (13-cm diameter × 14-cm height) at a density of one plant per basin. The plastic basins were filled with a modified Hoagland nutrient solution (Hoagland and Arnon 1950). The EC and pH of the nutrient solution were adjusted to 1.6 dS m^{-1} and 6.0, respectively. Solution was added every second or third day to maintain the EC and pH. Red and blue LED lamps (*WEN-T8H*, *WEGA Plant Lighting Company*, China) were used as light sources (R:B = 83:17%). The energy aggregation areas of the blue and red lights in the spectra were approximately at 457 and 632 nm, respectively. These two spectral regions are crucial for plant growth and nutritional quality (Landi *et al.* 2020). A PPFD of 250 μmol m^{-2} s^{-1}, relative humidity (of air) of 60–70%, and CO_2 concentration of 400 ± 50 ppm were maintained throughout the experiments.

Experimental design: Experiments were arranged as follows: three light/dark cycles of 12/12 h (C12), 6/6 h (C6), and 3/3 h (C3) were set, and the plants were subjected to the treatments for 30 d after transplanting. Each light/dark cycle treatment contained 18 samples. The plants were rearranged randomly every second or third day to avoid positional effects within the mini-PFALs.

Light-response curves: An open-flow gas exchange system (*LI-6400XT*, *Li-Cor*, Lincoln, NE, USA) with an integrated fluorescence leaf chamber (*LI-6400-40*, *Li-Cor*, Lincoln, NE, USA) was used to simultaneously measure leaf fluorescence and gas exchange. To avoid the effect of environmental fluctuations on gas-exchange measurements, all measurements were taken in an artificial climatic chamber with an air temperature of 24 ± 1°C, a PPFD at the leaf surface of 750 ± 50 μmol m^{-2} s^{-1}, and a relative humidity of 60–70%. LED arrays (*KW-ZWD06-T8*, *Kingwua Bright Inc.*, China) were used as light sources in the climatic chamber. Each lettuce sample exposed to light conditions in mini-PFALs for more than 1 h was sequentially moved into the artificial climatic chamber for measurement. The measurements were completed between 08:30 and 16:30 h. To minimize leaf position and age effects, the measurements were taken on the upper six fully expanded leaves after the plants had been acclimated to the room for approximately 30 min. Three plants were used

per treatment for the measurements. Before generating the light-response curve, the Chl content of the leaf was measured *via* the SPAD value, which was determined using a portable Chl meter (*SPAD-502*, *Konica Minolta*, Osaka, Japan). When full photoinduction of the lettuce plants was complete, an automatic program of light-response curves was run to measure the change in gas-exchange rate with a varied PPFD. The level of the PPFD was varied in the following order: 1,200; 1,000; 800, 600, 500, 350, 200, 150, 100, 80, 50, 0 μmol m^{-2} s^{-1}. Within the *Li-Cor* leaf chamber, the ambient CO_2 concentration was adjusted to 400 μmol s^{-1} with a CO_2 injection system, leaf temperature was maintained at 25°C, PPFD was 800 μmol m^{-2} s^{-1} with a 10:90 blue:red light, leaf-to-air vapor pressure deficit (VPD) was between 1.1 and 1.4 kPa, and the flow rate was 300 μmol s^{-1}. The light-response curves were modeled by fitting modified rectangular hyperbolas to the data as described by following formula (Ye and Yu 2008):

$$P_N = \alpha \frac{1-\beta\times PPFD}{1+\gamma\times PPFD} PPFD - R_D \quad (1)$$

where P_N is the net photosynthetic rate, α is the initial slope or apparent photosynthetic quantum yield [*i.e.*, the apparent quantum efficiency (AQY)], β and γ are constants independent of the PPFDs (dimensionless), and R_D is the dark respiration rate. The light-saturation point (LSP), light-compensation point (LCP), and P_{Nmax} were given by following formulae:

$$P_{Nmax} = \alpha\left(\frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma}\right)^2 - R_D \quad (2)$$

$$LSP = \frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma} \quad (3)$$

$$LCP = \frac{\alpha-\gamma\beta-\sqrt{(\gamma R_D-a)^2-4\alpha\beta R_D}}{2\alpha\beta} \quad (4)$$

CO_2-response curves: To estimate the relationship between the P_N and the intercellular CO_2 concentration (C_i), CO_2-response curves were generated. The temperature, PPFD, and CO_2 concentration in the leaf chamber were adjusted to 25°C, 800 μmol m^{-2} s^{-1}, and 400 mmol mol^{-1}, respectively. Measurements were taken to construct a CO_2-response curve by adjusting the ambient CO_2 concentration to 400, 300, 200, 100, 50, 400, 400, 500, 600, 800; 1,000; 1,200; and 1,500 mmol mol^{-1}. The CO_2-response curves were obtained by fitting the data to a modified rectangular hyperbola (Ye and Yu 2009).

$$P_N = J\frac{1-\theta\times C_i}{1+\omega\times C_i} C_i - R_P \quad (5)$$

where J is the initial slope [*i.e.*, initial carboxylation efficiency (CE)], θ and ω are constants (dimensionless), R_P is the photorespiration rate. The CO_2-saturation point (C_{isat}), CO_2-compensation point (Γ*), and CO_2-saturated net photosynthetic rate (A_{Nmax}) were given by following formulae:

$$C_{isat} = \frac{\sqrt{(\theta+\omega)/\theta}-1}{\omega} \quad (6)$$

$$\Gamma^* = \frac{J-\omega\theta-\sqrt{(\omega R_P-J)^2-4J\theta R_P}}{2J\theta} \quad (7)$$

$$A_{N\,max} = J\left(\frac{\sqrt{\theta+\omega}-\sqrt{\theta}}{\omega}\right)^2 - R_P \quad (8)$$

Fluorescence parameters: The Chl fluorescence parameters of lettuce under different light cycles were determined using the *Li-6400XT* gas-exchange system from 19:00–21:00 h. The variables measured in the experiment included the minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), variable fluorescence (F_v), and maximal quantum yield of PSII photochemistry (F_v/F_m). The measured blade and repetition number were consistent with the measured light-response curve.

Growth and biomass measurements: At the end of the experimental period, plant height, canopy area, LA per plant, leaf shape ratio, and specific leaf area (SLA, projected total leaf area per unit leaf dry mass) were determined. Photographs were taken of the top and side of each plant, and image processing was conducted using *ImageJ* software (National Institutes of Health, Bethesda, MD, USA) to obtain the plant height and canopy area. The LA and leaf shape ratio were obtained by scanning the leaves of each plant using a *LA2400* scanner. The leaf shape ratio was calculated as the ratio of leaf length to leaf width. Images were then acquired using the *WinFOLIA* software (*Regent Instruments Inc*., Quebec). The leaves and roots were separated and weighed collectively to determine the fresh mass (FM) using an electronic analytical balance with an accuracy of 0.1 mg. The dry mass (DM) of the leaves and roots was obtained by first drying them in an oven at 105°C for 1 h and then drying at 80°C for another 48 h. The root/shoot ratio was estimated as the ratio of below- to aboveground DM.

Statistical analysis: Results are expressed as the mean ± SD of three replicates in each of three individuals. The data were analyzed using a one-way analysis of variance (*ANOVA*) and a multiple comparisons test using *SPSS 20* software. Multiple comparisons between treatment means were conducted using the least significant difference (LSD) test at $p<0.05$. *Pearson*'s analysis (two-tailed) was used to evaluate the correlations between the SPAD and F_0. All the model parameters were evaluated with a nonlinear regression using *OriginPro 8* software. Graphs and tables were constructed using *Microsoft Excel 2013*.

Results

Leaf gas exchange: As shown in Fig. 1, the curves of the P_N showed parallel changes under different light cycles. In the range of 0–200 μmol(photon) m^{-2} s^{-1}, there was no

significant difference in the P_N between the three light/dark cycles treatments. Above 200 μmol(photon) m^{-2} s^{-1}, leaves in the C12 treatment showed a higher P_N at all PPFDs. The P_N value was the highest in the C12 treatment followed by the C6 treatment, and these two light/dark cycles had a similar effect on the light-use efficiency of lettuce plants. The P_N in the C3 treatment was significantly lower than that in the other light/dark cycles. The light-response curves were well fitted by the modified rectangular hyperbolic model as indicated by the R^2 values, which were greater than 0.998 (Table 1). There was no significant difference between the AQY in all light cycles. Lettuce in the C12 and C6 treatments had a higher and significantly different P_{Nmax} compared with the C3 treatment. The P_{Nmax} in the C6 and C12 treatments were 44 and 60% higher than that in the C3 treatment, respectively. A similar pattern was observed in R_D values, which were 18 and 30% higher in the leaves under the C6 and C12 treatments compared to the leaves under the C3 treatment. The highest LSP and LCP occurred in the C12 treatment and were significantly higher than that in the C6 and C3 treatments.

CO_2-response curve: There was a significant difference in the P_N values with regard to the different light/dark cycles applied. The P_N value was the highest in the C12 treatment followed the C6 and C3 treatments (Fig. 2). The R^2 values in these three treatments were all greater than 0.991, which indicates that the CO_2-response curves were well-fitted by the modified rectangular hyperbolic model (Table 2). The A_{Nmax} in the C12 treatment was significantly higher than that in the C6 and C3 treatments. There was no significant difference in the A_{Nmax} between the C6 and C3 treatments. The CE varied significantly with the light/dark cycle treatment. The CE was the highest in the C12 treatment and the lowest in the C3 treatment. In contrast, the change in Γ* was significantly higher in the C3 treatment than that in the C12 treatment. The C_{isat} decreased as the light cycle prolonged, however, there was no significant difference between the C_{isat} values under different light/dark cycles. The R_P was significantly different between the different light/dark cycle treatments and showed an increase with the lengthening of the light cycle. The R_P increased by 23 and 53% for the C6 and C12 treatments, respectively, compared to the C3 treatment.

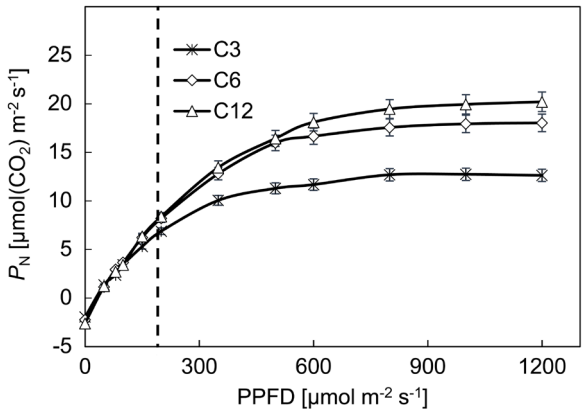

Fig. 1. Light response curve of lettuce under the light/dark cycles of C3, C6, and C12. P_N – net photosynthetic rate. *Dashed vertical line* indicates PPFD value of 200 μmol m^{-2} s^{-1}. Mean values with standard error of mean (n = 3).

Fluorescence parameters: As the length of the light cycle increased, the SPAD increased and reached its highest level in the C12 treatment (Table 3). The lowest SPAD was observed in the C3 treatment, but did not differ significantly from that observed in the C6 treatment. Similarly as the SPAD, the F_o and F_m increased with the lengthening of the light cycle and reached their highest levels in the C12 treatment. There was no significant difference in the F_0 between the C12 and C6 treatments. The F_m value in the C12 treatment was almost 15 and 24% higher compared to the C6 and C3 treatments, respectively. There was no significant difference in F_v/F_m between the three light/dark cycles. The F_v/F_m values in all light cycle treatments fluctuated within range of 0.81–0.83.

Plant performance: The growth of lettuce plants was significantly affected by light/dark cycles (Table 4). As the length of the light cycle increased, LA and fresh shoot mass increased significantly. LA increased by 11 and 31% for the C6 and C12 treatments, respectively, compared to the C3 treatment. Fresh shoot mass increased by 31 and 60% for the C6 and C12 treatments, respectively, compared to the C3 treatment. Plant height, canopy area, root FM, shoot DM, and root DM were the highest in the C12 treatment and the lowest in the C3 treatment. The root/shoot ratio and SLA were the highest in the C3 treatment and were significantly higher than that in the C6 and C12 treatments. No significant difference in the root/shoot ratio was observed between the C12 and C6 treatments.

Table 1. Characteristic parameters of the light response curve for lettuce under the light/dark cycles of C3, C6, and C12. AQY – apparent quantum efficiency; P_{Nmax} – light-saturated net photosynthetic rate; LSP – light-saturation point; LCP – light-compensation point; R^2 – coefficient of determination; R_D – dark respiration rate. *Different lowercase letters* indicate significant difference at p<0.05 in the LSD test. The results are presented as the mean ± SD (n = 3).

Treatment	AQY [μmol(CO_2) $μmol^{-1}$(photon)]	P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	LSP [μmol(CO_2) m^{-2} s^{-1}]	LCP [μmol(CO_2) m^{-2} s^{-1}]	R_D [μmol(CO_2) m^{-2} s^{-1}]	R^2
C12	0.074 ± 0.001[a]	20.223 ± 0.451[a]	1,055.917 ± 23.187[a]	36.237 ± 0.910[a]	2.540 ± 0.031[a]	0.999
C6	0.074 ± 0.000[a]	18.167 ± 1.448[a]	969.864 ± 5.955[b]	32.991 ± 1.471[b]	2.304 ± 0.127[a]	0.999
C3	0.071 ± 0.001[a]	12.653 ± 1.030[b]	928.788 ± 8.775[b]	30.183 ± 0.102[b]	1.959 ± 0.028[b]	0.998

Discussion

The photosynthetic characteristics exhibited contrasting trends in the photosynthetic capacity of lettuce in response to changes in the light/dark cycle in the mini-PFAL system. Among the three light/dark cycles, the lettuce grown in the longer light cycle had higher P_{Nmax}, LSP, and P_N values in the light-response curve, which demonstrates that lettuce plants under a longer light cycle had a greater photosynthetic capacity and could adapt to higher light intensity conditions. The reason for this relationship may be that leaf photosynthesis exhibits a delayed response in reaching its maximal photosynthetic rate when plants are illuminated after a prolonged exposure to darkness (Jun and Hong 2002). This phenomenon of photosynthesis lag is due to the photoactive opening of stomata, the accumulation of metabolites to a sufficient level and light-activation of stromal enzymes require a prior process. The prolonged light cycle actually reduced the proportion of photosynthetic lag time to total photosynthetic time. The longer light cycle provided more time for plant photoaccumulation. In addition, the previous studies have shown that the plant circadian rhythm is involved in coordinating responses of physiological and developmental processes, such as photosynthesis, respiration, and metabolism (Greenham and McClung 2015, Song *et al.* 2015). Circadian rhythm enables plants to fix more carbon, contain more Chl during the photoperiod and maximize photosynthetic rates, providing a competitive advantage (Dodd *et al.* 2005). According to the photosynthetic rates under all treatments, we observed that C12 and C6 treatments significantly increased photosynthetic capacity of leaves compared to C3. This might be due to that the plant circadian rhythms

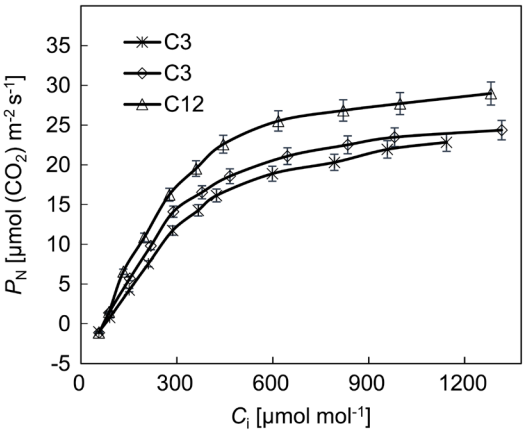

Fig. 2. CO_2-response curve of lettuce under the light/dark cycles of C3, C6, and C12. P_N – net photosynthetic rate; C_i – intercellular CO_2 concentration. Mean values with standard error of mean (n = 3).

Table 2. Characteristic parameters of the CO_2-response curve for lettuce under the light/dark cycles of C3, C6, and C12. CE – initial carboxylation efficiency; A_{Nmax} – CO_2-saturated net photosynthetic rate; C_{isat} – CO_2-saturation point; Γ* – CO_2-compensation point; R^2 – coefficient of determination; R_P – photorespiration rate. *Different lowercase letters* indicate significant difference at p<0.05 in the LSD test. The results are presented as the mean ± SD (n = 3).

Treatment	CE [mol(CO_2) m^{-2} s^{-1}]	A_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	C_{isat} [μmol mol^{-1}]	Γ* [μmol mol^{-1}]	R_P [μmol(CO_2) m^{-2} s^{-1}]	R^2
C12	0.183 ± 0.019^a	28.528 ± 2.165^a	1,184.096 ± 27.215^a	69.700 ± 2.656^b	10.418 ± 0.381^a	0.996
C6	0.141 ± 0.004^b	23.902 ± 0.416^b	1,235.669 ± 3.649^a	71.751 ± 0.745^{ab}	8.325 ± 1.566^b	0.997
C3	0.103 ± 0.004^c	22.624 ± 0.752^b	1,245.648 ± 54.580^a	76.387 ± 1.029^a	6.795 ± 0.344^c	0.991

Table 3. SPAD values and fluorescence parameters for lettuce under the light/dark cycles of C3, C6, and C12. F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; SPAD – unitless value obtained with the *SPAD-502* chlorophyll meter. *Different lowercase letters* indicate significant difference at p<0.05 in the LSD test. The results are presented as the mean ± SD (n = 3).

Treatment	SPAD	F_0	F_m	F_v/F_m
C12	27.58 ± 0.05^a	151.13 ± 7.84^a	881.27 ± 50.40^a	0.83 ± 0.00^a
C6	21.63 ± 0.35^b	142.70 ± 5.31^a	769.27 ± 19.63^b	0.81 ± 0.01^a
C3	20.22 ± 0.43^b	126.23 ± 4.42^b	711.60 ± 19.51^b	0.82 ± 0.00^a

Table 4. Effect of light/dark cycles on the growth and development of lettuce. DM – dry mass; FM – fresh mass; SLA – specific leaf area. *Different lowercase letters* indicate significant difference at p<0.05 in the LSD test. The results are presented as the mean ± SD (n = 3).

Treatment	Plant height [cm]	Leaf area [cm^2]	Canopy area [cm^2]	Shoot FM [g]	Root FM [g]	Shoot DM [g]	Root DM [g]	Root/shoot ratio	SLA [m^2 kg^{-1}(DM)]
C12	8.55 ± 0.48^a	602.40 ± 12.09^a	152.65 ± 4.17^a	43.14 ± 0.69^a	3.91 ± 0.08^a	2.42 ± 0.04^a	0.29 ± 0.01^a	0.12 ± 0.00^b	24.89 ± 0.94^b
C6	7.76 ± 0.14^{ab}	512.94 ± 3.53^b	143.23 ± 5.83^b	35.22 ± 0.89^b	3.60 ± 0.13^{ab}	1.99 ± 0.04^b	0.26 ± 0.02^a	0.13 ± 0.01^b	25.84 ± 0.28^b
C3	7.00 ± 0.64^b	460.34 ± 9.28^c	138.83 ± 5.54^b	26.91 ± 0.94^c	3.45 ± 0.19^b	1.62 ± 0.08^b	0.25 ± 0.01^a	0.15 ± 0.01^a	28.50 ± 0.80^a

in C12 and C6 were maintained more stable than that in C3, which maximized photosynthetic rates despite the environments changed. Plants no longer accumulate organic matter when the light intensity is lower than the LCP. The R_D value reflects the plant's consumption of photosynthetic products. Both the LCP and R_D were enhanced by the C12 treatment compared to the C6 and C3 treatments, implying that the long light cycle increased the consumption of photosynthetic products, resulting in a relatively inefficient use of resources (Zhang *et al.* 2015). There was no significant difference in the AQY between the three light cycles, indicating that the influence of light/dark cycles on the ability of lettuce to use low light energy was not notable. Leaf photosynthesis measured at different CO_2 concentrations significantly varied with light/dark cycles. The maximum A_{Nmax} and P_N values of the CO_2-response curve of lettuce under the C12 treatment showed that lettuce was more adaptable to a high CO_2 environment under the longer light cycle. The CE is a measure of the activity and efficiency of Rubisco. A higher CE value corresponds to a more complete carboxylation efficiency during photosynthesis (Liu *et al.* 2014). The CE was significantly enhanced under the longer light cycle, which shows that lettuce plants had more efficient CO_2 uptake under long light cycle conditions. In addition, the Γ* of lettuce decreased significantly as the light cycle increased, implying that lettuce plants in the longer light cycle treatments had a higher light-use efficiency under a low CO_2 environment. The peak R_P value of lettuce in the C12 treatment indicates that the long light cycle resulted in a high respiration rate in *Lactuca sativa.*

Leaf Chl content is one of the most important factors determining leaf photosynthetic capability and dry matter production. The leaf SPAD value is considered a useful indicator of leaf Chl content (Loh *et al.* 2002, Ling *et al.* 2011). The lower SPAD that we observed in the C3 and C6 treatments may partially explain the lower photosynthetic rates and dry shoot masses found in the plant leaves from those two treatments. The marked increase in SPAD in the C12 treatment demonstrates the plant's ability to enhance the light-harvesting capacity under long light cycle conditions. F_v/F_m value indicates the PSII maximum light conversion efficiency. In healthy organisms, the F_v/F_m value is approximately 0.8–0.84 in most C_3 plant species, but the value decreases significantly when plants are exposed to stress (Oxborough and Baker 1997, Baker 2008, Kalaji *et al.* 2014). The F_v/F_m values in all treatments were all above 0.8, indicating that different light cycle treatments would not cause loss of photosynthetic activity and poten-tial efficiency of PSII. Hence, the differences in the F_0 value or F_m value were not caused by any reversible inactivation of the PSII reaction center. It has been previously suggested that F_0 value was mainly determined by the difference in Chl content in leaves (Strasser *et al.* 2004, Fu *et al.* 2012). Among all treatments, a positive correlation was observed between the F_0 value and the SPAD (*Pearson*'s $r = 0.769$, $p<0.05$). A maximum F_0 value was found in C12 treatment. It might be hypothesized that the longer light cycle conditions resulted in the higher Chl content of plant leaves, the more light energy was absorbed, and so higher F_0 values were obtained after dark adaptation. However, the experimental results showed that although there was no significant difference in the SPAD under C6 and C3 treatments, the F_0 values under C6 treatment were significantly higher than those under C3 treatment. Therefore, other mechanisms are presumably responsible for the variation in F_0 observed in this study. According to several authors, this parameter was associated with the changes of the antenna size and the contribution of fluorescence originating from PSI (Dinç *et al.* 2012, Brestič *et al.* 2015). More likely, the antenna size and the PSI fluoresence could be maintained at higher levels under C6 treatment than those under C3, resulting in higher F_0 values under C6. The F_m denotes the maximum fluorescence yield in dark-adapted leaves, and reflects electron transfer through the PSII reaction center (Baruffo and Tretiach 2007). A block on the donor side of PSII is generally correlated with quenching of F_m due to the lack of electrons available to provide for the accumulation of the primary plastoquinone acceptor (Govindjee 1995). Similar conclusions are referred here. As the light cycle increased, the F_m increased significantly and reached its maximum in the C12 treatment. The increase in F_m values was probably due to the increase of electron accumulation on the donor side of PSII by the long light cycle.

Although the long light cycle increased the consumption of photosynthetic products, the fresh shoot mass, fresh root mass, dry shoot mass, and root mass increased with an increase in the length of the light cycle. This is consistent with the findings of Park *et al.* (2012) in lettuce plants. Compared to values in other light cycles, the plant height, LA, and canopy area increased significantly when the light cycle reached the C12 levels. This shows that the long light cycle was beneficial for lettuce growth under low light intensity. However, the root/shoot ratio decreased with an increase in length of the light cycle, which shows that more mass was allocated to aboveground tissues under long light cycle conditions. The SLA reflects the LA for light capture per unit mass. A high SLA was observed in plants grown in the C3 treatment. For the sunflower, Tardieu *et al.* (1999) found that the SLA increases if the environmental conditions suppress the expansion rate more strongly than the photosynthetic rate and *vice versa*. The current findings indicated a larger effect of short light cycle on leaf expansion than dry matter accumulation. As previously reported, the shortened light cycle made the leaves compact and rounder (Hang *et al.* 2019). Thus, the long light cycle in the mini-PFAL system favored higher biomass accumulation and was more likely to cause an increase in the carbohydrates used for physiological metabolism and growth.

Light/dark cycles have a significant effect on the effective absorption, transmission, and transformation of light energy in lettuce plants. Lettuce responds to changes in light/dark cycles by altering its photosynthetic physiology, allocation of resources and morphology. As indicated by F_v/F_m values, the lettuce plants grew without stress under the three light/dark cycles. However, the variety of F_m values indicated that the lengthened light cycle had a positive effect on electron transfer potential on the donor

side of PSII. In addition, the different light/dark cycles had a significant effect on lettuce photosynthesis and growth. The prolonged light cycle led to an increase in photosynthetic capacity and CO_2-uptake efficiency, which significantly enhanced the growth rate of lettuce. When the lettuce plants grew under the C12 conditions, plant height, LA, leaf FM, and leaf DM were maximized. The root/shoot ratio and the SLA increased as the light cycle was shortened. The shortened light cycle led to more mass being allocated to the root and to more compact leaves. The results showed that if light energy and CO_2 in mini-PFALs are to be fully utilized to achieve high yields, a long light cycle under low light conditions is a wise choice. However, given the time-specific charges in some areas, the energy consumption costs under different light and dark cycles should be calculated according to the local electricity tariff standards. Further studies are needed to determine the appropriate light and dark cycles by comparing the input/output ratio of the lettuce product.

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