Effects of environment lighting on the growth, photosynthesis, and quality of hydroponic lettuce in a plant factory

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Abstract: Leafy vegetable production under controlled environment using artificial lighting has many advantages over conventional greenhouses and open-field production. However, high initial investment and operation costs are restricting the wide application of this technology. In order to design an optimal artificial lighting environment for lettuce production, effects of different combinations of light intensity, photoperiod, and light quality on growth, quality, photosynthesis, and energy use efficiency of lettuce (*Lactuca sativa* L. cv Ziwei) were investigated under a closed plant factory. Lettuce transplants were grown under photosynthetic photon flux density (PPFD) at 150 μmol/m^2·s, 200 μmol/m^2·s, 250 μmol/m^2·s, and 300 μmol/m^2·s provided by fluorescent lamps (FL) with a red to blue ratio (R:B ratio) of 1.8 and light-emitting diode (LED) lamps with R:B ratio of 1.2 and 2.2, in combination with photoperiod of 12 and 16 h/d. In order to examine the "long term" photosynthetic characteristics, net photosynthetic rates of hydroponic lettuce leaves were continuously measured for 2 d (15th and 16th day after transplanting) before harvest. There was no difference in leaf fresh weight (FW) between PPFD of 250 μmol/m^2·s and 300 μmol/m^2·s with photoperiod of 16 h/d, regardless of light quality, and same results showed in contents of nitrate, soluble sugar, and vitamin C, respectively. The results of continuous measurements of net photosynthetic rate of lettuce leaves before harvest indicated that plants grown at PPFD of 250 μmol/m^2·s had consistently higher compared to those grown at PPFD of 300 μmol/m^2·s. Combining the results from growth, photosynthesis, quality, and energy consumption, it can be concluded that PPFD at 250 μmol/m^2·s with photoperiod of 16 h/d under LED with R:B ratio of 2.2 is a suitable light environment for maximum growth and high quality of commercial lettuce (cv. Ziwei) production under indoor controlled environment.

Keywords: plant factory, daily light integral, artificial light, photosynthetic photon flux density, net photosynthetic rate, energy use efficiency

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1 Introduction

The indoor controlled environment with artificial light, which is called plant factory, growth chamber, or plant production unit, has been increasingly used for commercial leafy vegetable production in addition to research purposes in plant science[1-4]. Lettuce (*Lactuca sativa* L.), which has a short growth cycle, high planting density, and low energy demand, is produced in large quantity in plant factory[5], and has been as the model crop for many researchers to study its response to light environment[6,7].

Fluorescent lamps (FL), high-pressure sodium, and light-emitting diode (LED) lamps are often used as a sole source or mixed lighting sources for lettuce production in plant factories[8,9]. Use of LEDs have been gradually increasing worldwide due to their small size, long life, selected spectral composition, and cool emitting temperature compared with other light sources. However, 48% of light fixtures in current use are FLs because they are the most economical and convenient in space limited closed plant factories[8]. Many researchers have used FLs as a standard light source to compare with other light sources using lettuce as a model crop to investigate the growth, quality, physiological parameters, and light use efficiency (LUE)[10,11]. Different ratios of red to blue light (R:B ratio) affect lettuce plant growth and photosynthesis. Leaf net photosynthetic rate and photosynthetic capacity increased with decreasing R:B ratio; however, shoot dry weight and LUE increased with increasing R:B ratio[12]. While a lot of studies have been conducted on the light quality related to lettuce plant growth and quality, no optimal light quality or R:B ratio has been determined.

Too low or too high light intensity can impact lettuce growth, leaf nitrate content, phytochemical accumulation, and even cause physiological disorders[13,14]. Higher photosynthetic photon flux density (PPFD) can achieve higher biomass, better plant morphology, shorter production cycle, and increased activities of anti-oxidative enzymes in lettuce[15-18]. Fu et al. reported that no photoinhibition occurred when plants were grown at light intensities of 100-600 μmol/m^2·s with the highest LUE at 200 μmol/m^2·s, while 600 μmol/m^2·s led to the highest fresh weight, indicating that 400-600 μmol/m^2·s is the suitable light intensity range recommended for lettuce production in a greenhouse[15,16]. In another study, under four PPFDs of 100 μmol/m^2·s, 150 μmol/m^2·s, 200μmol/m^2·s, and 300 μmol/m^2·s,

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dry mass, percent dry mass, and leaf numbers of lettuce transplants increased with increasing PPFD except for leaf area and hypocotyl length, and the authors suggested that minimum PPFD of 200 μmol/m^2·s was needed for the production of lettuce plug transplants[18]. Chang et al.[19] found no significant differences in leaf area, leaf number, and photosynthetic rate of lettuce, with the exception of fresh weight, when grown at PPFD of 215 μmol/m^2·s, 235 μmol/m^2·s, and 265 μmol/m^2·s provided by external electrode fluorescent lamps. Jeong et al.[20] compared morphology, fresh weight, and dry weight of lettuce plants grown under PPFDs of 200 μmol/m^2·s, 230 μmol/m^2·s, 260 μmol/m^2·s, or 290 μmol/m^2·s and concluded that higher PPFD with a shorter photoperiod of 6 h/2 h (light/dark) significantly increased lettuce growth and development in a plant factory.

Extending photoperiod can produce more carbohydrate, increase growth, and stimulate the photosensitive pigments in lettuce for inducing relative gene expression and improved nutrient absorption for better quality[13,21]. By comparing the electric energy use efficiency and photosynthetically active radiation use efficiency, the 16-20 h/d photoperiod was recommended to improve the LUE of lettuce grown in a plant factory[10]. Hiroki et al.[22] investigated the effects of lighting cycles (16/8, 16/4, 16/2 h, light/dark) and PPFD levels of 110 μmol/m^2·s and 170 μmol/m^2·s in a container-type growth chamber on lettuce. They determined that fresh weight of lettuce under the 16/2 h cycle was about 30% higher than those under the 16/8 h cycle. Extending the photoperiod from 16 h/d to 24 h/d would increase lettuce biomass by 20%, but had no more influence in decreasing the leaf nitrate content[23].

Commercial lettuce production in a closed plant factory uses a PPFD as low as 150 μmol/m^2·s in consideration of saving power consumption, thermal dissipation and lamp output capacity; however, this low light intensity causes lower yield. Although there are a lot of studies on the effects of light intensity, photoperiod, and light quality on the growth of lettuce, few researchers have examined these factors along with lettuce quality and the photosynthetic characteristics. In order to achieve the maximum LUE and economic benefits in lettuce production, it is necessary to optimize the lighting environment for both plant growth and quality. In this study, the effects of light intensity, photoperiod, and light quality on the growth and quality of hydroponically grown lettuce were investigated to find out the optimal light condition for commercial lettuce production in a controlled environment with artificial light. In addition, the photosynthetic property was examined continuously for two days under a combination of light intensity and photoperiod.

2 Materials and methods

2.1 Plant materials and growth conditions

Seeds of purple leaf lettuce (*Lactuca sativa* L. cv. Ziwei) were sown in sponge cube (23 mm ×23 mm×23 mm) in a hydroponic system. After germination, seedlings were grown for 18 days in a closed plant system (China Agricultural University, Beijing, China) under PPFD of 200 μmol/m^2·s provided by LEDs with R:B ratio of 1.2 (Beijing Lighting Valley Technology Co., Ltd., China) and a photoperiod of 16 h/d before treatment. Temperature was maintained at (22±1)°C and (18±1)°C (mean ± SD, day/night), relative humidity at (70±5)%/(65±5)% (day/night), and CO_2 concentration of (800±50) mol/mol. When the seedlings had 5 true leaves, they were transplanted to the hydroponic cultivation bed. The air temperature, relative humidity, and CO_2 concentration remained the same from transplanting to harvest. Figure 1 shows hydroponic lettuce cultivation in closed plant factory at transplanting and harvest stages.

Figure 1 Hydroponic lettuce cultivation in a closed plant factory at transplanting and harvest stages

Two environment controlled chambers, each with a dimension of 3.00 m×2.85 m×2.30 m were utilized in this study. In each chamber, there were ten cultivation beds, two shelves on each side of the room. Therefore, a total of 40 cultivation beds were used for this experiment. The cultivation bed consisted of ABS cultivation bench with a dimension of 1200 mm (L)×900 mm (W)×70 mm (H) and ABS board with 4 mm thickness as a bench cover with 24 planting holes (16 mm in diameter).

A Yamasaki lettuce solution was utilized with major nutrient elements of N, P, K, Ca, and Mg at 91.0, 15.5, 136.4, 40.0, and 12.2 mg/L, and the standard content of minor elements solution with (mg/L) 3.54 Fe, 0.52 Mn, 0.05 Zn, 0.02 Cu, 0.50 P, and 0.01 Mo. The pH of the nutrient solution was maintained in the range of 6.0-6.5 and electrical conductivity (EC) at 1.0-1.2 mS/cm. Nutrient solution was recirculated. Pure water was used for the first two days after seeding. Then a quarter strength of standard nutrient solution was used for the cotyledon stage and one third strength was used for the 1-2 true leaves stage. When lettuce had 2-3 true leaves, half strength nutrient solution was used. After transplanting, a full strength nutrient solution was applied and the nutrient solution was replaced every 10 d.

2.2 Treatments and experimental design

Twenty four treatments were created by a combination of four levels of PPFD at 150 μmol/m^2·s, 200 μmol/m^2·s, 250 μmol/m^2·s, and 300 μmol/m^2·s and two photoperiods of 12 h/d and 16 h/d (factorial design) under three kinds of artificial light with different R:B ratios as shown in Table 1. Fluorescent lamps (FLs) (T5-28W, Shanghai Flower and Biology Lighting Co., Ltd., China) with R:B ratio of 1.8 and LEDs (WR-16W, Beijing Lighting Valley Technology Co., Ltd., China) with R:B ratios of 1.2 and 2.2 were placed 35 cm above the cultivation bed. Figure 2 shows the spectral distribution of lamps determined for light wavebands ranging from 300 nm to 800 nm at 15 cm below the lamps with a PPFD of 250 μmol/m^2·s by fiber spectrometer (AvaField-2, Avantes Inc., Apeldoornb, The Netherland). In the treatment with FL, green light accounted for 35.5% of the total PPFD, and the infrared light was 24.8%. In the treatment with LED at R:B ratio of 1.2, green light accounted for 46% of the total PPFD, and the far-red light was 9.5%. For LED with R:B ratio of 2.2, green light accounted for 41.2% of total PPFD, and far-red light was 9.3%. The PPFDs were measured 15 cm above the bench top using a quantum sensor (LI-190SA, LI-COR Inc., Lincoln, NE, USA), which was connected to a data-logger (LI-1400, LI-COR Inc., Lincoln, NE, USA).

DLI was calculated as follows: DLI (mol/m^2·d) = PPFD (μmol/m^2·s) × photoperiod (h/d) × 3600 (s/h) / 10^6. In each treatment, one layer of cultivation shelves with a total of 18 plants was employed in experiments. Plant samples were selected randomly in each treatment. Six plants were measured for evaluating growth and quality properties. The third vigorously growing leaf from the top of the lettuce was measured in this experiment. The experiments were repeated three times, from 15 Jun. to 26 Jul., 22 Aug. to 3 Oct., and 12 Oct. to 23 Nov., 2016. Since the trends were similar, the data presented in this paper are from the third experiment.

Table 1 A list of treatments created by combinations of different photosynthetic photon flux density (P), photoperiod (H), and three different lamps with different R:B ratio (F or L)

Lighting source	R:B ratio	PPFD/ μmol·m^{-2}·s^{-1}	Photoperiod/ h·d^{-1}	DLI/ mol·m^{-2}·d^{-1}	Treatment symbol
Fluorescent lamp	1.8	150	12	6.48	F1.8-P150-H12
		200	12	8.64	F1.8-P200-H12
		250	12	10.80	F1.8-P250-H12
		300	12	12.96	F1.8-P300-H12
		150	16	8.64	F1.8-P150-H16
		200	16	11.52	F1.8-P200-H16
		250	16	14.40	F1.8-P250-H16
		300	16	17.28	F1.8-P300-H16
LED	1.2	150	12	6.48	L1.2-P150-H12
		200	12	8.64	L1.2-P200-H12
		250	12	10.80	L1.2-P250-H12
		300	12	12.96	L1.2-P300-H12
		150	16	8.64	L1.2-P150-H16
		200	16	11.52	L1.2-P200-H16
		250	16	14.40	L1.2-P250-H16
		300	16	17.28	L1.2-P300-H16
LED	2.2	150	12	6.48	L2.2-P150-H12
		200	12	8.64	L2.2-P200-H12
		250	12	10.80	L2.2-P250-H12
		300	12	12.96	L2.2-P300-H12
		150	16	8.64	L2.2-P150-H16
		200	16	11.52	L2.2-P200-H16
		250	16	14.40	L2.2-P250-H16
		300	16	17.28	L2.2-P300-H16

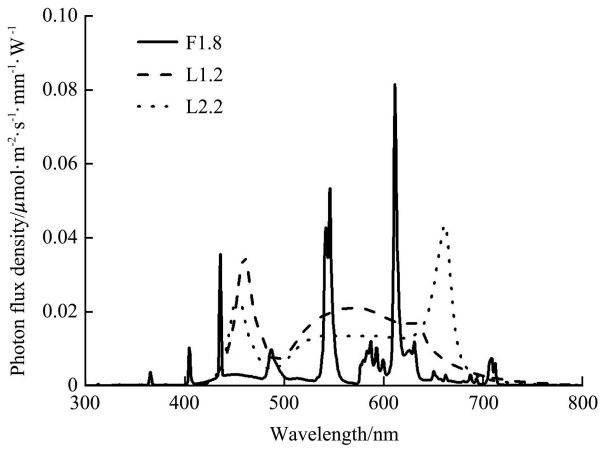


Figure 2 Spectral distribution of fluorescent lamp with R:B ratio of 1.8 (F1.8) and two LED lamps with R:B ratios of 1.2 and 2.2, respectively (L1.2 or L2.2)

2.3 Measurement

2.3.1 Growth data

At the end of each experiment, lettuce plants were separated into leaves and roots, and fresh weights (FW) of leaves and roots were recorded by an electronic analytical balance with accuracy of 0.1 mg (FA1204B, BioonGroup, Shanghai, China). The leaves and roots were dried in an oven at 105°C for 3 h and subsequently set to 80°C and dried for 72 h and dry weight (DW) of leaves and roots were recorded.

2.3.2 Continuous measurement of leaf net photosynthetic rates

Net photosynthetic rates of the lettuce leaves were measured continuously during the last two days before harvest in the plants grown under FL at PPFDs of 250 μmol/m^2·s and 300 μmol/m^2·s with photoperiod of 16 h/d (F1.8-P250-H12, F1.8-P250-H16, F1.8-P300-H12, and F1.8-P300-H16 treatments), one leaf per treatment, using two open path dynamic photosynthetic systems with four leaf chambers (MD-100A, Yizongqi Technology Co., Beijing, China). In this continuous photosynthesis measurement, the air was pumped (FML201.5, Chengdu Qihai E&M Manufacturing Co., Chengdu, China) into the air-tight rectangle acrylic cuvette (25 cm×15 cm× 6 cm) with a flow rate of 1.0 L/min, and the air inlet and outlet paths were switched by four solenoid valves for two leaf chambers measurement. The differential CO_2 concentrations of the inlet and outlet were measured automatically by an infrared gas analyzer (LI-7000, LI-COR Inc., Lincoln, NE, USA) for calculating and recording the net photosynthetic rate every 8 min[24]. The light environment, CO_2 concentration and temperature in the leaf cuvette were the same as those in the controlled environment.

2.3.3 Lettuce quality determination

On the seventeenth day after transplanting, six replicates per treatment were sampled to determine the contents of nitrate, vitamin C, soluble sugar, soluble protein, and anthocyanin.

Nitrate content was determined using the coloration method of sulfosalicyclic acid[25]. Leaf samples of 2-3 g were cut into small pieces and placed in a tube, and 10 mL deionized water was added. The tube was sealed and placed in boiling water for 30 min. After the solution cooled down, the remaining liquid was filtered into another bottle and distilled water was added to reach a final volume of 50 mL. A 0.1 mL was extracted from the 50 mL solution, mixed with 0.4 mL of 5% salicylic & sulfuric acid, and 9.5 mL 8% NaOH was added after 20 min. Absorbance of the solution sample was measured using a UV-VIS spectrophotometer (UV3150, Shimatsu Productions Co., Japan) at wavelength of 410 nm. A standard regression curve between absorbance and nitrate concentration was obtained and nitrate content was calculated based on the standard regression curve.

Vitamin C content was determined by the 2,6-dichlorophenol indophenol titration method[25]. Fresh leaf of 10 g was grinded with 5 mL 2% oxalic acid solution. The grinded mixture was filled with the oxalic acid solution into a flask achieving 100 mL solution after shaking and filtering. The 10 mL of filtrate was titrated by 2,6-dichlorophenol indophenol solution in evaporating dish until the color changed into pink for 30 s, then the amount of dye was obtained. Same method was used to titrate into 10 mL vitamin C solution and 2% of oxalic acid solution for titration degree and blank dye concentration. The titrate result and the sample's parameters were used for calculating vitamin C content.

The soluble sugar content was determined by the anthrone-sulfuric acid colorimetry method[25]. Briefly, 0.1-0.3 g of lettuce leaf was cut into pieces and mixed with 20 mL of distilled water in

a graduated tube. Then the sealed tube was put into boiling water for 30 min, and filtered into a new tube filled with distilled water up to 50 mL. The 0.5 mL extraction solution from above tube was added with 1.5 mL of distilled water as a test sample determined by above spectrophotometer using wavelength of 630 nm for absorbance. Using sample's absorbance, the soluble sugar content was calculated by standard sucrose content relative curve.

To determine the soluble protein content, Coomassie brilliant blue G-250 dye method was used[25]. Samples of 1.0 g fresh lettuce leaf with 8 ml distilled water were grinded into pulp and collected into centrifugal tube. The tube was stewing at 20°C to 25°C for 30 min, then put into centrifuge (DT5-2, Beijing Era Beili Centrifuge Co., Ltd., China) with 4000 r/min for 20 min. The supernatant combined with distilled water to the capacity of 10 mL was used as sample for testing. The sample of 0.1 mL volume was mixed with 5 mL of Coomassie brilliant blue G-250 solution. Absorbance at wavelength of 595 nm was used for measuring soluble protein content after 2 min.

The anthocyanin content was measured using a spectrometric method[26]. Two grams of tissue of the third leaf was mixed with pre-cold 1% hydrogen chloride methanol solution, which was grinded into pulp and collected into 20 mL of centrifugal tube. The tube was filled and mixed with 1% hydrogen chloride methanol solution to 50 mL, then placed in the refrigerator under 4°C for 20 min. The filtrate was measured by the above spectrophotometer using 1% hydrogen chloride methanol solution as blank reference for zeroing. The deference of absorbance under wavelengths of 530 nm and 600 nm was used to calculate the anthocyanin content.

2.3.4 Light and energy use efficiency

Smart metering (TP9004, Shenzhen Northmeter Co., Ltd., China) was used for monitoring the total power consumption of each treatment. The recorded power consumption was divided by the value of 100 g FW or 1 g DW. Light energy use efficiency (LEU) was determined as follows: LEU = $k \cdot D$/PAR, where k is the conversion coefficient from DW to chemical energy (about 20 MJ/kg); D is the increase of DW in specific growth time per unit area (kg/m^2); and PAR is photosynthetically active radiation (W/m^2). The electric energy use efficiency (EUE) was defined as: EUE = $h \cdot$LEU, where h is the conversion factor from electric energy to PAR energy, which is around 0.195 for FLs (R:B ratio of 1.8), 0.382 for LEDs with R:B ratio of 1.2, and 0.326 for LED lamps with R:B ratio of 2.2[3].

2.4 Statistical analysis

Analysis of variance was conducted to determine the significance of PPFD, photoperiod, and light quality on lettuce growth and quality at p=0.05 level. LSD's multiple range test was performed across all treatments. The above statistical analysis was performed using the SPSS 18.0 (IBM, Inc., Chicago, IL, USA). In order to quantify the relationship between FW or DW and daily light integral, linear regression was performed using the Origin 8.1 (Origin Lab Corporation, Northampton, MA, USA).

3 Results and discussion

3.1 Effects of artificial light on growth of hydroponic lettuce

The growth of hydroponic lettuce was significantly influenced by PPFD, photoperiod, and light quality (Table 2). Leaf FWs were highest in the treatments of F1.8-P250-H16 and F1.8-P300-H16. However, there were no differences in leaf FW between the two LED lamps, regardless of PPFD and photoperiod. With photoperiod of 12 h/d, leaf FWs were higher under FL than LED at PPFDs of 250 μmol/m^2·s and 300 μmol/m^2·s. Root FW was highest in L2.2-P250-H16, while there were generally no differences between FL and LED with R:B ratio of 1.2. No substantial differences were found in leaf FW between FL and LED with R:B ratio of 1.2, while some treatments in LED with R:B ratio of 2.2 had higher leaf DW compared to the other two lamps. Similar tendencies were observed for root DW among treatments.

Table 2 Effects of photosynthetic photon flux density (PPFD), photoperiod (H), and light quality (LQ) on fresh weight (FW) and dry weight (DW) of hydroponic lettuce at day 18 after transplanting (n= 6)

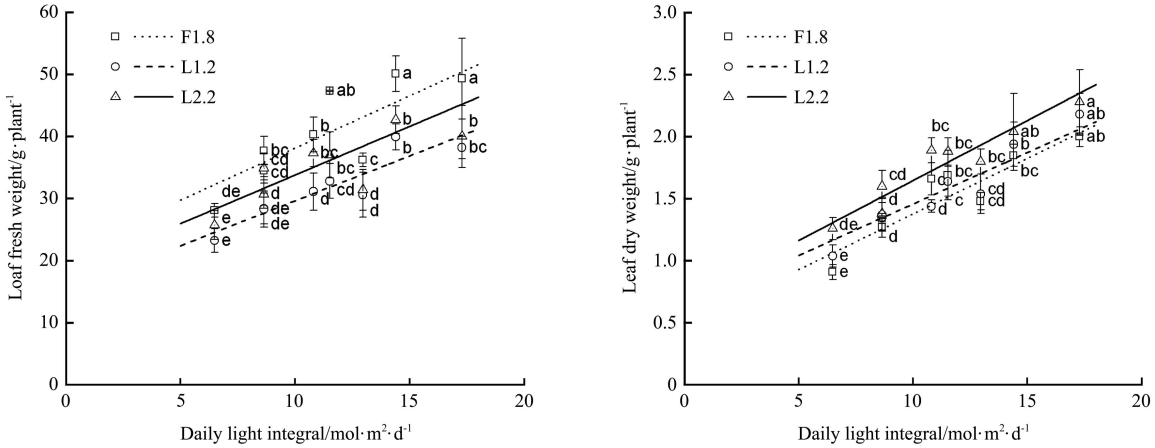
Treatment	Leaf FW	Root FW	Leaf DW	Root DW
	g per plant			
F1.8-P150-H12	28.1±1.1 de	3.06±0.30 f	0.91±0.06 e	0.14±0.01 e
F1.8-P200-H12	34.5±1.0 cd	3.90±0.25 e	1.34±0.04 d	0.17±0.01 de
F1.8-P250-H12	40.3±2.8 b	4.61±0.45 cd	1.66±0.13 c	0.19±0.02 d
F1.8-P300-H12	36.2±1.1 c	5.18±0.18 c	1.48±0.07 cd	0.23±0.01 c
F1.8-P150-H16	37.7±2.3 bc	3.82±0.13 e	1.27±0.08 d	0.16±0.01 e
F1.8-P200-H16	47.3±0.1 ab	5.32±0.25 c	1.69±0.20 bc	0.19±0.01 d
F1.8-P250-H16	50.1±2.9 a	6.01±0.34 b	1.85±0.09 bc	0.28±0.02 b
F1.8-P300-H16	49.3±6.5 a	6.41±0.40 b	2.00±0.08 ab	0.30±0.02 b
L1.2-P150-H12	23.2±1.9 e	2.94±0.28 f	1.04±0.09 e	0.11±0.03 f
L1.2-P200-H12	28.4±2.5 de	3.61±0.35 ef	1.35±0.01 d	0.15±0.02 e
L1.2-P250-H12	31.1±3.0 d	3.73±0.23 e	1.44±0.05 d	0.17±0.01 de
L1.2-P300-H12	30.6±3.6 d	5.14±0.52 c	1.54±0.16 cd	0.23±0.02 c
L1.2-P150-H16	28.3±2.9 de	4.23±0.30 d	1.37±0.13 d	0.21±0.02 cd
L1.2-P200-H16	32.8±2.8 cd	4.80±0.40 cd	1.64±0.12 cd	0.26±0.04 bc
L1.2-P250-H16	39.9±2.1 b	5.95±0.41 b	1.94±0.18 b	0.28±0.02 b
L1.2-P300-H16	38.2±1.8 bc	5.50±0.49 bc	2.18±0.17 a	0.27±0.04 b
L2.2-P150-H12	25.7±2.3 e	3.51±0.20 ef	1.26±0.09 d	0.14±0.01 e
L2.2-P200-H12	34.9±2.4 cd	4.64±0.11 cd	1.60±0.13 cd	0.22±0.02 cd
L2.2-P250-H12	37.3±2.2 bc	6.01±0.51 b	1.89±0.10 b	0.25±0.01 bc
L2.2-P300-H12	31.4±3.3 d	6.35±0.67 b	1.80±0.10 bc	0.25±0.02 bc
L2.2-P150-H16	30.7±2.3 d	5.02±0.64 c	1.38±0.13 d	0.16±0.02 e
L2.2-P200-H16	37.0±3.7 bc	5.93±0.60 b	1.88±0.11 b	0.18±0.01 de
L2.2-P250-H16	42.7±2.2 b	7.34±0.62 a	2.03±0.31 ab	0.29±0.04 b
L2.2-P300-H16	40.0±5.0 b	6.60±0.94 b	2.28±0.26 a	0.35±0.04 a
PPFD	*	*	*	*
H	*	*	*	*
LQ	*	*	*	*
LQ×PPFD	NS	*	NS	*
LQ×H	*	NS	*	*
PPFD×H	NS	*	*	*
PPFD×H×LQ	NS	*	NS	*

Note: different letters in the same columns were significantly different tested by LSD's multiple comparison at $p \leq 0.05$. NS, * represent no significant or significant difference at $p \leq 0.05$, respectively.

Increasing light intensity generally increases photosynthesis and hence plant growth[15,20], as shown in this study. The biomass of lettuce was higher under longer photoperiod with the same PPFD. Longer photoperiod leads to more carbohydrates used for physiological metabolism and growth. Longer photoperiod could stimulate the photosensitive pigments to induce gene expression in lettuce growth, quality, and nutrition uptake[27]. Based on the growth parameters, plants performed better in P250-H16 and P300-H16 than other treatments. Our results were slightly different from other studies. For example, Jeong et al.[15] reported no differences in lettuce growth among PPFDs of 200 μmol/m^2·s, 230 μmol/m^2·s, and 260 μmol/m^2·s, but PPFD of 290 μmol/m^2·s resulted in better growth and development of lettuce. No differences in fresh weight of romaine lettuce were found between

PPFDs of 400 μmol/m^2·s and 200 μmol/m^2·s[16]. These differences may be due to the different types or cultivars of lettuce used in these studies. Based on our results of biomass, a light intensity between 250-300 μmol/m^2·s was reasonable for lettuce production under artificial light.

In most treatments with both photoperiods, FW and DW were higher at PPFD in 200 μmol/m^2·s than in 150 μmol/m^2·s. With a photoperiod of 16 h/d, PPFD at 250 μmol/m^2·s resulted in similar or even higher FW and DW compared to PPFD in 300 μmol/m^2·s, except for leaf DW in L1.2 and root DW in the L2.2. Based on the relationship between DLI and leaf FW and DW, it is obvious that as DLI increased, leaf FW and DW increased linearly (Figure 3). Significant interactions were observed between PPFD and photoperiod on root FW, Leaf DW, and root DW; PPF and light quality on root FW and DW; light quality and photoperiod on leaf FW and DW and root DW; and PPF, photoperiod, light quality on root FW and DW (Table 2).



Note: Means with different letters in the same columns were significantly different tested by LSD's multiple comparison at $P \leq 0.05$. NS, * represent no significant or significant difference at $p \leq 0.05$, respectively.

Figure 3 Relationships between daily light integral and leaf fresh and dry weights of hydroponic lettuce

Dry mass accumulation was proportional to DLI which is the product of light intensity and photoperiod. A linear relationship between DLI and leaf fresh or dry weight was obtained, which was consistent with other report[28]. DLI has been used in managing light in greenhouse lettuce production for many years. The growth and quality of lettuce are closely related to DLI. Longer photoperiod could compensate for a low PPFD at the same DLI[18]. In commercial lettuce production, both suggested that a daily light integral of 17 mol/m^2·d should be maintained in the greenhouse[29]. Our experiments showed a lower DLI (14.4 mol/m^2·d) is sufficient. The DLI of 14.4 mol/m^2·d (P250-H16) had similar growth and quality compared with P300-H16, DLI of 17.28 mol/m^2·d.

3.2 Effects of artificial light on quality of hydroponic lettuce

Light intensity, photoperiod, and light quality had significant effects on the content of nitrate, vitamin C, soluble sugar, soluble protein, and anthocyanin (Table 3). Nitrate content was lower at higher light intensity and longer photoperiod, while vitamin C, soluble sugar, soluble protein, and anthocyanin contents were higher. The nitrate content was lowest in plants grown under higher PPFD (250 μmol/m^2·s and 300 μmol/m^2·s) with longer photoperiod. No differences in the contents of nitrate, vitamin C, and soluble sugar were found between treatments of P300-H16 and P250-H16, regardless of light quality. Contents of vitamin C, soluble protein, and soluble sugar were lower in FL treatments than those in LED with R:B ratio of 2.2. There were no differences in anthocyanin content between PPFD at 250 μmol/m^2·s and 300 μmol/m^2·s with photoperiod of 16 h/d except the treatment of F1.8-P300-H16 which had the highest content. Generally, the differences in anthocyanin among different light quality were small compared to vitamin C, soluble sugar, and protein contents. PPFD and photoperiod interactively affected contents of nitrate, vitamin C, and anthocyanin. PPFD, photoperiod, and light quality interactively affected soluble protein content but not for other quality parameters (Table 3).

Higher PPFD and longer photoperiod could achieve higher contents of vitamin C, soluble sugar, and soluble protein while reducing nitrate content, which agreed with other reports[30,31]. The activity of nitrate reductase was higher under high light intensity, which could decrease the nitrate accumulation. High light intensity would increase the photosynthetic production (sucrose concentration), which promote assimilation of nitrate ion[32]. Genetics, temperature, and light affected the anthocyanin content[33]. Our results on anthocyanin content was similar to other research which indicated that PPFD, photoperiod, and light quality significantly affected anthocyanin content[34,35]. Lettuce in P300-H16 treatments had the best quality and those in P250-H16 had similar contents of soluble sugar, vitamin C, and nitrate. Considering the higher power consumption in P300-H16 and P250-H16 was recommended for commercial lettuce production under artificial light.

Effects of light quality on lettuce growth, development, and quality have been investigated using monochromatic LED lights, a combination of red and blue LED lights, and other light sources such as white LED and FL[34,36-38]. Combination of red and blue lights can effectively promote the lettuce growth and development than monochromatic light. For example, Lin et al.[38] reported that combined red, blue, and white LEDs were better than red and blue LEDs and FL based on growth, nutrition, appearance, and the edible quality of lettuce plants grown in a growth chamber at PPFD of 210 μmol/m^2·s. Continuous light exposure at pre-harvest can effectively reduce nitrate accumulation and increase phytochemical concentrations in lettuce[36]. In this study, LED with R:B ratio of 1.2 and 2.2 had higher quality and lower power consumption than florescent lamp.

3.3 Net photosynthesis of hydroponic lettuce

The two-day continuous measurement of photosynthesis indicated that highest leaf net photosynthetic rates were observed in

F1.8-P250-H16, followed by F1.8-P300-H16 (Figure 4). The differences between the two PPFDs with shorter photoperiod (F1.8-P250-H12 and F1.8-P300-H12) were smaller compared to those with longer photoperiod. Although the treatment F1.8-P250-H16 had the greatest net photosynthetic rate, no statistical differences in either leaf FW or DW were observed between PPFDs at 250 μmol/m^2·s and 300 μmol/m^2·s, indicating that the last two-days do not necessarily reflect the whole production period. The daily accumulative net photosynthetic rates in F1.8-P250-H16 and F1.8-P300-H16, calculated based on the integration of the area under the curve of the instantaneous net photosynthetic rate, were 96% and 71% greater than F1.8-P250-H12 on the 15th day after transplanting. Same tendency was observed on the 16th day.

Table 3 Effects of different light parameters on contents of nitrate, vitamin C, soluble sugar, soluble protein, and anthocyanin of hydroponic lettuce at day 18 after transplanting (n=6)

Treatments	Nitrate content /mg·kg^{-1}	Vitamin C content/ mg·(100 g)$^{-1}$	Soluble sugar content/ %	Soluble protein content/ mg·g^{-1}	Anthocyanin content/ ΔOD·(g FW)$^{-1}$
F1.8-P150-H12	783.08±44.99 a	20.99±2.23 d	1.01±0.06 e	9.85±0.74 de	0.62±0.14 d
F1.8-P200-H12	697.54±182.86 bc	22.43±2.16 d	1.11±0.36 de	10.37±1.51 de	0.87±0.11 cd
F1.8-P250-H12	565.56±89.63 cd	22.06±3.75 d	1.48±0.13 d	9.17±1.16 e	0.95±0.18 c
F1.8-P300-H12	470.00±75.49 de	23.51±1.96 d	1.47±0.16 d	11.75±1.13 cd	1.31±0.28 bc
F1.8-P150-H16	756.17±103.95 bc	21.62±2.34 d	0.90±0.47 e	10.27±0.90 de	0.96±0.26 c
F1.8-P200-H16	563.22±79.06 cd	23.39±4.72 d	1.50±0.40 cd	11.56±0.74 d	1.09±0.30 bc
F1.8-P250-H16	292.51±43.07 e	30.34±2.02 cd	2.11±0.55 c	13.67±2.04 bc	1.35±0.09 b
F1.8-P300-H16	339.51±72.02 e	34.74±3.93 bc	2.16±0.66 c	11.61±1.06 d	2.30±0.61 a
L1.2-P150-H12	667.50±121.67 ab	24.36±3.73 cd	1.04±0.44 e	9.48±1.75 e	0.33±0.05 e
L1.2-P200-H12	626.55±98.98 bc	26.94±2.96 cd	0.94±0.15 e	11.92±0.87 cd	0.59±0.15 de
L1.2-P250-H12	589.73±121.67 bc	29.27±7.42 cd	1.28±0.45 de	12.38±1.61 cd	0.61±0.25 d
L1.2-P300-H12	546.27±73.74 cd	29.50±4.33 cd	1.88±0.64 cd	12.73±1.37 c	0.78±0.18 cd
L1.2-P150-H16	654.79±27.43 b	25.66±4.38 cd	1.08±0.33 de	12.78±1.81 c	0.57±0.17 de
L1.2-P200-H16	539.18±65.28 cd	32.02±3.79 c	1.55±0.28 cd	11.91±1.35 cd	0.73±0.13 cd
L1.2-P250-H16	528.35±20.14 cd	41.67±8.82 ab	1.92±0.17 cd	14.15±2.11 b	1.12±0.18 bc
L1.2-P300-H16	506.65±38.21 d	43.33±2.97 a	2.02±0.24 c	13.16±1.27 bc	1.50±0.58 b
L2.2-P150-H12	810.38±78.41 a	33.49±3.16 bc	1.55±0.31 cd	10.04±1.82 de	0.33±0.07 e
L2.2-P200-H12	797.76±119.69 a	34.11±6.92 bc	2.98±0.29 b	12.85±1.19 c	0.51±0.12 de
L2.2-P250-H12	779.25±39.60 ab	35.43±3.99 b	3.09±0.78 b	13.38±1.08 bc	0.83±0.09 cd
L2.2-P300-H12	520.78±90.13 cd	35.83±4.94 bc	3.96±0.72 a	14.32±1.35 b	0.82±0.07 cd
L2.2-P150-H16	624.66±70.01 bc	31.24±2.71 c	2.56±0.59 bc	13.27±0.83 bc	0.60±0.08 de
L2.2-P200-H16	590.68±110.07 c	35.60±6.20 b	4.01±1.24 a	14.10±2.09 bc	0.86±0.18 cd
L2.2-P250-H16	479.16±43.28 de	38.39±3.70 ab	4.27±0.60 a	15.58±1.21 b	1.47±0.30 b
L2.2-P300-H16	456.78±59.49 de	40.14±4.90 ab	4.26±0.44 a	18.85±1.71 a	1.34±0.26 b
PPFD	*^{y}	*	*	*	*
H	*	*	*	*	*
LQ	*	*	*	*	*
PPFD×H	*	*	NS	NS	*
PPFD×LQ	*	NS	*	*	*
H×LQ	NS	*	NS	NS	NS
PPFD×H×LQ	NS	NS	NS	*	NS

Note: Means with different letters in the same columns were significantly different tested by LSD's multiple comparison at p≤0.05. NS, * represent no significant or significant difference at p≤0.05, respectively.

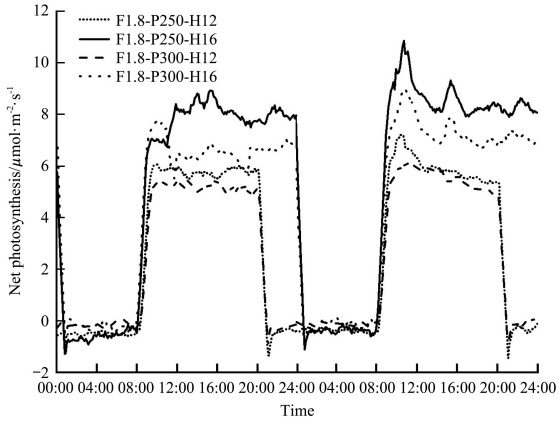


Figure 4 Continuous measurement of net photosynthetic rate of hydroponic lettuce leaves for two days before harvest under fluorescent lamps

3.4 Energy use efficiency of artificial light for lettuce production

Power consumption per 100 g fresh weight and 1 g dry weight were the highest under FL. LEDs had the advantage of higher LEU and EUE than FL (Table 4). The EUE of LEDs lighting was lower at higher PPFDs and longer photoperiod. The LEU for LEDs did not change substantially in the PPFD range of 150-300 μmol/m^2·s with photoperiod from 12 h/d to 16 h/d. For commercial lettuce production in a closed plant factory, light intensity, photoperiod, and light quality are controlled collectively in consideration of plant growth (or yield), energy consumption, initial and operational costs. Although many researchers have studied the effect of single (light intensity) or two factors (PPFD and photoperiod) on the growth and quality of lettuce, limited attention has been paid on the effects of multiple factors as shown in this study.

Table 4 Energy use efficiency of different lamps in lettuce production experiment

Treatment	Power consumption per 100 g fresh mass/ kWh·(100 g FW)$^{-1}$	Power consumption per 1 g dry mass/ kWh·(g DW)$^{-1}$	Light energy use efficiency (LEU)	Electric energy use efficiency (EUE)
F1.8-P150-H12	4.35	1.32	0.019	0.0038
F1.8-P200-H12	4.60	1.18	0.021	0.0041
F1.8-P250-H12	4.82	1.18	0.021	0.0041
F1.8-P300-H12	6.28	1.53	0.016	0.0031
F1.8-P150-H16	4.30	1.27	0.020	0.0039
F1.8-P200-H16	4.43	1.25	0.020	0.0039
F1.8-P250-H16	5.11	1.36	0.018	0.0036
F1.8-P300-H16	6.17	1.50	0.016	0.0032
L1.2-P150-H12	2.46	0.56	0.025	0.0096
L1.2-P200-H12	2.78	0.59	0.025	0.0091
L1.2-P250-H12	2.90	0.63	0.021	0.0086
L1.2-P300-H12	3.54	0.72	0.020	0.0076
L1.2-P150-H16	2.60	0.54	0.026	0.0101
L1.2-P200-H16	3.13	0.62	0.024	0.0087
L1.2-P250-H16	2.91	0.60	0.022	0.0090
L1.2-P300-H16	3.83	0.68	0.021	0.0079
L2.2-P150-H12	2.47	0.52	0.031	0.0099
L2.2-P200-H12	2.40	0.52	0.030	0.0097
L2.2-P250-H12	2.74	0.56	0.029	0.0092
L2.2-P300-H12	3.48	0.64	0.023	0.0080
L2.2-P150-H16	2.67	0.62	0.026	0.0082
L2.2-P200-H16	2.94	0.61	0.026	0.0083
L2.2-P250-H16	3.15	0.68	0.024	0.0075
L2.2-P300-H16	3.74	0.66	0.022	0.0078

Note: FW means fresh weight, DM means dry weight.

4 Conclusions

In commercial production of lettuce in indoor controlled environment, finding the lowest optimal light intensity is important to achieve acceptable yield with the lowest energy consumption. Based on growth, quality, and energy consumption, we recommend that PPFD of 250 μmol/m^2·s with photoperiod of 16 h/d provided by LED with R:B ratio of 2.2 is suitable for commercial production of lettuce (cv. Ziwei). LED light source has advantages in energy consumption and lettuce quality in artificial light plant factory, which can replace fluorescent lamp completely. However, the performance of LED light source should be evaluated with luminous efficiency, light quality, attenuation characteristics and life expectancy. Its performance evaluation and plant cultivation effects should be standardized test and evaluation. Further studies on the effect of other combination of LED lights to further enhance lettuce growth and quality may be necessary.

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