Laser flashing light as a radiation source for lettuce growth

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Abstract: Relatively lower light intensity and higher energy consumption of electrical lightings in plant factories encourage people to search for new light sources and illuminating patterns. In this study, a laser diffuse system was built to produce laser flashing light (LF) by making high-intensity red and blue light strips (light intensities were 4700 and 1200 μmol/m^2·s, respectively) swept reciprocally in the cultivation area. The growth of lettuces (*Lactuca sativa* var. capitata) was employed to evaluate the feasibility and efficiency of LF. The results showed that LF could maintain the growth of lettuce; however, the plants in this illumination pattern showed an obvious shade avoidance response and a significant decrease in growth compared with the LED continuous illumination (LEDC) control. After 32 d of growth, the leaf fresh mass in LF and LEDC were 32.9 g and 79.9 g, respectively. The leaf area of LF was only 40% of the value in LEDC. Leaf number, leaf width, and root length in LEDC were 40.2%, 78.6%, and 124.4% higher than LF, respectively. On the contrary, leaf length and stem length in LEDC were significantly 7.7% and 32% shorter than LF. Much lower light intensity equivalent to continuous light (66.6 μmol/m^2·s) in LF as well as low quantum absorption and utilization efficiency might be the main reason. Further studies are needed to optimize the illuminating pattern related to frequency and duty ratio, based on the photosynthesis parameters of lettuce. Also, the laser diffuse principle and system construction need to be improved to acquire high photon utilization efficiency and light dispersion.

Keywords: laser diffusion, high intensity, intermittent light, plant growth
DOI: 10.25165/j.ijabe.20241706.8579

Citation: Li K, Cheng R F. Laser flashing light as a radiation source for lettuce growth. Int J Agric & Biol Eng, 2024; 17(6): 86–92.

1 Introduction

Light is the key influencing factor for plant growth and development[1,2]. Plant growth rate, plant quality, and photosynthesis could be significantly changed through modified light intensity[3,4], light quality[5,6], and photoperiod[7,8]. Light parameters such as frequency and duty ratio also have been adjusted to acquire flashing light and have been widely used in inactivation of foodborne pathogens and microorganisms[9-15].

The positive effect of flashing light in plant growth has been known for many years. Algal experiments reported that the photosynthesis rate exposed to intermittent lighting was more than that under continuous illuminating[16,17]. An identical phenomenon was also observed in higher plants such as floating duckweed (*Lemna minor*), soybean (*Glycine max*), and lettuce (*Lactuca sativa*)[18-20]. It was also demonstrated that flashing light irradiation can ensure photosynthetic efficiency under low energy consumption[21]. Park et al.[22] even conclude that photosynthetic cells do not need continuous illumination.

Consistent with the research of Weller et al.[23], powerful average flashing has been confirmed to be a critical factor for such a positive effect, which has been proven to determine the photosynthetic rate[24,25]. Such high-intensity intermittent irradiation could be obtained mainly by significantly increasing the power input of light sources. Burlew[26] adopted a 1000 W incandescent lamp to provide intensity close to solar radiation to illuminate the algae, which was about 25×10^4 ergs/cm^2·s (about 250 W/m^2 heat flux density). Tennessen et al.[27] applied LED pulses of 5000 μmol/m^2·s to make an equivalent of 50 μmol/m^2·s illumination, resulting in the same photosynthesis with continuous treatment. Xue et al.[28] acquired an overall light intensity of 30 μmol/m^2·s by using a 150 000 μmol/m^2·s output LED. In addition, Hiramoto et al.[29] got high power electric pulses by shortening the inert gas flash lamp light duration.

However, it is uneconomical and impractical to achieve such high light intensity by using mainstream lighting technologies due to their enormous light source investment and energy consumption, which make them rarely used in actual production. In addition, the temperature under such light sources will be increased by extra power input, leading to negative effects on plant growth[10,14] and energy consumption in environmental control[30].

As a light source with a long history, light amplification by stimulated emission of radiation (laser) has continuously shown application advantages in various fields due to its higher optical output power[31,32], much greater power conversion efficiency[33,34], and higher directional emission property. Laser illumination was widely used in seedling pretreatment and cultivation for its induction of enzymatic activities, uptake and translocation of ions, as well as biochemical process[35,36]. Laser light was also applied in plant production. Takatsuji et al.[37] discussed the possibility of using laser diode (LD) in a plant factory through comparison of light quality, light intensity, lighting effectiveness, and price among the light sources. They concluded that 2500 yen per average output red and blue LDs with up to 30% light emitting efficiency can compete with the pressure sodium lamp in a plant factory. Tsuchiya et al.[38]

Received date: 2023-10-12 **Accepted date:** 2024-11-08
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also confirmed the possibility of using laser to cultivate lettuce by using 30 pieces of 500 mW, 680 nm LDs. Yamazaki et al.[39] found rice plants could complete their life cycle under red LD light supplemented with blue light, and they reached the harvesting stage earlier than control plants grown under high-pressure sodium (HPS) lamps. A laser projector with LDs (50-100 mW) combined at three wavelengths (450 nm, 570 nm, and 640 nm) was reported to be sufficient to grow radish sprouts by scanning the plants in a bi-directional pattern[40]. Ooi et al.[32] tested on the Arabidopsis (*Arabidopsis thaliana*) and noted that the plants appeared to be healthy under a laser illumination system with a single wave-length beam adjusted to a ratio of 9:1 (red 671 nm and blue 473 nm), giving an average total photon flux density of 90-100 μmol/m^2·s.

However, these LDs were low in power, were used without diffusion, and achieved low light intensity and small illuminating area. In this study, a laser diffuse device was developed to disperse the laser over a large area covering multiple mature lettuces. The availability and effectiveness of laser flashing light in lettuce growth were evaluated by analyzing the growth parameters as well as electricity consumption.

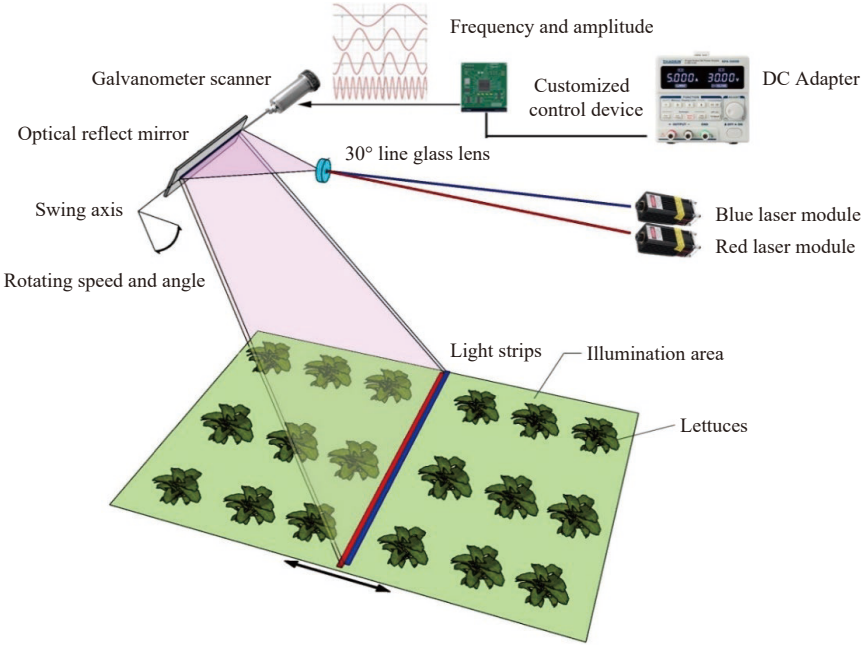

Figure 1 Schematic diagram of laser flashing light

2 Materials and methods

The possibility of using a laser flashing light (LF) in a plant factory was assessed in a practical experiment using "Flandria" lettuce (*Lactuca sativa* var. capitata) plants, by evaluating the growth differences between LF and LED continuous illumination (LEDC).

2.1 Laser flashing light system

By following the schematic diagram of LF (Figure 1), a laser diffuse system (Figure 2) was built as the experiment light source. It was composed of a laser lighting array and a laser diffuse unit. Red and blue laser modules emitted laser beams focused on the 30°-line glass lens, then formed two short laser strips laid at the optical reflect mirror and were reflected onto the cultivation board below. The mirror was driven by a galvanometer scanner in which rotation speed and angle were determined by adjusting the frequency and amplitude through a customized control device. Therefore, red and blue light strips swept reciprocally to illuminate the lettuces in the illumination area.

The laser lighting array consisted of one red laser module [150 mm×130 mm×120 mm (L×W×H), peak at 660 nm, output power 4030 mW, LSR660CP-4W, LASEVER Inc., Ningbo, Zhejiang] and one blue laser module [150 mm×90 mm×120 mm (L×W×H), peak at 460 nm, output power 4080 mW, LSR460CP-4W, LASEVER Inc., Ningbo, Zhejiang]. Their beam size at 500 mm is about 9 mm×9 mm. Both the modules were placed horizontally 300 mm above the cultivation board surface and the laser beams dropped at an identical point on the diffuse unit. In order to reduce the angle between the two laser beams and avoid the misalignment of the light strips, a distance of 400 mm was kept between the laser modules and diffuse unit. The angle between the laser modules and the distance away from the laser diffuse unit were adjusted intensively to make the red and blue laser beams focused on the line glass lens, then form two short laser strips lying at the laser reflect mirror in high coincidence (Figure 2).

The laser diffuse unit was mainly constituted of a 30°-line glass lens (diameter 9 mm, thickness 2 mm), a galvanometer scanner (Model 6860, Cambridge Technology, Bedford, MA, USA) with precise RGB optical reflect mirror (dielectric coated, 20 mm× 60 mm×1.1 mm, reflectance≥99%) designed for laser beam steering or scanning applications, and a direct current (DC) adapter (JMD20-D15, Honghai Technology Development Co., Ltd., Harbin, China). They were mounted to the cultivation shelf by customized holders and optical experiment holders (Hengyang Optics Inc., Guangzhou, China). The laser diffuse unit was assembled in the same plane as the laser lighting array (Figure 2).

Under reflection of the optical reflect mirror, red and blue laser strips occurred on the cultivation board (Figure 3), which were 400 mm long, and 10 mm and 8 mm wide, respectively. Because of the incidence angle of the two laser beams, there was a 30 mm dislocation at the ends of both strips, making the coincidence part of the beams 340 mm long. The coincidence area (cm^2) of red and blue strips could be determined by Equation (1). As a result, the red and blue strip areas were 34 cm^2 and 27.2 cm^2, respectively.

$$S = lw \tag{1}$$

where, S is coincidence area of red and blue strips, cm^2; l is coincidence length of red or blue laser strip, cm; w is width of red or blue laser strip, cm.

Photosynthetic photon flux density (PPFD) of the laser strips (μmol/m^2·s) on the cultivation boards was measured by a spectrometer (LI-1500 with LI-190R quantum sensor; LI-COR, Nebraska, USA). The values of the red and blue strips were 4700 and 1200 μmol/m^2·s, respectively.

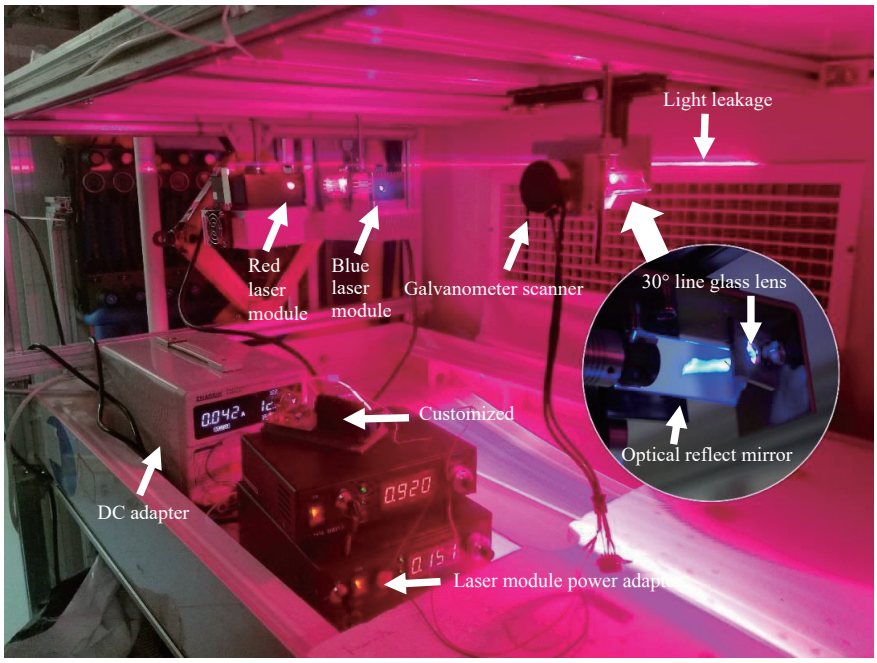

Figure 2 Composition of laser diffuse system

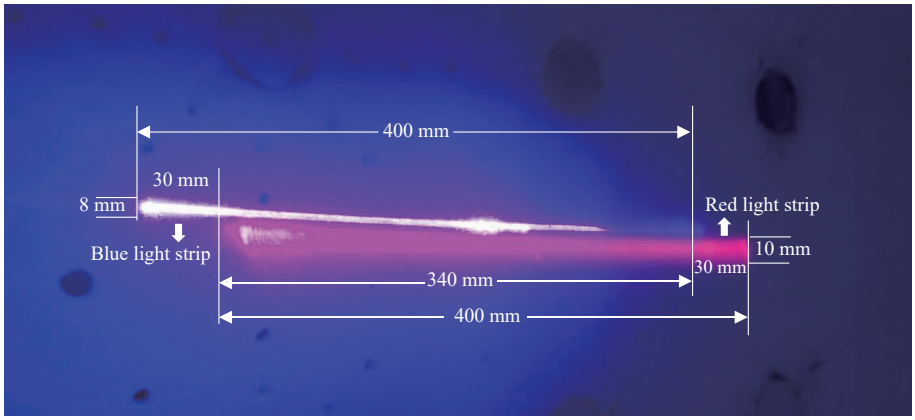

Figure 3 Red and blue laser strips on the cultivation board

Combined with the strip areas measured before, the photosynthetic photon flux (PPF, μmol/s) of the red and blue strips could be determined by Equation (2). As a result, the PPF of the red and blue stripes were 15.98 and 3.26 μmol/s, respectively. The red-blue ratio (R/B) was about 5:1.

$$I = \Phi S / 10\,000 \quad (2)$$

where, I is photosynthetic photon flux of red and blue strips, μmol/s; Φ is photosynthetic photon flux density of red and blue strips, μmol/m^2·s; S is coincidence area of red and blue strips, cm^2.

With the working of the galvanometer scanner, the laser strips swept reciprocally to illuminate the plant canopy (Figure 4). The rotating speed and amplitude of the galvanometer scanner motor were regulated individually by a customized control device to obtain various flash frequencies and illumination ranges. In our study, the illumination range was adjusted to 850 mm wide, acquiring a rectangular projection area of 340 mm×850 mm = 0.289 m^2, and covering 15 lettuces on the cultivation boards.

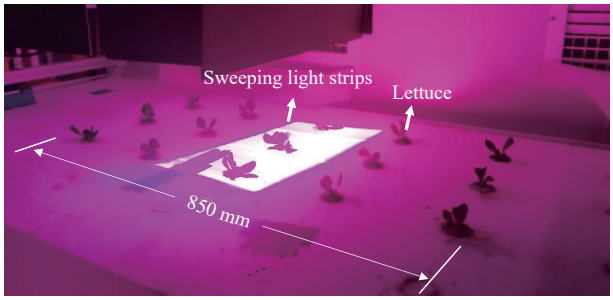

Figure 4 Red and blue laser strips swept reciprocally to illuminate the lettuces in the illumination area when the galvanometer scanner was working

Under this circumstance, the PPFD of the LF illumination area could be determined by Equation (3). As a result, the PPFD on the cultivation board of the LF illumination area was 66.6 μmol/m^2·s, equivalent to continuous light.

$$\Phi = I / A \quad (3)$$

where, Φ is the sum of photosynthetic photon flux density of red and blue strips, μmol/m^2·s; I is photosynthetic photon flux of red and blue strips, μmol/s; A is the area of laser flashing light illumination, cm^2.

The flash frequency was maintained at 10 Hz through a customized control device, while observing the waveform change of a digital oscilloscope (DS1052E, RIGOL Technologies, Inc., Suzhou, China). Since the laser strips fell on the cultivation area constantly, the equivalent PPFD was not influenced by flash frequency. Under a 10 Hz flash frequency, which means the light strips swept reciprocally to illuminate the plants 10 times in one second, it took 0.1 s for one-way light strips movement and 0.2 s for one cycle. Since the length of the projection area was 850 mm, each photosynthetic unit of lettuce (100 mm in diameter) were exposed to the 10 mm-wide laser strips for about 1/8.5 of 0.1 s (1.2 ms) during one flash, then experienced a much longer non-irradiation intermittent stage (0.088-0.176 s for the lettuces in the middle and the edge of the projection area) before the next flash (Figure 5).

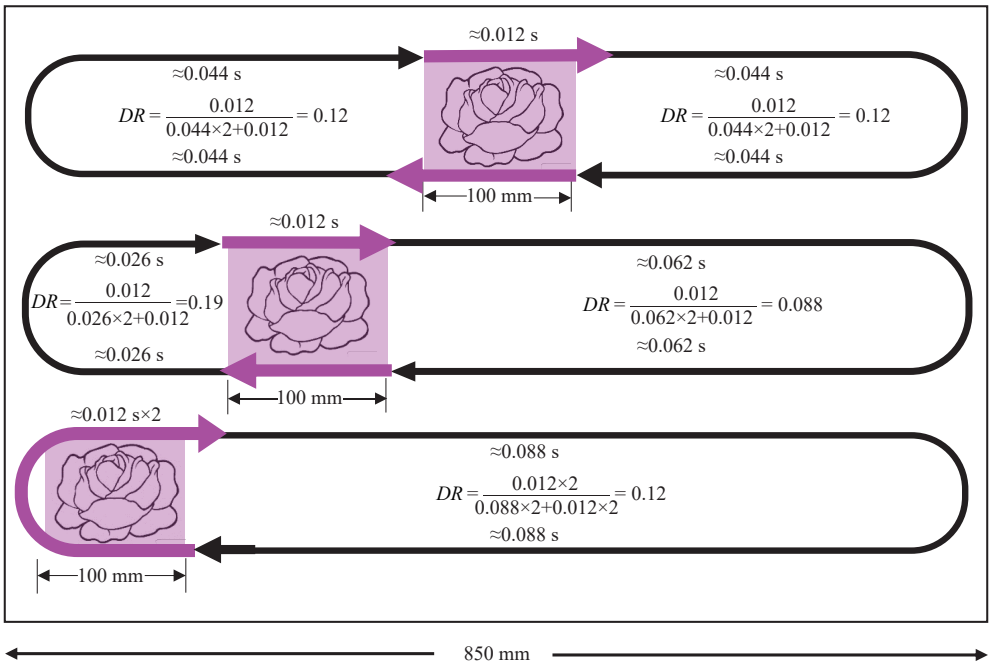

Figure 5 Sweeping pattern of the laser light strips

The illumination range was 850 mm wide, so under a 10 Hz flash frequency, it took 0.2 s for the light strips to sweep one cycle through the lettuce in the middle, secondary middle, and edge of the cultivation area. The red area and red arrows represent the light strips' movement over the lettuce canopy at corresponding positions. The black arrows represent the light strips' movement outside the corresponding lettuce canopy. The time data beside the arrows represent the time required for the light strips to complete the corresponding movement. DR is for duty ratio.

During the light period of LF, the plants experienced a relatively longer dark period after each flash. The duty ratio (DR) for the lettuce varied in different locations of the cultivation area. The DR of the lettuce illumination could be determined by Equation (4). As a result, the DR for the lettuce in the middle, secondary middle, and edge of the cultivation area were 0.088 to 0.19 for a 100 mm-diameter canopy (Figure 5).

$$D = t/(t_0 + t) \tag{4}$$

where, D is duty ratio; t is time for the light strips to sweep over the lettuce canopy, s; t_0 is time between leave and return of the light strips to the lettuce, s.

The one-cycle (0.2 s) time map of the arrival of light strips in LF at the lettuce in the middle, secondary middle, and edge of the cultivation area is shown in Figure 6.

In Figure 6, the laser light strips sweep one cycle from one end of the cultivation area to the other end and back again in 0.2 s. During this sweep, a total of 5900 μmol/m^2·s red and blue light strips illuminate the single plant twice for 0.012 s each time. Because of the different positions (middle, secondary middle, and edge) of lettuce in the cultivation area, the intervals between two exposures were different. For the lettuces located in the edge of the cultivation area, two exposures merged into one longer exposure (0.024 s).

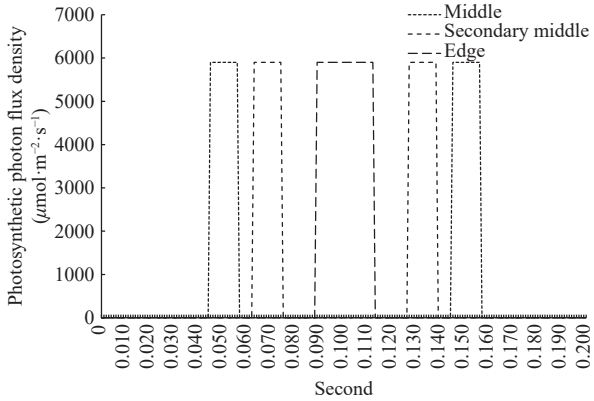

Figure 6 The one-cycle (0.2 s) time map of light strips in LF

2.2 LED continuous lighting system

In LEDC control, light panels (Bio-lighting Sciences and Technology Co. Ltd., Dongguan, China) consisting of red [peak at 660 nm, full width at half maximum (FWHM) at 70 nm] and blue (peak at 460 nm, FWHM at 40 nm) LEDs were located 300 mm above the cultivation area, covering 0.84 m^2 (completely covering

the growing area of the plants below). The R/B during the experiments was 5:1, identical to the LF. PPFD on the cultivation boards was adjusted to 150 μmol/m^2·s. The illumination time was 16 h/d (from 06:00 to 22:00) under a 16/8 h light/dark cycle. Different from LF, the direct current (DC) powered LEDs had no flash frequency.

The energy consumption of LEDC and LF were kept identical by adjusting the power input of red and blue laser power adapters, respectively. An electricity meter (UT230A-II; UNI-T Science and Technology Co., Guangdong, China) was employed to measure the electricity consumption of the power adapters.

Based on the PPFD equivalent to continuous light and the illumination area for each treatment, the daily light integral (DLI) of the lettuce could be determined by Equation (5). As a result, the DLI for the lettuce under LF and LEDC were 3.84 and 8.64 mol/m^2·d, respectively.

$$DLI = 3600\Phi T / 1\,000\,000 \quad (5)$$

where, DLI is daily light integral, mol/m^2·d; Φ is the photosynthetic photon flux density equivalent to continuous light, μmol/m^2·s; T is light period, h.

2.3 Plant material and growth conditions

Identical plant material and growth conditions were used in LF and LEDC. Lettuce (*Lactuca sativa* var. capitata) seeds (Flandria RZ, Rijk Zwaan De Lier, The Netherlands) were sown in sponge seedling blocks (25 mm×25 mm×25 mm) on a plastic seedling tray (57 cm×23.5 cm×4 cm) and germinated in an electrical light growth chamber (GLED-250PY, Luxi Technology Co., Ltd., Beijing, China). The light/dark cycle was 16/8 h and the temperature was maintained at (20±0.5)°C and (8±0.5)°C, respectively. Half-strength modified Hoagland's nutrient solution (NS, pH 6.3±0.1, EC 0.8±0.2 mS/cm) was employed during the seedling stage. Fifteen days after sowing, uniform seedlings were transplanted onto cultivation boards (polyethylene, 720 mm× 1300 mm×14 mm, 37 plants/m^2) in a fully closed plant factory located at the Chinese Academy of Agricultural Sciences (CAAS), Beijing, China (39°57′40.2″N, 116°19′34.6″E). They were cultivated with the deep flow technique[31] for 32 d using modified Hoagland's NS (pH 6.3±0.1, EC 1.6±0.2 mS/cm). The air temperature in the plant factory was (24±0.5)°C and (22±0.5)°C during the light and dark periods, respectively. The carbon dioxide (CO_2) concentration was maintained at 400±50 μL/L. The lighting and growth conditions are listed in Table 1.

Table 1 The lighting and growth conditions for laser flashing light (LF) and LED continuous illumination (LEDC) treatments

	Light wavelength/nm	Frequency/Hz	Duty ratio	Number of plants	Light period/h	PPF/(μmol·s^{-1})	Projection area/m^2	PPFD/(μmol·s^{-1}·m^{-2})	DLI/(mol·m^{-2}·d^{-1})	Lights power/W
LF	Red:660 Blue:460	10	0.088-0.19[a]	15	16	Red:15.98 Blue:3.26	0.29	66.3	3.84	90
LEDC	Red: 660 (FWHM:70 nm) Blue: 460 (FWHM:40 nm)	N/A	1	32	16	Red: 105 Blue: 21	0.84	150	8.64	90

[a]Based on the time for a whole plant (leaf radius 100 mm) to be swept illuminated by light strips.

2.4 Measurements

Growth parameters such as fresh mass, number of leaves, leaf length, and root length were measured 5 d, 12 d, and 32 d after transplanting (DAT) to analyze the physiological characteristics of shoots and roots. Five plants were collected at each time. Masses were measured by an analytical balance (GL6202-1SCN, Sartorius Lab Instruments GmbH Co. KG, Goettingen, Germany). Leaf areas were measured using a leaf area meter (LI-3100C; LI-COR, Nebraska, USA). Leaf length, leaf width, root length, and stem length were measured using a Vernier calliper. The three leaves with the largest leaf area in each plant were selected to measure the leaf length and leaf width. The length between the top surface of the sponge seedling blocks and the point where the latest leaf had grown was regarded as the stem length. The analysis of variance (ANOVA) was performed to determine the differences in these parameters between LF and LEDC based on the statistical significance.

3 Results and discussion

Lettuce production and morphological properties were significantly different between LF and LEDC. During 32 d of cultivation under the lighting conditions described in Table 1, all lettuce seedlings in both treatments survived and showed varying degrees of growth, inconsistent with the different surviving rates between the fluorescent lamps and laser projectors[41]. Obvious growth differences had emerged at DAT 5 (Figure 7a, Table 2).

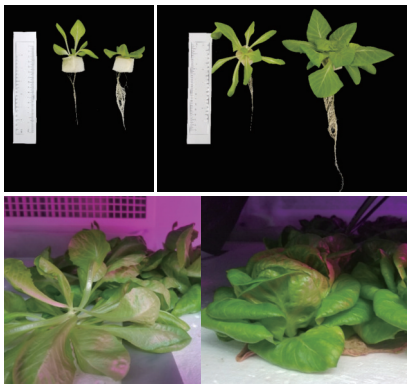

Figure 7 Lettuce growth under laser flashing light (LF) and LED continuous illumination (LEDC) control 5 d, 12 d, and 32 d after transplanting

Table 2 Changes in lettuce leaf and root morphological parameters 5, 12, and 32 days after transplanting (DAT) in laser flashing light (LF) and LED continuous illumination (LEDC) system

DAT	Treatments	FW/g	Leaf area/cm^2	Leaf number	Leaf width/mm	Leaf length/mm	Root length/mm	Stem length/mm
5	LF	1.8±0.2b	44.9±3.5b	5.4±0.6a	27.6±4.0b	55.4±4.0a	69.0±5.6b	N/A
	LEDC	2.4±0.4a	100.3±6.4a	5.2±0.5a	36.2±4.0a	45.4±3.5b	98.6±10.5a	N/A
12	LF	4.3±0.6b	134.2±15.9b	11.2±0.8b	33.8±2.9b	95.6±7.1a	155.6±11.6b	19.2±2.8a
	LEDC	12.2±2.5a	288.6±53.1a	14.4±1.1a	69.0±5.6a	93.2±8.2a	246.2±17.0a	15.6±3.1b
32	LF	32.9±2.3b	571.9±51.7b	20.4±1.7b	80.4±10.7b	179.2±9.1a	233.8±26.6b	56.8±8.7a
	LEDC	79.9±5.7a	1,427.8±180.2a	28.6±1.8a	143.6±16.9a	165.4±8.6b	524.6±85.4a	38.6±5.7b

Note: Data were analyzed by variance analysis and ±S.D. of the means according to the least significant difference test.

As listed in Table 2, the seedling fresh mass, leaf area, leaf width, and root length in LF were 25%, 55.2%, 23.8%, and 30% lower than the respective LEDC values. The leaf length in LF was 22% longer than LEDC, and there was no difference in leaf number. The stem length was too small to compare.

The differences between LF and LEDC had widened by DAT 12 (Figure 7b, Table 2). The fresh mass in LEDC was 9.8 g higher than before, while there was only a 2.5 g increase in LF. The leaf area, leaf width, and root length values in LEDC were 188.3 cm^2, 32.8 mm, and 147.6 mm higher than before, while only 89.3 cm^2, 6.2 mm, and 86.6 mm higher in LF, respectively. Leaf number increased rapidly during these days and started showing differences, and were 5.8 and 9.2 higher than 7 d before in LF and LEDC, respectively. The change of leaf length seemed to be less affected by light environment, and there was no significant difference between the treatments. Nevertheless, the lettuce under LF had relatively longer leaves. The stem length in LF was 19.2 mm, significantly 23% higher than LEDC.

At DAT 32, both treatments experienced a period of accelerated growth and even larger differences were observed (as shown in Figure 7c and Table 2). Fresh mass in LF and LEDC increased by 28.6 g and 67.7 g, respectively, resulting in 143% more yields in LEDC. The leaf area of LF was only 40% of the value in LEDC. Leaf number, leaf width, and root length in LEDC were 40.2%, 78.6%, and 124.4% higher than LF, respectively. However, leaf length and stem length in LEDC were significantly 7.7% and 32% shorter than LF.

Based on the results above, we found a PPFD of only 66.6 $\mu mol/m^2 \cdot s$ in LF, which was equivalent to the corresponding value in LEDC. The DLI of LF was 55.6% less than LEDC, and was probably too low for these plants, though the flashing light intensity was as high as 5900 $\mu mol/m^2 \cdot s$. The plants demonstrated an obvious shade avoidance response due to lack of light, including growth restriction and leaf and stem elongation, identical with the findings of previous research[40,42].

Another reason for the decrease in growth might be because of the shielding of laser light by the upper leaves. The high directivity laser beams could not reach the leaves at the lower canopy and bottom due to their high directivity and poor diffusion and penetration. This characteristic had already been recognized as a disadvantage of laser illumination in plant cultivation[40].

The frequency might be the most influential factor for plant development in LF. Both the irradiation and intermittent stages might be too long for proper photosynthesis. Under 5900 $\mu mol/m^2 \cdot s$ light intensity, a large number of photons arrived at the photosynthetic pigments instantaneously and lasted for 1.2 ms, which was much longer than the time within which the photons were absorbed[43], causing high non-photochemical quenching (NPQ), accumulation of reactive oxygen, and photooxidative stress, resulting in photoinhibition[44-46].

On the other hand, the absorbed photons would not support photosynthetic system operation to the next flash, since the carbon fixation step might have stopped in 5 ms[47,48], but the dark period after a flash was as long as 26-88 ms (Figure 5). Under this circumstance, the plants would have difficulties in the activation of carbon assimilation enzymes (such as Rubisco, etc.), the accumulation of intermediate products, and stomatal opening, resulting in photoinduction difficulties[49].

The different DRs seemed to have little influence on plant growth. This may be because the lower PPFD in LF treatment was the main limiting factor occupying an absolute dominant position, and the different DRs were not showing potential impact when the light requirement for fundamental growth was not fully satisfied.

In addition, the laser diffuse system had apparent light leakage (Figure 2 and Figure 8) because not all laser light fell on the lens. This is because the laser beam size at 500 mm was about 9 mm×9 mm, and the diameter of the 30°-line glass lens was 9 mm, bringing about light quantum loss and electric waste.

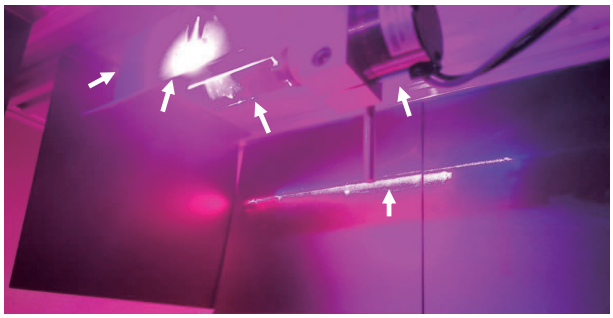

Figure 8 Light leakage from laser diffuse system

4 Conclusions

LF can be used to maintain the growth of lettuce. However, the plants in LF showed an obvious shade avoidance response and a significant decrease in growth because of the lower PPFD equivalent to continuous light as well as low quantum absorption and utilization efficiency, resulting in negative effects on photoinduction and enhanced photoinhibition. Further studies are needed to study the effects of different intensities, frequencies, and light/dark ratios of red and blue flashing light on the characteristics of photoinhibition and the light protection mechanism, combined with the effect on photosynthetic pigment content and microstructure of the leaves. Thereafter, it will be necessary to find out the response mechanism of photosynthetic induction to different flashing light parameters by studying the plant photosynthetic induction curve, as well as the quantitative analysis of the absorption, utilization, and distribution of light energy. This study will be helpful to more deeply understand the physiological nature of flashing light regulation of plant photosynthesis, and provide theoretical evidence and technical support for optimizing the application of highly efficient plant production under an electrical light cultivation environment.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 31701969). The authors also acknowledge the assistance of Guodong Wu, Qiang Wei, and Chengfeng Tong, the following members of the Institute of Environment and Sustainable Development in Agriculture, for their assistance during the study.

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