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The effect mechanism and model optimization of pulsed light dark duration on lettuce

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Abstract

Background: Despite its rapid development, the costs of crop artificial light source technology are still high. In addition, both the luminous efficiency and photosynthetic light supplement efficiency of the light source require further improvement. This study aims to improve the photosynthetic light supplement efficiency by altering the luminescence mode of the light source, transforming the conventional continuous supplementary light source into a pulse light source, and exploring how to further reduce energy consumption and improve the light supplement efficiency without influencing the light supplement effect of Lettuce.

Results: For this purpose, Lettuce (*Lactuca sativa* L.) was selected as the experimental material to investigate the effects of varying the duty ratio, frequency and dark duration on the Pn (net photosynthetic rate) of leaves. The results revealed that Pn values under each duty ratio treatment increased with frequency and gradually stabilized to a level similar to that of continuous light. At higher duty ratios, the lettuce leaf Pn under pulse light reached a stable state at a lower frequency, with Pn leveling showing an overall upward trend with the decreasing dark period duration and a large increase in the early stage. For dark period durations lower than a certain value (0.000683594 s), variations in Pn among treatments were minimal, with a gradual increasing trend until no significant differences are observed with continuous light (CK); Under the D₃ (weak light) condition, plants were easy to spindling (excessive growth) and exhibited narrow and slender leaves. Plants under the D₂ condition (The duration period duration was 0.000465468s) presented the strongest roots and stems, with wide leaves and a

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compact growth. The following trend in Pn was observed across all duty ratios $D_2 > D_1 (0.000046547s) > CK > D_3 (0.004654685s)$.

Conclusions: The dominant influencing factor of the plant net photosynthetic rate was determined as the ratio of the frequency and duty ratio (i.e., dark period duration). Compared with continuous light, pulsed light is more beneficial to plant growth and utilization.

Keywords: Pulsed light; dark period duration; net photosynthetic rate; model prediction; biomass; leaf form

Background

Light is a key factor in the plant growth process[1-3]. Increases in the global population, restricted land resources and the development of horticultural facilities have led to the emergence of a new semiconductor light source[4]. This light source, which has gradually become the dominant type of supplementary light, employs an artificial light source supplement and has replaced the traditional regulation of natural light for crop cultivation[5, 6]. This has consequently improved crop quality and yield. In particular, plants are observed to grow 2-4 times faster than typical outdoor plants when LEDs (lighting-emitting diodes) are used to autonomously control the internal environment, regardless of the outside climate[7, 8].

LEDs are widely employed in light cooperation, energy saving, the light morphogenesis of plants, and the photobiology of cells[9, 10]. Compared with fluorescent lamps (FL) and high pressure sodium lamps (HPS), LEDs are easier to install and operate, have a longer life span and a higher light conversion rate. The cost of supplementary light is also relatively low[11-13]. In addition, LEDs can also be used to study the effect of supplementary light on plant growth under different light intensities, quality, frequency, duty ratio and their combination[14, 15]. Plant supplementary light is typically a continuous light source, yet research on different frequencies and duty ratio pulse light applications reveals the potential of pulsed light to replace continuous

light[16]. As early as 1905, Brown and Escombe used a rotating disk to reduce the light intensity by 25% without changing the light quality, with no reductions in the photosynthetic rate[17]. Ki-ho Son demonstrated that a 75% duty ratio and low frequency pulsed LED did not result in any significant inhibitory effects on plant growth. This indicates the potential of pulsed LED irradiation technology in saving energy during plant production[18]. Other studies have revealed improvements in the photochemical efficiency of photosystem II and the electron transport efficiency of tomato plants under pulsed light[19]. Thus, pulsed light can be superior to continuous light in terms of plant growth.

Previous research has demonstrated that an adequately set pulsed light frequency (or duty ratio) that matches the photosynthetic reaction time of plants cannot only reduce light energy consumption and improve the utilization efficiency of light energy, but can also effectively avoid photoinhibition[20-22]. Weller and Franck (1941) observed that the P_n saturation values depend upon the number of flashes, i.e., the dark period duration[23]. Yoneda and Mori (2004) used pulsed light to measure the photosynthetic activity and fresh weight of lettuce plants and revealed an improvement in the photosynthetic capacity and fresh weight of lettuce leaves via a frequency flash treatment and an appropriate duty ratio combination (50% and 10 KHz). Furthermore, the authors demonstrated that the employment of more and higher pulsed light frequencies facilitates research on the effects of pulsed light frequency on plant growth[24]. Jao and Fang (2004) proposed that the influence of high frequency (>1000 Hz) LEDs on plant growth should be further studied in subsequent experiments[25]. Thus, observing the effects of pulsed light parameters on P_n can aid in revealing the complex dynamic laws of photosynthesis.

In the current study, an LED system was employed to provide different supplementary light environments for plants through pulse width modulation; and to explore the biological rules between the frequency, duty ratio and dark period duration of pulsed light and the P_n of plants. In addition, a relatively simple response model was built between the pulse light dark period length and the P_n of leaves based on a mathematical method and experimental

data. The proposed model is able to predict the Pn of leaves under different pulse lights. Verification tests were designed based on the results. The critical value of the optimal dark duration under any duty ratio was subsequently obtained using the model. The aims of the study were to: i) improve the photosynthetic light supplement efficiency by transforming the conventional continuous supplementary light source into a pulse light source, and ii) explore how to further reduce energy consumption and improve the light supplement efficiency without influencing the light supplement effect of Lettuce. Our work facilitates the selection of a highly efficient and energy saving artificial light source for plant factories and related facilities.

Methods

Materials and treatments

Experiments were conducted at the laboratories of the Agrobiological and Environmental Engineering Faculties, College of Horticulture, Northwest A&F University, US from May 2018 to May 2021. The lettuce (*Lactuca sativa* L.) variety 'Hong Kong glass' (which was originally sourced from the Qingxian Qingfeng Seed Industry Co. LTD) was selected as the test subject. Prior to transplanting, the seedling management was consistent. Following the acceleration of the lettuce seed buds, they were sown in a tray with 72 seedling holes and placed in an overhead LED light incubator for routine seedling management. Seedlings of an uneven growth were removed with a single leaf. When four leaves were in one mind (i.e., the fourth true leaf is fully unfolded), strong plants with a uniform growth were selected, and the plants in the nursery hole were transplanted into a 6 cm × 6 cm plastic cultivation tank. The day and night temperature, humidity and photoperiod in the overhead LED light incubator were set to $(23 \pm 1)^{\circ}\text{C} / (20 \pm 1)^{\circ}\text{C}$, 55%-60%, and 14 h/10 h, respectively. The light intensity of the LED lamp board was set to $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with a 20 cm × 30 cm lamp board. The plants were evenly placed under the lamp board. Fig. 1 presents the LED light source.

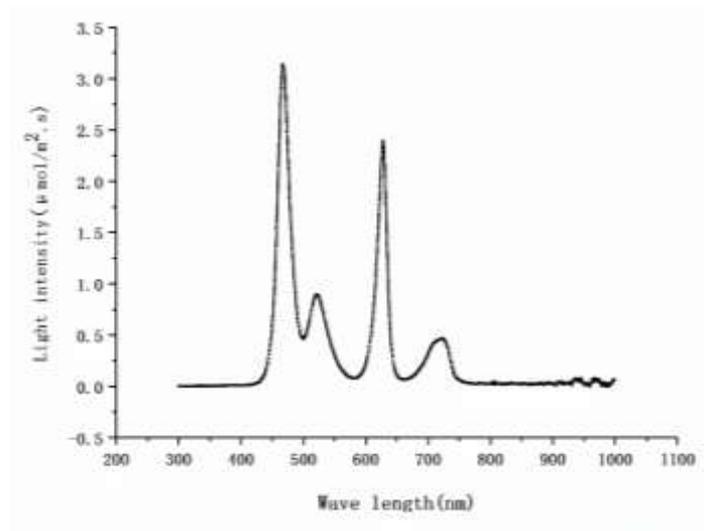


Fig. 1 LED light spectrum.

Five duty ratio levels and 14 frequency levels were initially set to explore the effects of pulsed light forms on the Pn of blades (Table 1). Based on the results, we selected four minimum combinations of the duty ratio and frequency that did not exhibit any significant differences from continuous light (20%+512 Hz; 40% + 512 Hz; 60% + 256 Hz; 80%+128 Hz). The dark period duration (0.001367188 s) was determined via Formula 1 and was set as the experimental parameter. The combination of five dark period durations and four duty ratios was set as the central value and continuous light was used as the control for a total of 21 treatments (Table 2). The results were then used to select the dark period duration value, which was taken as the experimental parameter of the long-term light treatment of LED plants. With this as the central value, three dark period durations were determined to further explore the effects of different dark period durations on the growth and photosynthetic characteristics of lettuce (Table 3).

$$\text{暗期时长} = \frac{1 - \text{占空比}}{\text{频率}}$$

Formula 1

Table 1 Parameter design based on different duty ratios and frequencies.

DR	F/Hz													
20%														
40%														
60%	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192
80%														
100%														

Table 2 Parameter design of different duty ratios and dark period durations.

DPD/s	F/Hz	CK	0.000341797	0.000683594	0.001367188	0.002734375	0.00546875
D							
20%			2341	1170	585	293	146
40%		DR:100%, DPD:0	1755	878	439	219	110
60%			1170	585	293	146	73
80%			585	293	146	73	37

Table 3 Led long-term illumination parameter design.

DPD/s	F/Hz	CK	0.000046547(D ₁)	0.000465468(D ₂)	0.004654685(D ₃)
DR					
20%			17187	1719	172
40%		DR:100%, DPD:0	12890	1289	129
60%			8593	859	86
80%			4297	430	43

Determination method

LED short-term light treatment: The transplanted plants were placed in the light incubator. After 14 days, the plants were placed one by one under the LED supplementary light board, and the light sources with different frequencies and duty ratios were illuminated on the plants through the LED light source controller.

A li-6800 type photosynthetic apparatus and transparent leaf chamber (LI-COR, an American company) were used to record the P_n of the fourth leaf at the plant growing point under different pulse lights and the duty ratios of 20%, 40%, 60%, 80% and 100% for 10 min. After each combination was measured, the plants were returned to the continuous light of the same instantaneous intensity for 10 min prior to the next set. The measurement was repeated three times in each experiment. The environmental parameters of the photosynthetic apparatus were set as

follows: carbon dioxide concentration of $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; gas flow rate of $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; and air relative humidity of 55%-60%.

LED light for long-term processing: Following the plant transplant engraftment, the plants were placed under the LED for supplementary light for 21 days. The lamp panel instantaneous intensity was maintained consistent, while the average light intensity changed over the duty ratio. Tests were performed every 7 d via object platform height adjustments to ensure that the top leaves received the light intensity. On day 21, three plants were randomly selected from each treatment, and their plant height, maximum leaf length, leaf width, stem diameter, dry and fresh weight above and below ground were measured. The fourth functional leaf below the growing point was taken to measure the photosynthetic related indexes. Photosynthetic parameters were measured using a LI-6800 type photosynthetic apparatus and a fluorescent leaf chamber with a $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ light intensity. Other environmental parameters of the photosynthetic apparatus were consistent with the previous experiment.

Data Analysis

The data analysis and parameter estimations were performed using DPS (Data Processing System) 7.5, while IBM SPSS Statistics 22.0 was used to calculate the mean data values and determine significant differences using analysis of variance. Origin 2017 (origin lab) and Microsoft Excel 2010 (Microsoft Corporation) were employed to create the graphs and tables, respectively.

Results

Analysis of the Pn effect on lettuce leaves

Leaf Pn under different duty ratios and frequencies

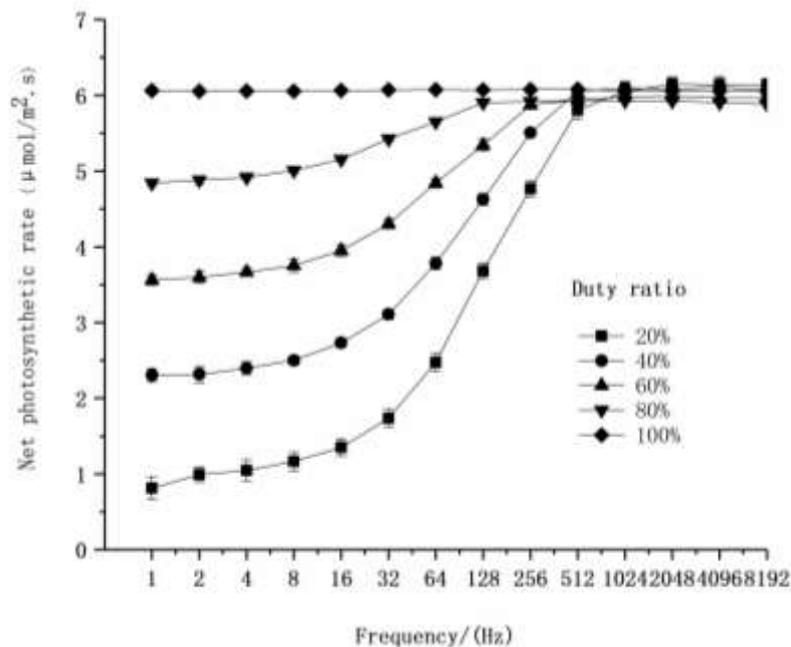


Fig. 2 Net photosynthetic rate under pulsed light with different frequencies and duty ratios.

Fig. 2 shows that at low frequencies, the Pn of lettuce leaves under pulse light is lower than that under continuous light. Furthermore, at the same frequency, the lettuce leaf Pn under the high duty ratio is consistently greater than that under the low duty ratio. Pn values under each duty ratio treatment are observed to increase with frequency and gradually stabilize to a level similar to that of continuous light. In addition, at higher duty ratios, the lettuce leaf Pn under pulse light reaches a stable state at a lower frequency. This suggests that the ratio of the frequency and duty ratio (i.e., dark period duration) may be the dominant influencing factor of the plant net photosynthetic rate.

Leaf Pn under different dark period duration and duty ratios

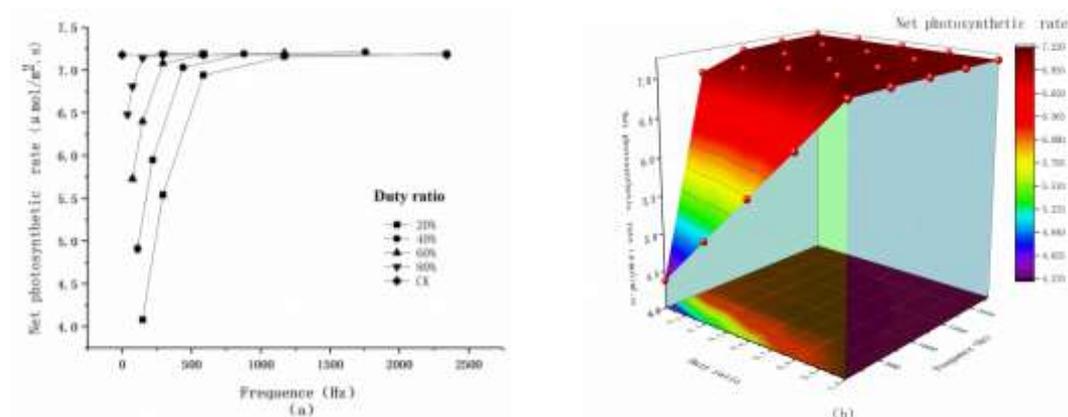


Fig. 3 Effects of coupling frequency with duty ratio and dark period duration on the net photosynthetic rate of lettuce. (a) Influence of duty ratio and frequency combination on Pn; (b) Influence of the interaction between the pulse optical frequency and duty ratio on Pb. A definite dark period duration can be obtained by combining the frequency and duty ratio.

Fig. 3 reveals that under the same duty ratio and at a low frequency, Pn increases significantly with frequency until the frequency reaches a certain value, whereby the growth amplitude of Pn drops and no further significant differences are observed with continuous light. When the frequency is fixed, an increase in the duty ratio induces the same trend in Pn values. No effects are observed between the different frequencies and the Pn of continuous light (Fig. 3a). When the frequency (duty ratio) increases, an increase in the duty ratio (frequency) will result in the gradual slowing down of the Pn growth trend, followed by stabilization (Fig. 3b).

Table 4 Net photosynthetic rate of lettuce under different treatments.

DR \ DPD/s	DPD/s					
	CK	0.000341797	0.000683594	0.001367188	0.002734375	0.00546875
20%	7.18±0a	7.19±0.024a	7.16±0.024a	6.94±0.013b	5.54±0.038c	4.08±0.017d
40%	7.18±0a	7.21±0.017a	7.19±0.018a	7.03±0.013b	5.95±0.028c	4.91±0.054d
60%	7.18±0a	7.2±0.005a	7.18±0.004a	7.08±0.003b	6.4±0.015c	5.73±0.036d
80%	7.18±0ab	7.2±0.022a	7.19±0.026ab	7.14±0.025b	6.81±0.013c	6.48±0.015d

Table 4 reports the significant difference analysis of lettuce Pn under different treatments. At the 20%, 40% and 60% duty ratios, the dark period duration is 0.000683594 s, and no significant differences are observed between the lettuce Pn and that under continuous light. At the 80% duty ratio, the dark period duration is 0.001367188 s. Although there is no significant difference between Pn and that of continuous light, it is significantly lower than that of 0.000341797 s; while when the dark period duration is 0.000683594s, there is no significant difference between Pn and that of 0.000341797 s. Therefore, 0.000683594 s is the optimal dark period duration amongst those tested, indicating that there is no significant difference between plant Pn and the continuous light treatment at this value. At high duty ratios and frequencies, the power consumption of the light source will increase, that is, the shorter the dark period, the more unfavorable the energy saving effect of the pulse light. Therefore, in order to further reduce the energy consumption and accurately calculate the specific value of the optimal dark period duration, we perform further analysis of the test data.

Model construction

Based on the Pn and dark period duration in Fig. 3, we selected the Unary nonlinear equation, with a similar change rule, in order to perform regression statistics on the photosynthetic data of leaves under varying duty ratios (20%, 40%, 60% and 80%) with different nonlinear equations of one variable (i.e., the relationship between dependent variables and independent variables is not linear). A total of 11 models with a high fitting degree were selected (Table 5).

Table 5 Statistical data of each model in the dark period duration and Pn regression calculations.

Model overview						
Non-linear model	20%			40%		
	R ²	F	P	R ²	F	P
Peal-Reed	1	5375.0884	0.0102	0.9998	1047.3859	0.0232
Polynomial Fitting	0.9987	523.4583	0.0019	0.9989	590.1411	0.0017
Yield Density	0.9803	74.5352	0.0028	0.9709	50.0897	0.005
Logistic	0.973	54.0575	0.0044	0.9667	43.5702	0.0061
Gompertz	0.9717	51.4942	0.0048	0.966	42.5906	0.0063
JohnsonSchumacher	0.9708	49.8702	0.005	0.9654	41.8978	0.0064
Hyperbola	0.9707	49.6399	0.005	0.9654	41.91	0.0064
Compartment Model	0.9705	21.9513	0.0439	0.9653	18.5611	0.0516
Bertalanffy	0.97	21.5809	0.0446	0.965	18.358	0.0521
Asymptotic	0.9692	47.1872	0.0054	0.9643	40.5275	0.0067
Richards	0.9557	14.3683	0.0658	0.9569	14.8002	0.064
Non-linear model	60%			80%		
	R ²	F	P	R ²	F	P
Peal-Reed	0.9998	1033.208	0.0233	0.9992	303.3707	0.043
Polynomial Fitting	0.999	634.3545	0.0016	0.9994	1130.1241	0.0009
Yield Density	0.9697	47.927	0.0053	0.9677	44.9495	0.0058
Logistic	0.9678	45.0708	0.0058	0.9669	43.7497	0.006
Gompertz	0.9675	44.6136	0.0059	0.9667	43.5898	0.0061
JohnsonSchumacher	0.9672	44.205	0.0059	0.9665	43.3366	0.0061
Hyperbola	0.9673	44.4094	0.0059	0.9669	43.7497	0.006
Compartment Model	0.9672	19.6675	0.0488	0.9667	19.3579	0.0495
Bertalanffy	0.9628	17.2441	0.0553	0.9637	17.7135	0.0539
Asymptotic	0.9662	42.8406	0.0062	0.9652	41.5989	0.0065
Richards	0.9619	16.8416	0.0566	0.9633	17.5223	0.0545

The R² values of all models is above 0.95, with the Peal-Reed and Polynomial Fitting models exhibiting the highest values. Larger F values indicate a more obvious effect (difference) between treatments; while smaller P values indicate a higher test accuracy. With the exception of the 80% duty ratio, $F_{\text{Peal-Reed}} > F_{\text{Polynomial Fitting}}$, while the Polynomial Fitting model exhibits the highest P value ($P < 0.01$). However, the P value of Peal-Reed is only at the significant level of $0.05 > P > 0.01$, indicating that this model is less accurate than other models.

Pn is observed to exhibit the same variation trend as the dark period duration across different duty ratios. In order to further verify the prediction performance of the Pn prediction model for unknown data, we selected the four most accurate models in terms of fitting degree. The actual and predicted Pn values were calculated with the Peal-Reed, Polynomial Fitting, Yield Density and Logistic models using the test data set (Fig. 4). The fitting result between the predicted and actual value of the Peal-Reed and Polynomial Fitting models is 1, with intercepts of

0.000003 and 0.00006, respectively. In comparison, the fitting degrees of the Yield Density and Logistic models are much lower.

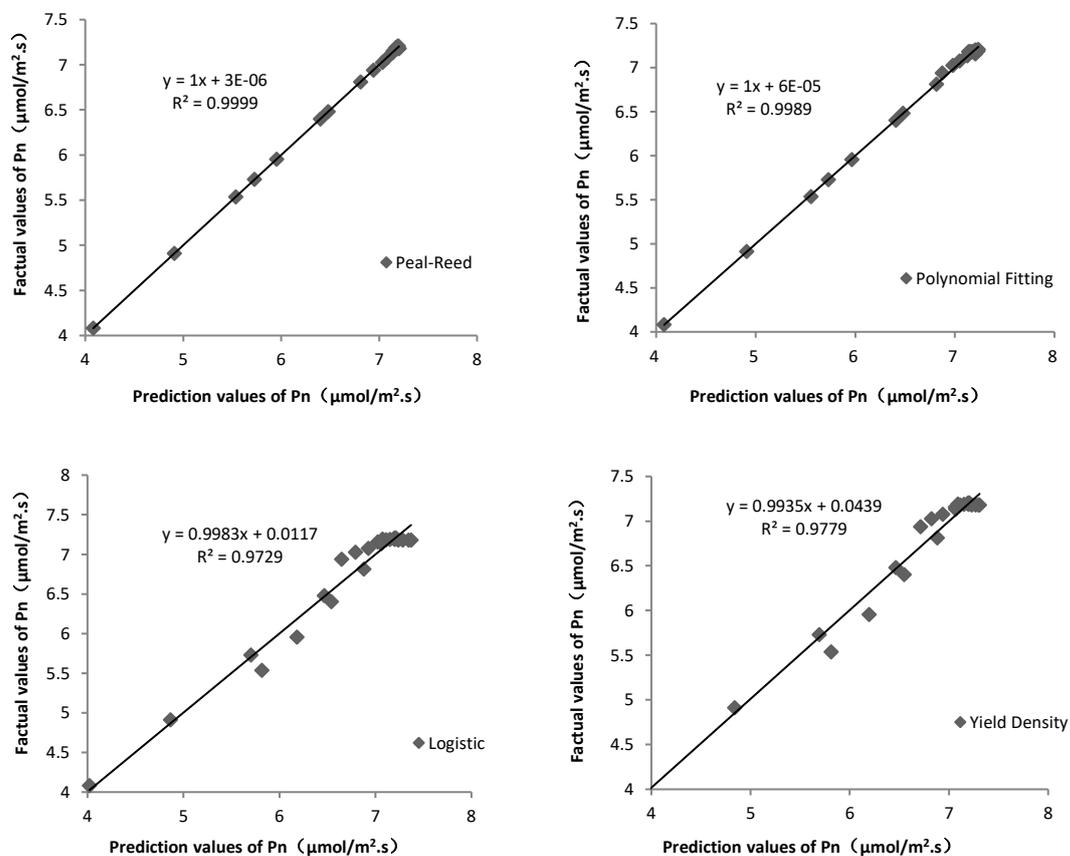


Fig. 4 Model fitting results.

The prediction results of the unknown data are highly correlated with the actual values. Our results reveal the high fitting degree of the Pn prediction model built with the Peal-Reed and Polynomial Fitting models, and is thus selected as the nonlinear foundation of the multivariate nonlinear model. **Error! Reference source not found.** reports the model parameters.

Table 6 Model parameters

Model	Duty ratio	Parameter				
		c ₁	c ₂	c ₃	c ₄	c ₅
Peal-Reed	20%	7.20180	0.00100	-3236.06020	480053.63450	-19925274.89120
	40%	7.20810	0.00060	-3382.14340	510345.38610	-20940211.71610
	60%	7.19990	0.00060	-2900.91180	377984.73940	-8846785.29720
	80%	7.19650	0.00040	-2612.35150	288710.13340	-8821.37210
Polynomial Fitting	20%	7.13853	455.68602	-571548.12257	70574028.97947	
	40%	7.15126	397.16277	-459758.80942	57087236.04810	
	60%	7.16324	239.54966	-285471.53674	35416218.57452	
	80%	7.17556	123.58461	-141394.18088	17467511.25099	

We assume that the parameters of the nonlinear models fitted with one variable under different duty ratios exhibit regular changes resulting from the duty ratio. Under this assumption, when the nonlinear equations of each duty ratio are synthesized into the multivariate nonlinear equation, the parameters can be replaced by the fitting equation of the duty ratio. Taking the model parameters in Table 6 as the dependent variable and the duty ratio as the independent variable, appropriate equations were selected for fitting (20%, 40%, 60%, 80% and 100% duty ratios were converted into 0.2, 0.4, 0.6, 0.8 and 1, respectively). Table 7 reports the optimal fitting equation.

Table 7 The fitting equation and goodness of fit parameter.

Model	ModelParameter	Fitting Equation	R ²	P
Peal-Reed	c ₁	$Y=1/(0.138976-0.000937*X+0.001170*X^2)$	0.7387	0.5112
	c ₂	$Y=1/(386.8101+3353.7542*X-1242.7235*X^2)$	0.9357	0.2535
	c ₃	$Y=-3077.6519-1540.3426*X+2716.5214*X^2$	0.9060	0.3066
	c ₄	$Y=441413.4216+394094.0269*X-747289.8687*X^2$	0.9309	0.2628
	c ₅	$Y=-18087029.0887-25596130.6883*X+61516215.5938*X^2$	0.9547	0.2128
Polynomial Fitting	c ₁	$Y=7.1264+0.061543X$	0.9999	0.0001
	c ₂	$Y=1/(0.003395-0.009738*X+0.018767*X^2)$	0.9982	0.0422
	c ₃	$Y=-730730.3875+732374.4305X$	0.9936	0.0032
	c ₄	$Y=90384019.7691-90495473.4892X$	0.9927	0.0037

The R² of all parameters in the model is above 0.99, and the fitting effect is significant. However, the fitting accuracy of the Peal-Reed model is poor. Thus, the Polynomial Fitting model is more suitable as the basic model

of this work. The model in Table 7 was substituted into the basic model to synthesize the multivariate nonlinear model using the following formula:

$$Y = \left(7.1264 + 0.061543X_1 + \frac{1}{0.003395 - 0.009738 \cdot X_1 + 0.018767X_1^2} * X_2 + \left((-730730.3875 + 732374.4305 * X_1) * X_2^2 \right) + (90384019.7691 - 90495473.4892 * X_1) * X_2^3 \right)$$

Formula 2

where Y is Pn; X₁ is the duty ratio; and X₂ is dark period duration. Fig. 5 compares the actual and predicted values Pn of the Polynomial Fitting-based multiple nonlinear regression model. The predictions of unknown data are highly correlated with the actual values. The results confirm the accuracy of the proposed Pn prediction model, as it can well reflect the relationship between the duty ratio of pulsed light, the dark period length and leaf Pn.

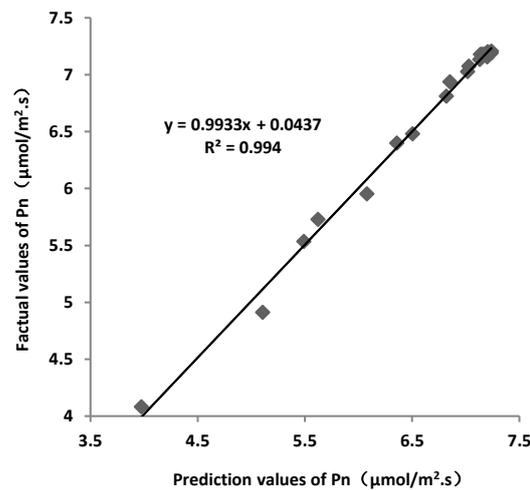


Fig. 5 Fitting results between the multivariate nonlinear model and measured data.

Analysis of marginal effect

The marginal photosynthetic rate can be used to determine the optimal parameters of each factor, as well as the influence of the parameter variations on Pn. The marginal function of the lettuce leaf Pn variations induced by the pulsed light duty ratio and the dark period length is obtained by differentiating X₁ and X₂ in Formula 2:

$$Y_{X_1}' : \frac{dY_1}{dX_1} = 0.061543 + \frac{X_2 \cdot (0.009738 - 0.037534X_1)}{(0.003395 - 0.009738 \cdot X_1 + 0.018767X_1^2)^2} + 732374.4305 * X_2^2 - 90495473.4892 * X_2^3$$

Formula 3

$$Y'_{X_2} \frac{dY_2}{dX_2} = \frac{1}{0.003395 - 0.009738 * X_1 + 0.018767 X_1^2} + (1464748.861 X_1 - 1461460.775) * X_2 + (271152059.3073 - 271486420.4676 X_1) * X_2^2$$

Formula 4

where Y'_{X_1} , Y'_{X_2} are the marginal functions of P_n to the duty ratio and dark period length, respectively. Fig. 6a and 6b plot Y'_{X_1} and Y'_{X_2} , respectively.

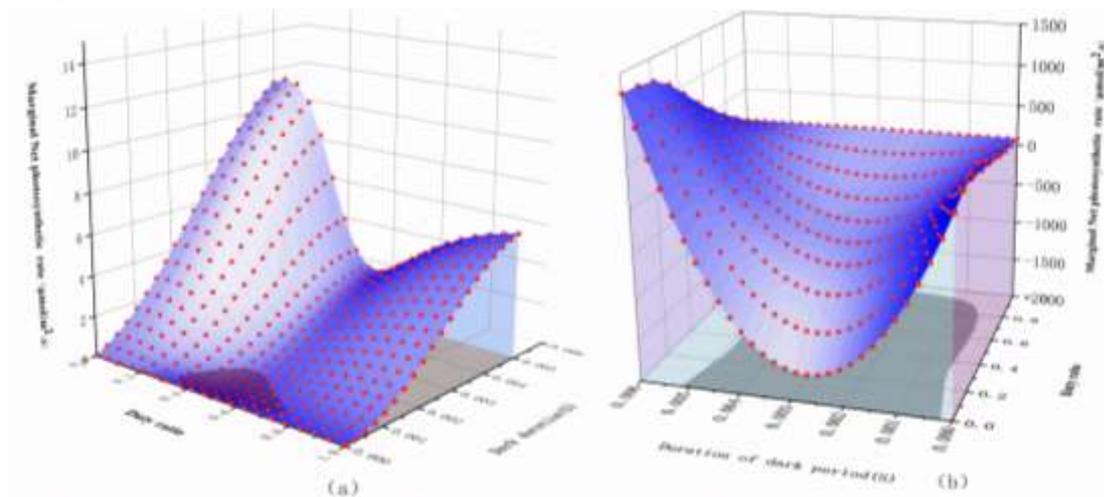


Fig. 6 Marginal net photosynthetic rate.

An ordinate greater (less) than zero indicates that the factors (duty ratio and dark period duration) promote (inhibit) the marginal photosynthetic rate. Fig. 6a reveals the marginal function to be constantly always greater than 0, indicating that the marginal net photosynthetic rate will increase with the duty ratio. Fig. 6b is a parabola with an upward opening, showing that as the dark period length increases, the photosynthetic rate is initially promoted, subsequently inhibited and promoted once again. Marginal function Y'_{X_2} is a quadratic with one variable and its original function has two inflection points. At $Y'_{X_2}=0$, the inflection points are equal to the intersection with the X-axis. We calculated the two inflection points U_1 and U_2 of the dark period length: the marginal photosynthetic rate was enhanced at the dark period length of $0-U_1$; when the dark period length was between U_1 and U_2 , the marginal photosynthetic rate was inhibited.

Dark period lengths longer than U_2 continued to promote the marginal photosynthetic rate. However, the actual measurements in this experiment reveal that for larger dark period lengths (i.e., low frequencies), the net

photosynthetic rate of plants decreased with the gradually increasing dark period length. As the dark period duration increases, the marginal photosynthetic rate should initially be promoted and subsequently inhibited, which is not consistent with the final stage of the marginal effect analysis. However, the research purpose of this experiment is to ensure that the Pn of lettuce leaves is not lower than that of the continuous light treatment by using pulse light treatment without affecting the normal growth of lettuce. Following an excessively long dark period duration, low frequencies reduce the Pn of the plants far lower than that of the continuous light treatment, which obviously fails to meet our purpose. Therefore, we omit the final stage analysis and only consider the first two stages in the subsequent sections, namely, the dark period duration is taken as $\in(0, U_2)$.

Formula 4 is used to determine U_1 and U_2 under different duty ratios, while the value of U_1 for the optimal dark period duration under different duty ratios is obtained from Table 8. After integration, the equation of the optimal dark period duration and duty ratio is as follows:

$$X_2 = 0.0071X_1^3 - 0.01X_1^2 + 0.0042X_1 - 0.00004, R^2 = 0.9617$$

Formula 5

where X_1 is the duty ratio; and X_2 is dark period duration. In the actual production process, it is only necessary to input the duty ratio based on Formula 5 to obtain the most optimal dark period duration and thus optimize the LED light supplement parameters. This provides a theoretical basis for the development of the most energy-saving and efficient plant growth light source under a combined frequency and duty ratio.

Table 8 U_1 and U_2 values under different duty ratios.

DR	U ₁	U ₂
10%	0.000309236	0.005079975
20%	0.000422456	0.004965994
30%	0.000498569	0.004888901
40%	0.000503447	0.004882716
50%	0.000466708	0.004917625
60%	0.000433278	0.004948307
70%	0.000431753	0.004945243
80%	0.000499879	0.004867912
90%	0.000843582	0.004496363

Analysis of the interaction effect between the duty ratio and dark period duration on the net photosynthetic rate of lettuce leaves

Fig. 7 reveals the influence of the interaction between the pulsed light duty ratio and dark period duration on Pn. At a constant dark period length, Pn will gradually increase and stabilizes with an increasing duty ratio. The longer the dark period, the larger the increase of Pn. When the duty ratio is constant, a reduction in the dark period length induces the same change trend on Pn. As the duty ratio gradually increases (dark period duration decreases), the changes in Pn resulting from the increased duty ratio (reduced dark period duration) become less obvious until no change occurs. Thus, the complete model of dark period duration, duty ratio and net photosynthetic rate is described as follows:

$$Y = \left(7.1264 + 0.061543X_1 + \frac{1}{0.003395 - 0.009738 \cdot X_1 + 0.018767X_1^2} * X_2 + ((-730730.3875 + 732374.4305 * X_1) * X_2^2) \right) + (90384019.7691 - 90495473.4892 * X_1) * X_2^3$$

$$0 < X_1 < 1, \quad 0 \leq X_2 < 0.005$$

The marginal function of the model reflects the change rule between Pn, the pulsed light duty ratio and dark period length. This is consistent with the measured value (Fig. 2), indicating that the model is highly accurate.

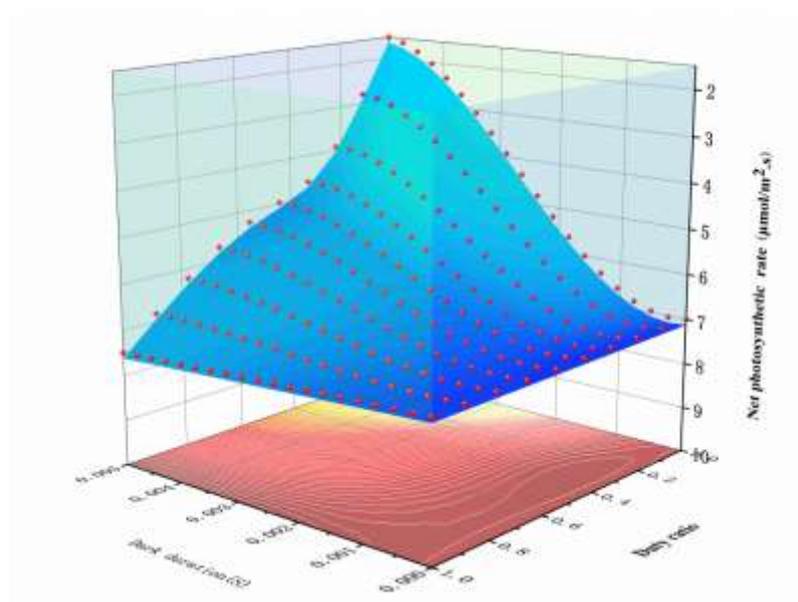


Fig. 7 Interactive effect of the duty ratio and duration of the dark period on the net photosynthetic rate.

Effects of long-term light tests on lettuce growth

We performed a long-term light treatment to validate the proposed model. Light supplement parameters were designed based on the results of the short-term pulsed light treatment. In particular, the plants were placed under a LED light supplement lamp board for a period of 21 days to explore the variations in plant apparent morphology and photosynthesis under different pulsed light conditions.

Effects of different pulsed light treatments on the appearance of lettuce

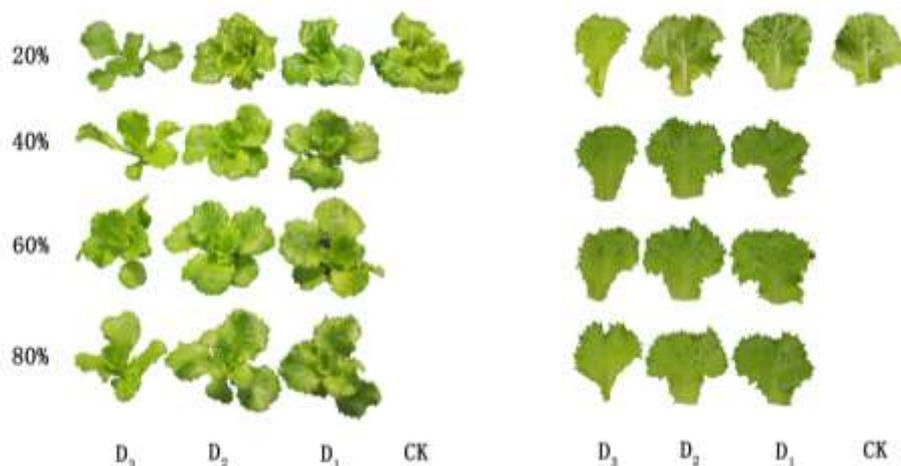


Fig. 8 Comparison of lettuce morphology under different treatments.

Unlike the other treatments, the leaves of plants in the high-dark period (D_3) treatment exhibited growth characteristics under weak light (e.g., excessive plant length, narrow and slender leaves, etc.) under all duty ratios. In the later growth period, the plant stems were not able to support the upright growth of plants in all directions, resulting in plant breakage. Under a short dark period duration, the roots and stems of the plants were stronger, the leaves were wider and thicker, the number of leaves was greater, the growth between true leaves was compact, and the overall growth of the plants was better. Compared with the short dark period treatment, no significant difference were observed in the appearance of plants under the D_1 , D_2 and CK treatments. In particular, plants of the short dark period treatment exhibited thicker roots and rhizomes compared to the D_3 treatment, and the plants maintained an upright and compact growth (Fig. 8).

Effects of pulse light under different dark period durations on lettuce growth

An increase in the dark period duration was observed to enhance the leaf length and plant height. Under low light conditions, the leaves appeared to be thinner. In contrast, the leaf width and dry and fresh weight of plants increased significantly during stages D_3 to D_2 with a reduction in the dark stage length, and subsequently stabilized after reaching critical point D_2 . Changes in the leaf width and dry and fresh weight were essentially equal under

different duty ratio conditions. At the dark period length of D₃, the leaf width and dry and fresh weight were at the lowest level and gradually increased as the dark period duration decreased. The leaf width and dry and fresh weight were maximized at dark period length D₂ and were slightly larger than those under D₁ and CK. Compared with CK, there was no significant difference among D₁, D₂ and CK, with the exception of the lowest duty ratio level of D₃ (Table 9). Furthermore, the stem diameter differences among treatments indicate plant growth to be most robust under D₂.

Table 9 Effects of duty ratio and dark period duration (s) on the growth of Hong Kong lettuce.

Duty ratio	Dark duration (S)	Plant Height (cm)	Leaf length (cm)	Leaf width (cm)	Stem diameter (mm)	Stem and leaf fresh mass (g)	root fresh mass (g)	stem and leaf dry mass (g)	root dry mass (g)
20%	D ₁	13.98±0.75bc	13.37±0.28b	12.53±0.38a	10.30±0.32a	23.43±0.61a	4.85±0.14a	1.81±0.28a	0.33±0.05a
	D ₂	13.58±0.69c	13.03±0.27b	12.74±0.18a	10.39±0.05a	23.99±0.16a	4.95±0.23a	1.84±0.21a	0.36±0.09a
	D ₃	15.99±0.30a	15.85±0.51a	10.06±0.32b	6.11±0.35d	13.74±0.87c	1.75±0.19c	0.79±0.09b	0.10±0.02b
40%	D ₁	13.41±0.20c	13.34±0.19b	12.40±0.35a	10.28±0.17a	23.72±0.45a	4.81±0.12a	1.82±0.17a	0.32±0.05a
	D ₂	13.54±0.28c	13.20±0.15b	12.48±0.11a	10.91±0.28a	24.32±0.43a	4.97±0.28a	1.90±0.30a	0.34±0.03a
	D ₃	15.21±0.39ab	15.00±0.29a	10.62±0.15b	6.86±0.20cd	15.25±0.19bc	2.21±0.36c	0.84±0.08b	0.14±0.01b
60%	D ₁	13.75±0.19c	13.29±0.17b	12.26±0.08a	10.07±0.12a	23.16±0.29a	4.79±0.09a	1.82±0.30a	0.34±0.06a
	D ₂	13.70±0.29c	13.13±0.25b	12.37±0.10a	10.29±0.51a	23.43±0.61a	4.98±0.10a	1.92±0.28a	0.34±0.03a
	D ₃	15.59±0.13a	15.27±0.35a	10.41±0.06b	7.71±0.20bc	16.19±0.56b	3.20±0.16b	0.93±0.07b	0.17±0.04b
80%	D ₁	13.71±0.34c	13.43±0.14b	12.14±0.23a	10.18±0.33a	23.04±0.34a	4.94±0.13a	1.80±0.16a	0.32±0.02a
	D ₂	13.68±0.27c	13.30±0.37b	12.22±0.17a	10.30±0.37a	23.46±0.54a	5.04±0.27a	1.84±0.30a	0.36±0.05a
	D ₃	13.78±0.76c	15.17±0.34a	10.21±0.71b	8.06±0.44b	16.13±2.21b	2.95±0.18b	1.03±0.11b	0.17±0.01b
CK	0	13.91±0.31bc	13.53±0.16b	12.09±0.33a	9.96±0.05a	23.25±0.20a	4.77±0.40a	1.80±0.16a	0.31±0.02a

Effects of pulse light under different dark period durations on the photosynthetic characteristics of lettuce

The P_n of glass lettuce gradually increased as the dark period duration decreased until saturation was reached. No significant differences were observed between the P_n of D₁, D₂ and the continuous light treatment (CK), while D₃ P_n values were significantly lower. In addition, the parameters of D₂ were slightly higher than those of the other treatments. The overall performances of the treatments were observed as D₂>D₁>CK>D₃ across all duty

ratios. There were no significant differences in intercellular carbon dioxide concentrations among treatments.

Transpiration rate and stomatal conductance showed no significant difference among D₁, D₂ and CK treatments

except that the D₂ treatment was significantly higher than the control when the duty ratio was 20%. Furthermore,

the plants of D₃ presented the lowest transpiration rate under any duty ratio as well as significant differences with

other dark ratio treatments (Table 10).

Table 10 Effects of different treatments on the photosynthetic parameters of leaves

Duty ratio	Dark duration (S)	T _r	Pn	C _i	G _s
20%	D ₁	4.65±0.97ab	12.66±0.67a	308.46±17.43a	0.34±0.08ab
	D ₂	5.74±0.21a	12.93±1.27a	320.43±6.98a	0.43±0.03a
	D ₃	0.90±0.01f	3.09±0.01c	322.39±12.56a	0.05±0.00e
40%	D ₁	4.83±0.86ab	13.27±0.55a	314.93±20.68a	0.35±0.07ab
	D ₂	5.30±0.18ab	13.83±0.26a	321.33±2.87a	0.35±0.02ab
	D ₃	1.42±0.02ef	5.03±0.04c	322.38±0.94a	0.09±0.00de
60%	D ₁	5.09±0.42ab	13.37±1.20a	320.95±4.82a	0.37±0.05ab
	D ₂	5.35±0.06ab	13.99±0.08a	325.67±2.02a	0.38±0.01ab
	D ₃	2.36±0.77de	7.74±0.64b	316.19±9.60a	0.16±0.06cde
80%	D ₁	5.34±0.08ab	13.35±0.02a	322.34±1.13a	0.35±0.01ab
	D ₂	5.37±0.33ab	14.43±1.54a	319.76±10.11a	0.38±0.04ab
	D ₃	3.15±0.10cd	9.40±0.27b	307.08±12.75a	0.21±0.01cd
CK	0	3.82±0.46bc	12.90±0.62a	301.06±18.40a	0.26±0.04bc

Discussion

LED light is closely related to photosynthesis and the light absorption, transformation and dissipation of plants[26-28]. In order to maximize the utilization of light and avoid the adverse effects of high/low light on plant growth and development, it is necessary to determine how to improve the optimal pulse light parameters in a controlled environment[29, 30]. Pulsed light is generally characterized by three parameters: average optical flux density (PPFD), frequency and duty ratio. The increase of Pn with the duty ratio is generally due to the characteristics of the pulsed light itself, in particular, PAR (photosynthetically active radiation) is proportional to

the duty ratio. While the response between frequency and P_n is related to the electron transport and energy conversion process in photosynthesis[31, 32]. Our results reveal that the frequency and duty ratio of pulsed light can affect the P_n of the blade under light. Under the same duty ratio, the P_n of the blade gradually increases with the frequency and tends to saturation, and no significant differences are observed with the continuous light at the later stage. This can be explained by the following. A photosynthetic intermediate buffer pool (PIs pool) exists in leaves, which can accumulate a certain amount of photosynthetic intermediate during the application of the light and maintains a continued carbon assimilation process when the light stops. However, the assimilation rate will gradually decrease with time, which may be related to the consumption and regenerated of the RuBP (Ribulose-1,5- biphosphate), ATP (Adenosine triphosphate) and NADPH (Nicotinamide adenine dinucleotide phosphate) supplies[33-35].

The majority of available information on the light-dark cycle (L/D) process of high-frequency pulsed light has been accumulated in historical experiments and greatly contributes to our current understanding of the photosynthetic intermediate pool. Warburg (1919) was able to increase the continuous light yield by 300-400% by shortening the light and darkness durations, suggesting that the photoperiod was too long to achieve a maximum efficiency. He also proposed that extending the dark cycle may increase the yield allowing for enough time for all intermediates formed in each flash to be removed before the next[36]. Kok (1956) estimated the maximum possible number of photosynthetic intermediates (pool size) for dark reactions by measuring the P_n of green microalgae under pulsed light[37]. In addition, Yustinadiar used the optimal light/dark cycle method to determine that the 45:15 min (light: dark) cycle significantly enhanced *Nanocloranopsis thaliana* yields[38]. Chen (YEAR) revealed intermittent light to be more suitable for plant growth compared to continuous light, and the lower light/dark cycle L/D) may lead to higher biomass productivity[39]. Zarmi (YEAR) also proposed that the photon efficiency of algae photosynthesis can be increased by 3 to 10 times compared with continuous light when pulse

light is correctly applied. The author also explained how the photonic efficiency enhancement depends on the optical pulse and the photon flux density of time scales. More specifically, the key is through the correct timing of the algae cell light/dark cycle to avoid photosynthetic pathway jams[40]. The results from the previous literature explain the relationship between leaf photosynthesis and the L/D cycle in the light pulse process by considering the PIs pool. Moreover, our results show that the higher the pulse light frequency, the shorter the stop time of light in a cycle, and the lower the degree of influence on Pn. This indicates the strong influence of a suitable dark period value on the photosynthetic morphogenesis of plants.

At present, the application of pulsed light in plants is rarely studied, and the majority of related work is based on algae research. In addition, reports on the dynamic response mechanism of plants under pulsed light are limited, and only focus on plant morphology, photosynthesis and other aspects of the surface, while the influence mechanism on plant physiology and photosynthesis remains at the theoretical stage. The innovation of the current paper lies in the integration of pulse light parameters, as well as the analysis of the effects of the duty ratio and frequency on Pn. Moreover, we introduced the dark period duration and proposed and verified the concept that "it is not frequency and duty ratio but their ratio (dark period duration value) that determines the net photosynthetic rate of plants". In practical applications of plant research, only the optimal dark period length can be used to select the most appropriate duty ratio and frequency, which can reduce energy consumption under the condition of ensuring the normal growth of plants.

In this study, we established a nonlinear model among the duty ratio of pulsed light, the length of the dark period and the Pn of the blade via a mathematical model. R^2 values of the simulations exceeded 0.99. All model parameters were significant, indicating that the assumptions before the model establishment were accurate and the model and optimization process were feasible. The relative error in the verification process was small, further highlighting the high accuracy of the model (Fig. 5) and proving its ability to effectively simulate the Pn of lettuce

leaves under different duty ratios of pulse light and dark periods. The model is relatively simple and only requires a few parameters. In practical applications, the model parameter values can be estimated using a small amount of data, allowing the P_n of blades under all pulsed light in the defined domain to be simulated. Following the derivation of the model, the limit value of P_n under different duty ratios and the corresponding optimal dark period duration can also be obtained. This provides a theoretical basis for the optimization of LED light filling parameters in the actual production.

In order to better utilize light in plant cultivation and optimize the photosynthetic process, it is necessary to synchronize the light (photiod) and dark (dark cycle) times of plants when using pulsed light technology. This study analyzed the effects of different pulsed light on lettuce in terms of the dark period duration and the physiological and photosynthetic morphological indexes of plants. The results reveal a more consistent effect in the dark period duration, irrespective of the short- and long-term pulse treatments. Plant matter accumulation is generally influenced by photosynthesis. Under different duty ratios, the photosynthetic level of pulsed light was similar to or even higher than that of continuous light following a reduced dark period duration. Thus, an appropriate shortening of the dark period duration can also achieve higher yields, which is consistent with the results of this experiment.

Compared with continuous light, pulsed light reduces the average PAR and light energy absorbed by the blade, and theoretically has a positive effect on reducing the heat dissipation of light energy. This can consequently reduce the photoinhibition and improve the efficiency of light energy use[41]. It is imperative to optimize plant growth under a pulsed light supplement and reduce the power costs for efficient indoor production of high-quality crops throughout the year. Tennessen et al. (YEAR) demonstrated that photons in pulses of 100 ps or shorter were absorbed and stored in the reaction center, and that pigments used in the dark ages to transport electrons to the lutein cycle were not affected by the lutein cycle. This indicates that the application of efficiently calculated

intermittent light can reduce energy consumption[42]. Song (year) also suggested that the implementation of a short-interval pulsed light strategy may reduce the energy requirements of growing crops in artificially lit environments[43]. With green and red skirt as the research object, Cho showed that LED pulse light can improve LUE and save energy more effectively compared with continuous light[44]. In the actual production process, although the combination of high frequency and duty ratio is more conducive to the utilization of crop light energy, the power consumption of a light supplement lamp will also increase to some extent[45]. Therefore, under the condition of high yielding plants (i.e., the P_n of plants under pulse light condition is not lower than continuous light), the effect of low consumption, high efficiency and energy saving can be achieved only by determining the optimal dark period duration.

Finally, it's a bit of a shame though our results reveal that P_n has a strong regularity for different pulse light environmental conditions, the proposed model is established on the basis of fixed environmental conditions (light intensity, temperature, humidity, gas flow rate) and a single plant. Whether the model adapt to different environmental conditions and response rules between the pulse light duty ratio, dark period and P_n of different plants remains to be verified. Future research will focus on the application of pulsed light in plant research, and the dark period will become a new research area of the effects of pulsed light on plant growth and development. More attention should be focused on different facilities (e.g., light intensity, CO₂, temperature, humidity, etc.) to continuously optimize the model. "Low consumption, high efficiency" is also a research hotspot in the field of plant factory production, as well as the application of light environments in agriculture facilities.

Conclusion

In the current study, according to Formula 1, any combination of duty ratio and frequency can obtain the corresponding dark period duration value. The lettuce leaf P_n under each duty ratio increases with the frequency

and gradually stabilizes to a level similar to that of continuous light. At higher duty ratios, the lettuce leaf Pn under pulse light reaches a stable state at a lower frequency. This indicates that the determining factor of the net photosynthetic rate may not be frequency and duty ratio, but rather their ratio, namely, the dark period duration.

Compared with continuous light, pulsed light is more beneficial to plant growth and utilization. In this study, the response relationship between the pulse light parameters (duty ratio and frequency) and Pn was used to elicit the dark period duration. The experiment was designed and the response model between the dark period duration and Pn of lettuce leaves was constructed based on the test results. Finally, the model was verified. Through the derivation of the model, the fitting equation between the best dark period duration and duty ratio was established, which provided the basis for exploring the best pulse light suitable for lettuce growth. This allows plant factories and facilities to select high efficiency and energy saving artificial light sources.

Supplementary information

Definitions of the abbreviations

DR: Duty Ratio; DPD: Dark period duration; F: Frequency; CL: Continuous light; Pn: Net Photosynthetic Rate; L/D: Period of light/dark; PPFD: Photosynthetic photon flux density

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YHB conceived and designed research, conducted experiments, analyzed data, and wrote manuscripts.

MST conception and design research, part of the experiment.

XXD, MLY, ZGZ, BYH, LY, WYJ revised the paper. All the authors have read and approved the manuscript.

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