

OPTIMAL LIGHT INTENSITY AND PHOTOPERIOD FOR THE GROWTH AND QUALITY OF *PLATOSTOMA PALUSTRE* IN A PLANT FACTORY

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ABSTRACT

Platostoma palustre (Blume) A. J. Paton is an important medicinal and edible plant in China and Southeast Asian countries with great market and economic value. In recent years, due to the high labor cost of cultivation and management of *P. palustre* and limited economic benefits, domestic farmers have been hesitant to plant it. This has led to an inadequate supply of raw materials for *P. palustre* in China, necessitating the import of large quantities from Southeast Asian countries. Consequently, alternative methods for planting and cultivating *P. palustre* beyond traditional field practices are needed. In this study, a hydroponic experiment was conducted to investigate suitable lighting conditions for *P. palustre* growth in a plant factory. The experiment involved a two-factor, four-level design incorporating red light intensities of 50, 100, 150, 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ and photoperiods of 8h/16h, 12h/12h, 16h/8h, 20h/4h Light/Darkness (L/D). The results showed that increasing red light intensity and duration promoted the whole plant weight and stem diameter of *P. palustre* to some extent, while extending light duration was beneficial for the leaf area. Light intensities ranging from 50 to 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ combined with a 20h/4h L/D photoperiod favored the accumulation of total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid. Prolonged photoperiods and higher light intensities facilitated the root growth of *P. palustre*. Light intensities between 100 and 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ with an 8h/16h L/D photoperiod were helpful for the accumulation of total pectin and soluble sugar content. Membership function analysis indicated that the T12 treatment (150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity and 20h/4h L/D photoperiod) was more suitable for the cultivation and quality production of *P. palustre* in the plant factory. The current study provided scientific data for plant factory or indoor large-scale cultivation and planting of *P. palustre*.

Keywords: *p. palustre*, light intensity, photoperiod, growth, quality

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INTRODUCTION

Platostoma palustre (Blume) A. J. Paton, also known as Liangfencao or Xiancao in Chinese, is an herbaceous plant of the genus *Platostoma* in the Lamiaceae family (Tang *et al.*, 2020, 2021, 2023a). It is a significant medicinal and edible plant, distributed in Guangxi, Taiwan, Guangdong, Zhejiang, Jiangxi, and other regions in China (Tang *et al.*, 2022a, 2022b; Quan *et al.*, 2022). *P. palustre* primarily comprises polysaccharides, phenolic acid, flavonoids, terpenoids, amino acids, etc, with high nutritional value and potential medical healthcare benefits (Tang *et al.*, 2022c, 2023b). Among them, polysaccharides are high in content and important bioactive components in *P. palustre*, with physiological functions such as antioxidant (He *et al.*,

2020), anti-hepatic injury (Wang *et al.*, 2019), and hypolipidemic (Wei, 2017), while the flavonoid content accounts for 5.47%-6.21% of the whole herb composition (Zhang and Xie, 2012), with antioxidant (Wei *et al.*, 2018) and anti-aging effects. It is mainly used for processing and making products such as bean jelly and herbal tea, which are popular among the public and have a broad market prospect.

In recent years, due to the development of China's economy and society and the decrease in the number of people engaged in crop farming, more efficient cultivation and management techniques are required for growing *P. palustre*. Due to the benefits of cultivation are not attractive enough, people are reluctant to engage in *P. palustre* cultivation, resulting in an inadequate supply of *P. palustre* within China, thereby

necessitating the importation of significant quantities of raw *P. palustre* materials from Southeast Asian nations (Tang *et al.*, 2022d). Plant factory or indoor cultivation may be a new direction in addition to traditional field farming (Tang *et al.*, 2021). *P. palustre* is a perennial plant, under the specific conditions of the plant factory, it could theoretically grow continuously and also be harvested multiple times. Therefore, using plant factories or indoor cultivation technology to research precision cultivation and targeted enrichment of *P. palustre* may have a positive role in promoting the development of *P. palustre* industry.

In a plant factory or indoor cultivation system, the environmental factors, such as illumination conditions, temperature, humidity, and nutrition supply can be controlled by electric-based equipment (Zha and Liu, 2018; Tang *et al.*, 2021). Light, as one of the basic elements for plant growth and development, can be greatly influenced by factors like light quality, intensity, and duration (Zhang *et al.*, 2018). Our previous study has shown that red light has a promoting effect on the morphogenesis of the root system and the growth and development of *P. palustre* plants (Tang *et al.*, 2021). However, the impact of varying red-light intensities and photoperiods on the growth and quality characteristics of *P. palustre* remains unexplored.

To further study the influence of the red-light intensities and photoperiods on the growth and quality characteristics of *P. palustre*, we adopted a complete combination of 2 factors and 4 levels, with the red light intensities of 50, 100, 150, 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ and the photoperiods of 8h/16h, 12h/12h, 16h/8h, and 20h/4h Light/Darkness (L/D), respectively. The growth, biochemical, and quality indicators were also measured.

This study provides scientific data for plant factory or indoor large-scale cultivation and planting of *P. palustre*.

MATERIALS AND METHODS

Preparation of plant materials: The robust and pest-free branches (length about 10 cm) with terminal buds from the mother plant of *P. palustre* were used as cuttings, and 4-6 leaves were retained in the upper part. The cuttings were fixed and placed on a floating board with a length of 30 cm, a width of 23 cm, and 20 holes. The fixed floating boards were placed in a plastic box for hydroponics. The hydroponic experiments were carried out in August 2022, in the plant factory within the Guangxi Botanical Garden of Medicinal Plants (Nanning, China). Before the cuttings took root, they were cultured in tap water. After 3 days, the cuttings took root and were cultured in nutrient solution [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, KNO_3 , $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, H_3BO_3 , $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $(\text{NH}_4)_6\text{Mo}_7\text{O} \cdot 4\text{H}_2\text{O}$]. The nutrient solution was changed every three days.

Experimental design: LED lights were employed for lighting treatment, with the red-light intensity of 50, 100, 150, 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ and the photoperiod of 8h/16h, 12h/12h, 16h/8h, and 20h/4h Light/Darkness (L/D), respectively. A complete combination of 2 factors and 4 levels was adopted in this experiment, with a total of 16 treatments (Table 1). Each treatment contained 20 cuttings. The experiment was conducted at a plant factory. The indoor temperature of the plant factory was 25 °C, the air humidity was 65%, and the CO_2 concentration was 400 $\mu\text{mol}/\text{mol}$.

Table 1. Experimental treatment settings for red light intensity and photoperiod.

Treatment	Light intensity	Photoperiod	Treatment	Light intensity	Photoperiod
T1	50	8/16	T9	150	8/16
T2	50	12/12	T10	150	12/12
T3	50	16/8	T11	150	16/8
T4	50	20/4	T12	150	20/4
T5	100	8/16	T13	200	8/16
T6	100	12/12	T14	200	12/12
T7	100	16/8	T15	200	16/8
T8	100	20/4	T16	200	20/4

The unit of light intensity: $\mu\text{mol}/(\text{m}^2\cdot\text{s})$; The unit of photoperiod: h/h light/darkness. N=20

Collection of agronomic trait data: After 30 days of experimental treatments, 10 plants with consistent growth were selected, and the height and stem diameter were accurately measured employing a Vernier caliper. The fresh weight of each plant was determined using an electronic balance. Leaf area measurements were conducted using the YMJ-B leaf area measuring

instrument (<http://www.tpykj.net/about/index/32.html>). We selected 5 plants with consistent root growth, cut the roots from the plants, wiped dry the roots with tissues, and measured the fresh weight of the roots using an electronic balance. Meanwhile, the root length, average root diameter, root surface area, and root volume were

measured and analyzed using a plant image analyzer system.

Determination of photosynthetic pigment content: The photosynthetic pigment was measured by 95% ethanol extraction method. The 3rd pair of fresh leaves were selected from the terminal bud downwards. The leaves from different plants were collected with a puncher ($\phi 6$ mm) and quickly put into a centrifuge tube containing 5 ml of 95% ethanol, covered tightly with 5 replications. All leaf samples were kept in the dark for 24 hours. After the leaves completely turned white, the measurements were carried out using a UV spectrophotometer at 665, 649, and 470 nm. The absorbance values (A) were recorded, meanwhile, the Chlorophyll a concentration (C_a), Chlorophyll b concentration (C_b), Carotenoid concentration (C_c), Chlorophyll a content (T_a), Chlorophyll b content (T_b), Total chlorophyll content (T_{a+b}), and Carotenoid content (T_c) were calculated according to the formula.

Chlorophyll a concentration (C_a) ($\text{mg}\cdot\text{L}^{-1}$)
 $=13.95\times A_{665}-6.88\times A_{649}$;

Chlorophyll b concentration (C_b) ($\text{mg}\cdot\text{L}^{-1}$)
 $=24.96\times A_{649}-7.32\times A_{665}$;

Carotenoid concentration (C_c) ($\text{mg}\cdot\text{L}^{-1}$) $=\frac{1000\times A_{470}-2.05\times C_a-114.8\times C_b}{245}$;

Chlorophyll a content (T_a)
 $(\text{mg}/\text{mm}^2)=C_a\times V_T\div(S\times 1000)$;

Chlorophyll b content (T_b)
 $(\text{mg}/\text{mm}^2)=C_b\times V_T\div(S\times 1000)$;

Carotenoid content (T_c)
 $(\text{mg}/\text{mm}^2)=C_c\times V_T\div(S\times 1000)$;

Total chlorophyll content (T_{a+b}) ($\text{mg}\cdot\text{L}^{-1}$) $=T_a+T_b$;

V_T represents the total volume of the extraction solution (ml), and S represents the leaf area (mm^2).

Determination of quality index: The plants with consistent growth were selected and the leaves were selected from the 3rd-4th pair of fresh leaves from the terminal bud downwards. Leaf samples were cryopreserved using liquid nitrogen and stored in a -80 °C ultra-low temperature refrigerator for subsequent indicator determination.

Determination of total pectin content: The 3rd pair of fresh leaves was selected from the terminal bud downwards and then frozen in liquid nitrogen immediately. 0.1g of the frozen leaves were weighted and the carbazole colorimetric method (Kyriakidis *et al.*, 2001) was used to determine the total pectin content according to the instruction of the total pectin content Kit (G0717F) (<http://geruisi-bio.com/>), with 5 replications. In brief, the samples were ground and mixed with 1.5 mL 80% ethanol. Then, the leaf homogenates were put into a water bath at 85 °C for 10 min, and subsequently centrifugated at 8000 rpm for 10 min to get the precipitates. Repeat the above steps to get the purified

precipitates. After mixed with 1 mL extraction solution, the solutions were water-bathed at 95 °C for 60 min. When cooling down, the solutions were centrifugated at 8000 rpm for 10 min and prepared to determine the total pectin content.

Determination of soluble sugar: The 3rd pair of fresh leaves was selected from the terminal bud downwards and then frozen with liquid nitrogen immediately. 0.1g of the frozen leaves were weighted and the anthrone colorimetric method (Zhang *et al.*, 2020) was employed to determine the soluble sugar content according to the instructions of the soluble sugar content Kit (G0501F) (<http://geruisi-bio.com/>), with 5 replications. In brief, the samples were ground and mixed with 0.8 mL 80% ethanol. Subsequently, the homogenates were put into a water bath at 50 °C for 20 min and then centrifugated at a speed of 12000 rpm for 10 min to get the supernatants (prepared for determination).

Total pectin content (mg/g) $=\frac{0.37\times(A_{\text{sample}}-A_{\text{ck}}+0.0141)}{W}$;

Soluble sugar content (mg/g) $=\frac{0.41\times(A_{\text{sample}}-A_{\text{ck}}-0.0203)}{W}$;

A represents the absorbance value, and W represents the sample weight, g.

Data analysis: The Office and SPSS 13.0 software were used for one-way analysis of variance (ANOVA), correlation analysis, and membership function analysis, and graphs were prepared using GraphPad Prism 5 software. Significant difference is at ($P \leq 0.05$) and ($P \leq 0.01$) (One-way ANOVA, Duncan test).

RESULTS

Effects of different red light intensities and photoperiods on the growth of *P. palustre*: It could be seen from Figure 1A, under the treatments of 12h/12h L/D photoperiod at 100 and 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity and 16h/8h L/D photoperiod at 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity, the plant height was significantly higher than the other treatments ($P \leq 0.05$) with an increase of 11.23-43.60%, and there was no significant difference in plant height among the other treatments ($P \geq 0.05$). At the same light intensity and photoperiod, the whole plant weight tended to increase by extending the light duration and increasing the light intensity (Figure 1B). At 20h/4h L/D photoperiod, the 100, 150, and 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity treatments and the 16h/8h L/D photoperiod treatment with 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity showed no significant difference in total plant weight but were significantly higher than the other treatments. Under the same light intensity, the total plant weight under a 20h/4h L/D photoperiod increased by approximately 71.95-132.62% compared to an 8h/16h L/D photoperiod. As shown in Figure 1C, under the same light intensity, the

number of leaves seemingly showed a trend of first increasing and then decreasing with the increase of photoperiod, reaching the highest at 16h/8h L/D photoperiod. The stem diameter almost showed an increasing trend with the extension of the photoperiod at the same light intensity (Figure 1D). The stems were thickest under the treatment of 20h/4h L/D photoperiod at

200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity, which was significantly higher than the other treatments ($P \leq 0.05$) and increased by 22.58%-90.44%. Under the 50, 100, and 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity, the leaf area showed an upward trend with the increase of photoperiod, while under the 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity, the leaf area seemed to increase and then decrease (Figure 1E).

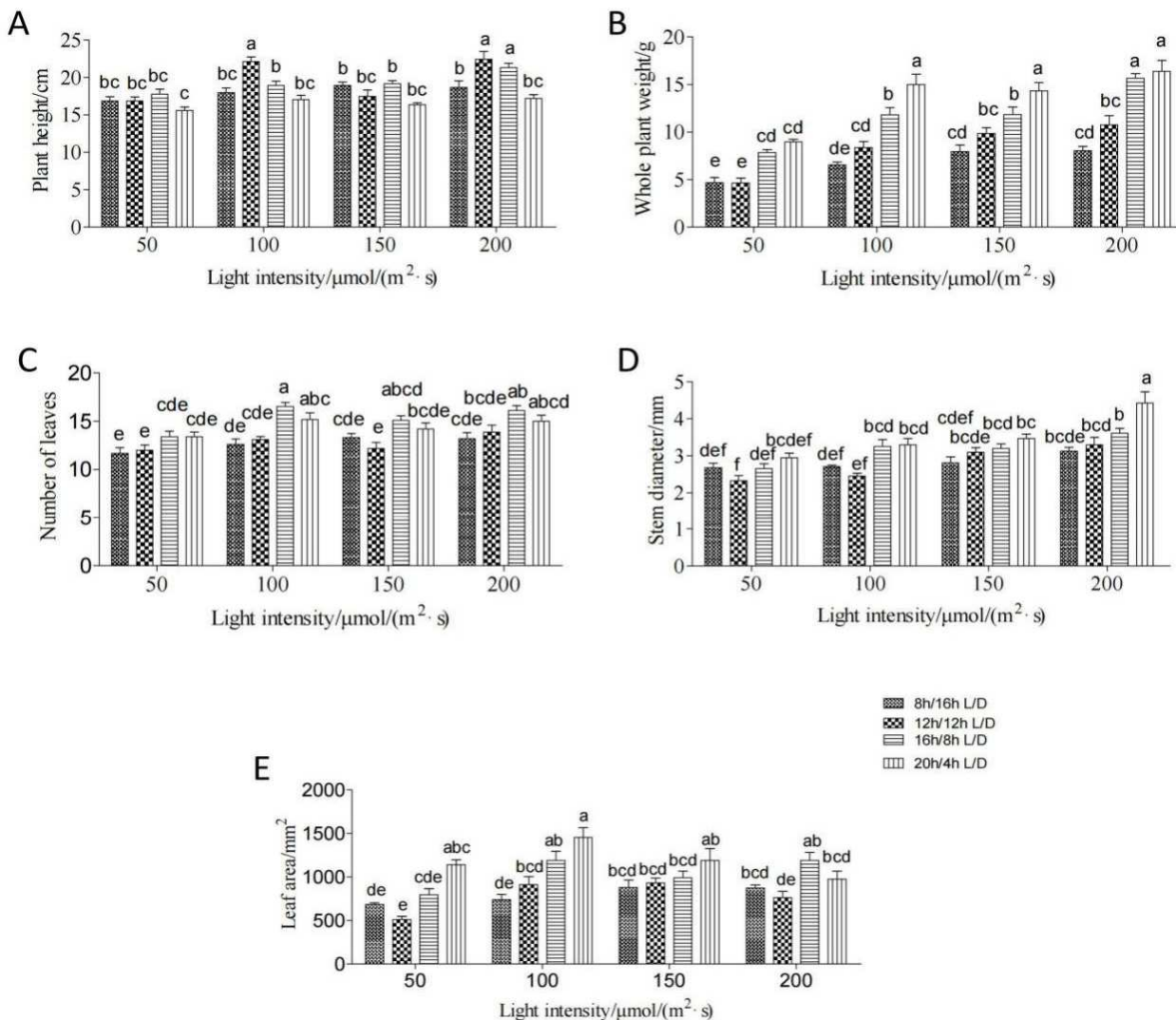


Fig. 1: Plant height, whole plant weight, number of leaves, stem diameter, and leaf area (A-E) under different red-light intensity and photoperiod conditions, respectively. Different letters indicated the significant differences ($P \leq 0.05$).

Effects of different red light intensities and photoperiods on the photosynthetic pigments of *P. palustre*: Under 50-150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity and 20h/4h L/D photoperiod conditions, the highest contents of the chlorophyll a (307.58 mg/mm^2) (Figure 2A), chlorophyll b (143.36 mg/mm^2) (Figure 2B), and total chlorophyll (Figure 2D) were obtained, meanwhile, the highest carotenoids content was observed at 50, 100, and 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity under 20h/4h L/D

photoperiod (39.25, 38.19, and 45.07 mg/mm^2 , respectively) (Figure 2C). In particular, the differences in chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll content were not significant among the four photoperiod treatments at 200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity ($P \geq 0.05$), but all were significantly lower than the treatments at 50, 100, and 150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity with 20h/4h L/D photoperiod ($P \leq 0.05$) with a decrease of 10.64-44.24%.

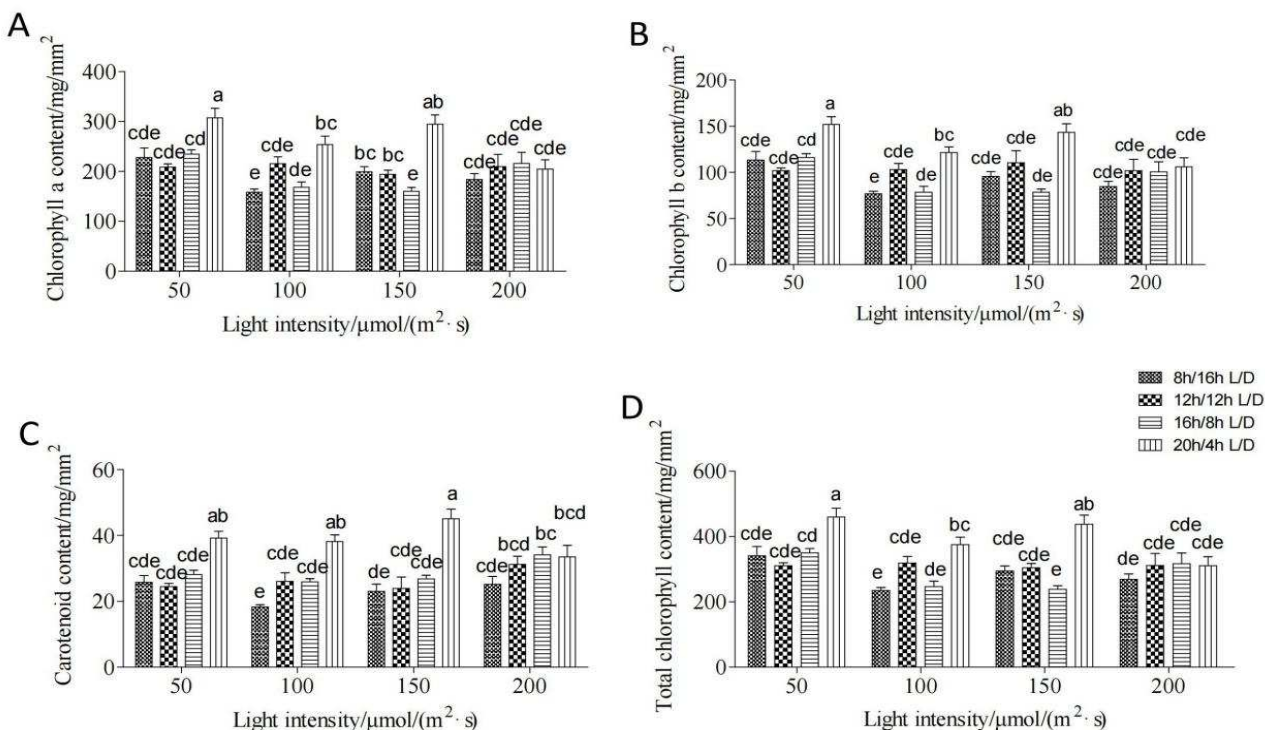


Fig. 2: The chlorophyll a content, chlorophyll b content, carotenoid content, and total chlorophyll content (A-D) under different red-light intensity and photoperiod conditions, respectively.

Different letters indicated significant differences ($P \leq 0.05$)

Effects of different red light intensities and photoperiods on the roots of *P. palustre*: As shown in Figure 3, under the same light intensity, increasing the illumination time resulted in an increasing trend in root weight, root volume, root length, root surface area, and average root diameter; Similarly, under the same photoperiod, increasing light intensity also lead to increases in root weight, root length, root surface area, root volume, and average root diameter. The root weight of the 20h/4h L/D photoperiod treatment under the conditions of 150 and 200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity exhibited a significant elevation compared to the other treatments with an increase of 24.40-751.00% (Figure 3A) ($P \leq 0.05$). The root was found the longest in the treatment under the 200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity and 16h/8h L/D photoperiod conditions (1756.74 cm) (Figure 3B). The root surface area of the treatment under the 200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 20h/4h L/D photoperiod presented a dramatic increase compared to the other treatments ($P \leq 0.05$), except for the 20h/4h L/D treatments under the 100 and 150 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity conditions (Figure 3C). The root volume of the treatments under the 12h/12h L/D, 16h/8h L/D, and 20h/4h L/D photoperiod conditions at 100 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity and the 20h/4h L/D photoperiod condition at 200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity were significantly higher than the other treatments (Figure 3D) ($P \leq 0.05$). The average root diameter was found the biggest in the

treatment under the 100 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity and 20h/4h L/D photoperiod conditions (Figure 3E).

Effects of different red light intensities and photoperiods on the contents of total pectin and soluble sugar in *P. palustre*: As shown in Figure 4A, the total pectin contents of the treatments under the 100 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 8h/16h L/D and 200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 8h/16h L/D were dramatically higher than the other treatments ($P \leq 0.05$), except for the treatments under 100 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 12h/12h L/D, 150 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 8h/16h L/D, and 150 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 20h/4h L/D conditions. The total pectin contents of these treatments increased by 34.68-126.77% compared to other treatments. Under the low light intensity (50 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$), photoperiod treatments seemed to have no significant effect on the total pectin content, but as light intensity increased, photoperiod seemed to have a negative impact on the total pectin content. In addition, the soluble sugar contents were found the highest in the treatments under the 100 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 8h/16h L/D, 150 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 8h/16h L/D and 12h/12h L/D, and 200 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity with 8h/16h L/D and 12h/12h L/D. They were 3.45, 3.20, 3.62, 3.33, and 3.38mg/g, respectively, which increased by 6.10-59.27% compared to other treatments.

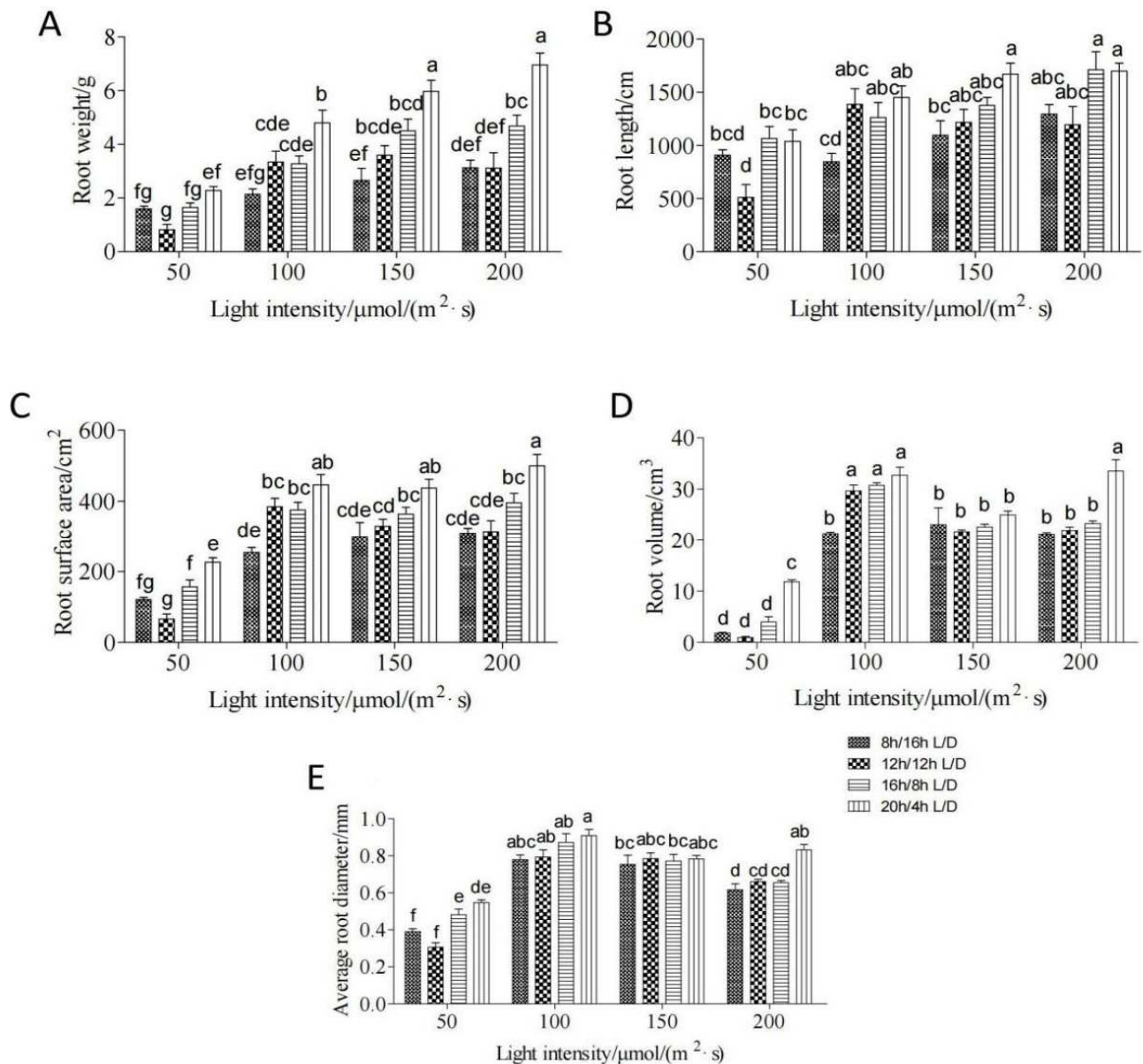


Fig. 3: The root weight, root length, root volume, root surface area, and average root diameter (A-E) under different red-light intensity and photoperiod conditions, respectively. Different letters indicated significant differences ($P \leq 0.05$)

The correlation analysis: In this study, the sixteen indicators mentioned above were used for correlation analysis (Table 2). The results showed that light intensity was positively correlated with plant height, whole plant weight, stem diameter, number of leaves, root weight, root length, root volume, root surface area, average root diameter, and soluble sugar content ($P \leq 0.01$), but negatively correlated with chlorophyll b ($P \leq 0.05$). Unexpectedly, photoperiod was significantly correlated with all sixteen indicators. Among these, photoperiod was negatively correlated with plant height, total pectin content, and soluble sugar content ($P \leq 0.01$), and

positively correlated with the other indicators ($P \leq 0.01$).

Analysis of the membership function values of various indicators: We further calculated the D values for each treatment using membership functions using the sixteen indicators. The higher the D value, the better the treatment. As shown in Table 3, the D value of the T12 treatment (150 $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ light intensity and 20h/4h L/D photoperiod) was the biggest, indicating that the T12 treatment was more suitable for the cultivation and quality production of *P. palustre* in comparison with the other treatments.

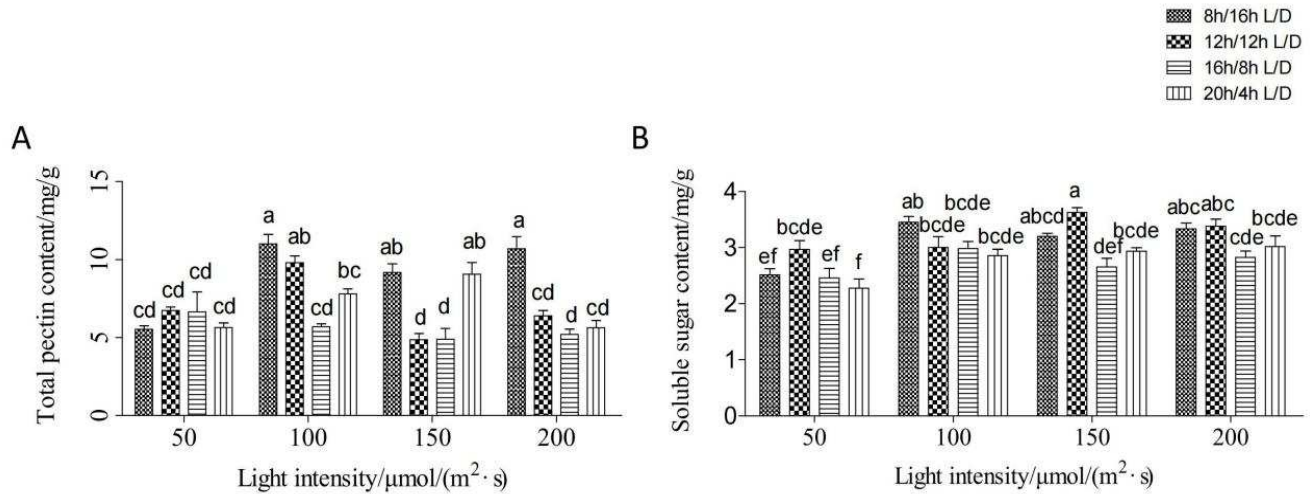


Fig. 4: The total pectin content (A) soluble sugar content (B) under different light intensity and photoperiod conditions.

Different letters indicated significant differences ($P \leq 0.05$)

Table 2. Correlation analysis of the growth and content indexes of *P. palustre* under different red-light intensities and photoperiods.

Factor	PH	WPW	NL	SD	LA	Ta	Tb	Tc
Light intensity	0.342**	0.510**	0.263**	0.513**	0.141	-0.200	-0.226*	0.170
Photoperiod	-0.207**	0.641**	0.397**	0.410**	0.484**	0.446**	0.429**	0.623**
Factor	Ta+b	RW	RL	RSA	RV	ARD	TPC	SSC
Light intensity	-0.214	0.604**	0.535**	0.623**	0.598**	0.444**	0.046	0.444**
Photoperiod	0.450**	0.554**	0.443**	0.459**	0.305**	0.285*	-0.360**	-0.393**

** : Correlation is significant ($P \leq 0.01$). * : Correlation is significant ($P \leq 0.05$). PH: Plant height; WPW: Whole plant weight; NL: Number of leaves; SD: Stem diameter; LA: Leaf area; Ta: Chlorophyll a content; Tb: Chlorophyll b content; Tc: Carotenoid content; Ta+b: Total chlorophyll content; RW: Root weight; RL: Root length; RV: Root volume; RSA: Root surface area; ARD: Average root diameter; TPC: Total pectin content; SSC: Soluble sugar content

Table 3. Comprehensive evaluation of different treatments.

Treatment	$\mu(x)$				D value	Ranking
	$\mu1$	$\mu2$	$\mu3$	$\mu4$		
T1	0.1496	0.6062	0.2735	0.3974	0.3220	14
T2	0.0000	0.4708	0.3827	0.3582	0.2102	16
T3	0.2938	0.6026	0.2982	0.6483	0.4134	12
T4	0.5056	1.0000	0.5127	0.5619	0.6665	3
T5	0.3034	0.0000	0.9205	0.3619	0.2744	15
T6	0.5579	0.2213	0.9300	1.0000	0.5159	6
T7	0.7310	0.1020	0.1679	0.6050	0.4659	7
T8	0.9332	0.5437	0.7477	0.5651	0.7685	2
T9	0.4519	0.2056	0.7872	0.5518	0.4144	11
T10	0.5316	0.2637	0.6142	0.0552	0.4267	10
T11	0.6664	0.1565	0.0000	0.5270	0.4276	9
T12	0.9210	0.7684	1.0000	0.4006	0.8497	1
T13	0.4711	0.1463	0.8145	0.4546	0.4026	13
T14	0.5639	0.2447	0.4684	0.6699	0.4592	8
T15	0.8668	0.3589	0.0821	0.7805	0.6196	5
T16	1.0000	0.3187	0.3284	0.0000	0.6549	4

DISCUSSION

Effects of different red light intensities and photoperiods on the growth and development of *P. palustre*:

Currently, research on the interactive effects of artificial light intensity and photoperiod on plant growth is mostly focused on vegetables, medicinal plants, and economic crops. The interaction between light intensity and photoperiod affects the agronomic traits of plants, such as plant height, biomass, branching, and leaf number. Different species of plants, as well as different varieties within the same species, exhibit varying responses to the interaction of light intensity and photoperiod. Under long photoperiods, increasing light intensity promotes the growth of red-leaf lettuce plants, and the effect of light intensity on leaf number shows a positive correlation. The fresh weight of individual plants tended to increase with prolonged photoperiods (Ji *et al.*, 2019). Conversely, extending the duration of light exposure and reducing light intensity significantly improved the plant height, leaf area, leaf number, stem thickness, and aboveground and underground fresh and dry weights of leaf lettuce (Zhou, 2022). In previous studies, the plant height of *Linum usitatissimum* and *Caragana korshinskii* showed an increasing trend with decreasing light intensity (Xu *et al.*, 2013; Yan *et al.*, 2015), nevertheless, the plant height did not show a clear trend in this study (Figure 1A). However, increasing light intensity and prolonging the photoperiod were found to enhance the whole-plant fresh weight, stem thickness, and leaf area of *P. palustre* (Fig. 1B-E), which was in line with the research conducted by Liu *et al.* (2019) and Lee *et al.* (2022). The number of leaves exhibited an initial increase followed by a decrease as the photoperiod increased while maintaining constant light intensity (Figure 1C), which was inconsistent with the study of Zhou (2022). In addition, increasing light intensity and lengthening the photoperiod favored the growth of roots of *P. palustre*. It was inconsistent with the study by Lee *et al.* (2022), in which low light intensity and short photoperiod promoted ginseng root growth. Overall, it was concluded that red light intensity and photoperiod had different effects on the growth and development indicators of *P. palustre*.

Effects of different red-light intensities and photoperiods on photosynthetic pigments of *P. palustre*:

Photoperiod and light intensity can jointly affect the photosynthetic characteristics of plants. Increasing light intensity resulted in a decrease in chlorophyll a, chlorophyll b, and total chlorophyll in lettuce, while an increase in carotenoids, and extending the photoperiod could significantly promote the chlorophyll and carotenoid content (Liu *et al.*, 2019). The content of chlorophyll a and chlorophyll b in American ginseng decreased with increasing light intensity, but

there was no significant difference in carotenoids (Liu *et al.*, 2022a). In the present study, the interactions between the medium-low light intensities and long light periods were beneficial for the accumulation of total chlorophyll, chlorophylls a and b, and carotenoids in *P. palustre* (Fig. 2). Under different photoperiods with high light intensity, there were no significant differences in chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll content of *P. palustre*, but they were all significantly lower than those under medium-low light intensity and long photoperiods. It was inconsistent with the study by Liu *et al.* (2022b). Therefore, these findings implied that the effects of light intensity and photoperiod on the photosynthetic pigment content of plants had not yet formed a uniform pattern and were highly influenced by factors such as species, growth period, experimental treatments, etc.

Effects of different red-light intensities and photoperiods on pectin content and soluble sugar content of *P. palustre*:

For medicinal plants, in addition to the requirement of yield, more attention is paid to the quality of medicinal materials. The effective ingredients of medicinal plants are the material basis for their clinical efficacy and an important criterion for assessing the quality of medicinal materials (Yao *et al.*, 2022). Light intensity and photoperiod have different effects on the accumulation of active ingredients in different medicinal plants. Cai (2008) found that a short photoperiod was more conducive to converting *Scutellaria baicalensis* polysaccharides into flavonoids. Sun *et al.* (2013) found that increasing light intensity could increase the content of secondary metabolites of *S. baicalensis*. The content of saponin components and total saponin in the rhizomes of *Paris polyphylla* var. *yunnanensis* exhibited an initial increase followed by a decrease as light intensity increased (Cao *et al.*, 2019). In this study, medium-high light intensity and short photoperiod treatments favored the accumulation of total pectin and soluble sugar contents. This was consistent with the study of Yuki *et al.* (2017) in *Stevia rebaudiana*.

Potential applications of light in *P. palustre*

production: As mentioned earlier, in recent years, due to the high labor cost of cultivation and management of *P. palustre* and the low economic benefits, farmers are reluctant to plant it, resulting in the insufficient supply of raw materials of *P. palustre* in China, which needs to be imported in large quantities from Southeast Asian countries. In addition to traditional field cultivation, plant factory or simulated cultivation of *P. palustre* may be a method that can be tried. Under the conditions of these methods, light is necessary for *P. palustre* growth and development. Moreover, in this study, light could significantly affect the root growth and development of *P. palustre*. Since *P. palustre* is generally propagated by cuttings (asexual reproduction), further exploration of the

effects of light on *P. palustre* rooting may have positive practical implications for future *P. palustre* seedling nurseries and seedling growth.

Conclusions: In this study, the results indicated that the T12 treatment (150 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ light intensity and 20h/4h L/D photoperiod) was more suitable for the cultivation and quality production of *P. palustre* in the plant factory. The current study provided scientific data for plant factory or indoor large-scale cultivation and planting of *P. palustre*.

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