Partial shade improved the photosynthetic capacity and polysaccharide accumulation of the medicinal plant *Bletilla ochracea* Schltr.

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Abstract

To study the light intensity suitable for *Bletilla ochracea* Schltr., morphology, photosynthetic parameters, and polysaccharide content of seedlings were evaluated under different light intensities. All shade treatments promoted plant growth and net photosynthetic rate while having no significant effect on transpiration rate. The maximum photochemical efficiency and potential photochemical efficiency reached the lowest values under full sunlight. The electron transport rate and photochemical quenching under shade were significantly higher than those under full light, while nonphotochemical quenching was the highest under full light. This indicated that the shade alleviated photoinhibition in summer and improved the utilization of light. *B. ochracea* could adapt to different light intensities, enhancing photosynthetic efficiency under low light by improving the electron transport and the degree of opened PSII reaction centers, and adapting to high light by increasing heat dissipation. Plant growth, photosynthesis, and polysaccharide accumulation of *B. ochracea* greatly increased under 76.4% shade.

Keywords: *Bletilla ochracea* Schltr.; chlorophyll fluorescence; photosynthesis; shade.

Introduction

Plants convert light energy into chemical energy *in vivo* through photosynthesis. The optimal light conditions can promote the growth of plants to accumulate dry matter and increase yield (Allakhverdiev 2020, Paradiso *et al.* 2020). The previous studies have reported that many plants suffer from photoinhibition and photodamage under a high-light environment (Zeng *et al.* 2017, Liu *et al.* 2019). Shade is a common gardening measure to protect plants from photoinhibition and oxidative damage caused by excessive light, but excessive shade can reduce yield and quality

(Zivcak *et al.* 2014). In addition, shade can also alleviate drought, high temperatures, and other adversities (Lopez *et al.* 2018, Bannister *et al.* 2021). However, the shade tolerance of plants varies with species and cultivars (Slot *et al.* 2019). Plants could adapt to varying light conditions through morphological and physiological regulation (Liu *et al.* 2016, Raza *et al.* 2020). The leaf is one of the most sensitive parts to light. The morphology and structure of the leaves are usually suitable for their physiological function (Zhang *et al.* 2016). Thus, the characteristics of leaves can reflect the response and adaptation of plants to the environment (Tang *et al.* 2020). Leaves under shade are

Highlights

- Shade promoted leaf growth and photosynthesis capacity of *Bletilla ochracea*
- The 76.4% shade was the most suitable for polysaccharide accumulation in *B. ochracea*
- Shade conditions relieved the photoinhibition that seedlings suffered in summer

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Abbreviations: Chl – chlorophyll; DM – dry mass; *E* – transpiration rate; ETR – electron transport rate; *F*₀ – minimal fluorescence; *F*_m – maximal chlorophyll fluorescence; FM – fresh mass; *F*_v – variable fluorescence; *F*_v/*F*₀ – potential photochemical efficiency; *F*_v/*F*_m – maximal quantum yield of PSII photochemistry; Mt – saturated leaf mass; NPQ – nonphotochemical quenching; *P*_N – net photosynthetic rate; *q*_p – photochemical quenching coefficient; *Y*_{II} – effective quantum yield of PSII photochemistry.

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usually larger and thinner than those under sunlight, and the contents of chlorophyll ($a+b$) [Chl ($a+b$)], especially Chl b , are higher (Lichtenthaler *et al.* 2007, 2013; Mathur *et al.* 2018). The photosynthetic rate of shade-tolerant plants can remain relatively high under low-light conditions. Shishido *et al.* (1987) has reported that *Cucumis sativus* L. can grow normally under shade conditions because it captures as much light energy as possible to facilitate CO₂ fixation and carbohydrate accumulation. Besides, shade-tolerant soybeans showed higher net photosynthetic rates than shade-sensitive soybeans in shade conditions, due to higher PSII activity and energy transport from PSII to PSI (Yao *et al.* 2017). High shade (90%) conditions also upregulated transcripts of antioxidant enzymes of *Pinellia ternata* to reduce photoinhibition and increased expression of light and auxin signals genes, which significantly increased tuber growth and photosynthesis efficiency as compared to full-light conditions (Xue *et al.* 2019).

Chl fluorescence parameters can reflect a series of important regulation processes in the photosynthetic apparatus through the measurement of light energy absorption, transmission, dissipation, and distribution in the photosynthesis so as to truly and accurately reveal the influencing mechanisms on photosynthetic processes (Alyemeni *et al.* 2018). F_v/F_m (maximal quantum yield of PSII photochemistry) refers to the efficiency of the open PSII reaction centers capture of excitation energy and the maximum photochemical efficiency of plants (Lichtenthaler *et al.* 2007). F_v/F_0 (potential photochemical efficiency) denotes the potential activity of PSII photoreaction centers and is an index to measure the photoenergy-utilization capability of plants (Lichtenthaler *et al.* 2007, Faseela *et al.* 2020). Higher F_v/F_0 and F_v/F_m mean the greater light energy utilization potential of plants (Maxwell and Johnson 2000). ETR (electron transport rate) indicates the rate of photosynthetic electron transfer, which is related to the photosynthetic rate of plants (Mathur *et al.* 2018). *Aeschynanthus longicaulis*, a shade-tolerant species, suffered from photoinhibition and photodamage under high light conditions and showed significantly lower ETR and F_v/F_m (Li *et al.* 2014). Photochemical quenching (q_p) reflects the proportion of opened PSII reaction centers. Higher q_p facilitates electron transfer and PSII yield (Mathur *et al.* 2018). Compared to shade-tolerant soybeans, the q_p of shade-sensitive soybeans showed a significantly higher decrease under 50 and 75% shade treatments (Yao *et al.* 2017). Under high or fluctuating light conditions, lesser light energy is consumed for photosynthetic electron transfer in PSII, and more energy is dissipated as heat, which is the most important photoprotection strategy for plants (Endo *et al.* 2014, Qiao *et al.* 2021). The redundant light energy dissipated harmlessly as heat is reflected by nonphotochemical quenching (NPQ). Previous studies have shown that plants grown in high light showed higher NPQ values than those grown in low light (Kalmatskaya *et al.* 2020). Y_{II} reflects the effective quantum yield of PSII photochemistry, which is the proportion of light that is absorbed by Chl for PSII

photochemical reaction (Murchie and Lawson 2013). Compared to normal conditions, adversities can decrease the photosynthetic quantum yield (Wan *et al.* 2011, Wu *et al.* 2019).

Bletilla ochracea Schltr., a traditional Chinese medicinal plant, is native to forests and shrubs canopy in high-altitude areas and is widely cultivated in China (Yang *et al.* 2012). There are nine species of *Bletilla* (Orchidaceae) in the world, including four species of China. The bioactive extracts of the genus are widely used in the pharmaceutical and chemical industries. Studies on chemical constituents and pharmacological activities of the genus were carried out in the 1970s (Jiang *et al.* 2020). The quality and output value of *Bletilla* were evaluated by determining the content of polysaccharides. Polysaccharides extracted from *B. ochracea* tubers, showing antitumor and antioxidation activities, are identified as substitutes for *Bletilla striata* polysaccharides in the cosmetic and medicine industries (Niu *et al.* 2020). Previous studies were mainly carried out to determine medicinal efficacy of *B. ochracea* (Yang *et al.* 2012, Li *et al.* 2018). To maximize its medicinal and economic value, the yield and quality of *B. ochracea* must be improved. To cultivate high-yield plants, it is necessary to provide a suitable light environment (Yi *et al.* 2020). Therefore, the growth process, chlorophyll fluorescence, photosynthetic characteristics, and polysaccharide content of *B. ochracea* plants under shade conditions were studied in order to provide a reference for the sustainably used resources of *Bletilla* in the future.

Materials and methods

Plant material and growth conditions: Seedlings of *Bletilla ochracea* were cultivated in Industrial Crop Research Institute of Sichuan Agricultural Sciences (China) on 20 December 2017. The two-year-old seedlings with uniform growth and no disease and insect pests were divided into four groups for each shade treatment. The same water and fertilizer management were used during the experiment.

Experiment design: On 20 February 2018, black sunshade nets were used to provide shade to *B. ochracea* seedlings and the open field-growing environments. The height of the shade nets was 1.5 m and the distance between each pot was 50 cm to avoid cross shade. Two seedlings per pot were grown. Four levels were set for different shade environments:

Treatment		Shade [%]	Transmittance [%]
CK	full illumination	0	100
A	one layer of dense black shade net	94.23	5.77
B	two layers of sparse black shade net	76.41	23.59
C	thin black sunscreen net	54.93	45.07

The illuminometer (1010A, VICTOR, China) was used to determine different shading degrees. We spent several years of research on the cultivation of *B. ochracea*. The vigorous growth period of *B. ochracea* is in late April. Therefore, we started measuring data on 28 April. From 28 April to 17 July, the leaves of seedlings were taken to measure their morphological and physiological indexes every 15 d.

Plant height and leaf traits: Plant height, leaf length, and leaf width were measured with a tape ruler. Ten plants were randomly selected, each of which was measured three times and the average values were taken.

The method of measuring leaf water content was following. First, the leaves were cut randomly without veins, and the surface water was absorbed with filter paper. Second, the leaves were weighed. The fresh mass (FM) of each sample was 0.3 g. Third, the leaves were soaked in ionic water for 5 h and then we weighed saturated leaf mass (Mt). Finally, leaves were kept in an oven at 105°C for 30 min, dried at 80°C for 24 h, and weighed for dry mass (DM). Relative leaf water content [%] = $[(FM - DM)/(Mt - DM)] \times 100$.

The spectrophotometer (UV1800, Shanghai Jinpeng, China) was used to determine chlorophyll content (Wellburn 1994).

Polysaccharide content: The tubers were harvested on 17 July, dried, ground, and passed through sieve ($250 \pm 9.9 \mu\text{m}$). The tuber powder was taken to determine of polysaccharide content of *B. ochracea*, based on the method of Li *et al.* (2018). First, the crude polysaccharide solution was extracted. The tuber powder (0.2 g) was weighed, placed in a round bottom flask, and 50 ml of water was added. The round bottom flask was weighed, solution heated and refluxed for 1 h, and then cooled. The flask was weighed for the second time, water was added to make up the reduced mass, well shaken, and filtered. To 5 ml of filtrate, 30 ml of absolute ethanol was added, shaken well, and kept for 1 h. Then the mixture was centrifuged at 3,000 rpm for 15 min, supernatant was removed, and precipitate was dissolved in hot water. The resultant solution was cooled, and the volume was made up to 100 ml in a volumetric flask.

Secondly, 100 g of solid glucose (analytical grade) was precisely weighed and dissolved in 100 ml of deionized water. Glucose solutions of 0.05, 0.1, 0.2, 0.4, 0.5, 0.8, and 1.0 ml of 1 mg(glucose) ml^{-1} were precisely measured and placed in 10-ml tube. Water was added to make up the volume to 2 ml for each solution and shaken well. To prepare anthrone sulfuric acid solution, 0.1 g of anthrone was accurately weighed and dissolved in 100 ml of 80% concentrated sulfuric acid. Then, 4.5 ml of anthrone sulfuric acid solution was precisely added and mixed well. Then, it was heated in boiling water bath for 10 min and taken out. It was quickly cooled in running water for 10 min. The absorbance was measured at 625 nm by UV-spectrophotometer (Evolution 260 Bio, Thermo Scientific, Germany). The standard curve was drawn with glucose concentration (0, 0.05, 0.2, 0.4, 0.5, 0.8,

1 mg g^{-1}) as the horizontal coordinate and the absorbance as the vertical coordinate. The linear regression equation is $y = 1.2651x + 0.1223$, $r = 0.9982$, showing a good linear relationship. After that, polysaccharide extract of *B. ochracea* was taken to measure the polysaccharide content.

Photosynthesis parameters: Photosynthetic rate and Chl fluorescence were measured from 8:00 to 18:00 h. Portable photosynthetic gas analyzer (Li-6100, LI-COR, USA) was used to measure net photosynthetic rate and transpiration rate. Plants grown under different shade degrees were randomly selected. The leaves with the same leaf position (three plants per treatment, three leaves per plant, and three times per leaf) were measured every 2 h between 8:00 to 18:00 h.

Chl fluorescence measurements: The Chl fluorescence parameters of leaves were measured by a portable plant Chl fluorescence analyzer (Junior PAM, Walz, Germany). Each time fluorescence parameters were determined in six leaves. The leaves were dark-adapted for 30 min before each measurement. For light-saturation curve measurement, the light intensity gradient was set at 90, 125, 190, 285, 420, 625, 820; and 1,150 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. The electron transport rate (ETR), photosynthetic quantum yield (Y_{II}), photochemical quenching coefficient (q_p), and the nonphotochemical quenching coefficient (NPQ) were calculated (Maxwell and Johnson 2000): $ETR = 0.5 \times \alpha \times PAR \times Y_{II}$, $Y_{II} = (F_m - F_t')/F_m'$, $q_p = (F_m' - F_t')/(F_m' - F_0')$, $NPQ = (F_m - F_m')/F_m'$.

Statistics analysis: The data is presented as mean $\pm$ standard deviation (SD) using Microsoft Excel (version 2010, Microsoft, USA). Photosynthesis parameters and parameters of Chl fluorescence were analyzed by one-way analysis of variance (ANOVA). Leaf characteristics, plant growth, Chl ($a+b$), and Chl a/b were analyzed by repeated ANOVA using SPSS software (version 19.0, SPSS, USA). All the significant differences between means were detected by Duncan's multiple range test ($P < 0.05$). All the graphs were drawn by Origin (version 8.0, OriginLab, USA).

Results

Leaf characteristics and plant growth: The plant heights under different shade treatments are shown in Table 1. The plants height under all treatments increased rapidly before 15 May and successively showed a stable increasing trend till 17 July. The plant heights under all the shade treatments were significantly higher than those under full-light treatment. Plant height reached the maximum under 76.4% shade. These data demonstrated that light intensity significantly affected plant height and the shade promoted plant elongation.

The growth of leaves length was first increasing rapidly and becoming stable gradually in all shade treatments (Table 2). Leaves under full-light conditions were significantly different from those under shade treatments

analyzed by one-way *ANOVA*. Leaves were also promoted by shade and the leaves under 76.4% shade were the longest.

Leaves of *B. ochracea* under shade conditions were wider than those under full light (Table 3). The width of leaves under shade treatments were significantly different from those under full light as analyzed by *ANOVA* for repeated measurement and the width under 76.4% shade was the highest. The width of leaves under different shade degrees were as following: 76.4% shade > 94.2% shade > 54.9% shade.

Leaf water content is an important indicator of leaf status. It can be seen from Table 4 that the water contents

of leaves under shade were higher than those under full light. Through *ANOVA* for repeated measurement, a significant difference between CK and shade treatments was found. The leaf water contents for each treatment under different shade degrees were as follows: 94.2% shade > 76.4% shade > 54.9% shade.

Chl content: Under shade conditions, the Chl (*a+b*) contents were significantly higher than those under full-light condition (Table 1S, *supplement*). The Chl (*a+b*) contents under all the conditions decreased rapidly on 15 May compared to the values on 28 April, since then they successively increased until 30 May, and then they

Table 1. Changes of plant height [cm] of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD. Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight).

Treatment	28 April	15 May	30 May	14 June	2 July	17 July
A	12.13 $\pm$ 0.13 ^b	17.70 $\pm$ 0.31 ^a	21.56 $\pm$ 0.48 ^b	23.58 $\pm$ 1.16 ^a	25.19 $\pm$ 1.36 ^a	25.94 $\pm$ 2.98 ^{ab}
B	13.84 $\pm$ 0.34 ^a	18.40 $\pm$ 0.86 ^a	27.34 $\pm$ 0.53 ^a	27.48 $\pm$ 2.29 ^a	28.53 $\pm$ 1.10 ^a	30.23 $\pm$ 1.64 ^a
C	11.34 $\pm$ 0.64 ^b	16.99 $\pm$ 1.09 ^a	21.97 $\pm$ 0.42 ^b	23.14 $\pm$ 1.39 ^a	24.23 $\pm$ 0.60 ^a	25.20 $\pm$ 1.02 ^b
CK	10.51 $\pm$ 0.30 ^c	15.87 $\pm$ 0.39 ^b	18.50 $\pm$ 0.87 ^c	20.94 $\pm$ 1.36 ^b	21.79 $\pm$ 1.74 ^b	22.06 $\pm$ 2.03 ^b

Table 2. Length of leaves [cm] of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD. Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight).

Treatment	28 April	15 May	30 May	14 June	2 July	17 July
A	8.17 $\pm$ 0.11 ^b	11.38 $\pm$ 0.65 ^a	18.33 $\pm$ 0.72 ^a	20.61 $\pm$ 0.06 ^b	21.78 $\pm$ 0.51 ^b	22.23 $\pm$ 1.63 ^a
B	10.08 $\pm$ 0.28 ^a	12.52 $\pm$ 1.08 ^a	18.14 $\pm$ 2.16 ^a	20.26 $\pm$ 1.23 ^a	22.34 $\pm$ 0.24 ^a	23.94 $\pm$ 0.85 ^a
C	9.48 $\pm$ 0.24 ^a	12.46 $\pm$ 0.44 ^a	17.83 $\pm$ 0.83 ^a	17.92 $\pm$ 0.62 ^c	20.24 $\pm$ 1.43 ^a	22.69 $\pm$ 0.53 ^a
CK	7.37 $\pm$ 0.19 ^c	10.37 $\pm$ 0.37 ^b	16.47 $\pm$ 0.55 ^b	16.88 $\pm$ 0.04 ^c	18.29 $\pm$ 1.01 ^b	20.46 $\pm$ 1.51 ^b

Table 3. Width of leaves [cm] of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD. Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight).

Treatment	28 April	15 May	30 May	14 June	2 July	17 July
A	1.30 $\pm$ 0.17 ^a	1.53 $\pm$ 0.03 ^a	1.74 $\pm$ 0.24 ^a	1.88 $\pm$ 0.02 ^a	1.99 $\pm$ 0.12 ^a	2.17 $\pm$ 0.12 ^a
B	1.44 $\pm$ 0.26 ^a	1.52 $\pm$ 0.11 ^a	1.67 $\pm$ 0.13 ^a	1.71 $\pm$ 0.01 ^a	1.84 $\pm$ 0.26 ^a	1.92 $\pm$ 0.08 ^a
C	1.41 $\pm$ 0.14 ^a	1.80 $\pm$ 0.23 ^a	1.90 $\pm$ 0.10 ^a	2.07 $\pm$ 0.09 ^a	2.08 $\pm$ 0.06 ^a	2.17 $\pm$ 0.88 ^a
CK	0.83 $\pm$ 0.13 ^b	1.30 $\pm$ 0.08 ^b	1.36 $\pm$ 0.06 ^b	1.62 $\pm$ 0.06 ^b	1.68 $\pm$ 0.04 ^b	1.72 $\pm$ 0.09 ^b

Table 4. Leaf water contents [%] of *Bletilla ochracea* under different shade treatment. Data are means $\pm$ SD. Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight).

Treatment	28 April	15 May	30 May	14 June	2 July	17 July
A	83.51 $\pm$ 0.12 ^a	79.29 $\pm$ 0.36 ^a	80.57 $\pm$ 1.37 ^a	76.39 $\pm$ 0.71 ^a	80.64 $\pm$ 1.21 ^a	74.14 $\pm$ 0.82 ^a
B	80.03 $\pm$ 0.16 ^b	77.66 $\pm$ 1.02 ^a	77.16 $\pm$ 1.41 ^b	75.97 $\pm$ 0.71 ^a	75.75 $\pm$ 0.60 ^b	72.17 $\pm$ 1.11 ^a
C	77.29 $\pm$ 0.11 ^c	74.02 $\pm$ 0.57 ^b	75.38 $\pm$ 1.94 ^b	72.41 $\pm$ 0.87 ^b	73.60 $\pm$ 1.90 ^b	69.60 $\pm$ 1.07 ^b
CK	76.49 $\pm$ 0.16 ^c	72.07 $\pm$ 1.64 ^b	71.40 $\pm$ 0.79 ^c	66.85 $\pm$ 0.49 ^c	70.36 $\pm$ 0.31 ^c	72.35 $\pm$ 0.29 ^a

showed a steady trend. Through *ANOVA* for repeated measurements, there is a significant difference between CK and other shade treatments. It can be seen from Table 2S (*supplement*) that the trend was not the same throughout the determination period. On 28 April, the value of Chl *a/b* in CK was higher than the values under shade treatments and further increased on 15 May. From 30 May onwards, the Chl *a/b* decreased steadily in CK. The value of Chl *a/b* for CK was higher than shade treatments at all data points except two, on 15 May and 30 May. The change of the ratio of Chl *a/b* is an adaptation to the different shade environments. The Chl *a/b* reached the highest value in the middle of May.

Photosynthesis parameters: The changes in P_N were of unimodal type under different shade treatments, without midday depression of photosynthesis (Fig. 1A). From 8:00 to 12:00 h, the values of P_N were in order of 76.4% shade > 94.2% shade > 54.9% shade > CK. From 14:00 to 18:00 h, the values of P_N were in order of 94.2% shade > 76.4% shade > 54.9% shade > CK.

P_N values in treatments A and B were always significantly higher than those in full-light treatment and there was no significant difference between treatments A and B. From 10:00 to 16:00 h, there was a significant difference between full light and 54.9% shade. All treatments reached the peak value at 12:00 h. The lowest peak value was in the full-light treatment, which was significantly lower than shade treatments. At 12:00 h, the maximum P_N was under 76.4% shade, which was 1.43 times higher than that under CK. The results showed that shade treatment significantly promoted photosynthesis capacity of *B. ochracea*. As shown in Fig. 2B, the transpiration rates (*E*) under all the treatments showed the similar tendencies and reached the highest value at 12:00 h and then showed a downward trend. In the morning, the *E* values under CK were significantly higher than those under shade treatments. The minimum values appeared at 18:00 h (except for 94.2% shade). The *E* values showed no significant difference between all treatments at 18:00 h.

From the results, it can be inferred that shade had a little effect on *E* of *B. ochracea*.

Chl fluorescence: Parameters of Chl fluorescence can quickly and sensitively detect the effects of environment variations on plant photosynthesis. Under different light conditions, the values of F_v/F_m showed the V-shaped change. It can be seen from Fig. 2A that the F_v/F_m values under different shade treatments decreased in the forenoon and then increased slowly. Throughout the day, the F_v/F_m value of each treatment was the highest at 8:00 h and the lowest at 12:00 h. Normally, the F_v/F_m values of higher plants reach 0.75–0.85 (Maxwell and Johnson 2000). F_v/F_m values under shade treatments were significantly higher than those under full-light condition. F_v/F_m values under 94.2% and 76.4% shade conditions have been relatively steady and maintained at a relatively high level, while there was no significant difference in F_v/F_m values between three shade conditions. F_v/F_m under full-light treatment decreased to 0.44 at 12:00 h, which was much lower than those under shade treatments. According to these data, full light caused photoinhibition of seedlings and shade conditions were helpful to alleviate the photoinhibition under strong light in summer.

Under different shade treatments (Fig. 2B), the F_v/F_0 values decreased from 8:00 to 12:00 h and then increased till 18:00 h. The F_v/F_0 value under 94.2% shade treatment was the highest at noon, while that under CK was the lowest. The values of F_v/F_0 under full light were always lower than those under shade treatments. During the day, there was no significant difference in the F_v/F_0 values between 76.4% and 54.9% shade treatment. Before 14:00 h, 94.2% shade treatment had the highest F_v/F_0 values and was significantly higher than other treatments. At 16:00 h, the F_v/F_0 values of three shade treatments were significantly higher than full sunlight ($P < 0.05$). At 18:00 h, the F_v/F_0 value in 54.9% shade reached the highest value in the whole experiment, which was 1.88 times and 1.36 times of that in CK and 94.2% shade, respectively.

Fig. 1. Photosynthetic parameters of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD ($n = 3$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight). Parameters of photosynthesis on 17 July: (A) photosynthetic rate (P_N) and (B) transpiration rate (*E*). Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). The n.s. indicates no significant difference between treatments.

Fig. 2. Changes in photochemical efficiency of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD ($n = 3$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight). Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). The n.s. indicates no significant difference between treatments. F_v/F_0 – potential photochemical efficiency; F_v/F_m – maximal quantum yield of PSII photochemistry.

As photosynthetic effective radiation increased, ETR continued to increase (Fig. 3A). Under all light intensities, the ETR values under shade conditions were significantly higher than those under full light. The electron transport capacity of seedlings grown under full light was lower than that of plants grown under shade conditions. The q_p of each treatment decreased with the increase of photosynthetic effective radiation, the values of q_p were in order of 94.2% shade > 54.9% shade > 76.1% shade > CK (Fig. 3B). Starting from $125 \mu\text{mol}(\text{photon}) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the values of q_p under 94.2% shade were always significantly higher than those under treatments B and C. There was no significant difference between treatments B and C. Shade raised the openness of PSII reaction centers. The Y_{II} of *B. ochracea* decreased with increasing photosynthetic effective radiation (Fig. 3C). The Y_{II} of 54.9% and 76.4% shade was higher than that under treatments A and CK. There was no significant difference between CK and 94.2% shade, at all light intensities, and it was always significantly lower than those under 54.9% shade. At $1,150 \mu\text{mol}(\text{photon}) \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, the Y_{II} in 54.9% shade was the highest, and the minimum Y_{II} was 1.60 times higher than that in CK. The NPQ values under 54.9% shade were the lowest and the NPQ values under CK were the highest (Fig. 3D). There was no significant difference between A and B treatments, but it was significantly higher than those under 54.9% shade.

Polysaccharide content: The polysaccharide contents were 51.2 (94.2% shade), 70.4 (76.4% shade), 60.7 (54.9% shade), and 60.1% (CK) of total sugar, respectively (Fig. 4). The polysaccharide content at 76.4% shade significantly increased by 17.1% compared with CK. The polysaccharide content in the medicinal material may be related to the light intensity.

Discussion

The change of morphological traits is obvious when plants develop under different light environments. In our

experiment, the shade also had a tremendous effect on photosynthetic process in plants. The plants height, leaf length, and leaf width increased under all shade conditions (Tables 1, 2, and 3), which could be attributed to the increase of photosynthetic activity (Qiao *et al.* 2021). The growth rates under 76.4% and 94.2% shade were more rapid compared to CK (Table 1), showing that moderately and highly shaded environments promoted the growth of *B. ochracea*, similar to *Leymus chinensis* (Yang *et al.* 2018) and *Phoebe bournei* (Tang *et al.* 2020). In this experiment, the increasing trend of plant height, leaf length, and leaf width slowed down since 2 July (Tables 1, 2, and 3). The possible reason could be that morphological adaptation in weak light environment has already occurred (Terashima *et al.* 2011). Meanwhile, high light and temperature in summer were not conducive to plant growth under shade conditions (Takahashi and Murata 2008). Some studies have reported that photoinhibition may lead to the decrease of photosynthesis causing the decrease of plant growth rate under conditions of excessive light intensity (Fang *et al.* 2012). In the study, compared with full-light treatment, 76.4% and 94.2% shade conditions obviously enhanced the plant heights and leaf growth. The earlier research has shown that in the lower canopy of the rainforest, only by evolving larger plant leaves and horizontal leaf orientation, plants can capture light energy to the maximum extent (Lichtenthaler *et al.* 2007). Therefore, under low-light conditions, some plants will allocate photosynthetic products to leaf growth to promote photosynthesis, and ultimately ensure normal plant growth (Terashima *et al.* 2011). Shade not only changed the light intensity but also changed the air moisture and velocity in plant microenvironment (Lopez *et al.* 2018). Ahemd *et al.* (2016) showed that shade conditions could lower greenhouse temperature and humidity of greenhouse air to reduce energy and water consumption. Leaf water content is an important plant functional trait which can show water status and the degree of metabolism in leaves (Wang *et al.* 2019, Zhou *et al.* 2021). Our results showed that shade could increase the water content of leaves to keep plants

Fig. 3. Changes in fluorescence parameters of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD ($n = 3$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight). Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). The n.s. indicates no significant difference between treatments. The change of Chl fluorescence parameters with increasing photosynthetic effective radiation: (A) Electron transport rate (ETR); (B) photochemical quenching (q_p); (C) actual photosynthetic quantum yield (Y_{II}); (D) nonphotochemical quenching (NPQ).

Fig. 4. Changes in polysaccharide content of *Bletilla ochracea* under different shade treatments. Data are means $\pm$ SD ($n = 3$). Different lowercase letters represent significant differences between treatments by Duncan's test ($P < 0.05$). A, B, C, and CK indicate that corresponding shade rates are 94.2%, 76.4%, 54.9%, and 0% (full sunlight).

in a good water status. In general, shade facilitates the cultivation of seedlings in summer.

Chlorophylls are the most important pigments in plants with the function of absorbing and transmitting light. The content and proportion of Chls are important indexes for plants to adapt to different environmental factors. Generally, leaves under shade tend to have a larger light-harvesting complex, and thus lower Chl a/b values and higher Chl ($a+b$) contents (Yang *et al.* 2019). Chl ($a+b$) content depends not only on the environmental conditions but also on the plant species (Lichtenthaler *et al.* 2013, Zivcak *et al.* 2014, Zhang *et al.* 2016). In this experiment, shade environments increased the Chl ($a+b$) content of *B. ochracea* (Table 1S). A large number of studies have reported that the Chl ($a+b$) content of shade-intolerant plants could be lowered under weak-light conditions (Lopez *et al.* 2018, Mathur *et al.* 2018). With the increasing shade degree, the Chl ($a+b$) content of *B. ochracea* increased. Higher Chl ($a+b$) contents and thinner leaves help them capture light energy to survive under shade. One of the important properties of Chls is the selective absorption of light. Chl a and Chl b have different absorption spectra. The absorption of Chl a in red light (longer light wavelength) is wider than Chl b , while

the absorption of Chl *b* in blue and purple light is wider than Chl *a* (Lü *et al.* 2013). The decrease of Chl *a/b* values can improve the utilization efficiency of blue and purple light and adapt to the growth in shade (Lü *et al.* 2013). In this study, the Chl *a/b* values of leaves decreased with the increase of shade degree and the Chl *a/b* values were significantly lower than that under full light since 15 May (Table 2S). The results showed that under the conditions of high shade, the varying pigment contents were helpful for plants to improve the light trapping and utilization capability of chlorophylls. It is one of the adaptation mechanisms of plants induced in the shade environments.

B. ochracea is native to forests and shrubs canopy at high-altitude areas in China, growing in shady and cool habitat. In order to choose the light intensity conducive to the seedlings' photosynthesis, we measured the photosynthetic rate and transpiration rate in mid-July when there was high temperature and strong light. Our results showed that *B. ochracea* could tolerate both full light and weak light environment. Besides, shade environments facilitated the photosynthesis activity and plant growth. A large number of studies have reported that shade conditions could optimize photosynthesis capacity of plants, such as *Myrica rubra* (Zeng *et al.* 2017), *Torreya grandis* (Lin *et al.* 2019), and *Phoebe bournei* (Tang *et al.* 2020). However, different plants tolerate different shade degrees (Shao *et al.* 2014, Yang *et al.* 2018). In this study, the diurnal curves of P_N were of a unimodal type (Fig. 1A), which indicated that there was no midday depression of photosynthesis during the treatments. Contrastingly, some earlier studies have shown that excessive radiation leads to photoinhibition and reduction of PSII activity, and even midday depression of photosynthesis, making the photosynthetic curve bimodal (Zhao *et al.* 2012, Liu *et al.* 2019, Yang *et al.* 2019). Considering medicinal plants yield and irrigation costs, evapotranspiration can play a guiding role in the cultivation of medicinal plants (Ahemd *et al.* 2016). High light induced leaf temperature rise and excessive transpiration can lead to water deficit, decrease of photosynthetic rate and even plant death (Liu *et al.* 2019, Yang *et al.* 2019). Over-shading also limits transpiration, which can limit photosynthetic rate and affect plant growth, too (Shao *et al.* 2014). In our study, the diurnal variation curves of E were consistent with the diurnal variation curves of P_N (Fig. 1B). Besides, shade significantly increased the photosynthetic rate of seedlings, but had no significant effect on transpiration rate. According to our study, shade environments facilitated the improvement of photosynthesis, and high shade conditions (94.2% and 76.4% shade) were more effective (Fig. 1A). The possible reasons are (1) shade increases light energy-utilization efficiency and electron transport rate; (2) shade reduces photoinhibition.

Plants have evolved a variety of photoprotection strategies to adapt to fluctuating and high-light conditions. Photoinhibition occurs during the day and the degree of photoinhibition increases with light intensity (Ruban *et al.* 2012). Photodamage of photosynthetic apparatus is

inevitable, and photosynthetic system is in the process of constant damage and repair cycle (Takahashi and Murata 2008). Previous studies have shown that under a weak light, the repair rate of D1 protein is higher (Allakhverdiev and Murata 2004). Thus, we speculated the seedlings under shade treatment were less photoinhibited and had a higher electron transfer rate, which resulted in higher P_N . A strong light environment is always accompanied by high temperature in summer. High temperature stress could limit photosynthesis activity due to decreased electron transfer rate and CO_2 fixation (Ashraf and Harris 2013). Moderate shade can improve the plant microenvironment to protect plants from oxidative damage under high temperature and strong light, thereby protecting the PSII reaction centers, avoiding midday depression of photosynthesis and leaf burns (Liu *et al.* 2019). Shade can also improve the utilization rate of light energy and improve the photosynthetic activity (Chen *et al.* 2017). In this experiment, the diurnal variation curves of F_v/F_m and F_v/F_0 were in the V shape (Fig. 2), indicating that utilization rate of light energy was lowest at 12:00 h. In high sunlight (noon), stomata also close leading to reduced CO_2 exchange and reduction in photosynthesis. F_v/F_m and F_v/F_0 values under full light were always the lowest, which is similar to *Citrus reticulata* (Medina *et al.* 2002). Shade conditions improved the utilization rate of light energy and were helpful to alleviate the photoinhibition. Plants have a strong photoprotection capability, probably owing to the photoprotection measures like NPQ, reactive oxygen scavenging, photorespiration, and cyclic electron flow (Ruban *et al.* 2012). The NPQ increased with the increase of photosynthetic effective radiation (Fig. 3D). With the increase of light intensity, more light energy absorbed by leaves was dissipated as heat, which reduced the light energy used for photosynthetic electron transfer. In order to reduce the damage of active oxygen to photosynthetic apparatus, the increase of NPQ can form a protective mechanism to dissipate excess light energy and avoid the damage of photoreaction centers (Gururani *et al.* 2015). Under full light, the q_p values were the lowest and the NPQ values were the highest (Fig. 3B,D), which demonstrated that *B. ochracea* adapted to the strong light environment by dissipating heat under full-light condition. Thus, *B. ochracea* could not only adapt to weak-light condition, but also tolerate full-light condition.

The ETR values under full light were significantly lower than those under shade treatments (Fig. 3A). It is shown that *B. ochracea* leaves grown under shade conditions improved the ability of utilization of absorbed light energy. It is also possible that shade improved the openness of the PSII reaction centers to increase the total amount of light energy obtained by leaves (Gururani *et al.* 2015). With the increase of light intensity, q_p showed the reverse trend to that of ETR, *i.e.*, it decreased which indicated that the open part of PSII reaction centers was lower and had lower light energy-conversion efficiency (Fig. 3B). With the increase of photosynthetic active radiation, the photoinhibition reduced the quantum yield

of PSII electron transfer, thus limited photosynthesis and led to the decrease of photochemical efficiency of PSII. According to the results, the Y_{II} reduction rate in 54.9% and 76.4% shade was higher than that in full sunlight and 94.2% shade (Fig. 3C), indicating that the photoinhibition was relatively weaker under 54.9% and 76.4% shade and highly shaded seedlings were susceptible to photoinhibition. Photosynthetic quantum yield is affected not only by light intensity but also by light quality; quantum yield of blue and purple light is lower than that of red and yellow light (Hogewoning *et al.* 2012). The light quality and light intensity under shade conditions both are different from that under full-light conditions (Yoshimura 2010). The proportion of blue and purple light increased under shade conditions. In conclusion, *B. ochracea* could adapt to a variety of light intensities, while suitable shade could improve the ability of seedlings to use light energy and reduce photoinhibition.

The accumulation and transportation of photosynthetic products in plants affect the formation of plant yield and quality (Dong *et al.* 2019). By changing the canopy temperature, photosynthetic rate, heat dissipation, and pigment profile, the photosynthetic products of plants under shade treatment increased that affected the content and quality of polysaccharides as reported earlier (Xu *et al.* 2020). The polysaccharide content of *Codonopsis pilosula* increased significantly under 44.8% illumination intensity (Wang *et al.* 2017). Zheng *et al.* (2012) reported that *Dendrobium candidum* under 88% shade treatment optimized photosynthesis capacity and highly promoted polysaccharide accumulation. In our study, the polysaccharide content of *B. ochracea* under 76.4% shade treatment was the highest, which was significantly higher than that under full light. The possible reason is that shade improves photosynthetic capacity, promotes sugar accumulation, and provides more precursors for the synthesis of polysaccharides. Our results indicated that high shade (94.2%) environment and full-light conditions were not conducive to polysaccharides accumulation in *B. ochracea*. Variation in light conditions cause changes in the yield of photosynthetic products, which can indirectly affect the accumulation of polysaccharides (Yang *et al.* 2017, Ye *et al.* 2017). For example, adjusting light quality improved the photosynthetic capacity and glucose metabolism of *Dendrobium officinale*, thus increasing the polysaccharide accumulation in protocorm of *Dendrobium officinale* (Lei *et al.* 2021). The 76.4% shade treatment was the most beneficial for the accumulation of polysaccharides in *B. ochracea*.

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