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Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg

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ABSTRACT

Tetrastigma hemsleyanum Diels et Gilg was grown under full sunlight and moderate and high levels of shade for one month to evaluate its photosynthetic and chlorophyll fluorescence response to different light conditions. The results showed that *T. hemsleyanum* attained greatest leaf size and P_n when cultivated with 67% shade. Leaves of seedlings grown with 90% shade were the smallest. Leaf color of plants grown under full sunlight and 50% shade was yellowish-green. The P_n value increased rapidly as PPFD increased to 200 μmol m^{-2} s^{-1} and then increased slowly to a maximum, followed by a slow decrease as PPFD was increased to 1000 μmol m^{-2} s^{-1}. P_n was highest for the 67% shade treatment and the LSP for this shade treatment was 600 μmol m^{-2} s^{-1}. Full sunlight and 50% shade treatments resulted in significant reduction of ETR and qP and increased NPQ. Chl *a*, Chl *b* and total chlorophyll content increased and Chl *a*/*b* values decreased with increased shading. Results showed that light intensity greater than that of 50% shade depressed photosynthetic activity and *T. hemsleyanum* growth. Irradiance less than that of 75% shade limited carbon assimilation and led to decreased plant growth. Approximately 67% shade is suggested to be the optimum light irradiance condition for *T. hemsleyanum* cultivation.

1. Introduction

Tetrastigma hemsleyanum Diels et Gilg, belonging to the family Vitaceae, is a herbaceous perennial species native to China. It is distributed in the eastern, central, southern and southwestern provinces of China and is best suited to shady and moist hillsides and valleys. The entire herb and its root tubers possess anti-inflammatory, analgesic, and antipyretic properties. It also is used in Chinese folk medicine for dispelling phlegm and improving blood circulation. It is used for the treatment of high fever, infantile febrile convulsion, pneumonia, asthma, hepatitis, rheumatism, menstrual disorders, sore throat, and scrofula (Liu et al., 2002). In addition, it recently has been reported to work well in improving the immune system and for anti-cancer properties (Feng et al., 2006; Xu et al., 2006; Ding et al., 2005). Thus, in recent years, it has become an important species in China for its medicinal properties and economic value. However, due to human overexploitation coupled with its specific environmental growth requirements for cultivation, *T. hemsleyanum* has become an endangered species.

Climate, soil nutrients, and water have long been understood to be primary factors influencing agricultural productivity (Boyer, 1982; Fischer and Turner, 1978; Novoa and Loomis, 1981). It is relatively easy to control water and nutrient supplies through irrigation and fertilization. In contrast, light intensity (one of the most important plant growth requirements) is more difficult to control (Wang et al., 2007). Through the process of photosynthesis light energy is used to produce ATP and NADPH in the light reaction and subsequently, in the light-independent reaction, carbon is fixed into carbohydrates and oxygen is produced. Under high irradiance, however, the photosynthetic apparatus absorbs excessive light energy, resulting in the inactivation or impairment of the chlorophyll-containing reaction centers of the chloroplasts (Bertaminia et al., 2006). As a consequence, photosynthetic activity is depressed by photoinhibition (Osmond, 1994). In contrast, under low irradiance, insufficient ATP is produced to allow for carbon fixation and carbohydrate biosynthesis. This leads to reduced plant growth. Although *T. hemsleyanum* has been reported to be a shade-preferring plant (based on its primary occurrence in the shaded understory) (Xu et al., 2006), no studies have determined the optimum light intensity for its growth.

Abbreviations: Pn, net photosynthetic rate; PPFD, photosynthetic photon flux density; Yield, effective quantum yield of photochemical energy conversion; ETR, relative rate of electron transport through PSII; qP, photochemical quenching; NPQ, nonphotochemical quenching; *E*, transpiration rate; WUE, water use efficiency; LSP, light saturation point; Chl, chlorophyll; Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*.

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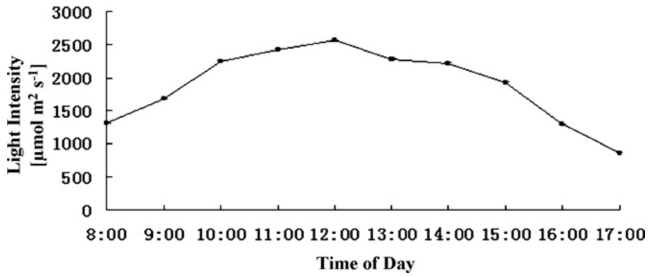

Fig. 1. Curves of diurnal variation of full sunlight during September 2007 in Jinhua.

The objective of the present study was to determine the optimum light intensity for the growth of *T. hemsleyanum* by quantifying the effects of different shade treatments on chlorophyll content, chlorophyll fluorescence, and photosynthetic capacity.

2. Materials and methods

2.1. Plants and growth conditions

T. hemsleyanum plants (with root tubers) were collected from the North Mountain area of Jinhua City and planted in the Zhejiang Normal University campus greenhouse in September 2006. Plantlets were obtained by layering and planting in pots containing a mixture of peat, sand, and humic soil (1:2:1). Plants were grown under five shade treatments (0, 50, 67, 75, and 90% of natural incident irradiance) with 3 replications. Diurnal variation of September full sunlight in Jinhua, measured with a TES-1332 Digital Lux Meter (*TES*, Taiwan), is displayed in Fig. 1. Shading was accomplished by using one or two layers of commercial black cloth shade for 30 days beginning on September 1, 2007. Daily maximum air temperatures were between 36 and 40 °C. Irrigation was provided manually to saturation at 18:00 h each day.

2.2. Photosynthetic parameters

Photosynthetic photon flux density (PPFD) response curves were developed using a GFS-3000 portable photosynthesis system (*WALZ*, Effeltrich, Germany). The parameters were measured on fully expanded leaves from 09:00 to 17:00 h on a clear, cloudless day. The air cuvette temperature and the air CO_2 concentration were maintained at 25 °C and 750 μL L^{-1}, respectively. PPFD was increased from 0 to 1000 μmol m^{-2} s^{-1} (0, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 μmol m^{-2} s^{-1}). Water use efficiency (WUE) was calculated as P_n/E (μmol CO_2 μmol^{-1} H_2O) (Galmés et al., 2007). Assimilation was recorded at each of the 10 light levels following a 10 min acclimation period. Five replications were used for each plant.

2.3. Chl fluorescence

Chl fluorescence was measured with a MINIPAM (pulse-amplitude modulation) fluorometer (*WALZ*, Effeltrich, Germany). Fluorescence measurements were taken simultaneously with gas exchange measurements since the fiber optic bundle of the fluorometer was fitted with a gas-tight seal within the gas exchange cuvette. Leaves were light-adapted for approximately 10 min prior to measurements of the effective quantum yield of photochemical energy conversion (Yield), photochemical (qP) and nonphotochemical (NPQ) quenching of chl fluorescence. Measurements were obtained over a range of PAR values between 0 and 1455 μmol m^{-2} s^{-1}. The relative effective quantum yield of photochemical energy conversion at steady-state photosynthesis was calculated as Yield = (Fm′ − Fs)/Fm′, where Fs and Fm′ are the fluorescence at steady-state photosynthesis and maximum fluorescence in the light, respectively. qP was calculated as (Fm − Fm′)/(Fm′ − F_0). NPQ quenching was calculated as (Fm − Fm′)/Fm′ (Genty et al., 1989). The relative rate of electron transport through PSII (ETR) was calculated as Yield × PPFDa × 0.5 (Krall and Edwards, 1992), where PPFDa is the absorbed light (μmol photons m^{-2} s^{-1}) by leaf (measured using an integrating sphere).

2.4. Chl contents

Following the final measurements described above, leaves were collected for determination of chlorophyll content (Chl *a*, Chl *b*, Chl *a* + *b*). Chlorophyll pigments were extracted by grinding leaves in 80% acetone in the dark at room temperature and were expressed as mg/gfm from the equations of Porra (2002).

2.5. Data analysis

All experiments were conducted in a completely randomized block design replicated three times. Significance at $P \leq 0.05$ was assessed by ANOVA using SAS version 9.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Leaf morphology

Light conditions had significant effects on *T. hemsleyanum* leaf morphology. Leaves grown under 67% (Fig. 2c) and 75% (Fig. 2d) shade were larger than leaves from other treatments. Leaves from the 90% shade treatment (Fig. 2e) were the smallest. Leaf color of plants grown under 67% (Fig. 2c) and 75% shade (Fig. 2d) were dark green, while those grown under full sunlight (Fig. 2a) and 50% shade (Fig. 2b) were yellowish-green.

3.2. Photosynthesis

Regardless of shading treatment, the P_n value increased rapidly as PPFD increased to 200 μmol m^{-2} s^{-1} and then increased slowly to a maximum, followed by a slow decrease as PPFD was increased to 1000 μmol m^{-2} s^{-1} (Fig. 3a). The light compensation points (LCP) in full sunlight and 90% shade treatment plants were a little lower than the ones in 50%, 67%, and 75% shade treatment plants (Fig. 3a). The light saturation points (LSP) in 0% and 50% shade treatments were lower than for other treatments (Fig. 3a). Both P_n and maximum P_n varied significantly ($P < 0.05$) with light intensity treatments. The P_n value increased with increased shading; the highest P_n was observed in the 67% shade treatment plants and the LSP for this shade treatment was 600 μmol m^{-2} s^{-1}. Lower light intensities reduced the P_n value. The P_n–PPFD curves for natural light intensity and 90% shade treatments were almost coincident beyond 200 μmol m^{-2} s^{-1}.

Transpiration rate (*E*)–PPFD curves of the five light intensity treatments are shown in Fig. 3b; *E* values varied significantly ($P < 0.05$) with light intensity treatments. The *E* values of full sunlight and 50% shade treatment plants were lower and *E*–PPFD curves appeared to be a marked single-peak curve. The *E* values of full sunlight treatment plants (when PPFD was >200 μmol m^{-2} s^{-1}) were always lower than that from the 50% shade treatment plants. The *E* values from the other three treatments increased as PPFD increased. The average *E* in 90% shaded plants was always the highest with increasing PPFD. The average *E* value of 67% shade plants was lower than that of 75% shade plants. When PPFD was increased above 800 μmol m^{-2} s^{-1}, however, the *E* value was higher than that of the 75% shade treatment plants.