Bangladesh J. Bot. **52**(2): 395-402, 2023 (June) DOI: https://doi.org/10.3329/bjb.v52i2.67057

DIURNAL VARIATIONS OF PHOTOSYNTHESIS AND LEAF ANATOMICAL CHARACTERISTICS OF *CORNUS WALTERI* WANGER SEEDLINGS UNDER DIFFERENT LIGHT REGIMES

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Keyword: Light, *Cornus walteri*, Photosynthetic characteristics, Anatomical structure

Abstract

Diurnal variations of photosynthesis and leaf anatomical structure characteristics of *Cornus walteri* seedlings under three light conditions, including L_0 (under full light radiation), L_1 (under natural tree shade), L_2 (under one layer of shading) were studied. Results showed that, P_n under different light regimes showed asymmetric convex linear changes. The variation of P_n under L_0 was the strongest; the maximum of P_n appeared at 12:00 AM, that of G_s was so. Photosynthetic Active Radiation (PAR) under L_2 was insufficient, so P_n decreased significantly, and the peak was at 10:00 AM. P_n and G_s under L_1 changed gently.The daily average sequence of P_n was $L_0 > L_2 > L_1$ and C_i showed the reverse sequence. Shading had a significant effect on photosynthetic response parameters of *Cornus walteri* seedlings, which caused a significant decrease of LCP, LSP, and decline of P_{nmax}, AQY and R_d was so. Under shading, leaves of *Cornus walteri* seedlings became thinner, thickness of epidermal cells (EP) decreased significantly, thickness of both PT and ST reduced. *Cornus walteri* was intolerant tree species, which has certain physiological regulation ability to weak light, but severe shading will hinder seedling growing. So it was suggested to raise seedlings under full light, and create a favorable condition for seedlings growing.

Introduction

Light is the basis of plants growth and development, and the most important environmental factor affecting plants distribution and productivity. Shading can change the growth environment of plants, affect the photosynthetic physiological characteristics, and growth, development and morphology (Brodribb and Robert 1997, Franklin 2005). In summer, strong light, high temperature and drought are often the limiting factors for the seedlings growth of many plants, and become the key to whether seedlings can successfully through summer. In recent years, more attention has been paid to the population renewal and breeding of light environment affecting specific populations, especially some precious. For example, early study showed that insufficient light was the main limiting factor to population renewal of *Parrotia subaequalis*, which led to a sharp reduction of medium diameter individuals in the population (Yan 2008) . Guo *et al*. (2017) had researched the relationship between photosynthetic characteristics of *Emmenopterys henryi* seedlings and environmental factors in the young stage, and concluded that *E. henryi* was shade-tolerant plant with the low shade-tolerant ability. It is suggested to reduce the light density and to ensure rapid growth of the seedlings. Therefore, the study of the demand and adaptability of trees to light, especially to some rare species, will help to understand the shade -tolerance of species, explore how to take reasonable management measures to improve the competitiveness of seedlings, and increase the efficiency of *Cornus walteri* Wanger. (Cornaceae) is a deciduous tree which mainly grows in the valley areas and hills of Korea and China (Lee *et al*. 2017). Moreover, *C. walteri* is a woody oil tree, with very high oil contents in fruits (Paul and Don 2005), the application and cultivation of this species has a rapid expansion in China recently.

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At present researches on *C. walteri* mainly focused on resource collection (Tan *et al.* 2010), plus tree selection (Zhao *et al*. 2012, Li *et al*. 2014), flowering and breeding physiology (Xue *et al*. 2012, Bao *et al*. 2017, Zhang *et al.* 2021), oil composition of fruits (Wu *et al*. 2017, Liu *et al*. 2021), bioactive substances from stems and leave (Lee *et al*. 2017, 2018, Wang *et al*. 2022), and so on, while there is little reference on the adaptive physiological aspects of the light closely related to their seedling cultivation and population renewal. Hence, further investigations and researches on understanding possible responses, adaptations and physiological mechanisms of *C. walteri* under different light conditions are required. Thus the present study aimed to study Diurnal variations of photosynthesis and leaf anatomical characteristics of *C. walteri* seedlings under different light regimes.

Materials and Methods

Experiments were performed at horticultural experimental station in Jinling Institute of Science and Technology (JIT), Nanjing, China (lat 31°51’41”N, long 118°46’41”E). Typical monsoon climate, annual temperature 16.1℃, extreme maximum temperature 43.0℃, extreme minimum temperature 13.0℃, annual rainfall 1100 mm, annual sunshine hours 1912 h, annual relative humidity 80%, frost-free period 224 d.

Seeds of *C. walteri* were collected from Zijin Mountain National forest Park, Nanjing, China (lat 32°03’53”N, long 118°49’29”E) in Apr 2019, sow and cultivated seedlings in spring the later year. In Mar 2020, 200 robust seedlings with comparable specifications and good growth were selected to transplant into cylindrical ceramic containers, 35 cm diameter and 40 cm height. The culture material in containers was a mixture of loess and nutrient soil (mixing ratio 1:1), with a uniform mass of 10 kg. The surface of container soil was covered with sawdust to ensure soil moisture, and carried out normal water and fertilizer management. Seedlings were treated from Jun to Sep, and three light environments were set, repeated three times, and one-factor random blocks were designed to eliminate location effects. 30 basins of container seedlings were placed for each treatment, and protective rows were set up to prevent cross influence. L_1 was under natural tree shade, L_2 was under one sunshade cover, L_0 was under full light, that was control.

With Li-cor 6400 photosynthetic apparatus to determine relevant photosynthesis indexes. Three well-developed seedlings were selected, the 3 well-developed leaves, 4^{th} from top, were selected as experiment sample. Since the leaf photosynthetic rate usually present remarkable daily variation, in order to minimize the error caused by the change of light condition, all the measurement conducted within 1 h (10: 00~11: 00 AM) on a clear day. Air temperature was 34.09~34.42℃, CO_2 concentration 396.88~410.50 $\mu mol \cdot mol^{-1}$. The artificial light source, standard leaf chamber (2 cm × 3 cm) were used. The leaves were light-induced 30 min in advance, and photo-synthetically active radiation (PAR) was controlled at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, stopped 40~60s per leaf. Measured indexes included: Net photosynthesis rate (P_n), stomatal conductance (G_s) , intercellular CO_2 concentration (C_i), air CO_2 concentration (C_a), transpiration rate (T_r), relative humidity(*RH*), water use efficiency (WUE), WUE = P_n / T_r.

P_n—PAR indexes was finished by means of Auto-prog program of Li-6400 photosynthesizer, equipped with red and blue light source. P_n—PAR curve was fitted using a non-right-angle hyperbolic model (Ye and Li 2010), and obtained following parameters: Optical compensation point (LCP), optical saturation point (LSP), maximum net photosynthesis efficiency (P_{nmax}), dark respiration rate (R_d), apparent quantum efficiency (AQY) (Liu *et al*. 2011; Richardson 2002).

Making paraffin sections according to Li (1987), leaves were picked after transverse sections along the middle vein, the locks were cut approximately for 5 mm×5 mm, fixed with FAA, dehydrated in ethanol and xylene series, embedded in paraffin, stained with red-solid green, and sealed with neutral gum. Sections were observed and photographed under a OLYMPUS-BX61 light microscope, and various microscopic parameters were measured with the help of Image-Pro Plus 6.0 (Cai *et al*. 2016).

Figures were made with excel 2003, ANOVA was conducted with SPSS17.0, treatment means were compared by LSD test at 99% and 95%, confidence interval to estimate their significance under different treatments. The results were represented by Means ± SD.

Results and Discussion

As could be seen from Fig. 1, PAR showed different curve between different treatments. PAR maximum under L_0 was 1759 μmol·m^{-2}·s^{-1} (12:00 AM), which was significantly stronger than L_1 and L_2, and that accounted for 9.31 and 15.92% of the former respectively. Air temperature showed a similar trend, peak appeared at 2:00 PM. The change law of *RH* between different treatments was not obvious because of wind.

Change of P_n under different treatments shown in Fig. 2A. As could be seen, P_n showed different curves under three light conditions, with asymmetric convex linear changes, however, the amplitude of change, the emergent time of the peak occurrence, and the daily mean were significantly different. In comparison, the change of P_n under L_0 was the most obvious, with the peak occurring at 12:00 AM (14.891 μmol·m^{-2}·s^{-1}), and then drop sharply until it became negative at nightfall.

Change tendency of P_n under L_2 was similar to L_0, especially the change before 10:00 AM, but P_n after 10:00 decreased significantly. Variation of P_n under L_1 was relatively gentle, with the maximum at 12:00 (5.787 μmol·m^{-2}·s^{-1}). ANOVA shown that P_n under both L_1 and L_2 was significantly smaller than the control ($P<0.05$), variation of daily mean value of P_n between different light conditions was significant too ($P<0.05$, Table 1).

Table 1. Daily mean value of photosynthesis parameters of *C. walteri* seedlings under different light conditions.

Treatment	P_n /(μmol·m^{-2}·s^{-1})	G_s /(mol·m^{-2}·s^{-1})	C_i /(μmol·mol^{-1})	T_r /(mmol·m^{-2}·s^{-1})	WUE /(μmol·mmol^{-1})
L_0	9.640±1.021a	0.192±0.025a	318.553±9.812b	2.488±0.234a	3.494±0.412a
L_1	3.556±0.671c	0.117±0.021c	359.418±9.102a	1.481±0.128b	2.155±0.180c
L_2	4.608±0.232b	0.123±0.011b	339.360±8.891a	1.699±0.167b	2.414±0.234b

Different lowercase letters indicate significant differences between two treatments at 0.05 level $P<0.05$).

Generally, G_s reflects the ability of the atmospheric CO_2 and water vapor into plant leaves. The diurnal variation of G_s under different light conditions was shown in Fig. 2B, and three curves were slightly different. The variation of G_s under L_0 was similar to P_n, from 8:00 to 12:00 AM G_s has always changed at a high level, and the maximum was 0.309 mol·m^{-2}·s^{-1}, began to drop then, and fell to the lowest at 6:00 PM.

The change tend of G_s under L_2 was similar to L_0, but the maximum occurred at 10:00 AM. G_s always under L_1 keep declining. ANOVA showed that variation of daily mean value of G_s between different treatments was significant too ($P < 0.05$).

The change of C_i under different treatments was basically same, and curves was close to the bottom shape of the pot, the lowest value appeared at 2:00 PM (Fig. 2C). The order of daily mean value of C_i was $L_1>L_2>L_0$. ANOVA showed that there were significant differences in C_i between different treatments ($P < 0.05$), but no between L_1 and L_2 ($P > 0.05$).

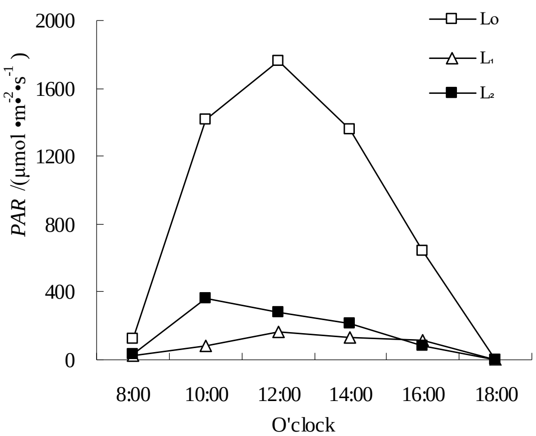

Fig. 1. Diurnal variation of photosynthetically active radiation under different light conditions.

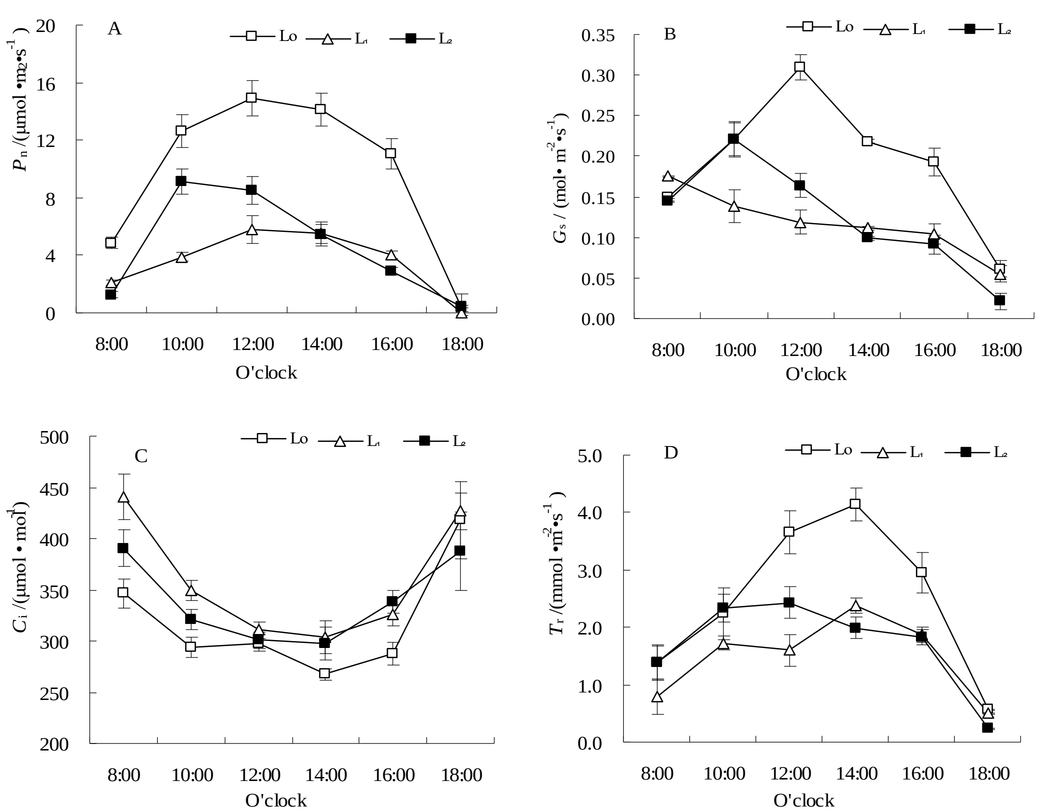

Fig. 2. Diurnal variation of net photosynthesis rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), transpiration rate (T_r) of *C. walteri* seedlings under different light conditions.

As shown in Fig. 2D, the change trend of T_r was convex shape, the peak of L_0 and L_1 occurred at 2:00 PM, but that of L_2 at 12:00 AM. The daily mean value order of T_r was $L_0 > L_2 > L_1$ (Table 1). That of WUE was consistent with the P_n. Correlation analysis showed that there were a significant positive correlation between WUE and P_n ($P < 0.05$). It can be seen that shading caught improve water use efficiency of seedlings and increased drought resistance ability. This was in agreement with previous reports in other species, such as *Cercidiphyllum japonicum* (Li *et al.* 2019), *Cucumis sativus* (Xu *et al.* 1993), oil peony (Cai *et al.* 2016), and *Rubus* subgenus (Yang *et al.* 2005).

The P_n—PAR curves of *C. walteri* seedlings under different light conditions were shown from Fig. 3, which showed a consistent trend, and the inflection point was around at 200 μmol·m^{-2}·s^{-1}. That was, when PAR was less than 200 μmol·m^{-2}·s^{-1}, P_n rose sharply, and difference of P_n between different treatments at same point was slight. However, when PAR was more than 200 μmol·m^{-2}·s^{-1}, the rise of P_n became relatively slow and gradually became horizontal. Non-right-angle hyperbolic fitting of P_n—PAR curves of *C. walteri* seedlings under different light environments could be seen from Table 2 (Ye and Li, 2010). It was clear that light conditions significantly affected light response parameters. The ANOVA showed that the variance of LCP, LSP, P_{nmax} and R_d of *C. walteri* seedlings under different light conditions were significant ($P < 0.05$).

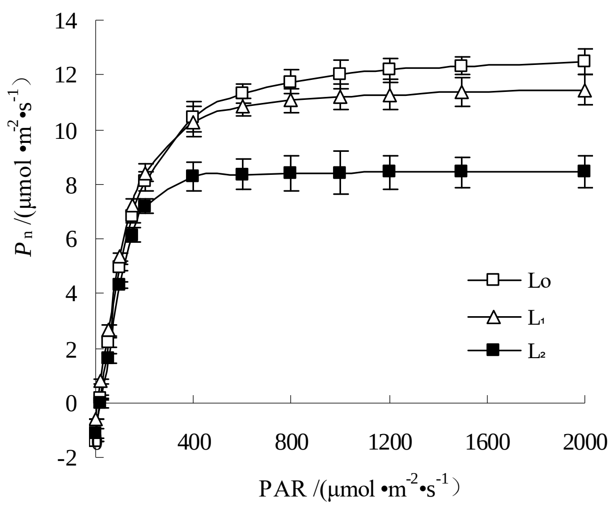

Fig. 3. P_n—PAR response curves of *C. walteri* seedlings under different light conditions.

Table 2 show that LCP and LSP were extremely sensitive to the light, with LCP under L_1 and L_2 was 0.388 and 0.462 times control respectively, and LSP 0.777 and 0.799 times control, respectively. Thus, low light caused a significant decrease of LCP and LSP. In general, this was the physiological regulation mechanism of seedlings under low-degree light, because low LCP and R_d can improve the photosynthetic efficiency of plants in low light to make up for the consumption of nutrients in normal growing (Pearcy and Sims *et al.* 1994), which was the inevitable result of plants adaptation to the low light environment, and also showed that *C. walteri* has a certain ecological adaptability to low light. At same time strong low light caused a significant decrease of P_{nmax}, R_d and AQY, so that reduced the photosynthesis efficiency (Onwueme and Johnston 2000).

Mesophyll was a place for the photosynthesis of plants, but also a physiological tissue sensitive to the environment, and the thickness of palisade tissue (PT) and sponge tissue (ST), cell layers, cell morphology, chloroplast distribution showed certain adaptive adjustment with the change of external light intensity (James *et al*. 2000), this has been confirmed by previous reports in other species, such as *Liquidambar formosana* (Wang *et al*. 2007), *Cercidiphyllum japonicum* (Li *et al*. 2019), *Trifolium repens* (Yang *et al*. 2015) and so on.

Table 2. Characteristic parameters of P_n—PAR response curves of *C. walteri* seedlings under different light conditions.

Treatment	LCP /(μmol·m^{-2}·s^{-1})	LSP /(μmol·m^{-2}·s^{-1})	P_{nmax} /(μmol·m^{-2}·s^{-1})	R_d /(μmol·m^{-2}·s^{-1})	AQY /(μmol·μmol^{-1})	R^2
L_0	13.633±1.171a	322.511±8.168a	14.650±0.028a	1.458±0.192a	0.088±0.016a	0.998±0.001a
L_1	5.286±0.452b	250.66±22.750b	12.647±0.820b	0.641±0.092b	0.074±0.007b	0.997±0.002a
L_2	6.295±1.541b	257.818±23.231b	9.382±1.021c	0.258±0.081c	0.038±0.005c	0.915±0.003a

Different lowercase letters indicate significant differences between two treatments at 0.05 level (P<0.05).

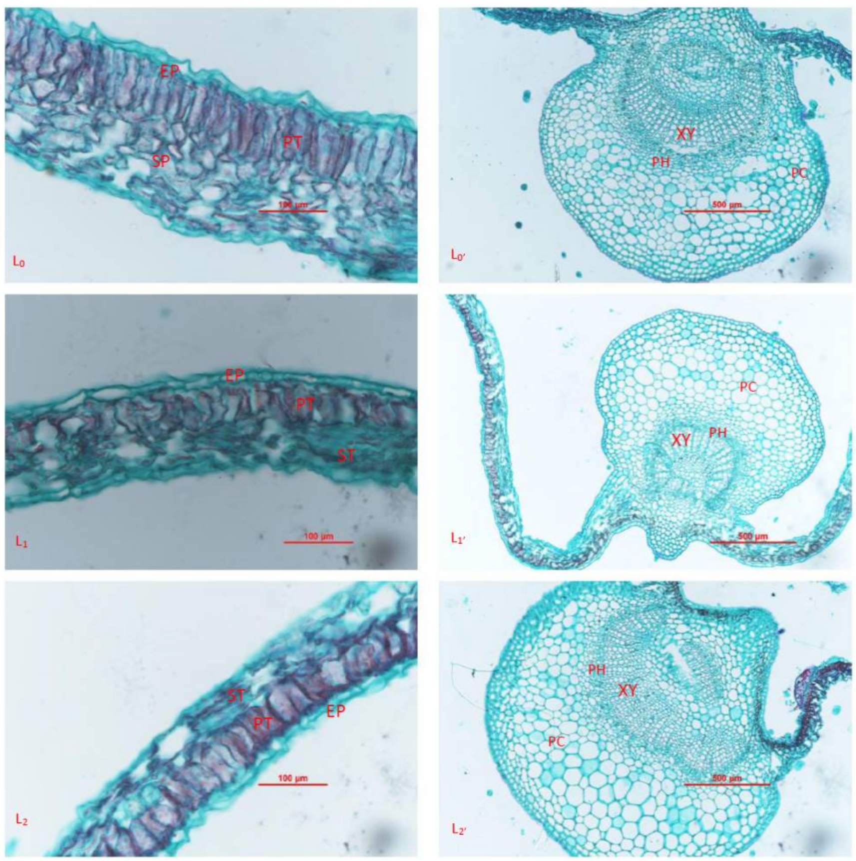

Fig. 4. The cross section structure of *C. walteri* leaves under different light conditions.
L_0 ,L_1,L_2: the cross section of leaves under different light conditions (scale 50 μm); $L_{0'}$, $L_{1'}$,$L_{2'}$: the cross section of main vein under different light conditions (scale 100 μm). EP, epddemal cell; PT, palisade tissue; ST, sponsy tissue; XY, xylem; PH, phloem; PC, Parenchyma cell.

The observation of paraffin sections showed that *C. walteri* were typical heterofacial plants with different upper and lower surface structures (Fig. 4). Epidermal cells (EP) were located on the surface of leave,which could be divided into upper and lower epidermis. EP showed light green, and cells were closely arranged. Mesophyll could be divided into PT and ST, in which distributed fully developed vascular bundles, and xylem (XY) and phloem (PH) were clear. PH was located outside XY, XY was almost heart-shaped, and multiple layers of parenchyma cells (PC) encircled XY and PH. The results showed that under the low light conditions, the leaf thickness (LT) decreased, the number of EP decreased, and thickness of both PT and ST decreased, which showed a physiological characteristics conducive to low light utilization.

To sum up, *C. walteri* has a certain physiological regulation ability to the adverse light environment, showed a certain light preference in the seedling period, and has a certain physiological regulation for low light, so shading had not strong impact on the normal growth of seedlings. However, severe light deficiency will cause a significant decrease in the photosynthesis efficiency, hinder seedling growing. It is suggested to seedling raising should be under full-light conditions as possibly, increase ventilation and light transmission, and create favorable conditions for the robust growth of seedlings.

Acknowledgements

The authors are thankful to Mengfan Cui, Lixin Huang, Wenhui Pei *et al*. JIT, for their helps in experiments. This research was jointly supported by Nanjing Gardens Green Bureau Science and Technology Project (YLKJ202109JH), and the Doctor Foundation (40610108) from JIT, China.

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(Manuscript received on 04 April, 2022; revised on 10 June, 2023)