COMPARISON OF LEAF PHOTOSYNTHESIS AND STRUCTURE OF JUVENILE AND ADULT SPECIES OF THE ENDANGERED PLANT *TSOONGIODENDRON ODORUM*

PAN, X. F.[#*] – TANG, J. M.[#] – JIANG, H. D. – ZOU, R. – YANG, Y. S. – WEI, X.[*]

Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, China

[#] *These authors contributed equally to this work*

**Corresponding author*
e-mail: weixiao@gxib.cn; panxinfeng@fafu.edu.cn

(Received 10th Nov 2023; accepted 18th Jan 2024)

Abstract. *Tsoongiodendron odorum* is a rare and characteristic plant species with an extremely limited population. Understanding the photosynthetic and physiological properties of this species is crucial for implementing proper protective cultivation methods. No reports have yet been made on the study of photosynthetic physiological characteristics at different growth stages. In this study, the photosynthetic responses of the leaves of *Tsoongiodendron odorum* juvenile and adult were examined and the chlorophyll content and anatomical characteristics of the leaf surface were compared. The results showed that: the rate of net photosynthetic (P_n) of adult trees was significantly higher than that of juvenile under various light intensity, and there was light inhibition in juvenile species under high light intensity; the maximum net photosynthetic rate (P_{max}) and light saturation point (LSP) of adult tree was significantly higher than those of juvenile, while the dark respiration rate (R_d) and light compensation point (LCP) of juvenile were higher than those of significant adult. Stomatal conductance (G_s) and transpiration rate (T_r) were higher in adult trees than in juveniles under high light intensity. Additionally, adult trees had significantly higher levels of chlorophyll b and total chlorophyll compared to juveniles, and their tissues differed significantly. Results of correlation and redundancy analysis showed that photosynthetic rate was positively correlated with leaf area, palisade cell tissue thickness, palisade-spongy ratio, xylem thickness, phloem thickness, and vascular cambium thickness. In conclusion, it is suggested that shading treatment should be carried out, and the shading degree should be gradually decreased with the growth of the plants to prevent the growth of *Tsoongiodendron odorum* from being limited by too strong or too weak light environment.

Keywords: *chlorophyll, photosynthesis differences, leaf structure, cultivation, protection*

Abbreviations: Car, carotenoid; Chl a, chlorophyll a; Chl a/Chl b, chlorophyll a to chlorophyll b; Chl b, chlorophyll b; G_s, stomatal conductance; LA, leaf areas; LCP, light compensation point; LET, lower epidermis thickness; LSP, light saturation point; PAR, photosynthetically active radiation; PH, phloem thickness; P_{max}, maximum net photosynthetic rate; P_n, net photosynthetic; PT, palisade tissue thickness; PT, palisade tissue thickness; PT/ST, ratio of palisade tissue to spongy tissue; PT/ST, ratio of palisade tissue to spongy tissue; R_d, dark respiration rate; SLA, specific leaf area; ST, spongy tissue thickness; ST, spongy tissue thickness; T_r, transpiration rate; UET, upper epidermis thickness; VC, vascular cambium thickness; VD, vein duct; VD, vein duct; WUE, water use efficiency; XY, xylem thickness

Introduction

Photosynthesis is the base of plant growth and development (Vernon and Avron, 1965). It involves the conversion of light energy into chemical energy, generating organic matter in plants, algae, and some microorganisms (Maurino and Weber, 2013; Vecchi et al., 2020; Häder, 2022). Process of photosynthesis is crucial for plants and even the entire ecosystem. This process does not remain the same throughout the life of the plant, but changes dynamically with the change of tree age (Bond, 2000). For woody plants, prereproductive and aft-reproductive whether the plant is adult or juvenile. The notion that there exists a substantial disparity in photosynthetic capacity between young and adult trees is a subject of ongoing controversy in the field. Several studies have uncovered significant variations in leaf physiological parameters, such as water utilization strategy, stomatal conductance, leaf water potential, and photorespiration, between young and adult plants (Mediavilla, 2003; Velikova et al., 2008a; Chondrogiannis and Grammatikopoulos, 2016). However, several studies have also suggested that there are no significant morphological transformations evident from juvenile to adult, and indicated that numerous parameters exhibit no significant differences between juvenile and adult trees (Franck et al., 2006; Li et al., 2007). Nonetheless, several studies have investigated the potential correlation between leaf photosynthetic capacity and other readily measurable quantitative parameters, but the few studies on the differences of photosynthetic characteristics and leaf anatomical structure among trees of different ages.

T. odorum (*Tsoongiodendron odorum*) is an evergreen arbor of the Magnolia family, mainly distributed in southern China and northern Vietnam (Liu, 2004). *T. odorum* was often extracted chemicals and essential oils from its leaves and woods for use in chemical raw materials and bio-pharmaceuticals (Huang et al., 2011; An et al., 2023). The wild population of *T. odorum* is currently experiencing a significant decline in both number and size, resulting in a near-threatened status as designated by the International Union for Conservation of Nature (IUCN) and identified extremely small populations (PSESP) in China (IUCN, 2014; Qin et al., 2017). Therefore, the genetic protection of *T. odorum* is imminent. *T. odorum* has been developed 12 microsatellite loci to assess the current population genetic processes influencing population sustainability, and to further understanding of its conservation genetics (Jiang et al., 2011). According to growth rate and leaf functional traits of four broad-leaved species under planted in Chinese fir plantations with different tree density levels, *T. odorum* suitable for planting under low-density stands with low shading. Up to now, the photosynthetic physiological characteristics of *T. odorum* at different growth stages have not been reported (Xu et al., 2022).

This study examined the variations in leaf structure, anatomical traits, and photosynthesis of juvenile and adult *T. odorum*. The aim was to reveal the relationship between leaf structure, photosynthetic physiology, and age. Correlation and redundancy analyses were employed to identify internal connections between the two stages. The study provides valuable insights into the differences between the two stages. These findings offer a scientific and theoretical foundation for introducing and cultivating *T. odorum*, as well as direction for its meticulous cultivation on a larger scale, based on two different stages.

Materials and methods

Study site

The experimental site is located in Guangxi Institute of Botany, which is located at 25°01’ north latitude, 110°17’ east longitude, 180 ~ 300 meters above sea level, and belongs to the subtropical monsoon climate. The average annual temperature is 19.2℃, the extreme minimum temperature is -4.2℃, and the average annual minimum temperature is consistently above 0℃ in typical years (*Fig. 1*). The average annual rainfall is about 1800 mm, and the average annual relative humidity is 78%. The entire Botanical Garden is composed of undulating hills with considerable fluctuation, creating numerous microclimatic environments and an exemplary ecological environment, which has unique advantages for accumulating and preserving plant resources in Guangxi and the subtropics.

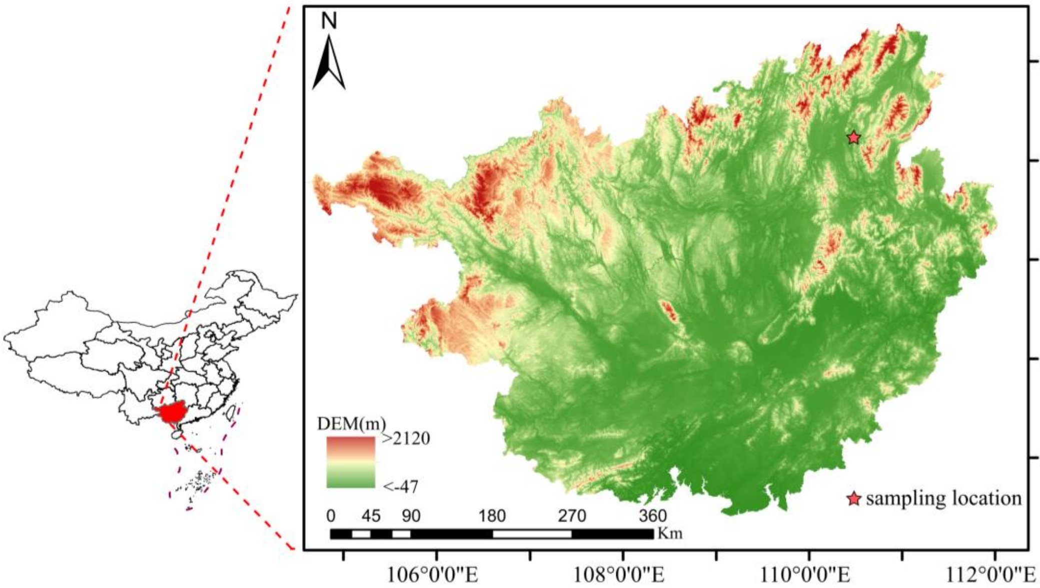


Figure 1. *map of the study area and indicate the position of the T. odorum tree*

Three *T. odorum* trees, each with a minimum age of one year, were selected as juvenile trees, while three *T. odorum* trees, each with a minimum age of ten years, were selected as adult trees. Healthy growing leaves from juvenile and adult trees were selected for measurement of leaf structure, anatomical characteristics, and photosynthetic characteristics parameters.

Determination of light response curve

The net photosynthetic rate (P_n) of foliage was quantified by the LED red and blue light source leaf chamber of the Li-6400 portable photosynthesis instrument in the morning of late September among the hours of 9:00 and 11:00. Three healthy and intact leaves were selected from each of the adult and young trees for determination. The leaves to be tested were subjected to a light intensity of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 15 min prior to measure. Our air flow rate was adjusted to 500 $mL \cdot min^{-1}$ and the leaf temperature was maintained at 28°C. The light intensity gradient was established at 2000, 1800, 1500, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with each intensity sustained for 160-200 s.

Determination of chlorophyll content

Juvenile and adult trees were chose respectively, and 3 leaves with good growth were selected for each tree. After cleaning the leaves, 0.2 g was accurately weighed and immersed in 95% ethanol for 24 h in the dark. Absorbance values were measured at wavelengths of 470 nm, 646 nm and 663 nm with a UV-visible spectrophotometer Alpha 1502 (Shanghai Puyuan Instrument Co., LTD.). The contents of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) were calculated according to Lichtenthaler's method (Lichtenthaler and Buschmann, 1987), as well as the ratio of chlorophyll a to chlorophyll b (Chl a/Chl b) and the ratio of chlorophyll to carotenoid (Car/Chl).

Leaf parameter determination

Fifty leaves were selected from each of the juvenile and adult trees, and the leaf areas (LA) were measured by Li-3000 leaf area instrument, and then treated at 110°C for 30 min and dried at 80°C for 24 h. The dry weight was weighed by electronic balance, and the specific leaf weight (SLW) and specific leaf area (SLA) were calculated.

The leaves of *T. odorum* juvenile and adult trees were cut crosswise along the back and midvein, the slices were 25 mm×25 mm, fixed with FAA fixing solution (70% ethanol: formalin: acetic acid = 90:5:5), dehydrated with ethanol and xylene series, paraffin embedded, toluidine blue dyed, and neutral gum sealed. The slices were observed and photographed under a light microscope, and the microscopic parameters were measured with the help of the graphical analysis software CaseViewer. The measurements were: Upper epidermal cell thickness (UET), lower epidermal cell thickness (LET), leaf thickness (LT), palisade tissue thickness (PT), spongy tissue thickness (ST), ratio of palisade tissue to spongy tissue thickness (PT/ST), diameter of the middle vein duct (VD), xylem thickness (XY), phloem thickness (PT) and vascular cambium thickness (VC). Thirty visual fields were used to measure the parameters.

Data processing and analysis

The parameters of photosynthetic curve, chlorophyll content and leaf structure were preliminarily analyzed by Excel. The light response curve was fitted by hyperbolic modified model, the abscissa is photosynthetically active radiation (PAR), and the ordinate is P_n and the apparent quantum efficiency (AQY), maximum net photosynthetic rate (P_{max}), light saturation point (LSP), light compensation point (LCP) and dark respiration rate (R_d) were calculated. Origin 2021 software was used to make relevant charts, SPSS Statistics 26 was used for one-way ANOVA, and Canoco 5.0 was used for redundancy analysis.

Results

Comparison of light response curves

The P_n of juvenile of *T. odorum* was significantly different from that of adult trees (*Fig. 2*). The P_n of adult trees of *T. odorum* was higher than that of juvenile under each light intensity. There was a great difference between the photosynthetic response curves of juvenile and adult trees of *T. odorum* with the changing trend of light intensity. The photosynthetic active radiation intensity of adult trees increased rapidly with the increase of light intensity in the range of 0-600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while the rapid increase trend stopped at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, the P_n remained stable with increasing light intensity. The P_n of juvenile decreased slowly with increasing of light intensity after reaching the LSP at about 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Comparison of characteristic parameters of light response

The AQY, P_{max}, LSP, LCP and R_d of *T. odorum* juvenile and adult were calculated based on the corrected right angle hyperbola model (*Table 1*). As shown in *Table 1,*

P_{max} (4.7331 ± 0.5698 > 2.8507 ± 0.1901) and LSP (1374.9095 ± 314.0331 > 564.9098 ± 44.7357) of adult trees were significantly higher than those of juvenile ($p < 0.05$). LCP (10.3517 ± 1.9928 < 3.6320 ± 1.8174) and R_d (0.6521 ± 0.1272 < 0.1233 ± 0.1707) of adult trees were significantly lower than those of juvenile ($p < 0.05$). There was no significant difference in apparent quantum efficiency between adult and juvenile.

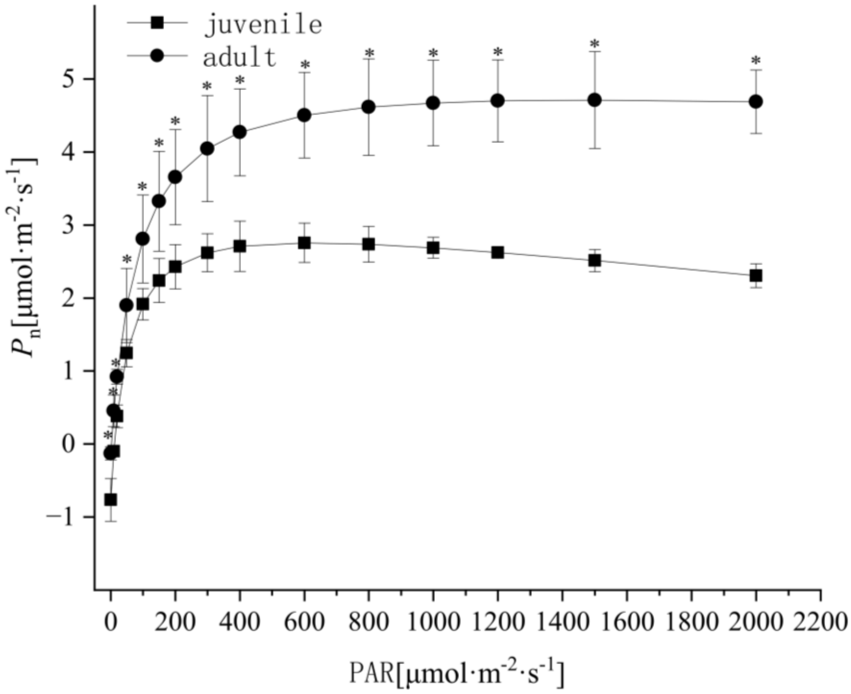


__Figure 2.__ Photosynthetic response curves of T. odorum juvenile and adult plants

__Table 1.__ Comparison of photosynthetic indexes of between T. odorum juvenile and adult trees

Type	AQY/(mol·mol^{-1})	P_{max}/(μmol·m^{-2}·s^{-1})	LSP/(μmol·m^{-2}·s^{-1})	LCP/(μmol·m^{-2}·s^{-1})	R_d/(μmol·m^{-2}·s^{-1})
Juvenile	0.0253 ± 0.0005a	2.8507 ± 0.1901a	564.9098 ± 44.7357a	10.3517 ± 1.9928a	0.6521 ± 0.1272a
Adult	0.0278 ± 0.0044a	4.7331 ± 0.5698b	1374.9095 ± 99.0331b	3.6320 ± 1.8174b	0.1233 ± 0.1707b

Values with superscript letter a and b are significantly different across columns ($P < 0.05$)

Analysis of light response curves for G_s, T_r, C_i and WUE

The G_s of both juvenile and adult trees demonstrated an escalating trend with the augmentation of photosynthetic radiation intensity (*Fig 3*). Nevertheless, the augmentation trend of G_s of juvenile was slower than that of adult, but G_s was higher than adult at the phase of low light conditions, and the Gs of adult trees was superior to that of juveniles after 1200 μmol·m^{-2}·s^{-1}. WUE of both juvenile and adult trees demonstrated a trend of swift increase under low-light environment. With the augmentation of light intensity, WUE of adult trees showed a relatively swift decline after 50 μmol·m^{-2}·s^{-1}, while that of juvenile showed a relatively moderate decline after 400 μmol·m^{-2}·s^{-1}. The transpiration rate of juvenile and adult trees fluctuates with photosynthetically active radiation intensity in a similar manner as G_s fluctuates with photosynthetically active radiation intensity, and Gs of adult trees after 300 μmol·m^{-2}·s^{-1} is superior to that of juvenile. The C_i value of juvenile was higher than adult trees under the photosynthetic radiation of 10-2 000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the C_i value of *T. odorum* juvenile and adult trees was the greatest under the effective radiation intensity of 0-50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the slope of response curve was the largest. After more than 50 µmol, C_i values of adult trees and juvenile trees decreased rapidly. The alteration of C_i of juvenile tended to be gradual under the effective radiation intensity of 100-1800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For adult trees, C_i increased gradually at the concentration of 50-2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

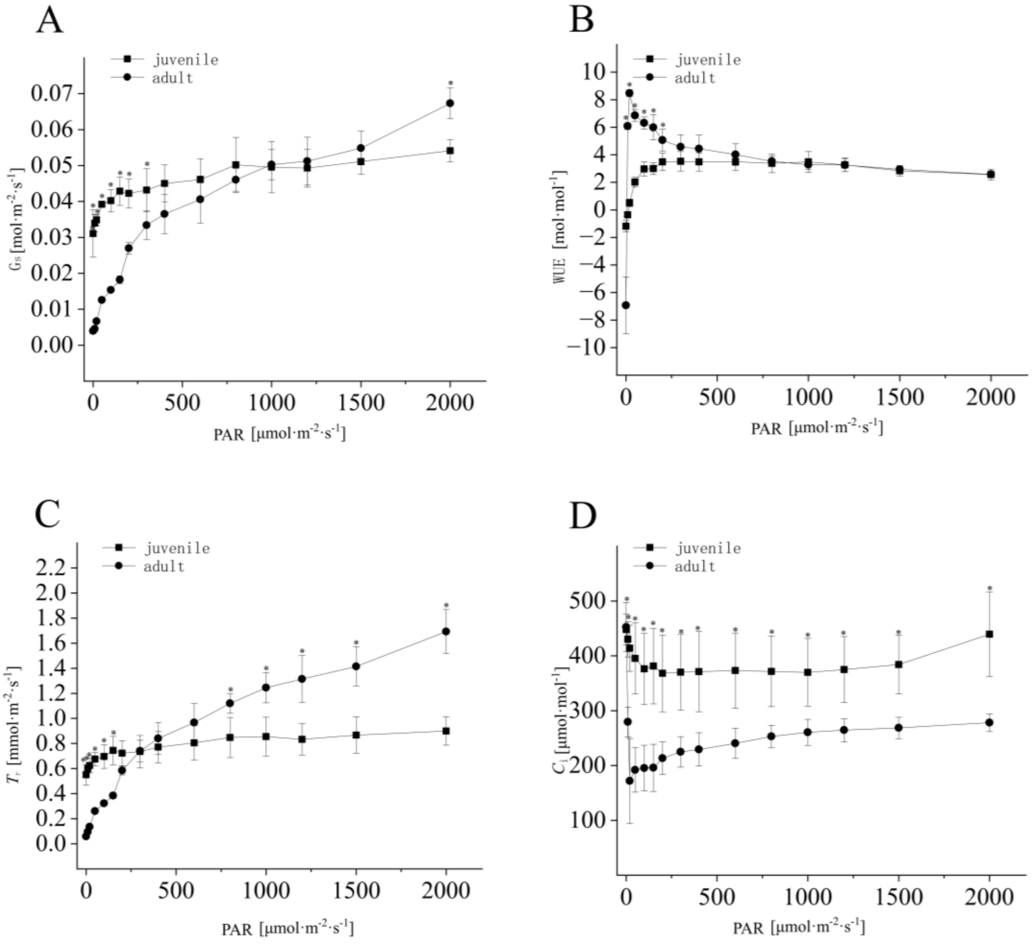


Figure 3. *Light response curves for G_s, T_r, C_i and WUE. (A) Line graph of stomatal conductance at different light intensities. (B) Line graph of water utilization rate at different light intensities. (C) Line graph of transpiration rate at different light intensities. (D) Line graph of intercellular CO_2 concentration at different light intensities*

Comparison of chlorophyll content

As shown in *Table 2*, diverse chlorophyll contents of juvenile and adult trees of *T. odorum* are different. In adult trees, Chlb (1.1409 ± 0.07804 > 1.5408 ± 0.1608) and total chlorophyll (3.4918 ± 0.1680 > 3.9382 ± 0.2806) were significantly higher than those of juvenile ($p < 0.05$). Car (1.0518 ± 0.0524 > 0.8454 ± 0.0552), Chla/Chlb (2.0765 ± 0.2704 > 1.5644 ± 0.0822) and Car/Chl (0.2312 ± 0.0055 > 0.2146 ± 0.0039) were significantly lower than that of juvenile ($p < 0.05$).

Table 2. *Chlorophyll content of leaves of T. odorum juvenile and adult*

Type	Chla ($mg\cdot g^{-1}$)	Chlb ($mg\cdot g^{-1}$)	Car ($mg\cdot g^{-1}$)	Chl (a+b) ($mg\cdot g^{-1}$)	Chla/Chlb	Car/Chl
Juvenile	2.3508±0.1969 a	1.1409±0.07804 a	1.0518±0.0524 a	3.4918±0.1680 a	2.0765±0.2704 a	0.2312±0.0055 a
Adult	2.3974±0.1201 a	1.5408±0.1608 b	0.8454±0.0552 b	3.9382±0.2806 b	1.5644±0.0822 b	0.2146±0.0039 b

Values with letter a and b are significantly different across columns ($P < 0.05$)

Comparison of anatomical structure characteristics of leaves

As illustrated in *Figure 4A–C,* the cells in the juvenile leaves are fewer and irregularly arranged, the PT is not fully developed, the differentiation of PT and ST is not distinct, and the vascular bundle is delicate. The cells in the adult leaves, PT was completely developed and arranged in regular fascicles, and PT and ST were distinctly differ and established an irregular ventilation system. The vascular bundle matured and augmented in diameter, and formed multi-circle layer parenchyma cells in adult leaves. As depicted in *Figure 4B–D*, there were fewer cells in XY and PH of vascular bundle in the primary vein of juveniles, and no outward derivation occurred. In adult leaves, XY, PH, and cambium of vascular bundles was significantly differentiated, and the number of cells augmented, and XY and PH extended to the periphery. As shown in *Tables 4* and *5*, PT, PT/ST, XY, PH and VC of adult trees were significantly higher than those of juveniles, while LT, UEC and LEC of mature trees and juveniles were not significantly divergent.

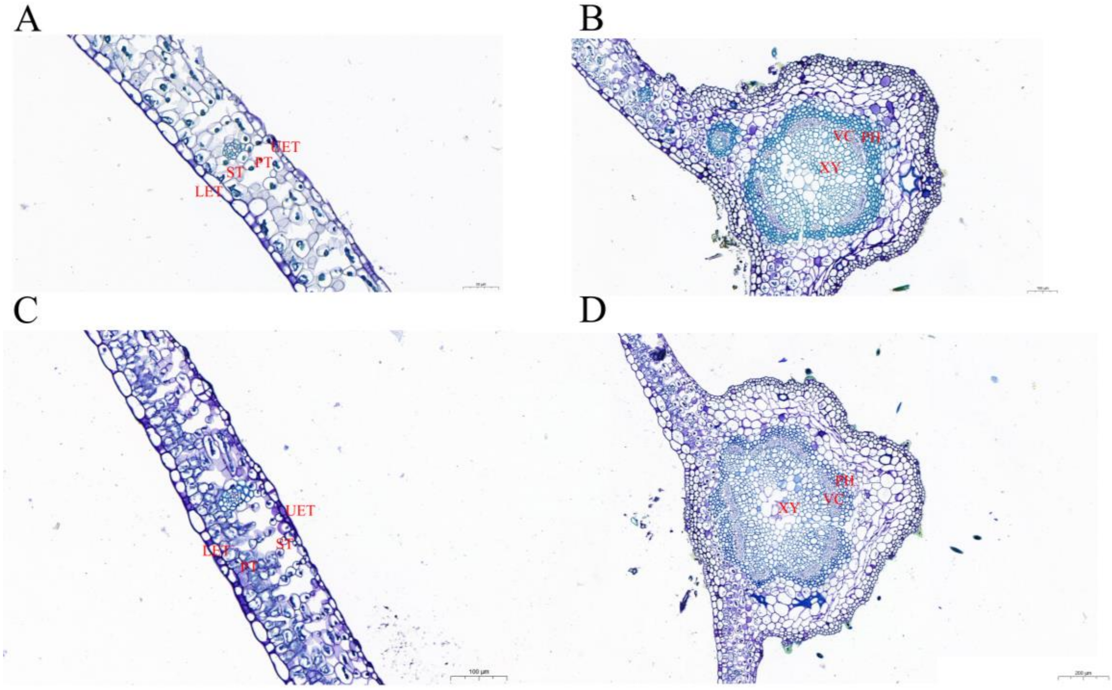


Figure 4. *Paraffin sections of T. odorum juvenile and adult trees. (A_ Cross-sectional paraffin section of juvenile leave. (B) Cross section of main vein of juvenile. (C) Cross section paraffin sections of adult tree leave. (D) Cross-sectional paraffin sections of the main veins of adult tree leave. UET stands for upper epidermis thickness, ST stands for spongy tissue thickness, PT stands for palisade tissue thickness, LET stands for lower epidermis thickness, PH stands for phloem thickness, VC stands for vascular cambium thickness, XY stands for xylem thickness*

Table 4. *Characteristic parameters of leaf anatomical structure*

Type	**LT (μm)**	**UET (μm)**	**LET (μm)**	**PT (μm)**	**ST (μm)**	**VD (μm)**	**PT/ST**
Juvenile	120.01 ± 5.24 a	20.83 ± 0.86 a	13.54 ± 1.03 a	23.20 ± 2.92 a	46.04 ± 2. 46a	34.54 ± 3.34 a	0.50 ± 0.09 a
Adult	121.50 ± 5.99 a	20.45 ± 0.71 a	13.55 ± 1.27 a	46.67 ± 1.53 b	46.9 ± 1.53a	59.74 ± 5.62 b	0.99 ± 0.04b

Values with letter a and b are significantly different across columns ($P < 0.05$)

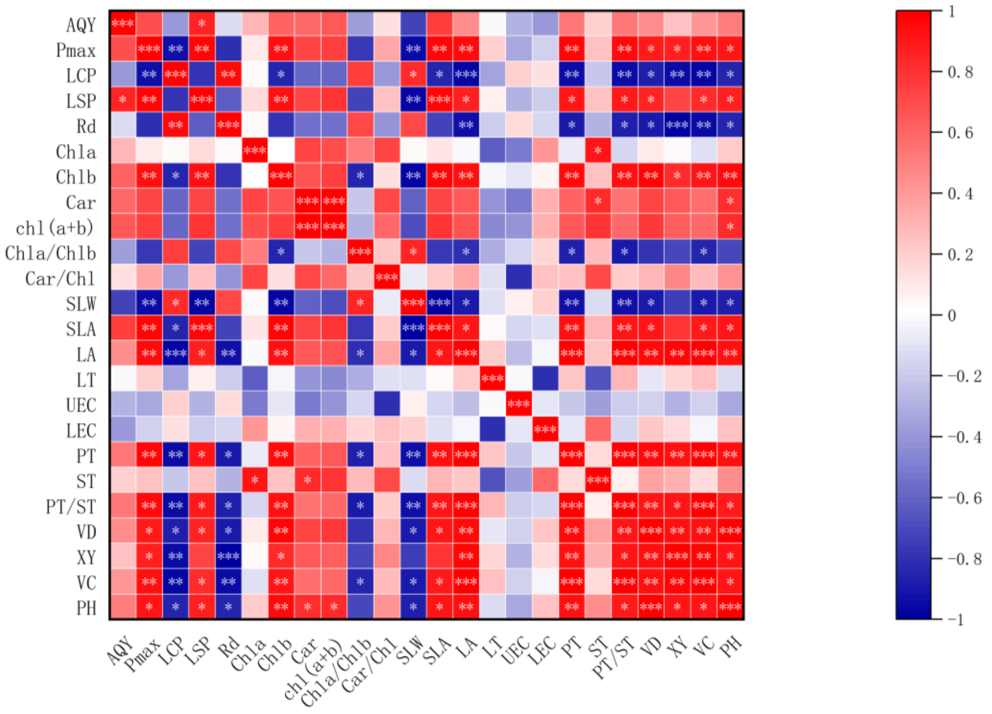
Table 5. *Leaf anatomical structure characteristic parameters and leaf characteristic parameters*

Type	**XY (μm)**	**PH (μm)**	**VC (μm)**	**LA (cm^2)**	**SLW ($g \cdot m^{-2}$)**	**SLA ($m^2 \cdot g^{-1}$)**
Juvenile	332.04 ± 6.18a	18.43 ± 2.01a	35.62 ± 3.30a	73.5466 ± 3.9261 a	71.2576 ± 1.4132 a	0.0142 ± 0.000 a
Adult	460.53 ± 28.70b	33.79 ± 1.00 b	50.74 ± 2.35b	149.3733 ± 4.8730 b	60.6123 ± 3.6608 b	0.0164 ± 0.000 b

Values with letter a and b are significantly different across columns ($P < 0.05$)

Correlation analysis of photosynthetic

Figure 5 illustrates the heat map of correlation analysis between leaf characteristics and photosynthetic physiology of juvenile and adult trees of *T. odorum*. Specifically, photosynthetic parameters P_{max} and LSP exhibited a positive correlation with leaf parameters LA and SLA. Pigment content Chl; leaf structural parameters PT, PT/ST, VD, XY, VC, and PH demonstrated positive correlations. A noteworthy positive correlation was also identified between P_{max} and LSP. Negative correlations were observed between Pmax and LCP and SLW, as well as between LSP and SLW.



* p<=0.05 ** p<=0.01 *** p<=0.001

Figure 5. *Heat map of correlation analysis between leaf structure and photosynthetic physiology of T. odorum*

DOI: http://dx.doi.org/10.15666/aeer/2205_43954408

In order to further evaluate the most influential indices affecting photosynthetic physiological alterations from *T. odorum*, chlorophyll content, leaf anatomical structure parameters, leaf structure parameters were used as explanatory variables, and 5 photosynthetic physiological indicators were used as response variables to do redundancy analysis for visualized with Canoco 5 software redundancy analysis (RDA), the results showed that the explanatory variables of axis 1 and 2 were 78.36% and 20.92%, respectively, and the first two axes accounted for 99.18% of the photosynthetic physiological variability of juvenile and mature trees of *T. odorum* (*Fig. 6*). As evident from *Figure 5*, the arrows of 5 traits, including SLW, PT, VC, Chlb, XY and UEC, were elongated, indicating that they could more effectively explain the variation of photosynthetic physiology of juvenile and mature *T. odorum* trees. The angle between P_{max} and SLA, Chlb, and PT/ST is small (acute), but with Chla/Chlb and SLW is wide (obtuse), indicating that Pmax is positively correlated with SLA, Chlb, and LA, and negatively correlated with Chla/Chlb and SLW. LCP was positively correlated with Chla/Chlb and SLW, and negatively correlated with SLA, Chlb and LA. There was a positive correlation between LSP and LA, Chlb and SLA, and a negative correlation between Chla/Chlb and SLW. It can be observed that the RDA and heat map analyses are consistent.

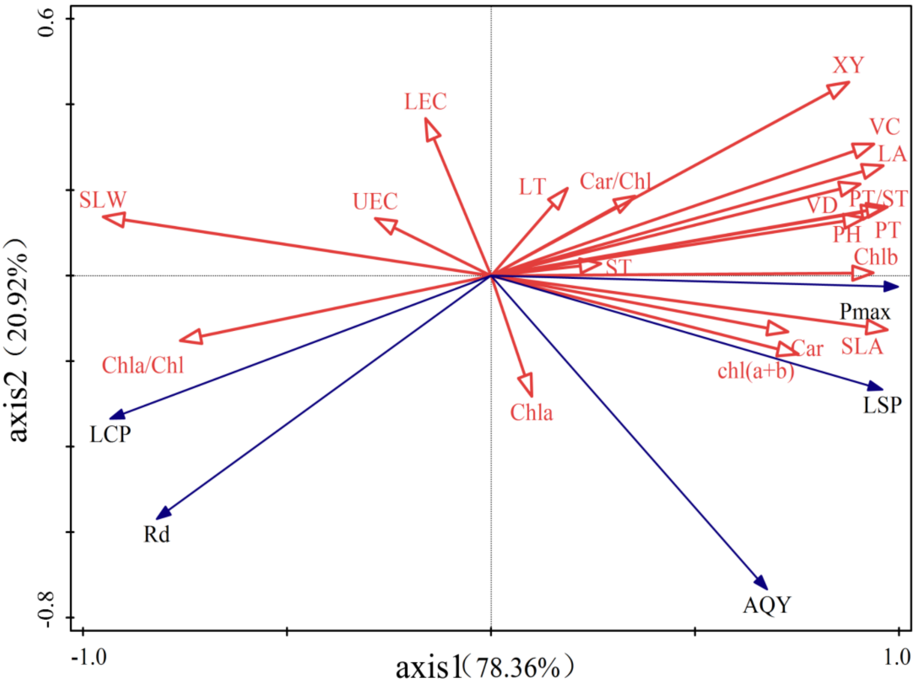


Figure 6. *RDA diagram of leaf structure and photosynthetic physiology of T. odorum juvenile and adult trees*

Discussion

Photosynthesis plays a crucial role in plant growth and development (Pollmann and Reinbothe, 2010; Yamori and Shikanai, 2016). In the studies of *Pseudotsuga menziesii* (Thomas and Winner, 2002), Mediterranean pines (Kuusk, Niinemets and Valladares, 2018), *Eucalyptus globulus* (Velikova et al., 2008b), highlight the significance of this process in different plant species. In *Figure 6*, significant differences were observed in leaf photosynthesis between juvenile and adult *T. odorium* trees. The study compared various parameters, including P_{max}, LSP, LCP, and R_d, using light response curves to elucidate the unique photosynthetic characteristics of adult and juvenile *T. odorium* trees. Under the same light intensity, the P_n of adult trees of *T. odorum* was always higher than that of juvenile, indicating that adult trees had higher photosynthetic capacity than juvenile under both low light and high light. In this study, we found that the net photosynthetic rate of adult trees did not decrease significantly with the increase of light intensity after reaching the light saturation point at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating that adult trees did not exhibit photoinhibition. Juvenile leaves are sensitive to high irradiance, however, after reaching the light saturation point, the Juvenile showed photoinhibition. The P_{max} of shade-loving plants is only slightly decreased under low light intensity (Reich et al., 1998), and it was found that strong light conditions could easily lead to photoinhibition, causing a decrease in photosynthetic capacity. Additionally, due to poor physiological and morphological plasticity, photosynthetic organs can become damaged. (Lovelock and Osmond, 1994). It is speculated that in order to avoid the damage of photosynthetic organs and reduce the water loss, the temporary closure of stomata in juvenile leads to the decrease of P_n. In this study, the G_s and transpiration rate of juvenile did not increase significantly under high light intensity, which supported this view. Therefore, when cultivating and transplanting *T. odorum* juvenile, attention should be paid to the light environment in the area. LSP and LCP of plants are important indicators to evaluate the adaptability of plants to light intensity. The LSP of adult trees was significantly higher than that of juvenile, indicating that the strong light adaptability of adult trees was stronger than that of juvenile, and the LCP of adult trees was lower than that of juvenile, so the adaptability of juvenile to low light was also lower than that of adult trees. This may be one of the reasons why *T. odorum* became an endangered species.

G_s, T_r, C_i, and WUE are important parameters that affect photosynthetic capacity (Yoshie, 1986). The direction of change of C_i concentration is the main reason for the variation of photosynthetic rate and the necessary basis for determining whether stomatal factors are related to photosynthesis (Farquhar and Sharkey, 1982). The C_i concentration of adult trees increased with the increase of light intensity, but there was no significant change in juvenile. This phenomenon indicated that the T_r rising rate of adult trees was higher than that of juvenile, which was one of the reasons for the higher P_n of leaves. The trend of change in T_r is similar to that of G_s. Numerous studies have shown that the correlation between photosynthetic rate and G_s is influenced by both stomatal and non-stomatal factors (Hernandez-Santana et al., 2016; Guo et al., 2019). It is concluded that the juvenile and adult trees of *T. odorum* may be mainly affected by stomatal factors. Plants regulate stomatal opening to control water vapor exchange with the outside world, thereby adjusting P_n and T_r to meet the growth requirements of conditions (Hernandez-Santana et al., 2016). Our results suggest that adult trees are more capable of stomatal regulation than juvenile. Under high light intensity, adult trees of *T. odorum* can open stomata to absorb enough CO_2 for photosynthesis, thus increasing net photosynthetic rate. Therefore, to prevent water loss under high light intensity, the G_s of juvenile increases slowly, and the growth rate of transpiration rate is also slower than that of adult trees, which may be one of the reasons for the lower net photosynthetic rate of juvenile leaves. WUE can reflect the water use efficiency of plant, and vary with different growth environments and growth periods in the same species (Velikova et al., 2008; Medlyn et al., 2017). In the low light environment, WUE of adult trees is higher than that of juvenile, indicating that the adaptability of adult trees to low light is higher than that of juvenile. In high light environment, the water utilization rates of both trees are similar, indicating that *T. odorum* may tend to adjust stomata aperture to maintain a higher photosynthetic rate.

The level of chlorophyll content can be used as one of the important indicators of plant photosynthetic capacity and ecological environmental changes (Cutolo et al., 2023). The results showed that the total chlorophyll content of adult trees was significantly higher than that of juvenile, and the P_n of adult trees was also higher than that of juvenile under different light intensity, indicating that chlorophyll content could effectively reflect the photosynthetic capacity of plants. Study has shown that Chlb is a key substance of the light-trapping antenna of leaves, and Chlb can increase the proportion of the light-harvesting antenna of leaves under low light (Bailey et al., 2001). In the same habitat, the Chlb in the leaves of adult trees was higher to capture more light energy to meet the needs of plant growth. Therefore, it is speculated that adult *T. odorum* may be affected by shading to some extent. Low light environment and low G_s and T_r can seriously affect the photosynthetic rate and the generation of photosynthesis of adult *T. odorum*, indicating that the environment with insufficient light is not conducive to the survival of adult *T. odorum* (Voitsekhovskaja and Tyutereva, 2015). The carotenoid content of juvenile is high and the Pn under strong light is low, indicating that juvenile cannot absorb more light energy. Therefore, carotenoids are required to accept excess excited chlorophyll molecules, thus avoiding the generation of reactive oxygen species and playing a role in photoprotection. In *Machilus chinensis* and *Cryptocarya chinensis,* similar results were found (Lin et al., 2022).

The leaf is the primary organ responsible for light energy absorption in plants, and leaf parameters can greatly influence photosynthesis (Houter and Pons, 2012; Chondrogiannis et al., 2023). Our findings revealed that adult trees possess a significantly larger leaf area than their juvenile counterparts, suggesting that trees need a larger leaf area to attain a higher photosynthetic capacity to sustain plant growth. The specific leaf weight of juvenile trees was significantly higher than that of adult trees, suggesting their high input cost per unit leaf area, slow growth rate, and low photosynthetic efficiency. Fajardo’s (2016) study demonstrated an inverse proportionality between specific leaf weight and leaf area within the same environment, implying that plants can modify their own leaf structural characteristics for adapting to a poor growth environment. The study found that adult trees had a higher leaf surface area and lower leaf weight, indicating strong adaptability to the environment. Leaf structure varies greatly at different developmental stages to fulfill photophysiological needs. The thickness, cell count, and chloroplast distribution in leaves are influenced by both external light and internal growth cycles (Gao et al., 2011; Kuusk et al., 2018; Chondrogiannis and Grammatikopoulos, 2021). In this study found that the cell number, PT and ST in young leaves were significantly lower than those in adult trees, indicating that the thickness and proportion of fence tissue have an important effect on photosynthesis. PT is the main place for the existence of chloroplast, and the high ratio of palisade to sea indicates that more chloroplast can be accommodated in the same leaf area, and the content of chlorophyll also increases accordingly (Evans and Vogelmann, 2006; Gotoh et al., 2018). The content of chloroplast in the leaves of adult trees in this study confirmed this view. In the leaves of higher plants, the vascular bundle is connected with the vascular bundle in the stem through the vein, and then the photosynthate is transported (Wang et al., 2020; Polutchko et al., 2021). Therefore, the degree of development of the vascular sheath cell organization and the density of the vascular bundle cell rich area directly affects the photosynthetic intensity of plants. In this study, the xylem thickness, phloem thickness and vascular cambium thickness of adult trees were significantly higher than those of juvenile, and adult trees could transport photosynthetic products more efficiently, while reducing the photoinhibition caused by the accumulation of photosynthetic products. The results are consistent with those in *Sphagnum* mosses (Murray et al., 1993) and *Arabidopsis* (Hu et al., 2021). According to PCC and RDA analysis, it was found that photosynthetic rate was strongly correlated with LA, PT, PT/ST, XY, ST and VT, suggesting that these six leaf structure parameters could be used as reliable indicators of photosynthetic physiological variation of *T. odorum*, and could also be the main driving factors for photosynthetic physiological growth of *T. odorum*. It could be used as an important reference index for screening the photosynthetic capacity of *T. odorum* in the future.

Conclusion

The LSP and P_{max} of adult *T. odorum* were significantly higher than those of juvenile, and the differences in chlorophyll content and leaf structure were the main reasons for the differences in photosynthetic rates. Therefore, in the introduction and cultivation of *T. odorum*, it is suggested that a certain shade mode should be adopted when cultivating juvenile, and the shade degree should be gradually reduced with the growth of plants, so as to prevent the growth of *T. odorum* from being restricted by too strong or too weak light environment.

Acknowledgments. The research is supported by the National Key Research and Development Program (No.2022YFF1300703), Chinese Academy of Sciences ‘Light of West China’ Program (2022), Guangxi Forestry Science and Technology Promotion Demonstration Project (2023LYKJ03 and[2022]GT23); The Key Research and Development Project of Guangxi (GuiKeAB22080097).

REFERENCES

[1] An, N. T. G., Chau, D. T. M., Huong, L. T., Dai, D. N., Ogunwande, I. A. (2023): Chemical constituents and antimicrobial activity of Tsoongiodendron odorum and Manglietia chevalieri essential oils from Vietnam. – Chemistry of Natural Compounds 59(3): 587-590.
[2] Bailey, S., Walters, R. G., Jansson, S., Horton, P. (2001): Acclimation of Arabidopsis thaliana to the light environment: the existence of separate low light and high light responses. – Planta 13(5): 794-801.
[3] Bond, B. J. (2000): Age-related changes in photosynthesis of woody plants. – Trends in Plant Science 5(8): 349-53.
[4] Chondrogiannis, C., Grammatikopoulos, G. (2016): Photosynthesis in developing leaf of juveniles and adults of three Mediterranean species with different growth forms. – Photosynthesis Research 130(1-3): 427-444.
[5] Chondrogiannis, C., Grammatikopoulos, G. (2021): Transition from juvenility to maturity strengthens photosynthesis in sclerophyllous and deciduous but not in semi-deciduous Mediterranean shrubs. – Environmental and Experimental Botany 181: 104265.
[6] Chondrogiannis, C., Kotsi, K., Grammatikopoulos, G., Petropoulou, Y. (2023): Seasonal differences in leaf photoprotective potential between adults and juveniles of two mediterranean perennials with distinct growth forms: a comparative field study. – Plants 12(17): 3110.

[7] Cutolo, E. A., Guardini, Z., Dall'Osto, L., Bassi, R. (2023): A paler shade of green: engineering cellular chlorophyll content to enhance photosynthesis in crowded environments. – New Phytologist 239(5): 1567-1583.

[8] Evans, J. R., Vogelmann, T. C. (2006): Photosynthesis within isobilateral eucalyptus pauciflora leaves. – The New Phytologist 171(4): 771-82.

[9] Fajardo, A., Siefert, A. (2016): Temperate rain forest species partition fine-scale gradients in light availability based on their leaf mass per area (LMA). – Annals of Botany 118(7): 1307-1315.

[10] Farquhar, G. D., Sharkey, T. D. (1982): Stomatal conductance and photosynthesis. – Annual Review of Plant Biology 33: 317-345.

[11] Franck, N., Vaast, P., Génard, M., Dauzat, J. (2006): Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown Coffea arabica. – Tree Physiology 26(4): 517-25.

[12] Gao, Y., Fang, J., Zhang, J., Ren, L., Mao, Y., Li, B., Zhang, M., Liu, D., Du, M. (2011): The impact of the herbicide atrazine on growth and photosynthesis of seagrass, Zostera marina (L.), seedlings. – Marine Pollution Bulletin 62(8): 1628-1631.

[13] Gotoh, E., Suetsugu, N., Higa, T., Matsushita, T., Tsukaya, H., Wada, M. (2018): Palisade cell shape affects the light-induced chloroplast movements and leaf photosynthesis. – Scientific Reports 8(1): 1472.

[14] Guo, Q. Q., Li, H. E., Gao, C., Yang, R. (2019): Leaf traits and photosynthetic characteristics of endangered Sinopodophyllum hexandrum (Royle) Ying under different light regimes in Southeastern Tibet Plateau. – Photosynthetica 57(2): 548-555.

[15] Häder, D. P. (2022): Photosynthesis in plants and algae. – Anticancer Research 42(10): 5035-5041.

[16] Hernandez-Santana, V., Fernández, J. E., Rodriguez-Dominguez, C. M., Romero, R., Diaz-Espejo, A. (2016): The dynamics of radial sap flux density reflects changes in stomatal conductance in response to soil and air water deficit. – Agricultural and Forest Meteorology 218-219: 92-101.

[17] Houter, N. C., Pons, T. L. (2012): Ontogenetic changes in leaf traits of tropical rainforest trees differing in juvenile light requirement. – Oecologia 169(1): 33-45.

[18] Hu, C., Nawrocki, W. J., Croce, R. (2021): Long-term adaptation of Arabidopsis thaliana to far-red light. – Plant, Cell & Environment 44(9): 3002-3014.

[19] Huang, Y., Zeng, Q., Fu, J. J., Kou, Z. J., Chang, R. J., Jin, H. Z., Zhang, W. D. (2011): Chemical constituents from Tsoongiodendron odorum Chun. – Biochemical Systematics and Ecology 39(3): 209-212.

[20] IUCN (2014): Magnolia odora: Rivers, M. C., Wheeler, L. – IUCN Red List of Threatened Species. IUCN, Gland.

[21] Jiang, J., Wang, J., Kang, M., Sun, W., Huang, H. (2011): Isolation and characterization of microsatellite loci in Tsoongiodendron odorum (Magnoliaceae). – American Journal of Botany 98(10).

[22] Kuusk, V., Niinemets, Ü., Valladares, F. (2018): Structural controls on photosynthetic capacity through juvenile-to-adult transition and needle ageing in Mediterranean pines. – Functional Ecology 32(6): 1479-1491.

[23] Li, W. D., Duan, W., Fan, P. G., Yan, S. T., Li, S. H. (2007): Photosynthesis in response to sink-source activity and in relation to end products and activities of metabolic enzymes in peach trees. – Tree Physiology 27(9): 1307-18.

[24] Lichtenthaler, H. K., Buschmann, C. (1987): Chlorophyll fluorescence spectra of green bean leaves. – Journal of Plant Physiology 129(1): 137-147.

[25] Lin, W., Yu, Z., Luo, Y., He, W., Yan, G., Peng, C. (2022): Photoprotection differences between dominant tree species at mid- and late-successional stages in subtropical forests in different seasonal environments. – International Journal of Molecular Sciences 23(10): 5417.

[26] Liu, Y. H. (2004) Magnolias of China. – Science Press, Beijing.

[27] Lovelock, C. E., Osmond, C. B., Jebb, M. (1994): Photoinhibition and recovery in tropical plant species: response to disturbance. – Oecologia 97(3): 297-307.

[28] Maurino, V. G., Weber, A. P. M. (2013): Engineering photosynthesis in plants and synthetic microorganisms. – Journal of Experimental Botany 64(3): 743-51.

[29] Mediavilla, S. (2003): Stomatal responses to drought of mature trees and seedlings of two co-occurring mediterranean oaks. – Forest Ecology and Management 187: 281-294.

[30] Medlyn, B. E., De Kauwe, M. G., Lin, Y., Knauer, J., Duursma, R. A., Williams, C. A., Arneth, A., Clement, R., Isaac, P., Limousin, J., Linderson, M., Meir, P., Martin-StPaul, N., Wingate, L. (2017): How do leaf and ecosystem measures of water-use efficiency compare? – New Phytologist 216(3): 758-770.

[31] Murray, K. J., Tenhunen, J. D., Nowak, R. S. (1993): Photoinhibition as a control on photosynthesis and production of sphagnum mosses. – Oecologia 96(2): 200-207.

[32] Polutchko, S. K., Stewart, J. J., Adams Iii, W. W., Demmig-Adams, B. (2021): Photosynthesis and foliar vascular adjustments to growth light intensity in summer annual species with symplastic and apoplastic phloem loading. – Journal of Plant Physiology 267: 153532.

[33] Qin, H. N., Yang, Y., Dong, S. Y., He, Q. (2017): Threatened species list of China's higher plants – Biodiversity Science 25(7): 696-744.

[34] Reich, P. B., Walters, M. B., Tjoelker, M. G., Vanderklein, D. W., Buschena, C. (1998): Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. – Functional Ecology 12: 395-405.

[35] Reinbothe, C., Pollmann, S., Reinbothe, S. (2010): Singlet oxygen signaling links photosynthesis to translation and plant growth. – Trends in Plant Science 15(9): 499-506.

[36] Thomas, S. C., Winner, W. E. (2002): Photosynthetic differences between saplings and adult trees: an integration of field results by meta-analysis. – Tree Physiology 22(2-3): 117-127.

[37] Vecchi, V., Barera, S., Bassi, R., Dall'Osto, L. (2020): Potential and challenges of improving photosynthesis in algae. – Plants (Basel, Switzerland) 9(1): 67.

[38] Velikova, V., Loreto, F., Brilli, F., Stefanov, D., Yordanov, I. (2008a): Characterization of juvenile and adult leaves of eucalyptus globulus showing distinct heteroblastic development: photosynthesis and volatile isoprenoids. – Plant Biology 10(1): 55-64.

[39] Velikova, V., Loreto, F., Brilli, F., Stefanov, D., Yordanov, I. (2008b): Characterization of juvenile and adult leaves of Eucalyptus globulus showing distinct heteroblastic development: photosynthesis and volatile isoprenoids. – Plant Biology 10(1): 55-64.

[40] Vernon, L. P., Avron, M. (1965): Photosynthesis. – Annual Review of Biochemistry 34: 269-96.

[41] Voitsekhovskaja, O. V., Tyutereva, E. V. (2015): Chlorophyll b in angiosperms: functions in photosynthesis, signaling and ontogenetic regulation. – Journal of Plant Physiology 189: 51-64.

[42] Wang, X., Wang, Y., Ling, A., Guo, Z., Asim, M., Song, F., Wang, Q., Sun, Y., Khan, R., Yan, H., Shi, Y. (2020): Rationale: photosynthesis of vascular plants in dim light. – Frontiers in Plant Science 11.

[43] Xu, R., Wang, L., Zhang, J., Zhou, J., Cheng, S., Tigabu, M., Ma, X., Wu, P., Li, M. (2022): Growth rate and leaf functional traits of four broad-leaved species underplanted in Chinese fir plantations with different tree density levels. – Forests 13(2): 308.

[44] Yamori, W., Shikanai, T. (2016): Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. – Annual Review of Plant Biology 67(1): 81-106.

[45] Yoshie, F. (1986): Intercellular CO_2 concentration and water-use efficiency of temperate plants with different life-forms and from different microhabitats. – Oecologia 68(3): 370-374.