COMPARATIVE STUDY OF LEAF MATERIAL PROPERTIES IN *CYCLOCARYA PALIURUS* FROM DIFFERENT PROVENANCES: FLAVONOID CONTENT, PIGMENT VARIATION, AND PHOTOSYNTHETIC EFFICIENCY

YANG, W. X.[1,2*] – ZHUANG, J. Q.[1] – DING, S. Y.[1] – ZHANG, M.[2] – TIAN, Y.[1] – WAN, S. Y.[1]

[1]*Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing Jiangsu 210037, P. R. China*

[2]*College of Forestry and Grassland, Nanjing Forestry University, Nanjing Jiangsu 210037, P. R. China*

[*]*Corresponding author*
e-mail: yangwanxia@njfu.edu.cn

(Received 6th Nov 2024; accepted 4th Feb 2025)

Abstract. As a unique species in China, the leaves of *Cyclocarya paliurus* are rich in various active ingredients, which have significant potential for development and utilization. *Cyclocarya paliurus* is widely distributed and exhibits notable variation. In this study, samples of *Cyclocarya paliurus* from the Jiulianshan and Xiushui provenanceXiushui provenance were selected to analyze their phenotypic diversity. The total flavonoid content of phenotypic traits with obvious optical classification characteristics was measured and compared. The study found significant differences in the polysaccharide content between different sources, with the Xiushui provenance showing notably higher polysaccharide content than the Jiulianshan source. In addition, there were considerable differences in the polysaccharide content of *Centella paliurus* leaves with different phenotypic traits. Except for the leaves with different leaflet types, where the difference in polysaccharide content was not significant, differences between other types were significant. The main findings of this paper are as follows: This discrepancy suggests that the absorption at 500–550 nm may be influenced by other pigments or compounds, and further investigation is needed to confirm the specific flavonoid absorption profile. In fresh plant extracts, due to the absence of interfering substances in the absorption region of total flavonoids, the total flavonoid content can be accurately measured by determining the absorbance at an appropriate wavelength. However, the study should specify the exact wavelength used for measurement to ensure reproducibility.
Keywords: *Cyclocarya paliurus leaves, total flavonoid content, phenotypic diversity, polysaccharide content, optical properties, plant extracts*

Introduction

Cyclocarya paliurus, a unique species endemic to China, is one of the nationally protected endangered plants. It is primarily distributed in mountainous areas, valleys, or limestone mountains of regions such as Jiangxi, Zhejiang, Jiangsu, Anhui, Fujian, Taiwan, Hubei, Sichuan, and Guizhou, with altitudes ranging from 420 m to 1100 m (eastern regions) and up to 2500 m (western regions). However, the current resources of *Cyclocarya paliurus* in China are limited, mainly consisting of natural forests, which are mostly found in deep mountainous ancient forests and some nature reserves. There is still a lack of widespread artificial breeding and cultivation experience, and relevant research is scarce, severely affecting the development and industrialization of *Cyclocarya paliurus*. As a woody medicinal plant, *Cyclocarya paliurus* has a long history of use in Chinese folk medicine. Its leaves contain various physiologically active substances beneficial to human health, such as flavonoids and triterpenoids. The development and utilization of

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_33853399

Centella paliurus first face the dilemma of limited natural resources. Furthermore, environmental conditions, such as light, have a significant impact on its growth and the accumulation of secondary metabolites, but detailed related studies are currently lacking (Mi et al., 2009; Fang et al., 2011; Liu et al., 2018).

Centella paliurus is characterized by rapid growth and strong germination ability, with trees reaching heights of 30-45 meters. The main usable part is the leaves. To better cultivate *Centella paliurus* forests for medicinal purposes, dwarfing and dense planting are inevitable development trends. Under this cultivation model, the impact of changes in light conditions on the growth and metabolism of *Centella paliurus* requires further research. Photosynthesis is a complex biochemical process involving numerous enzymatic reactions. Changes in external temperature not only directly affect the rate of enzymatic reactions and material diffusion in plants but also result in changes in other physiological parameters, such as transpiration rate and stomatal conductance (Feng et al., 2009; Wang et al., 2013). The relationship between photosynthesis and temperature is not only reflected in the need for an appropriate temperature for photosynthesis but also in the plant's ability to adapt to environmental temperature. The response curve of plant photosynthesis to temperature generally follows a bell shape, with the optimal temperature at the peak. Both high and low temperatures can inhibit photosynthesis (Wu et al., 2009). Water is one of the primary raw materials for photosynthesis, but excessive water consumption typically comes from transpiration. Therefore, the study of photosynthesis focuses more on the impact of water content on transpiration and stomatal limitation, especially the effect of changes in atmospheric humidity (Bai et al., 2018). Water deficiency reduces the rate of photosynthesis, but excessive water also inhibits photosynthesis. Photosynthesis is the process of utilizing light energy in nature, and light is one of the necessary external elements for photosynthesis. Therefore, as light intensity changes, the photosynthetic rate will also change accordingly. When photosynthesis and respiration reach a dynamic balance, the light intensity corresponds to the light compensation point, and further increasing the light intensity leads to the light saturation point, where the photosynthetic rate increases slowly or may even exhibit light inhibition (Zexin, 2007; Xie et al., 2018; He et al., 2022).

Flavonoids are a source of various bioactive compounds and are widely present in the plant kingdom. They have enormous potential in human health and disease prevention, including anti-aging, lipid-lowering, immune regulation, and other biological activities. As an important active component in *Centella paliurus* leaves, the extraction and measurement of flavonoids are of great significance for the development of *Centella paliurus* resources. Currently, the extraction of flavonoids from *Centella paliurus* leaves mainly uses hot solvent extraction, but this method has drawbacks such as high solvent consumption, long extraction time, and high costs, along with a low extraction rate of effective components. Therefore, developing new extraction methods will help improve traditional processes and enhance the utilization of active ingredients in *Centella paliurus* leaves (Xie et al., 2015; Xu et al., 2016).

The growth characteristics of *Centella paliurus* leaves are closely related to their medicinal value, with the chemical composition of the leaves varying under different environmental conditions. *Cyclocarya paliurus* leaves also have high medicinal value, and studies have shown that their leaf extracts offer various physiological benefits to the human body. Safety studies of *Cyclocarya paliurus* leaf extracts have shown no mutagenicity or teratogenicity, indicating a high development potential (Xie et al., 2015; Liu et al., 2016; Qiang and Chen, 2019). The chemical components of these plants have

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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positive effects on antioxidant, antibacterial, and antihypertensive properties, especially the extraction and utilization of flavonoids, which occupy an important position in related plant chemical research (Piątczak et al., 2020; Wang et al., 2021; Zhang et al., 2022).

Research has shown significant differences in the phenotypic traits and medicinal components among plants from different sources. A study by Jiang Naiyi and others on the diversity of 9 sources of medicinal material found that the polymorphism differed greatly between sources, with the Guangxi source exhibiting smaller polymorphism and the Hubei and Sichuan sources showing larger polymorphism (Xie et al., 2015). Chen Yi and others studied the relationship between seed traits and geographic factors and found a strong correlation between seed traits and annual average temperature (Liu et al., 2022). In addition, the content of secondary metabolites in *Centella paliurus* leaves also varies significantly across different regions (Xie et al., 2015; Liu et al., 2018; Zhang et al., 2020; Saedodin et al., 2021). In the context of plant chemical research and its impact on human health, studies have shown that plant compounds have significant medicinal potential, such as flavonoids extracted from medicinal plants, which have shown therapeutic potential for various health issues (Kottawa-Arachchi et al., 2019; Yin and Li, 2022; Wang and Zhang, 2022; Hong, 2023; Yang et al., 2023).

This paper analyzes the phenotypic diversity of *Cyclocarya paliurus* from two sources, Jiulianshan and Xiushui, and investigates their photosynthetic characteristics and total flavonoid content. It explores the differences between the two sources. The innovation of this study lies in the analysis of the differences in seedling growth traits, photosynthetic characteristics, and medicinal components from different sources, revealing the characteristics of geographical variation and annual dynamic changes, providing new insights for the development and application of *Centella paliurus* resources.

The effect of light intensity on the photosynthetic characteristics of the leaves

Light is one of the key environmental factors that influence plant growth, development, metabolism, and other physiological activities. During the growth process, plants are able to adjust their leaf morphology, the composition and function of photosynthetic organs in response to changes in light intensity in their environment, thereby exhibiting a series of ecological, biochemical, and biological adaptive changes to adapt to specific light conditions. Typically, plants growing in high light environments exhibit the typical characteristics of heliophytes, while plants in low light environments show traits of sciophytes. The leaves of heliophytes generally feature well-developed palisade tissues, higher rates of photosynthetic carbon dioxide fixation, greater biomass, and abundant accumulation of antioxidants. In contrast, the leaves of sciophytes have lower photosynthetic capacity but often contain more chlorophyll, with a higher proportion of chlorophyll b per unit weight. Their photosystem II (PSII) also exhibits higher quantum yield. Plants adapt to variations in environmental light intensity by adjusting their morphology and photosynthetic functions, which enables them to effectively utilize available light energy, enhancing their ecological adaptability.

So far, research on how plants adjust their growth, photosynthetic physiology, leaf morphology, anatomy, and ultrastructure of photosynthetic organs in different light environments is relatively limited. A comprehensive and systematic study of plant responses to light intensity not only helps deepen our understanding of how plants adapt to their growth environments but also provides an important theoretical foundation for the management and development of medicinal plant resources.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Experimental materials and experimental design

This study selected samples of *Medicago sativa* (alfalfa) from the Jiulianshan and Xiushui provenanceXiushui provenance in Jiangxi Province. These two sources are located under different ecological conditions and exhibit significant geographical differences, representing the impact of varying environments on the growth and photosynthetic characteristics of alfalfa. The main reason for choosing these two sources is their considerable differences in climate, soil, and light conditions, which provide a diverse ecological background to study the effects of light intensity on the photosynthetic properties of alfalfa. Field observations were conducted at these two sources, and mature plant samples were directly collected. All samples were taken from naturally growing alfalfa plants in their native environments, without being subjected to controlled seed collection or propagation conditions.

The study established three different shading intensities: full light (A1), 50% shading (A2), and 80% shading (A3). The shading net used was made of black polyethylene material with a shading rate of 60%, and the net was placed 2 meters above the ground. Treatment group A was covered with one layer of shading, while treatment group B was covered with two layers. This experiment included three treatment groups, each with three repetitions, and each repetition contained 10 alfalfa seedlings, totaling 90 seedlings.

Determination of chlorophyll mass fraction

First, mature fresh leaflets from labeled plants were collected, cleaned, and dried to a constant weight before being chopped into pieces for the determination of chlorophyll mass fraction. After measuring photosynthetic and fluorescence parameters and collecting samples for chlorophyll mass fraction, the labeled plants were uprooted and divided into root, stem, and leaf parts. These parts were dried at 70°C to a constant weight, and the dry weight of each part was then measured. To improve the extraction efficiency of total flavonoids, an optimized extraction method was used, which involved a mixture of ethanol and water as solvents, with adjustments to extraction temperature and time to maximize the recovery of active components. This method significantly improved the extraction efficiency of flavonoid compounds compared to traditional methods.

In the experiment, mass spectrometry analysis was performed for qualitative identification of the extracted plant samples, and compounds were identified by matching with a database. While the mass spectrometry results provided preliminary qualitative analysis, further separation and purification, combined with Nuclear Magnetic Resonance (NMR) spectroscopy, are required for more accurate structural confirmation.

The photosynthesis-light response curve was measured in a leaf chamber equipped with red and blue light sources. The leaf chamber model used was the "Q-Box Light Chamber" (Photon Systems Instruments, Czech Republic), which provides uniform light conditions and is equipped with precise temperature and humidity control systems. The temperature was controlled at (20 ± 0.5) °C, the CO_2 molar fraction was 380 μmol/mol, and the relative humidity was (40 ± 5) %.This experiment set up 12 different light intensity gradients, including: 1800, 1600, 1400, 1200, 1000, 800, 500, 200, 100, 50, 20, and 0 $\mu mol \cdot m^2 \cdot s$. Each light intensity gradient was maintained for 5 minutes during measurements. Prior to measurement, the leaves from each treatment were light-induced for 20 minutes under 1800 $\mu mol \cdot m^2 \cdot s$ light intensity, and during measurements, efforts were made to keep the leaves in their original position on the plant.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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To fit the photosynthesis-light response curve, this experiment used the leaf floating right-angle hyperbolic correction model, as expressed in the formula.

$$P_n(I) = \alpha \frac{1-\beta I}{1+\gamma I} I - R_d \tag{Eq.1}$$

where, P_n is the net photosynthetic rate, I is the light intensity, and α is the slope of the photosynthesis-light response curve at 0, that is, the initial slope of the light response curve.

The light saturation point is shown in (2).

$$L_{s,p} = \frac{\sqrt{(\beta+\gamma)/\beta - 1}}{\gamma} \tag{Eq.2}$$

The maximum net photosynthetic rate is shown in (3).

$$P_{n\max} = \alpha\left(\frac{\sqrt{(\beta+\gamma)/\beta - 1}}{\gamma}\right)^2 - R_d \tag{Eq.3}$$

Chlorophyll fluorescence parameters were measured on the labeled leaflets of A. The leaf-floating right-angle hyperbolic correction model is calculated by photosynthesis.

All data were statistically analyzed using SPSS software (Version 25, IBM Corp., USA). The significance of differences between groups was tested using one-way analysis of variance (ANOVA), and post-hoc comparisons were performed using Tukey's Honestly Significant Difference (HSD) test. Differences were considered statistically significant when the P value was less than 0.05.

The effect of shading treatment on C. chinensis

Shading treatments not only altered the light environment of lilies but also affected environmental factors such as temperature and humidity. As the shading intensity increased, the air temperature gradually decreased, while the air relative humidity gradually increased. The main environmental factors at different shading levels are shown in *Table 1*.

Table 1. *Effects of environmental factors at different shade levels during the day*

Shade level	Relative humidity/%	Temperature/℃
Full light	30.5	69.5
50% Light transmission	27.2	78.8
20% Light transmission	25.6	85.3

Shading treatment significantly affected the chlorophyll mass fraction and composition in the leaves of *C. chinensis* seedlings, as shown in *Table 2*.

The photosynthesis-light response curve was fitted by the leaf-floating right-angle hyperbolic correction model, as shown in *Figure 1*.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Table 2. *Growth conditions and chlorophyll composition of Phellodendron amurense leaves under different shading treatments*

Deal with	Plant height/cm	Ground diameter/cm	Total chlorophyll mass Number/mg/g	Chlorophyll a: Chlorophyll b
Full light	135.5	1.77	1.68	4.21
50% Light transmission	152.8	1.56	2.35	3.57
20% Light transmission	88.7	1.15	2.41	3.48

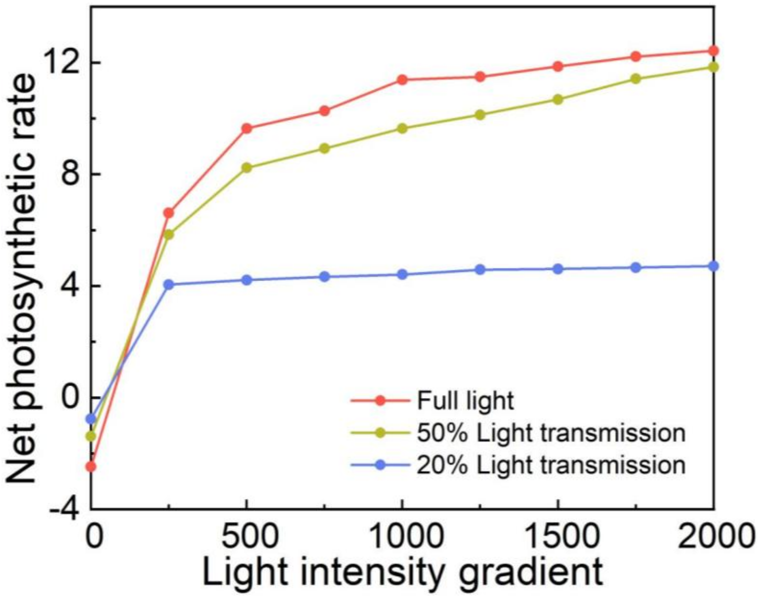

Figure 1. *Fitted photosynthetic rates of Phellodendron amurense under different light intensity gradients and shading treatments*

As shown in *Figure 1*, the changes in photosynthetic capacity and light energy utilization efficiency under different shading intensities indirectly reflect the impact of shading on photosynthetic pigments and their composition. Mild shading had no significant effect on the photosynthetic potential of *Medicago sativa*, while heavy shading significantly inhibited its photosynthetic potential. As light intensity decreased, the net photosynthetic rate sharply declined, with the net photosynthetic rate under A2 and A3 treatments being only 61.7% and 21.3% of that under the A1 treatment, respectively. Moreover, under low light conditions, the respiratory consumption of *Medicago sativa* seedlings significantly decreased, with the light saturation point for A2 and A3 treatments being 47.9% and 19.8% of the A1 treatment, respectively. These results suggest that, in low light environments, *Medicago sativa* seedlings adapt to low light conditions by reducing the consumption of organic matter, thereby improving their survival ability.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Correlations between total flavonoids content and light intensity of *Cyclocarya paliurus* from different provenances

Total flavonoids

Total flavonoids initially referred to a class of compounds whose backbone is based on 2-phenylchromone. Nowadays, the term generally refers to a series of compounds formed by two benzene rings connected through three carbon atoms. The parent structures are shown in *Figures 2 and 3*.

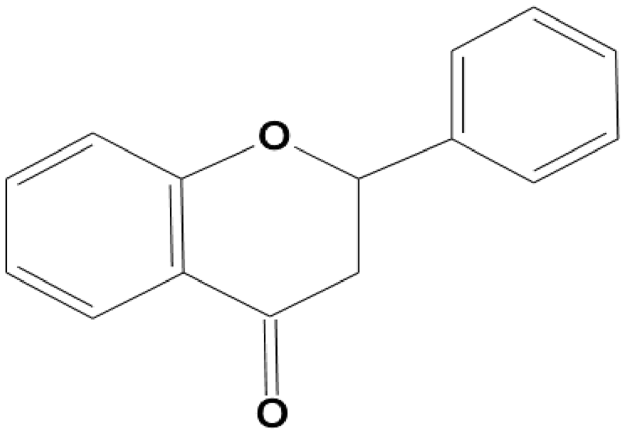

Figure 2. *Chemical structure of the parent flavonoid compound*

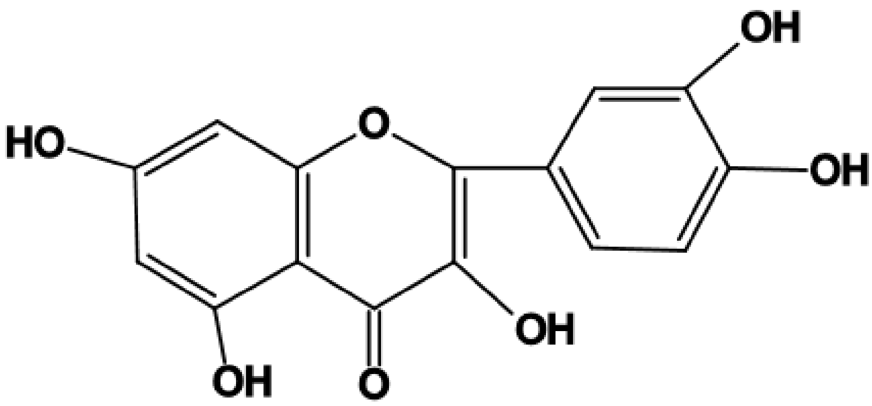

Figure 3. *Structural formula of flavonol*

Flavonoids are a class of polyphenolic compounds with various biological activities, widely distributed in the plant kingdom. In mature boxwood trees, there is significant variation in flavonoid content across different canopy layers. The southern-facing leaves, which receive ample sunlight, have the highest flavonoid content, while leaves on the east and west sides, as well as those in the interior of the canopy, have lower flavonoid content. Different drying temperatures significantly affect the extraction rate of flavonoids from leaves, with sunlight exposure time and altitude being the primary influencing factors.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
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Difference analysis of total flavonoids content in leaves of Cyclocarya paliurus under different light conditions

There are significant differences between the various *Medicago sativa* (alfalfa) species, with 8 different traits showing varying degrees of diversity across species. In the Jiulianshan source in Jiangxi, the coefficient of variation for sexual differentiation is the largest, reaching 46.184%, indicating that sexual differences among individual plants are widespread. The coefficients of variation for other traits are as follows: bark cracking, leaf color, leaflet count, stem diameter, number of primary branches, branch angle, and tree height. Comparing the phenotypic traits of the Jiulianshan source and the Xiushui provenance in Jiangxi, it was found that primary branch count, stem diameter, branch angle, and tree height showed considerable variation, while traits such as leaf size, sexual differentiation, leaf color, leaflet count, bark cracking, and fruit diameter showed greater intra-population variation. This suggests that these traits exhibit clear differentiation within the population and are suitable as characteristic traits for selecting *Medicago sativa*.

Before analyzing the differences in total flavonoid content among different trait types, we first measured the total flavonoid content of the leaves under different light conditions. Based on the results, we determined the sampling time for investigating the differences in total flavonoid content among these trait types. During leaf development, from juvenile to mature leaves, there is a process of gradual accumulation and redistribution of organic matter, which ultimately leads to leaf abscission. Studying the differences in total flavonoid content under different light conditions helps deepen our understanding of the dynamic changes in total flavonoid content throughout the entire leaf development process, providing a basis for the scientific and rational extraction of valuable compounds. Starting from the 10th day of leaf germination, leaf samples were collected every 10 days to measure the effect of light conditions on total flavonoid content, with the results shown in *Figure 4*.

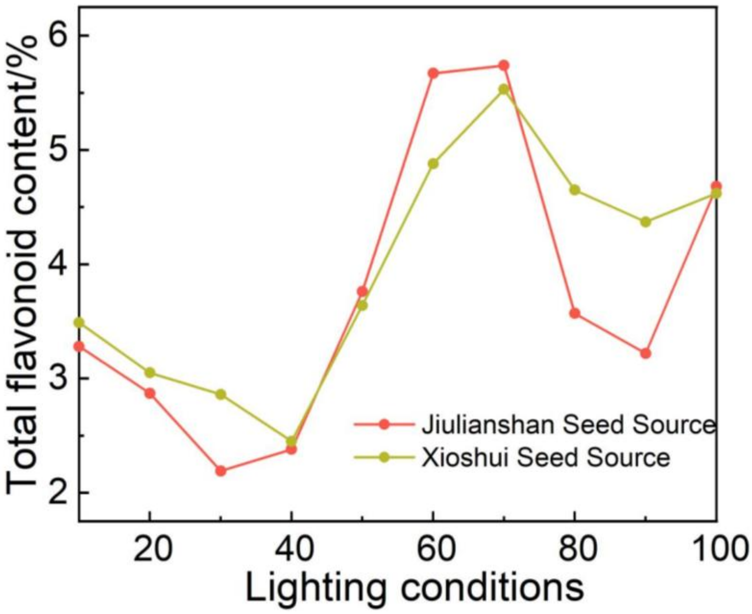

Figure 4. *Change curve of total flavonoid content in leaves under different light conditions*

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DOI: http://dx.doi.org/10.15666/aeer/2302_33853399

The observation results show that under different light conditions, there are certain differences in the total flavonoid content of *Centella paliurus* leaves. Overall, the total flavonoid content exhibits a bimodal trend. From 10 to 30 days, the total flavonoid content in the young leaves gradually decreases, a trend consistent with the changes in total flavonoid content in young *Centella paliurus* leaves. This phenomenon may be due to the temporary decrease in flavonoid content in *Centella paliurus* leaves during growth and flowering. Comparing different sources, between 40 and 70 days under different light conditions, the total flavonoid content in *Centella paliurus* leaves shows a downward trend. The flavonoid content in the Xiushui provenance gradually decreased to 4.275%, while the Jiulianshan source's flavonoid content decreased to 3.08%. Under the 100-day light condition, the total flavonoid content and trend in both sources were almost identical.

From the figure, it can be seen that there is a significant difference in total flavonoid content between the different sources. Analysis of variance shows that the differences between the two sources have reached a significant level, with P values less than 0.05, indicating that the differences in total flavonoid content are statistically significant and related to sexual differentiation in source A. On one hand, sexual differentiation promotes the biosynthesis of total flavonoids; on the other hand, it may also lead to the degradation of total flavonoids. Under the influence of light, acylated disaccharides are more stable than non-acylated disaccharides, and non-acylated disaccharides are more stable than monoside.

The maximum absorption range for total flavonoids is between 500 and 550 nm, while the maximum absorption range for flavonoid compounds closest to this range is between 350 and 380 nm. In fresh plant extracts, because there are few interfering substances in the maximum absorption range of total flavonoids, the content of total flavonoids can be quantitatively measured by absorbance at an appropriate wavelength using the Beer-Lambert method. After condensation or condensation with other organic substances, the stability of total flavonoids may be enhanced or reduced depending on the environmental conditions. Compounds such as polyhydroxyflavonoids and isoflavones can resist the photodegradation of total flavonoids. This is because the negatively charged sulfonic acid group and the positively charged flavonoid molecules attract each other, leading to the formation of complexes between these molecules and total flavonoids.

From *Table 3*, it can be observed that there are differences in the flavonoid content in the leaves of two different sources with different sexes. Specifically, in the Jiulianshan source, the flavonoid content in Atractylodes leaves showed a large variation range, with the highest flavonoid content found in the leaves of hermaphroditic plants, reaching 4.138%. This was followed by the female plants, with the lowest content found in male plants at 2.485%. The flavonoid content in Atractylodes leaves ranged from 2.858% to 4.291%, with male plants having the highest flavonoid content, followed by female plants, while hermaphroditic plants had the lowest content.

Table 3. *Differences in flavonoid compounds in the leaves of Cercis chinensis with different seeds and sexes*

Gender type	**Jiulianshan Seed Source**				**Xioshui Seed Sourc**			
	1	**2**	**3**	**Average**	**1**	**2**	**3**	**Average**
Female plant	2.38	2.51	2.42	2.44	3.36	3.36	3.36	3.36
Male plant	3.29	3.31	3.28	3.30	4.13	4.32	3.86	4.10
Hermaphrodite	4.13	4.13	4.13	4.13	2.81	2.82	2.83	2.82

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Analysis of leaf pigments and photosynthetic characteristics of *Cercis chinensis* from different sources

The stability of pigments to light

Light is a key environmental factor influencing the color changes in ornamental-leaved plants. It affects the synthesis of photoreceptor pigments and regulates the activity of related enzymes, thereby influencing the coloration and growth of these plants. Light intensity directly impacts the content and ratio of chlorophyll, carotenoids, and anthocyanins, thus affecting the color of the leaves. To investigate the changes in pigments under different light conditions, this study measured the photostability of pigments. The experiment set up three treatment conditions: direct light, diffuse light, and dark treatment, as shown in *Figure 5*.

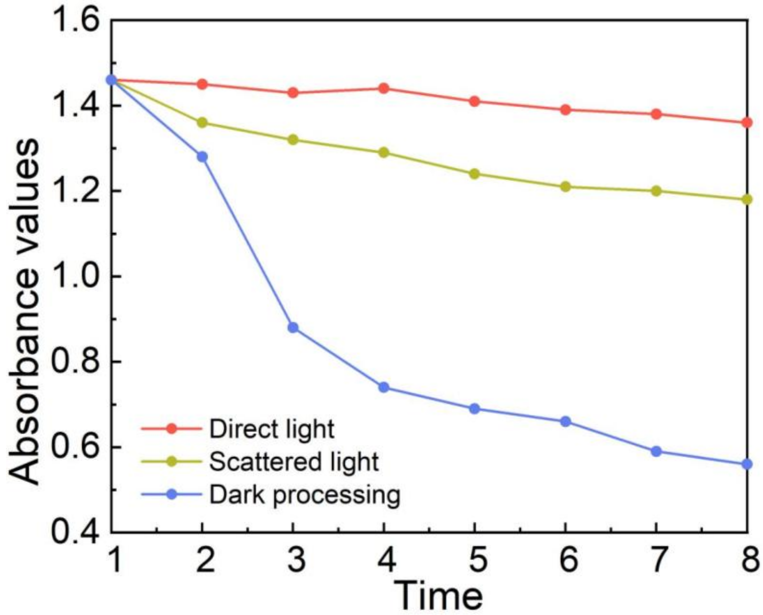

Figure 5. *Stability of chlorophyll in Astragalus membranaceus leaves under different light conditions*

As shown in *Figure 5*, under direct light, diffuse light, and dark conditions, the absorbance values of chrysanthemum pigment extracts all show a downward trend, with the greatest decrease observed under direct light, indicating the fastest reduction in chlorophyll content. Compared to the maximum and minimum values measured during the day, the absorbance of chrysanthemum leaves decreased by 41.91%. The next highest decrease occurred under diffuse light conditions, where absorbance decreased by 14.19%. The smallest decrease was observed in the dark condition, at only 4.75%. The trend in chlorophyll changes is similar to that of anthocyanins, with the greatest chlorophyll degradation occurring under direct light.

Determination of photosynthesis

The daily variation in photosynthesis of *Medicago sativa* (alfalfa) follows a bimodal curve, with a significant decline in net photosynthetic rate during the midday period on sunny summer days. This phenomenon is considered a way for plants to adapt to drought

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conditions, possibly achieved through stomatal and non-stomatal regulatory mechanisms. Studies on the daily variation of photosynthetic rate (Pn) in many plants have commonly found that the decrease in leaf Pn during midday is related to reduced stomatal openness. The daily variation in net photosynthetic rate of *Medicago sativa* is shown in *Figure 6*.

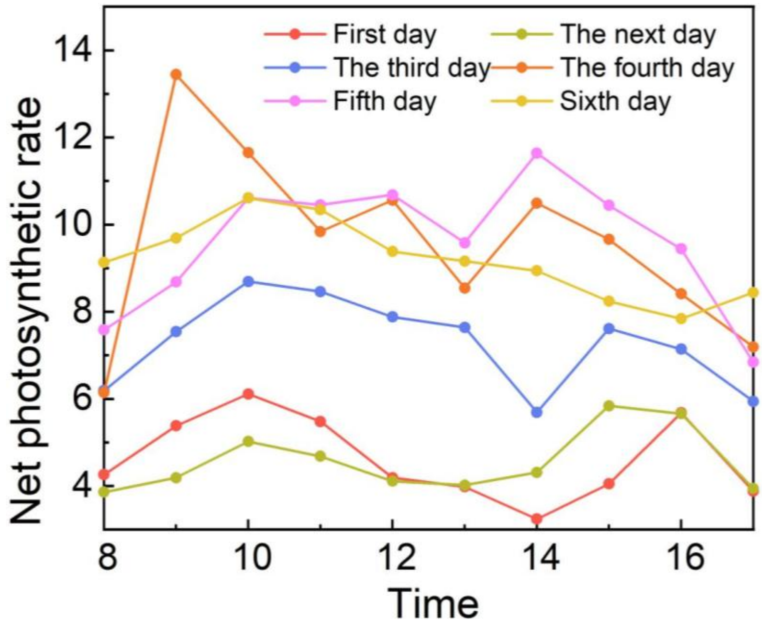

Figure 6. *Daily variation in net photosynthetic rate of Astragalus membranaceus*

As shown in the figure, the variation follows an asymmetric bimodal curve. In the morning, as light intensity increases, the photosynthetic rate rises rapidly, reaching the first peak around 10:00. Following this, as light intensity and atmospheric temperature continue to increase, the intercellular CO_2 concentration decreases, and the stomatal limit value increases, causing some stomata to close and leading to a decline in the net photosynthetic rate. During the midday period, the photosynthetic rate rebounds, reaching a second peak, approximately 75% of the first peak. The decrease in net photosynthetic rate at midday is closely related to the increase in light intensity, temperature, and transpiration rate, indicating that the decline in photosynthetic rate is directly associated with these factors. In contrast, the photosynthetic rate of mature trees shows no significant variation, suggesting that the increase in leaf pigment content has not led to a decrease in photosynthetic capacity.

The effect of light intensity on chlorophyll a and chlorophyll b

The fumigation treatment used a simple static fumigation system with a chamber volume of 10.8 m^3, dimensions of 3.6 m in length, 2.0 m in width, and 1.5 m in height, welded with steel bars. The top cover was made of polyethylene plastic film, with a shading net having a light transmittance of 50%. The temperature in the fumigation chamber was controlled between 25 and 32°C, and the humidity was maintained at 80%.

In the experiment, leaves were first cleaned, then punched into small pieces using a hole puncher, weighed to 0.1g, and placed in an Erlenmeyer flask. A 30 ml volume of distilled water was added to the flask, which was then placed in a vacuum desiccator. After 8 minutes of evacuation, air was slowly introduced into the chamber, and the

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mixture was allowed to stand for 20 minutes with gentle stirring using a glass rod. The treatment lasted for 15 days, with fumigation starting every morning at 8:00. Once the desired concentration was reached, fumigation was stopped, and the chamber was reopened for 20 minutes the next morning. After closing the gas chamber, fumigation continued until the set requirements were met.

Figure 7 shows the effect of light intensity on the content of chlorophyll a and chlorophyll b in the leaves of four ornamental-leaved trees.

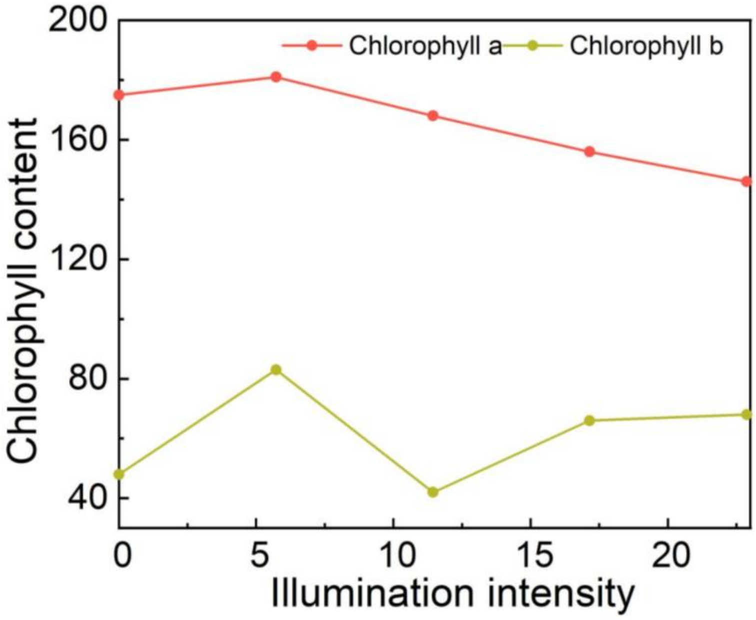

Figure 7. *Daily variation in net photosynthetic rate of Astragalus membranaceus*

When the light intensity reached 5.72, the content of chlorophyll a reached its maximum, being 106.42% and 110.98% of the control group. Afterward, the content of chlorophyll a decreased with increasing light intensity. As the light intensity further increased, the chlorophyll a content peaked again at a light intensity of 11.433, reaching 145.31% and 161.66% of the control group. Afterward, the chlorophyll a content continued to decrease, eventually reaching 22.857. Regarding the changes in chlorophyll b, there was an initial increase followed by a decrease, with the curve displaying two peaks at light intensities of 5.718 and 17.163.

From the discussion of the various photosynthetic parameters, it can be concluded that mature leaves, with better development, larger size, and green color, and a moderate growing period, exhibit stronger resistance to environmental factors, contributing significantly to the photosynthesis of poplar trees. The ranking of new and old leaves may vary slightly depending on the selected leaves or season. Generally, old leaves are more stable and have greater vitality than new leaves. Although old leaves are not as vigorous as mature leaves, they remain the second most significant contributors. New leaves, being in the growth phase, are smaller, bright green in color, and have not yet reached optimal physiological and biochemical states, resulting in the weakest resistance to stress.

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DOI: http://dx.doi.org/10.15666/aeer/2302_33853399

Conclusions

The study on leaf photosynthetic characteristics revealed that the daily variation in net photosynthetic rate follows an asymmetric bimodal curve, with the highest peak occurring around 10:00 AM and a smaller second peak around 3:00 PM. The daily variation in transpiration rate follows a unimodal curve, peaking around 1:00 PM, and the trend in stomatal conductance is generally consistent with the net photosynthetic rate. The close coupling between stomatal conductance and net photosynthetic rate helps maintain high water use efficiency. The annual variation in net photosynthetic rate follows a bimodal curve, with the strongest photosynthetic capacity observed when the leaf reaches its maximum area 40 days after leaf unfolding. This gradually decreases and then slightly recovers as the weather cools. The annual variation in transpiration rate follows a unimodal curve, increasing with rising temperatures, peaking in summer, and reaching its minimum just before the leaves completely fall.

This study also emphasizes the importance of optimizing the cultivation and propagation methods for *Paeonia chinensis*, a species that is currently endangered and faces challenges in artificial propagation. The results from this experiment, particularly the impact of light intensity and environmental conditions on photosynthetic efficiency and growth, provide valuable insights into improving propagation techniques. By understanding how light conditions influence photosynthetic rates and overall plant health, we can optimize these environmental factors to enhance the survival and growth rates of *Paeonia chinensis* in cultivation settings, ultimately contributing to the conservation efforts for this species.

One limitation of this study is that while the chemical components in the crude extract of *Astragalus membranaceus* (North Astragalus) were preliminarily analyzed and identified, the qualitative analysis using mass spectrometry combined with the database can only provide initial results. Further structural analysis, including separation, purification, and spectral analysis, is needed to obtain more accurate and reliable results.

Acknowledgements. This work was supported by the project funded by the youth science and technology innovation project of Nanjing Forestry University (No.CX2018007), the Key Research and Development Program of Jiangsu Province (BE2019388) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). Wanxia Yang and Jiaqi Zhuang equally contributed to this work.

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DOI: http://dx.doi.org/10.15666/aeer/2302_33853399