

# STUDY ON THE DIFFERENCES IN PHOTOSYNTHETIC CHARACTERISTICS AND LEAF ANATOMICAL STRUCTURE AMONG VARIOUS SPECIES OF *ORMOSIA* PLANTS

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**Abstract.** In order to explore the photosynthetic characteristics and leaf anatomical structure of the *Ormosia* genus, a series of measurements were taken on three endangered species: *Ormosia xylocarpa*, *Ormosia henryi* and *Ormosia microphylla*. These measurements included the light response curve, the CO<sub>2</sub> response curve, the leaf anatomical structure, the leaf epidermal characteristics and the chlorophyll content. The aim of this study was to identify the factors influencing plant photosynthesis. Results showed: (1) The three *Ormosia* species were light-favoring tree species. *O. henryi* had strong photosynthetic characteristics, and the maximum photosynthetic rate ( $P_{max}$ ) was  $15.1354 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , which was much higher than the other two *Ormosia* species. Among the three species of *Ormosia*, *O. henryi* has the strongest weak light utilization ability and CO<sub>2</sub> utilization rate, and *O. xylocarpa* has a strong CO<sub>2</sub> utilization range. (2) Among the three *Ormosia* species, *O. xylocarpa* had the thickest leaves, but the thickness of palisade tissue was *O. henryi* > *O. xylocarpa* > *O. microphylla*. (3) The single stomatal area of *O. henryi* was significantly higher than that of *O. xylocarpa* and *O. microphylla*, and the stomatal density of *O. xylocarpa* was significantly higher than that of *O. henryi* and *O. microphylla*. (4) The Chl a of the three *Ormosia* species was higher than Chl b, which was similar to the results of photosynthetic characteristics. The Chl a, Chl b and Chl (a+b) of *O. henryi* were significantly higher than those of *O. xylocarpa* and *O. microphylla*. The Car content of *O. microphylla* was significantly lower than that of *O. henryi* and *O. xylocarpa*. In conclusion; the leaf microstructure, chlorophyll content and epidermal characteristics of the three *Ormosia* species are significant importance with regard to their photosynthetic capacity. During the cultivation process, increasing CO<sub>2</sub> concentration in the short term can increase the photosynthetic rate of the aforementioned three *Ormosia* species; in seedling management, appropriate increase in light conditions for *Ormosia* plants has been demonstrated to facilitate their growth.

**Keywords:** *Ormosia*, *Ormosia xylocarpa*, *Ormosia henryi*, *Ormosia microphylla*, photosynthesis, leaf structure

## Introduction

*Ormosia* is a genus of the Fabaceae (Papilionaceae), comprising approximately 130 species. These are primarily distributed in Southeast Asia, northwest Australia, and tropical America. In China, there are 37 species of *Ormosia*, with Guangdong, Guangxi,

and Yunnan being the main regions of occurrence. China has the highest number of *Ormosia* species distributed in Asia (Wu et al., 2010). *Ormosia microphylla* is an endemic species in China, mainly distributed in Guangxi, Guizhou, Fujian and Guangdong provinces. It is also known as 'Xiaoye *Pterocarpus indicus*' or 'Guangxi *Pterocarpus indicus*' because of its similar material characteristics and color to *Pterocarpus indicus*. Its sapwood is light yellowish brown, heartwood is dark purple. The texture is straight and the material is hard. The primary applications of this material are in the production of high-end furniture and art crafts. The high value of the logs has led to a significant increase in illegal logging, which has resulted in extensive damage to the wild resources and a reduction in the distribution areas. It was listed in the 'National Key Protected Wild Plants List' by China in 2021. *Ormosia henryi* is similar to *O. microphylla* because of its material, which is called 'Daye *Pterocarpus indicus*' in the folk. It is also the top material for making furniture, so it is cut down in large quantities. Unlike *O. microphylla*, *O. henryi* is widely distributed in China, with a distribution extending southwards to the vicinity of the Yangtze River. Its roots, root barks, stems and leaves can be used as medicines, which have the effects of promoting blood circulation and removing blood stasis, dispelling wind and detumescence. It can be used for greening and fire prevention tree species in garden landscapes. It is currently listed as a secondary protected wild plant in China's 'National Key Protected Wild Plants List'. *Ormosia xylocarpa* is also endemic to China, mainly distributed in Jiangxi (southern), Fujian, Hunan (southern), Guangdong, Hainan, Guangxi, Guizhou (eastern). Its hard texture, wear-resistant, is used for craft carving and furniture materials. Different from the two *Ormosia* species mentioned above, *O. xylocarpa* is an excellent garden greening tree species with large leaves, thick leathery leaves and shaded canopy. It is also one of the three famous trees in Hainan. In addition, the seeds of *O. xylocarpa* can be used as medicine to treat eye diseases. At present, it is currently listed as a secondary protected wild plant in China's 'National Key Protected Wild Plants List'. It can be seen that *Ormosia* has excellent materials and a wide range of uses. It can be used as furniture, construction, agricultural tools and papermaking raw materials. Some tree species have good landscape effects and can be used for landscaping. Its seed shape is beautiful and is widely used in handicraft manufacturing (Tang et al., 2023). In addition, *Ormosia* is also rich in alkaloids, flavonoids, triterpenes and many other compounds. It has excellent pharmacological activity (Zhang et al., 2021).

The process of photosynthesis represents the primary mechanism through which plants accumulate organic matter, and it is a crucial factor influencing their growth and development. Furthermore, photosynthetic characteristics indirectly reflect the ability of plants to obtain resources, and thus serve as important indicators of plant growth and metabolism (Ou et al., 2023). The study of photosynthetic characteristics in plants is of great significance for the understanding of the optimal conditions for plant growth. The study of photosynthesis in endangered plants and the factors that influence it represents an important method for accurately assessing the optimal habitat conditions. It can ascertain the most conducive environmental conditions, such as light and water requirements, for their growth and identify the optimal cultivation site (Smith et al., 1993; Smith and Houppis, 2004). Secondly, endangered plants are extremely sensitive to the external environmental factors. In comparison to the widely distributed species of the same genus, they have the characteristics of poor survival ability, relatively low adaptability and weak photosynthetic capacity. Wei et al. (2008) compared the endangered species *Camellia nitidissima* with the widely distributed species *C. sinensis*,

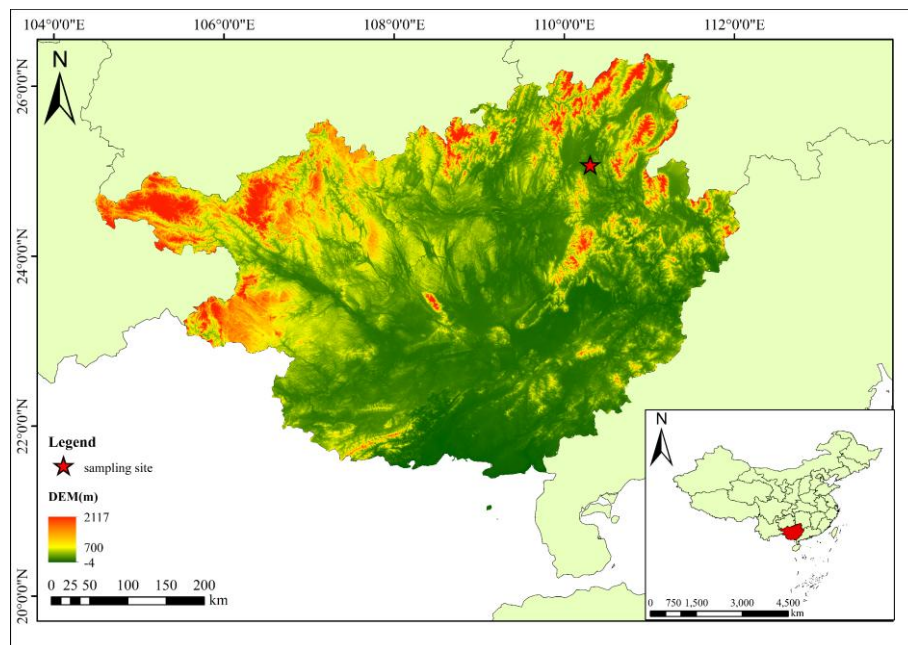
and found that they were significantly different in physiological metabolic rates such as photosynthesis, mutual photosynthesis, respiration, and transpiration, and there were significant differences in the living habits of the two plants. Therefore, by comparing the narrow distribution of *O. microphylla* with the more widely distributed *O. henryi*, the influencing factors affecting the distribution of *Ormosia* can be explored. Chen et al. (2024a) studied four endangered species of *Dendrobium scoriarum*, *D. lituiflorum*, *D. lohohense* and *D. aduncum*, and found that the maximum net photosynthetic rate ( $P_{max}$ ) of plants was significantly correlated with mesophyll thickness ( $P < 0.05$ ). Accordingly, an investigation into the photosynthetic characteristics and leaf structure of *O. xylocarpa*, which possesses thicker leaves, can facilitate a more comprehensive understanding of the intrinsic mechanisms underlying the endangered status of such plants. This understanding can then inform the formulation of targeted protection strategies that are tailored to the specific characteristics of the species in question. Furthermore, this approach can provide a foundation for the large-scale introduction and cultivation of these species.

*Ormosia* exhibits a slow natural regeneration and a precipitous decline in wild populations, attributable to its low breeding rate and anthropogenic destruction. The survival rate of sexual reproduction of the three *Ormosia* species is low in the context of artificial introduction and cultivation, with a concomitant high mortality rate during the transition from seedlings to adult plants. Furthermore, a systematic breeding process has yet to be established. Photosynthetic rate of plants is affected by the plant's own factors and the external environment. The higher the chlorophyll content, the stronger the photosynthetic rate of leaves. The photosynthetic rate of plants in different growth cycles is also different, and different light intensity, ambient temperature, mineral element content and air humidity will also affect the photosynthetic rate of plants (Pan et al., 2012). At present, there are few reports on the photosynthetic characteristics and leaf anatomical structure of *O. xylocarpa*, *O. henryi* and *O. microphylla*. Only Wang (2012) studied the daily process of photosynthesis in *O. henryi*, and did not study the key factors such as photosynthetic response and CO<sub>2</sub> response. *O. xylocarpa* and *O. microphylla* have not been reported. In this study, the photosynthetic characteristics, leaf anatomical structure and leaf pigment content of *O. xylocarpa*, *O. henryi* and *O. microphylla* were compared, and the photosynthetic characteristics of three *Ormosia* species under different light intensities were explored. The relationship between leaf structure, pigment content and photosynthetic characteristics offers a foundation for the conservation of *Ormosia* germplasm resources.

## Materials and methods

### General situation of test site

The experimental site is located in Guangxi Institute of Botany, Guilin City, Guangxi Zhuang Autonomous Region. It is located in 110°18.13'E, 25°04'N, 165 m-180 m above sea level, and belongs to the mid-subtropical monsoon climate zone. The region has abundant rainfall and mild climate. The annual sunshine is 1553.09 h, the annual rainfall is 1894 mm, the annual average temperature is 18.8°C, and the annual extreme minimum temperature is -3.0°C (Fig. 1).



**Figure 1.** Map of the study area and indicate the location of the test sample

## Materials

The test materials were introduced and cultivated perennial *O. xylocarpa*, *O. henryi* and *O. microphylla*. Each plant selected three species with the same orientation, good growth status and the same growth status for determination. The test plants were 5 years old, and the plant height was about 1.5 m.

## Method

### Determination of light response curve

The photosynthetic response parameters of leaves were measured by Li-6800 portable photosynthesis instrument (LI-COR USA) red and blue light source leaf chamber (6800-01F) on a clear and cloudless morning from August to September 2024. Before measurement, the leaves were induced for 15 min under PAR of  $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  to fully open the stomata. The  $\text{CO}_2$  concentration was controlled to  $400 \mu\text{mol}\cdot\text{m}^{-1}$  by  $\text{CO}_2$  vial, Flow was set to  $500 \mu\text{mol}\cdot\text{m}^{-1}$ ,  $\Delta P$ : 0.1 kPa,  $\text{H}_2\text{O}$  was opened, RH<sub>air</sub> was set to 60%, Mixing fan speed was set to 10000 rpm. Select the Light\_Response automatic measurement program and set the light intensity ( $Q_n$  values) from high to low. There are  $1800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $1600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $1400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $1200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $50 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively. Each gradient is maintained for 120 ~ 180 s. Finally, the measured data were fitted to the  $P_n$ -PFD curve by the following equation, and the light response curve was drawn with PFD as the horizontal axis and  $P_n$  as the vertical axis (Chen et al., 2024b).

$$P_n = AQY \frac{1-\beta PFD}{1+\gamma PFD} PFD - R_d \quad (\text{Eq.1})$$

In the equation:  $P_n$  is the net photosynthetic rate;  $AQY$  is the performance of quantum efficiency;  $\alpha$ ,  $\beta$  and  $\gamma$  are coefficients,  $PFD$  is photon flux density, and  $R_d$  is dark respiration rate. Through the adaptability test, the fitting effect is good, and then the following formulas are used to calculate  $LSP$ ,  $P_{max}$  and  $LCP$ :

$$LSP = \frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma} \quad (\text{Eq.2})$$

$$P_{max} = AQY \left( \frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma} \right) - R_d \quad (\text{Eq.3})$$

$$LCP = \frac{AQY - \gamma R_d - \sqrt{(\gamma R_d - AQY)^2 - 4\beta * AQY * R_d}}{2\alpha\beta} \quad (\text{Eq.4})$$

#### Determination of $CO_2$ response curve

In August-September 2024, a clear and cloudless morning was selected. The  $CO_2$  response parameters of leaves were measured using a Li-6800 portable photosynthesis system (LI-COR USA) with a red-blue light source chamber (6800-02). Leaves with intact leaves and no pests and diseases were selected during the measurement. Before the measurement, the leaves were induced for 15 min under PAR of  $1000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , so that the stomata were fully opened. The  $CO_2$  small rigid flask was used to provide  $CO_2$  gas with different concentrations for  $CO_{2\_s}$ . The Flow was set to  $500 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $\Delta P$ : 0.1 kPa,  $H_2O$  was turned on, RH<sub>air</sub> was set to 60%, Mixing fan speed was set to 10 000 rpm, and the light intensity (Setpoint) was set to  $1200 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ , Color Sepc: r90 (90% red light). The  $CO_2$ \_Response automatic determination program was selected.  $CO_2$  Values began with the environmental concentration, first reduced the concentration, then returned to the environmental concentration, and finally increased to the highest concentration. They were  $400 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $300 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $200 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $100 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $50 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $0 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $400 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $600 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $800 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $1\ 000 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $1\ 200 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $1\ 600 \mu\text{mol} \cdot \text{m}^{-1}$ ,  $2\ 000 \mu\text{mol} \cdot \text{m}^{-1}$ , respectively. Each gradient stayed 120-180 s. Finally, the measured data were fitted to the  $P_n$ - $C_i$  curve by the following equation, and the  $CO_2$  response curve was drawn with  $C_i$  as the horizontal axis and  $P_n$  as the vertical axis (Ye, 2010).

$$P_n = \alpha \frac{1-\beta C_i}{1+\gamma C_i} C_i - R_p \quad (\text{Eq.5})$$

In the equation:  $P_n$  is the net photosynthetic rate;  $C_i$  is intercellular  $CO_2$  concentration;  $\alpha$  is the initial carboxylation efficiency of the  $CO_2$  response curve;  $\gamma$  and  $\beta$  are photorespiration rates. When  $P_n$  is 0, the  $CO_2$  compensation point ( $CDCP$ ) can be obtained:

$$CDCP = \alpha \frac{\alpha - \gamma R_p - \sqrt{(\alpha - \gamma R_p)^2 - 4\alpha\beta R_p}}{2\alpha\beta} C_i - R_p \quad (\text{Eq.6})$$

The  $CO_2$  saturation point ( $CDSP$ ) of plants can be obtained by the following equation:

$$CDSP = \frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma} \quad (\text{Eq.7})$$

The potential maximum net photosynthetic rate ( $A_{max}$ ) of plants is:

$$A_{max} = \alpha \left( \frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma} \right)^2 - R_p \quad (\text{Eq.8})$$

### *Leaf epidermal characteristics*

The mature leaves with good growth status in the same direction were selected from the plants for photosynthetic determination, and the central part of the leaf margin to the main vein was cut into small squares with an area of about 5 mm×5 mm, and immediately fixed in 2.5% glutaraldehyde fixative. After being taken to the laboratory, ethanol was used for step-by-step dehydration immediately. The dehydration gradient was 30%, 50%, 70%, 80%, 90%, 100%, 100%, 100% ethanol, and each step was dehydrated for 15 min. After dehydration, the leaves were subjected to critical point drying and gold plating. The upper epidermis, lower epidermis and stomatal apparatus of the leaves were observed under vacuum electron scanning electron microscope (ZEISS EVO18). Each sample was randomly observed in 10 fields. The long axis (SL), short axis (SW), stomatal density (SD) and single stomatal area (SA) of stomatal apparatus were measured by Axio Vision SE64 Rel.4.8 scanning electron microscope supporting software. Stomatal density (SD) = the number of stomata in the field of vision / the area of visual field; single stomatal area (SA)= $\pi \times$  stomatal long axis (SL)  $\times$  stomatal short axis (SW)/4,  $\pi = 3.14$ .

### *Leaf anatomical structure*

Refer to Li (1987) to make paraffin sections of plant leaves. Three leaves in the same direction and in good condition were picked from each plant. Transversely cut along the middle vein, cut into 10 mm  $\times$  10 mm, and fixed with FAA fixative (70% ethanol: formalin: glacial acetic acid = 90:5:5). The leaves were placed in a dehydration box and dewaxed with ethanol and xylene series. After waxing, the plant tissues were embedded in paraffin, sliced, dewaxed to dehydration, stained with toluidine blue, and sealed with neutral gum. The sections were observed and photographed under an optical microscope, and the microscopic parameters were measured using the graphics analysis software Case Viewer. The measurement indexes included upper epidermal cell thickness (UET), lower epidermal cell thickness (LET), leaf thickness (LT), palisade tissue thickness (PPT), spongy tissue thickness (SPT), and the ratio of palisade tissue thickness to spongy tissue thickness (PPT/SPT). Ten visual fields were randomly selected to determine the parameters of each index.

### *Determination of photosynthetic pigment content in leaves*

Three leaves with the same maturity, leaf position and leaf size were collected from photosynthetic plants. Each sample was accurately weighed 0.2 g intact leaves into a 25 mL volumetric flask, using 95% ethanol volume. Immersed in dark conditions for 24 h, using UV-visible spectrophotometer (Alpha 1502 Shanghai Spectrum Instrument Co., Ltd.) at 470 nm, 649 nm and 665 nm wavelengths to determine the absorbance value; the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll (Chl a+b), carotenoid

(Car), the ratio of chlorophyll a to chlorophyll b (Chl a/Chl b) and the ratio of carotenoid to chlorophyll (Car/Chl a+b) were calculated by referring to the method of Li (2000).

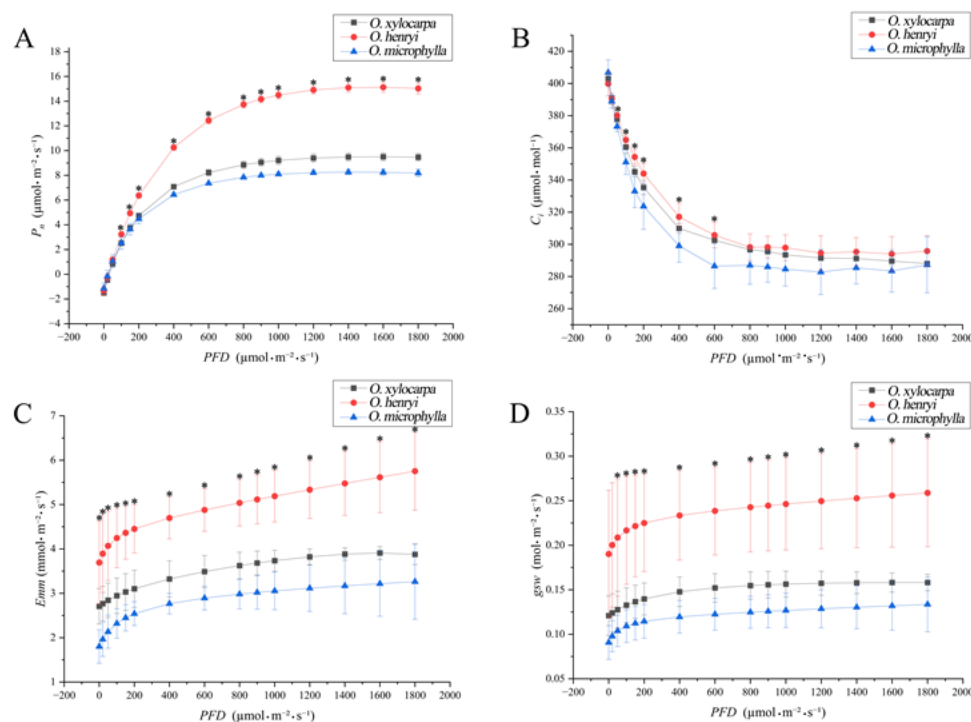
## Data processing

Excel 2016 was used to process the above test results, SPSS 27.0 was used for one-way analysis of variance, Duncan method was used for multiple comparisons, and photosynthetic model fitting software was used to fit and calculate the corresponding photosynthetic parameters. The Origin 2024 software was used to analyze the correlation between light and characteristic parameters, chlorophyll content, leaf anatomical structure and leaf epidermal characteristics, and the data in this paper were visualized.

## Results and analysis

### Light response curve parameters

The photosynthetic rate ( $P_n$ ) of *O. henryi* was significantly higher than that of *O. xylocarpa* and *O. microphylla* (Fig.2A). The  $P_n$  of *O. henryi*, *O. xylocarpa* and *O. microphylla* increased with the increase of PFD. When PFD was 0-600  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the  $P_n$  values of *O. henryi*, *O. xylocarpa* and *O. microphylla* increased significantly, and tended to be flat after 600  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Gradually reached the light saturation point (LSP), and there was no obvious photoinhibition. As shown in Fig. 2B-D, the intercellular  $\text{CO}_2$  concentration ( $C_i$ ), transpiration rate ( $E_{\text{mm}}$ ) and stomatal conductance ( $g_{\text{sw}}$ ) of *O. henryi* were higher than those of *O. xylocarpa* and *O. microphylla* ( $O. henryi > O. xylocarpa > O. microphylla$ ).



**Figure 2.** Three kinds of *Ormosia* species light response curves. Note: \* Significance  $<0.05$ . (A) Photosynthetic response curve. (B) The line chart of transpiration rate under different light intensity. (C) The line chart of stomatal conductance under different light intensity. (D) The line chart of intercellular  $\text{CO}_2$  concentration under different light intensity

The  $C_i$  of *O. henryi*, *O. xylocarpa* and *O. microphylla* decreased with the increase of  $PFD$ . When the  $PFD$  was 0-600  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the  $C_i$  showed a linear downward trend. When the  $PFD$  reached 600  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , it gradually became gentle and tended to be stable.  $Emm$  showed an upward trend, among which *O. henryi* showed a significant upward trend, which was significantly higher than that of *O. xylocarpa* and *O. microphylla*. The  $gsw$  showed an overall upward trend. When the  $PFD$  was 0-200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , the  $gsw$  response was obvious, and the *O. xylocarpa* showed a slight decrease when the  $PFD$  reached 1200  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

It can be seen from Table 1 that the maximum photosynthetic rate ( $P_{max}$ ) and apparent quantum yield ( $AQY$ ) of *O. henryi*, *O. xylocarpa* and *O. microphylla* showed significant differences ( $P<0.05$ ). The maximum photosynthetic rate ( $P_{max}$ ) was 15.1354  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 9.5089  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 8.0292  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively. The  $P_{max}$  and  $AQY$  of *O. henryi* were significantly higher than those of *O. xylocarpa* and *O. microphylla*, and there were significant differences in  $P_{max}$  between *O. xylocarpa* and *O. microphylla* ( $P<0.05$ ).

**Table 1.** Comparison of photosynthetic parameters of three *Ormosia* species

| Species               | $R_d$ /<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | $P_{max}$ /<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | LSP/<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | LCP/<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | AQY/<br>( $\text{mol}\cdot\text{mol}^{-1}$ ) |
|-----------------------|--------------------------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------------|
| <i>O. xylocarpa</i>   | 1.5054<br>$\pm 0.0436a$                                            | 9.5089<br>$\pm 0.3252b$                                                | 1567.8833<br>$\pm 79.4495a$                                     | 30.0549<br>$\pm 1.6719a$                                        | 0.0316<br>$\pm 0.001b$                       |
| <i>O. henryi</i>      | 1.3791<br>$\pm 0.3197a$                                            | 15.1354<br>$\pm 0.3918a$                                               | 1535.59<br>$\pm 65.2947a$                                       | 25.7183<br>$\pm 5.5684a$                                        | 0.0388 $\pm$<br>0.0012a                      |
| <i>O. microphylla</i> | 1.1461<br>$\pm 0.3963a$                                            | 8.0292<br>$\pm 0.5972c$                                                | 1394.7267<br>$\pm 183.6211a$                                    | 24.2127<br>$\pm 10.4619a$                                       | 0.0310<br>$\pm 0.0017b$                      |

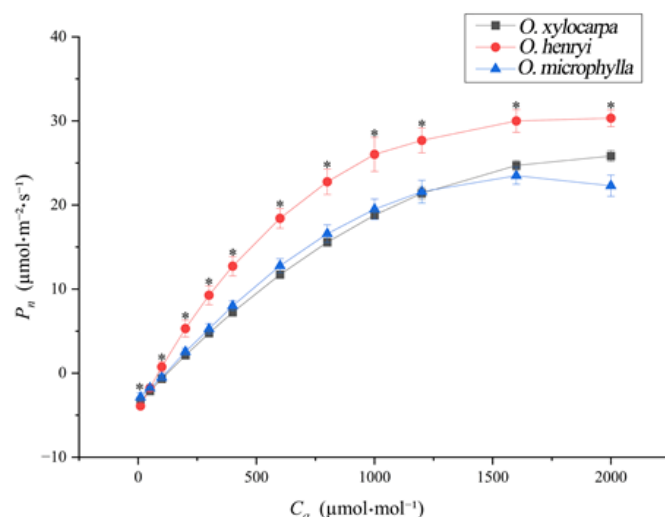
Note: Different lowercase letters in the same column indicate significant difference,  $P<0.05$ .  $R_d$  is dark respiration rate;  $P_{max}$  is the maximum photosynthetic rate; LSP is the light saturation point; LCP is the light compensation point; AQY is the apparent quantum efficiency

### *CO<sub>2</sub> response curve parameters*

It can be seen from Fig. 3 that the photosynthetic rate ( $P_n$ ) of *O. henryi* was higher than that of *O. xylocarpa* and *O. microphylla*, and *O. xylocarpa* was equivalent to *O. microphylla*. The variation trends of  $\text{CO}_2$  response curves of *O. henryi*, *O. xylocarpa* and *O. microphylla* were basically the same. The  $P_n$  of the three *Ormosia* species increased with the increase of  $C_a$ . When  $C_a$  reached 1600  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , *O. microphylla* began to reach  $\text{CO}_2$  saturation concentration, and  $P_n$  began to decrease slowly.

The maximum net photosynthetic rates ( $A_{max}$ ) of *O. henryi*, *O. xylocarpa* and *O. microphylla* were 30.9214  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , 28.9181  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 27.4316  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively (Table 2), and there was no significant difference among them ( $P>0.05$ ). The three parameters of  $\alpha$ ,  $R_p$  and  $CDSP$  of *O. henryi* were significantly different from those of *O. xylocarpa* and *O. microphylla* ( $P<0.05$ ). The  $CDSP$  of *O. xylocarpa*, *O. henryi* and *O. microphylla* were 2005.3467  $\mu\text{mol}\cdot\text{mol}^{-1}$ , 1758.9667  $\mu\text{mol}\cdot\text{mol}^{-1}$  and 1642.9633  $\mu\text{mol}\cdot\text{mol}^{-1}$  respectively, showing significant differences ( $P<0.05$ ) (Table 2).





**Figure 3.** Three kinds of *Ormosia* species  $CO_2$  response curves. \* Significance  $<0.05$ .  $P_n$  is the photosynthetic rate;  $C_a$  is the  $CO_2$  concentration in the leaf chamber

**Table 2.** Three *Ormosia* species  $CO_2$  response parameters

| Species               | $\alpha/$<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | $R_p/$<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | $A_{\max}/$<br>( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) | CDSP/<br>( $\mu\text{mol}\cdot\text{mol}^{-1}$ ) | CDCP/<br>( $\mu\text{mol}\cdot\text{mol}^{-1}$ ) |
|-----------------------|----------------------------------------------------------------------|-------------------------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| <i>O. xylocarpa</i>   | 0.0318<br>$\pm 0.0016b$                                              | 2.3273<br>$\pm 0.2331b$                                           | 27.4316<br>$\pm 3.0037a$                                               | 2005.3467<br>$\pm 52.4374a$                      | 123.285<br>$\pm 2.9857a$                         |
| <i>O. henryi</i>      | 0.0558<br>$\pm 0.0071a$                                              | 3.1014<br>$\pm 0.086a$                                            | 30.9214<br>$\pm 1.4229a$                                               | 1758.9667<br>$\pm 77.6354b$                      | 86.2781<br>$\pm 11.7751b$                        |
| <i>O. microphylla</i> | 0.0359<br>$\pm 0.0065b$                                              | 2.2231<br>$\pm 0.406b$                                            | 28.9181<br>$\pm 9.6305a$                                               | 1642.9633<br>$\pm 18.1708c$                      | 109.963<br>$\pm 6.9956a$                         |

Note: Different lowercase letters in the same column indicate significant difference,  $P < 0.05$ .  $\alpha$  is the initial carboxylation efficiency;  $R_p$  is the photorespiration rate;  $A_{\max}$  maximum net photosynthetic rate; CDSP is the saturated  $CO_2$  concentration; CDCP is the  $CO_2$  compensation point

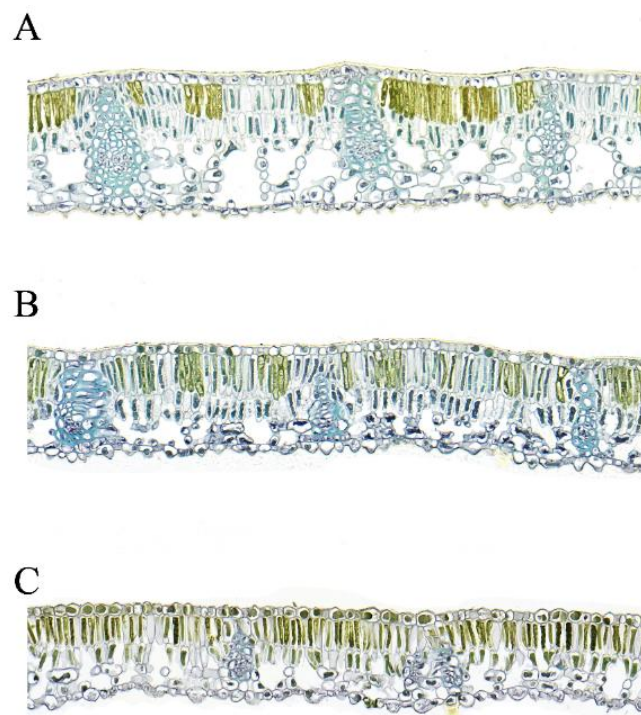
## Comparison of leaf structure

### Leaf anatomical structure characteristics

As shown in Fig. 4, *O. xylocarpa*, *O. henryi* and *O. microphylla* are all heterophyllous plants, and the internal structures of the two sides of the leaves are obviously different. The upper leaves are closely arranged with high light efficiency palisade tissue, and the lower leaves are sponge tissue with low light efficiency. The upper and lower epidermis are composed of monolayer cells. The palisade tissues of *O. xylocarpa* and *O. henryi* were densely arranged, while the cells of *O. microphylla* were loosely arranged with a certain gap. The palisade tissues of the three *Ormosia* species were composed of two layers of cells. The spongy tissue of *O. xylocarpa* is looser than that of *O. henryi* and *O. microphylla*.

As shown in Table 3, there were significant differences in leaf thickness (LT) and upper epidermal cell thickness (UET) among *O. xylocarpa*, *O. henryi* and *O. microphylla* ( $P < 0.05$ ). LT and UET of *O. xylocarpa* were higher than those of *O. henryi* and

*O. microphylla*. LT of *O. henryi* was higher than that of *O. microphylla*, but UET was lower than that of *O. microphylla*. The LET of *O. henryi* was significantly higher than that of *O. xylocarpa* and *O. microphylla* ( $P<0.05$ ). In addition, there were significant differences in palisade tissue thickness (PPT) and spongy tissue thickness (SPT) among *O. xylocarpa*, *O. henryi* and *O. microphylla* ( $P<0.05$ ). The SPT of *O. henryi* and *O. microphylla* was much lower than that of *O. xylocarpa*. The thickness ratio of palisade tissue to spongy tissue (PPT/SPT) of *O. xylocarpa* was significantly different from that of *O. henryi* and *O. microphylla* ( $P<0.05$ ). The difference is that the PPT/SPT of *O. xylocarpa* is 0.9628, which is close to 1, indicating that the PPT of *O. xylocarpa* is similar to SPT. The PPT/SPT of *O. henryi* and *O. microphylla* were 2.4495  $\mu\text{m}$  and 2.2387  $\mu\text{m}$ , respectively, and there was no significant difference ( $P>0.05$ ).



**Figure 4.** Anatomical structure of three *Ormosia* species leaves. Note: (A) Paraffin sections of *O. xylocarpa*; (B) Paraffin sections of *O. henryi*; (C) Paraffin sections of *O. microphylla*

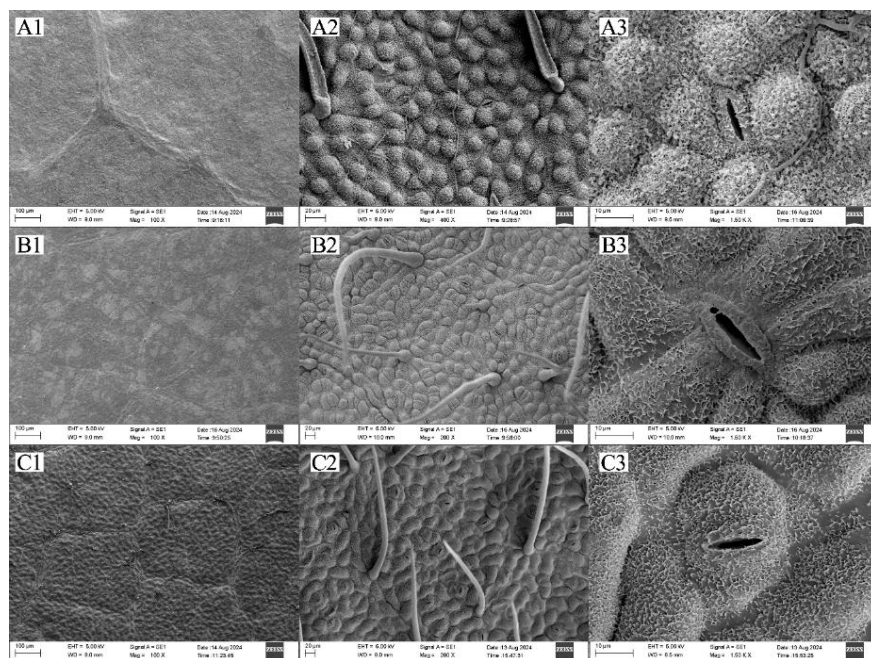
**Table 3.** Leaf cross-sectional anatomical structure parameters of three *Ormosia* species

| Species               | LT ( $\mu\text{m}$ )             | UET ( $\mu\text{m}$ )           | LET ( $\mu\text{m}$ )           | PPT ( $\mu\text{m}$ )           | SPT ( $\mu\text{m}$ )           | PPT/SPT                        |
|-----------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------------------------|
| <i>O. xylocarpa</i>   | 226.7333<br>$\pm 3.6352\text{a}$ | 22.3167<br>$\pm 1.925\text{a}$  | 14.55<br>$\pm 1.8534\text{b}$   | 89.5667<br>$\pm 2.4647\text{b}$ | 93.1<br>$\pm 3.5894\text{c}$    | 0.9628<br>$\pm 0.0323\text{b}$ |
| <i>O. henryi</i>      | 163.8333<br>$\pm 4.5067\text{b}$ | 16.6167<br>$\pm 0.3656\text{c}$ | 17.9167<br>$\pm 1.1409\text{a}$ | 93.55<br>$\pm 2.217\text{a}$    | 38.2667<br>$\pm 1.5526\text{b}$ | 2.4495<br>$\pm 0.1422\text{a}$ |
| <i>O. microphylla</i> | 135.1833<br>$\pm 2.7629\text{c}$ | 19.0333<br>$\pm 1.9552\text{b}$ | 15.3167<br>$\pm 1.8627\text{b}$ | 75.55<br>$\pm 2.7559\text{c}$   | 33<br>$\pm 2.3503\text{a}$      | 2.2987<br>$\pm 0.1742\text{a}$ |

Note: Different lowercase letters in the same column indicate significant difference,  $P<0.05$ . LT is leaf thickness; UET is the thickness of upper epidermal cells; LET is the thickness of lower epidermal cells; PPT is the thickness of fence tissue; PPT is the thickness of sponge tissue; PPT/SPT is the thickness ratio of palisade tissue to spongy tissue

### Leaf epidermal characteristics

By observing the leaf epidermis of the three *Ormosia* species, it was found that there was no stomatal distribution on the upper epidermis of the three *Ormosia* species, and the stomata were distributed on the lower epidermis of the leaves. It can be seen from the figure that the wax on the upper epidermis of *O. xylocarpa* and *O. henryi* showed a network, and the wax on the upper epidermis of *O. microphylla* was granular (Fig. 5 A1, B1, C1). The wax on the lower epidermis of *O. xylocarpa*, *O. henryi* and *O. microphylla* was granular, the lower epidermis of *O. xylocarpa* was flat with protuberant hairs, and *O. henryi* and *O. microphylla* were cylindrical hairs (Fig. 5 A2, B2, C2). The pore shapes of the three *Ormosia* species leaf are all elliptical. The outer arch is covered with waxy layer, and the inner edge of the outer arch is shallow wavy. (Fig. 5 A3, B3, C3).



**Figure 5.** The epidermal characteristics of three *Ormosia* species. Note: A1, A2 and A3 are the upper epidermis, lower epidermis and stomata of *O. xylocarpa*, respectively. B1, B2 and B3 were the upper epidermis, lower epidermis and stomata of *O. henryi*, respectively. C1, C2 and C3 are the upper epidermis, lower epidermis and stomata of *O. microphylla*, respectively

It can be seen from Table 4 that there were significant differences in stomatal long axis (SL), stomatal short axis (SW), stomatal density (SD) and single stomatal area (SA) among *O. xylocarpa*, *O. henryi* and *O. microphylla* ( $P < 0.05$ ). The SL, SW and SA of *O. henryi* were higher than those of *O. xylocarpa* and *O. microphylla*, and *O. xylocarpa* was the minimum ( $O. henryi > O. microphylla > O. xylocarpa$ ). However, the SD of *O. xylocarpa* is much higher than that of *O. henryi* and *O. microphylla*.

### Comparison of chlorophyll content

There were significant differences in chlorophyll a content (Chl a), chlorophyll b content (Chl b), chlorophyll content (Chl(a+b)) and the ratio of chlorophyll a to chlorophyll b (Chl a/Chl b) among the three *Ormosia* species ( $P < 0.05$ ). Among them, Chl a, Chl b and Chl(a+b) of *O. henryi* were higher than those of *O. xylocarpa* and

*O. microphylla*; the Chl a/Chl b of *O. xylocarpa* was higher than that of the other two species, and that of *O. henryi* was the lowest. The carotenoid content (Car) of *O. microphylla* was significantly different from that of *O. xylocarpa* and *O. henryi* ( $P<0.05$ ). There was no significant difference between *O. henryi* and *O. xylocarpa* ( $P>0.05$ ). The ratio of carotenoids to chlorophyll (Car/Chl(a+b)) was significantly different between *O. xylocarpa* and *O. henryi*, *O. microphylla* ( $P<0.05$ ), and there was no significant difference between *O. henryi* and *O. microphylla* ( $P<0.05$ ) (Table 5).

**Table 4.** Three *Ormosia* species stomatal indices

| Species               | SL<br>( $\mu\text{m}$ ) | SW<br>( $\mu\text{m}$ ) | SA<br>( $\mu\text{m}^2$ ) | SD<br>( $\text{PCS}\cdot\text{mm}^{-2}$ ) |
|-----------------------|-------------------------|-------------------------|---------------------------|-------------------------------------------|
| <i>O. xylocarpa</i>   | 12.1233 $\pm$ 0.7061c   | 2.3467 $\pm$ 0.2103c    | 22.3650 $\pm$ 2.7772c     | 307.4536 $\pm$ 58.3240a                   |
| <i>O. henryi</i>      | 18.6200 $\pm$ 1.080a    | 8.6017 $\pm$ 0.5364a    | 125.6269 $\pm$ 9.4658a    | 256.4900 $\pm$ 29.8565b                   |
| <i>O. microphylla</i> | 14.8617 $\pm$ 0.7077b   | 5.2917 $\pm$ 0.5569b    | 61.8328 $\pm$ 8.2229b     | 136.1428 $\pm$ 8.7588c                    |

Note: Different lowercase letters in the same column indicate significant difference,  $P<0.05$ ; SL is the long axis of stomatal apparatus; SW is the short axis of stomatal apparatus; SD is stomatal density; SA is the area of a single stomatal apparatus

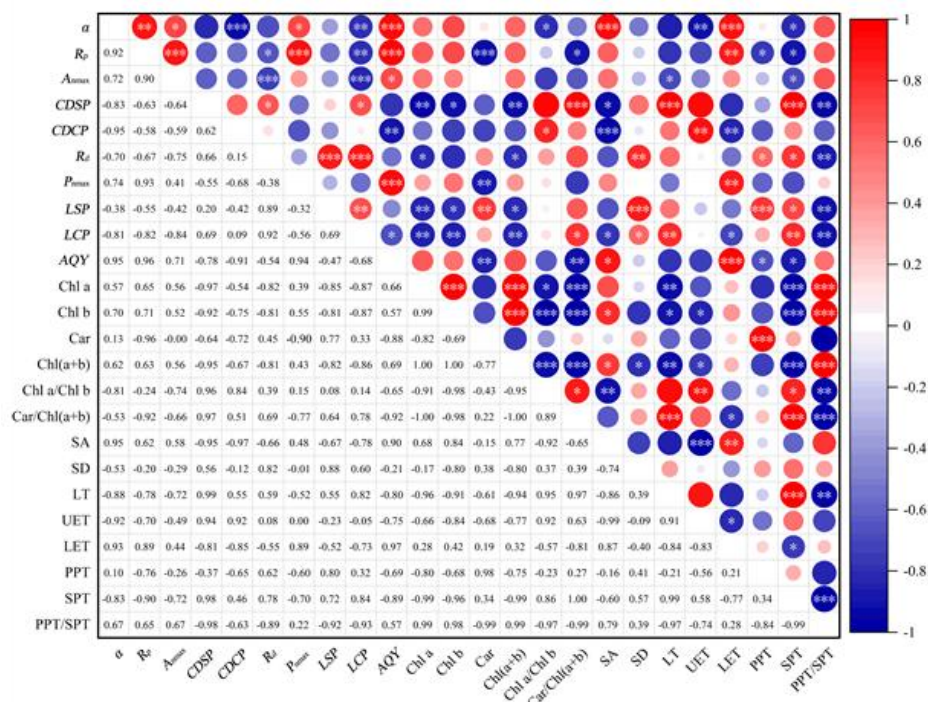
**Table 5.** Chlorophyll content of three *Ormosia* species

| Species               | Chl a                    | Chl b                    | Car                     | Chl(a+b)                 | Chl a/Chl b             | Car/Chl(a+b)            |
|-----------------------|--------------------------|--------------------------|-------------------------|--------------------------|-------------------------|-------------------------|
| <i>O. xylocarpa</i>   | 16.3479<br>$\pm$ 0.0061c | 7.4303<br>$\pm$ 0.0098c  | 2.9292<br>$\pm$ 0.001a  | 23.7782<br>$\pm$ 0.0044c | 2.2002<br>$\pm$ 0.0037a | 0.1232<br>$\pm$ 0.0001a |
| <i>O. henryi</i>      | 24.4022<br>$\pm$ 0.0185a | 14.4608<br>$\pm$ 0.0233a | 2.9556<br>$\pm$ 0.0324a | 38.863<br>$\pm$ 0.0049a  | 1.6875<br>$\pm$ 0.004c  | 0.0761<br>$\pm$ 0.0008b |
| <i>O. microphylla</i> | 23.9447<br>$\pm$ 0.0127b | 12.7774<br>$\pm$ 0.0092b | 2.7991<br>$\pm$ 0.0195b | 36.7221<br>$\pm$ 0.005b  | 1.874<br>$\pm$ 0.0023b  | 0.0762<br>$\pm$ 0.0005b |

Note: Different lowercase letters in the same column indicate significant difference,  $P<0.05$ ; Chl a is the content of chlorophyll a; Chl b is the content of chlorophyll b; Chl(a+b) is the chlorophyll content; Car is carotenoid content; Chl a/Chl b is the ratio of chlorophyll a to chlorophyll b; Car/Chl a+b is the ratio of carotenoids to chlorophyll

### **Correlation analysis of leaf structure characteristics, chlorophyll content and photosynthetic physiological and ecological parameters**

As shown in Fig. 6, there is a certain correlation between the leaf structure parameters and photosynthetic physiological and ecological parameters of the three *Ormosia* species. There were significant correlations between photosynthetic parameters *CDSP*, *LSP*, *LCP* and pigment content Chl a, Chl b, Chl(a+b), leaf parameters SPT, PPT/SPT. Among them, *CDSP* was significantly correlated with Car/Chl(a+b), LT and SPT ( $P<0.001$ ). *LSP* was significantly correlated with SD and PPT ( $P<0.001$ ). The difference is that *LCP* has no significant correlation parameters with pigment content and leaf parameters, but there are the most significant parameters, reaching 10 parameters. *CDSP* was only significantly correlated with Chl a/Chl b, SA ( $P<0.001$ ), UET and LET. *A<sub>max</sub>* was only significantly correlated with LT and SPT ( $P<0.05$ ). *P<sub>max</sub>* was only significantly correlated with pigment content Car and leaf parameter LET ( $P<0.01$ ).



**Figure 6.** Correlation analysis of leaf structure characteristics, chlorophyll content and photosynthetic parameters of three *Ormosia* species. Note: \* indicates  $P < 0.05$ ; \*\* means  $P < 0.01$ ; \*\*\* means  $P < 0.001$

## Discussions

### Photosynthetic characteristics

Photosynthesis is the main way for plants to accumulate material and energy (Zeng and Zhu, 2023). The response of  $P_n$ ,  $C_i$ ,  $Emm$  and  $g_{sw}$  under different  $PFD$  is an important photosynthetic physiological and ecological index for us to evaluate the adaptability of plants to the environment. Under different light intensities, the  $P_n$  of *O. henryi* was significantly stronger than that of *O. xylocarpa* and *O. microphylla*, which indicated that *O. henryi* had stronger material accumulation ability and stronger competition ability in wild communities. In the photosynthetic-light response curve, the  $P_n$  of the three *Ormosia* species did not decrease significantly, indicating that the three *Ormosia* species did not show obvious photoinhibition. Plant leaves balance  $CO_2$  and water evaporation in leaves by regulating stomatal opening and closing, thereby regulating photosynthesis and transpiration, helping plants maintain stable physiological functions in different environments and making them more adaptable (Wu et al., 2020; Li et al., 2024). Plant  $g_{sw}$  is often proportional to  $P_n$ . In this study, the  $Emm$  of the three *Ormosia* species increased with the increase of  $g_{sw}$ , and the upward trend was similar to  $g_{sw}$ . Under the same light intensity, the  $Emm$  and  $g_{sw}$  of *O. henryi* were significantly higher than those of *O. xylocarpa* and *O. microphylla*. Therefore, *O. henryi* was significantly higher than *O. xylocarpa* and *O. microphylla*, and the results were similar to the above views. Judging whether the weakening of plant photosynthesis is limited by stomatal factors or non-stomatal factors can be judged by changes in leaf  $C_i$  (Sharma et al., 1995). The  $C_i$  of the three *Ormosia* species decreased with the increase of  $PFD$ , and the  $C_i$  of *O. henryi* was similar to that of *O. xylocarpa*. With the increase of  $PFD$ , the  $P_n$  of the three *Ormosia*



leaves increased continuously, and the demand of photosynthesis for intercellular  $\text{CO}_2$  increased continuously. The  $g_{sw}$  could not meet the demand of leaf photosynthesis for  $\text{CO}_2$ , resulting in the decrease of  $\text{CO}_2$  content in leaf cells. Because the  $P_n$  of *O. henryi* was stronger than that of *O. xylocarpa* and *O. microphylla*, the consumption of intercellular  $\text{CO}_2$  was greater, so that even if the  $g_{sw}$  of *O. henryi* was significantly higher than that of *O. xylocarpa* and *O. microphylla*,  $C_i$  was similar to *O. xylocarpa*. This phenomenon that  $C_i$  is directly controlled by  $g_{sw}$  is similar to that of *O. pinnata* (Zhao et al., 2000) and *Yulania denudate* (Zhu et al., 2024).

Photosynthetic parameters can reflect the adaptability of plants to different light environments, which can help us study the adaptability of plants to the environment (Yokoya et al., 2007). The higher the  $P_{max}$  of plants, the stronger the carbon sequestration capacity, which is conducive to the accumulation of more organic matter (Vona et al., 2018; Mahmud et al., 2018). The  $P_{max}$  of *O. xylocarpa*, *O. henryi* and *O. microphylla* were  $9.5089 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $15.1354 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and  $8.0292 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively.  $LCP$ ,  $LSP$  and  $AQY$  can reflect the weak light utilization ability of plants, which can be used to judge the shade tolerance of plants (Ding et al., 2020). Under normal conditions, the  $LCP$  of shade-tolerant tree species was less than  $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and the  $LSP$  was  $500\text{--}1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The  $LCP$  of the positive tree species was  $50\text{--}100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , and the  $LSP$  was greater than  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Jiang, 2004). There was no significant difference in  $LCP$  and  $LSP$  between *O. xylocarpa*, *O. henryi* and *O. microphylla* ( $P>0.05$ ).  $LCP$  was higher than  $20 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $LSP$  was higher than  $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , *O. xylocarpa* and *O. henryi* were slightly higher than  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The three *Ormosia* species belong to the light-favoring tree species, which is similar to the light-favoring characteristics of *O. saxatilis* (Li et al., 2015). When the light intensity reaches the  $LCP$  of the plant, the organic matter produced by photosynthesis is equal to the organic matter consumed by respiration. The lower the  $LCP$ , the stronger the ability of plants to use weak light;  $LSP$  reflects the adaptability of plants to strong light. The higher the  $LSP$ , the normal growth and development of plants under stronger light conditions (Ye et al., 2016). Among the three *Ormosia* species, the adaptation range of *O. xylocarpa* and *O. henryi* to light is similar, and *O. microphylla* is lower, so the adaptation range of *O. xylocarpa* and *O. henryi* to light is wider than that of *O. microphylla*. In this study,  $g_{sw}$  and  $AQY$  of *O. henryi* were significantly higher than *O. xylocarpa* and *O. microphylla*, so *O. henryi* had stronger adaptability and utilization ability to low light than *O. xylocarpa* and *O. microphylla*.  $R_d$  can reflect the consumption of organic matter under dark conditions (Li, 2017). There was no significant difference in  $R_d$  among the three *Ormosia* species, which proved that *O. xylocarpa*, *O. henryi* and *O. microphylla* had the same ability to consume organic matter under dark conditions.

$\text{CO}_2$  is the main raw material for plant photosynthesis, and its content changes will directly affect the photosynthetic characteristics of plants. Increasing  $\text{CO}_2$  concentration can increase the combination of  $\text{CO}_2$  and carboxylase active sites to increase  $P_n$  (Li et al., 2016; Ye et al., 2018). The  $A_{max}$  of *O. xylocarpa*, *O. henryi* and *O. microphylla* were  $27.4316 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ,  $30.9214 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and  $28.9181 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively. It can be seen that there was no significant difference among the three, and the  $\text{CO}_2$  utilization ability of *O. henryi* was slightly stronger than that of *O. xylocarpa* and *O. microphylla*. The  $\alpha$  value of *O. henryi* was  $0.0558 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , which was significantly higher than that of *O. xylocarpa* ( $0.0318 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and *O. microphylla* ( $0.0359 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), indicating that *O. henryi* had a relatively strong ability to utilize low-concentration  $\text{CO}_2$ . Plant  $CDCP$  can reflect the  $\text{CO}_2$  concentration in the balance of respiration consumption

and photosynthesis, and *CDSP* reflects the ability of plants to utilize high concentration of  $\text{CO}_2$ . Generally, the photosynthetic efficiency of plants is evaluated by these two indicators (Shen et al., 2021). Among the three species of *Ormosia*, *O. henryi* had the lowest *CDCP*, which was  $86.2781 \mu\text{mol}\cdot\text{mol}^{-1}$ , indicating that *O. henryi* had better  $\text{CO}_2$  utilization rate and stronger dry matter accumulation ability in low concentration  $\text{CO}_2$  environment. The *CDSP* of *O. xylcarpa* is  $2005.3467 \mu\text{mol}\cdot\text{mol}^{-1}$ , which has a high  $\text{CO}_2$  utilization range. On the whole, the  $P_{\text{max}}$  of *O. xylocarpa*, *O. henryi* and *O. microphylla* were much lower than  $A_{\text{max}}$ , indicating that  $\text{CO}_2$  supply was a factor affecting the  $P_n$  of the three *Ormosia* species. Therefore, increasing  $\text{CO}_2$  concentration in the short term can increase the photosynthetic rate of the above three *Ormosia* species, which is similar to that of *Ormosia sinensis* (Wei et al., 2023). However, whether photosynthetic down-regulation will occur in the long-term high-concentration  $\text{CO}_2$  environment remains to be verified.

### **Leaf structure and pigment content**

In the process of plant growth, leaves are the most sensitive organs of plants to environmental changes, and have strong plasticity, and their physiological structure is easily affected by the natural environment (Aasamaa et al., 2001). The differentiation degree of palisade tissue and spongy tissue in leaves can directly reflect the adaptability of plants to light environment. Under normal circumstances, the higher the palisade tissue and the ratio of palisade tissue to spongy tissue, the higher the photosynthetic efficiency (Li et al., 2019; Dong et al., 2022). Among the three *Ormosia* species, *O. henryi* has the highest PPT/SPT, which is consistent with its higher  $P_{\text{max}}$ .

However, it is worth noting that the PPT/SPT of *O. xylocarpa* is close to 1 (0.9628), indicating that the thickness of palisade tissue is similar to that of sponge tissue, which is much lower than that of *O. henryi* and *O. microphylla*; however, the  $P_n$  of *O. xylocarpa* was not the lowest among the three plants, but it was *O. microphylla* with no significant difference between PPT/SPT and *O. henryi*. Therefore, PPT/SPT was not the influencing factor of plant  $P_n$ . The PPT of the three *Ormosia* leaves was *O. henryi* > *O. xylocarpa* > *O. microphylla* ( $93.55\mu\text{m}$  >  $89.5667\mu\text{m}$  >  $75.55\mu\text{m}$ ), so the palisade tissue was one of the main factors affecting the photosynthetic characteristics of the three *Ormosia* species.

The stomata of the three *Ormosia* species only exist in the lower epidermis, which is similar to the results obtained by Luo et al. (2004) to observe the epidermal characteristics of *Ormosia*. The non-stomatal distribution of the upper epidermis can reduce the water loss and improve the drought resistance of the plants (Liu et al., 2022; Zhang et al., 2024). Among the three sainfoin plants, the SA of *O. xylocarpa* was only  $22.365\mu\text{m}^2$ , and the SD was  $307.4536 (\text{PCS}\cdot\text{mm}^{-2})$ , indicating that *O. xylocarpa* needed more stomata to maintain its photosynthetic capacity due to the small area of single stomata. Among the three species of *Ormosia*, the SA of *O. microphylla* was larger than that of *O. xylocarpa*, but its SD was much lower than that of *O. xylocarpa*, which may be the reason why the  $P_n$  of *O. microphylla* was finally slightly lower than that of *O. xylocarpa*. In this experiment, *O. henryi* SA was significantly higher than *O. xylocarpa* and *O. microphylla*, and SD remained at about the same level as *O. xylocarpa*, which also made it have strong photosynthetic capacity in three *Ormosia* species. It can be inferred that SA and SD are one of the factors affecting the photosynthetic capacity of *Ormosia*. Studies have shown that plant photosynthesis is closely related to leaf chlorophyll content. Plant Chl a/Chl b can affect the ability of chloroplasts to reduce 2,6-dichlorophenol indophenol, which in turn affects plant  $P_n$  (Nishimura et al., 1964; Qiu et al., 2019). The content of Chl a and

Chl b can determine the ability of plants to use weak light. Plants with high Chl a have photophilia, while plants with high Chl b content show strong shade tolerance (Zou et al., 2020). Car in plant leaves has an auxiliary effect on leaf photosynthesis, which can absorb light waves that cannot be absorbed by chlorophyll, and transfer these an energy to chlorophyll to promote photosynthesis, thereby improving photosynthetic efficiency (Simkin et al., 2022). In this experiment, the three *Ormosia* species Chl a were higher than Chl b, which was similar to the results of photosynthetic characteristics. The Chl a, Chl b and Chl (a+b) of *O. henryi* were significantly higher than those of *O. xylocarpa* and *O. microphylla*, which may be one of the reasons why *O. henryi* has strong ability to utilize low light. The Car content of *O. henryi* and *O. xylocarpa* was significantly higher than that of *O. microphylla*, which may be one of the reasons why the  $P_n$  of *O. microphylla* was lower than that of the other two *Ormosia* species.

## Conclusion

Studies have shown that *O. xylocarpa*, *O. henryi* and *O. microphylla* have light-loving habits, with high light saturation point and CO<sub>2</sub> saturation point. Therefore, during the cultivation process, the CO<sub>2</sub> concentration can be increased in the short term to increase the photosynthetic rate of the three *Ormosia* species, so that they accumulate more organic matter needed for growth. In the management of *O. xylocarpa*, *O. henryi* and *O. microphylla* wild communities, *Ormosia* plants should be appropriately 'windowed' to provide good light conditions for plants, which is conducive to plant growth and development. Among the three *Ormosia* species in this study, *O. henryi* has strong photosynthetic physiological and ecological indicators, and its adaptability to the environment is better than that of *O. xylcarpa* and *O. microphylla*, which may be one of the reasons for its wider distribution in the field. The thickness of leaf palisade tissue, chlorophyll content, stomatal size and density were all factors affecting the photosynthetic characteristics of red bean, but they did not directly affect the relationship with leaf thickness.

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