COMPARATIVE STUDY ON LEAF ANATOMICAL STRUCTURE AND PHOTOSYNTHETIC CHARACTERISTICS OF WILD AND RETROGRESSION POPULATIONS OF *PAPHIOPEDILUM EMERSONII*

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Abstract. This study systematically compared the differences in photosynthetic physiological characteristics and leaf anatomical structure between the population of the original habitat and the regression population of *Paphiopedilum emersonii*.The results showed that the in situ plants showed significant advantages in photosynthetic performance, including higher net photosynthetic rate, maximum photosynthetic rate (P_{max}), light saturation point (LSP) and apparent quantum yield (AQY), but their chlorophyll content was lower than that of the regression plants.The anatomical structure showed that the leaves of the regression plants were thicker, and the thickness of palisade tissue and sponge tissue and stomatal area were significantly larger than those of the regression plants, which was positively correlated with their higher chlorophyll content. It is worth noting that photosynthetic parameters (P_{max}, LSP, AQY) were significantly positively correlated with pigment parameters (Car/Chl a + b) and upper epidermis thickness (UET). Based on these findings, it is recommended to select a moist habitat with shading when returning to the introduction to alleviate photoinhibition and maintain water balance, thereby increasing the survival rate of the returning plants.
Keywords: *Paphiopedilum emersonii, photosynthetic physiological characteristics, adaptability*

Introduction

Paphiopedilum Pfitz.belongs to Orchidaceae, Subfam.Cypripedioideae. The lips of *Paphiopedilum* Pfitz.are pocket-shaped, and it is also known as slipper orchid or fairy orchid. It has high ornamental value because of its unique flower type, gorgeous color and long flowering period. According to the literature, *Paphiopedilum* is bitter, slightly sweet in taste, cold, and has effects such as nourishing yin, moistening the lungs, relieving cough and resolving phlegm. It can also treat lung-heat cough, yin-deficiency night sweat, diabetes and other diseases (Ran et al., 2012; Huang et al., 2012; Si, 2015). In recent years, wild *Paphiopedilum* has been excavated and illegally traded on a large scale, and most of the *Paphiopedilum* plants live in the fragile ecological environment of the Gaster region. Habitat destruction caused by humans seriously threatens the survival and reproduction of *Paphiopedilum* plant populations (Wang, 2000; Yang et al., 2021). At present, all wild

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Paphiopedilum species are listed in *Appendix I* of the Convention on International Trade in Endangered Species of Wild Fauna and Flora and are prohibited from trading (Wang et al., 2021). *Paphiopedilum emersonii* (*P. emersonii*) is an endemic species in China (Liu et al., 2008). Its flowers are large, pure and bright white. It is a rare aromatic species in the genus *Paphiopedilum* and has extremely high ornamental value. Its habitat is fragile and narrow and situated at altitudes of 600-700 m, thus, it is considered an extremely small population wild plant. It is listed as a critically endangered species in the newly issued ' National Key Protected Plant List ' and belongs to the national first-class key protected plant (Li et al., 2020; Qin et al., 2022; Tian, 2023).

Return is the reintroduction of endangered or wild-extinct rare species into their native or semi-natural habitats through artificial reproduction to establish new populations with genetic diversity and self-sustainability. It is an important strategy for species conservation (Maunder, 1992; Zhou and Gao, 2011; Ren et al., 2014). Studies have shown that the photosynthetic characteristics of endangered species may be one of the main internal factors affecting the distribution of species and the degree of endangerment. The ecological needs of plants and the most suitable environmental conditions can be judged by photosynthetic characteristics (Smith et al., 1993; Chai et al., 2015; Peng et al., 2024). Photosynthetic efficiency under low light conditions reflects the survival competitiveness of species in shaded environments (Xu et al., 2024). The intensity of light can affect the morphological structure and chlorophyll content of plants. As the main organ of photosynthesis, the microstructure and pigment content of leaves will affect the photosynthetic characteristics of leaves (Dong et al., 2022; Ni et al., 2022; Kim et al., 2022). In recent years, there have been many studies on the photosynthetic characteristics of endangered plants. Xu et al. (2024) found that the influencing factors of photosynthesis of three kinds of *Geodorum* plants may be leaf thickness, chlorophyll content, etc.; Qin et al. (2023) found that strong light could cause photoinhibition and hinder photosynthesis by comparing the photosynthetic characteristics and chlorophyll of *Paphiopedilum emersonii* under different shading intensity treatments. By comparing the photosynthetic characteristics of three kinds of *Paphiopedilum*, Sun et al. (2022) also found that strong light could inhibit photosynthesis and affect the growth and development of plants. At present, the research on *P. emersonii* mainly focuses on ecological characteristics (Qin et al., 2013; Tang et al., 2021), population structure (Qin et al., 2012), genetic diversity (Qin et al., 2022), tissue culture breeding (Wang et al., 2010), etc. Although preliminary studies have investigated the ecological environmental factors and chlorophyll fluorescence characteristics of its reintroduction habitat (Zhou et al., 2018), systematic reports on differences in photosynthetic physiological traits between native and reintroduced plants are still lacking. Therefore, this study compares the photosynthetic characteristics and leaf morphological structures of *P.emersonii* from both native and reintroduced habitats, analyzes the differences in their photosynthetic physiology, and aims to provide a theoretical basis and practical guidance for the reintroduction and population restoration of this species.

Materials and methods

Materials

The *Paphiopedilum emersonii* materials involved in the study included wild plants and regression plants. The wild plants are located in the middle and upper evergreen broad-leaved forest in the karst limestone mountain area of Jinchengjiang District, Hechi City,

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Guangxi Zhuang Autonomous Region, China (550-650 m above sea level, canopy density > 60 %),characterized by highly exposed rocks and soil predominantly consisting of black limestone soil. The reintroduced plants were derived from aseptic tissue-cultured seedlings of *Paphiopedilum emersonii*. After one year of acclimatization in a nursery, the seedlings were transplanted in June 2023 to a reintroduction site that matched the ecological conditions of the wild habitat for rock crevice planting. The reintroduction site, located in the Karst Medicinal Botanical Garden in Jinchengjiang District, Hechi City, Guangxi (24.694°N, 108.085°E), has an altitude of 500 m and is in a subtropical monsoon climate zone with an average annual temperature of 20.4 °C and an average annual precipitation of 1,479.6 mm. It features a dense canopy layer, low light transmittance, and a shaded, humid, humus-rich environment. To ensure the reintroduced plants had fully adapted to the field environment, all photosynthetic physiological measurements were conducted in the 12th month after transplantation (June 2024).

Methods

Determination of light response curve

The light response curve was measured using a Li-6400 (LI-COR, USA) portable photosynthesis system with an LED red-blue light source leaf chamber. The measurements were conducted between 9:00 AM and 12:00 PM on clear days in late June 2024. Healthy, intact leaves free from pests and diseases were selected for the measurements, each group had at least 3 replicates. Photosynthetic active radiation (PAR) was set as follows :1 400 μmol·m^{-2}·s^{-1}, 1 200 μmol·m^{-2}·s^{-1}, 1 000 μmol·m^{-2}·s^{-1}, 800 μmol·m^{-2}·s^{-1}, 600 μmol·m^{-2}·s^{-1}, 400 μmol·m^{-2}·s^{-1}, 200 μmol·m^{-2}·s^{-1}, 150 μmol·m^{-2}·s^{-1}, 100 μmol·m^{-2}·s^{-1}, 80 μmol·m^{-2}·s^{-1}, 60 μmol·m^{-2}·s^{-1}, 50 μmol·m^{-2}·s^{-1}, 40 μmol·m^{-2}·s^{-1}, 30 μmol·m^{-2}·s^{-1}, 20 μmol·m^{-2}·s^{-1}, 0 μmol·m^{-2}·s^{-1}. Other environmental measurement conditions were controlled as relative humidity of 65 %, leaf chamber temperature of 25 °C, and leaf chamber CO_2 concentration of 400 μmol·mol^{-1}. Before the measurement, the leaves were induced under 600 μmol·m^{-2}·s^{-1} light intensity, and the maximum and minimum waiting time were set to 120 s and 200 s, respectively, and then the measurement began. The calculation formula is as follows:

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

The light saturation point is denoted as *LSP*:

$$LSP = \frac{\sqrt{(\beta+\gamma)/\beta}-1}{\gamma}$$

The maximum net photosynthetic rate is denoted as P_{max}:

$$P_{max} = \alpha \left(\frac{\sqrt{\beta+\gamma}-\sqrt{\beta}}{\gamma} \right) - R_d$$

In the equation, P_n represents the net photosynthetic rate; I denotes the light intensity (PAR);β and γ are the coefficient, I is photosynthetically active radiation,α indicates the

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slope of the light-response curve at I = 0, known as the apparent quantum efficiency; P_{max} refers to the maximum net photosynthetic rate; and R_d corresponds to the dark respiration rate.

Leaf anatomical structure

Reference Li (1987) method of making paraffin sections of plant leaves. After the end of the photosynthetic determination, three leaves with the same orientation and good growth status were taken from each plant, and cut transversely along the middle vein. The cut pieces were 10 mm × 10 mm and fixed with FAA fixative (70 % ethanol : formalin : glacial acetic acid = 90 : 5 : 5). The leaves were placed in a dehydration box using ethanol and xylene series to dehydrate and soak wax. After waxing, the plant tissue was 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 graphical analysis software CaseViewer. The following parameters were measured: upper epidermal thickness (UET), lower epidermal thickness (LET), leaf thickness (LT), palisade parenchyma thickness (PPT), spongy parenchyma thickness (SPT). Each sample was randomly observed 10 visual field statistical values.

Foliar surface features

The leaves of each plant were selected 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 put into 2.5 % glutaraldehyde fixative. Immediately after being brought to the laboratory, ethanol was used for step-by-step dehydration. The dehydration gradients were 30 %, 50 %, 70 %, 80 %, 90 %, 100 %, 100 % ethanol, and each stage was dehydrated for 15 min. After dehydration, the leaves were dried at the critical point and gilded. 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 for 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) = π × stomatal long axis (SL) × stomatal short axis (SW) / 4, π = 3.14.

Determination of photosynthetic pigment content in leaves

Three leaves with the same maturity, leaf position and leaf size were collected from the plants for photosynthetic determination. Each sample was accurately weighed and 0.2 g of intact leaves were placed in a 25 mL volumetric flask and diluted with 95 % ethanol. The volume was constant with 95 % ethanol and soaked in darkness for 24 h. The absorbance was measured at 470 nm, 649 nm and 665 nm using an ultraviolet-visible spectrophotometer (Alpha 1502 Shanghai Spectrum Instrument Co., Ltd.). 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 according to the method of Li (2000).

$$\text{Chla concentration}\left(C_a,\ \text{mg}\cdot L^{-1}\right):\ C_a = 13.95_{A665} - 6.88_{A649}$$

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$$\text{Chlb concentration}\left(C_b,\ \text{mg}\cdot L^{-1}\right):\ C_b = 24.96_{A649} - 7.32_{A655}$$

$$\text{Carotenoid concentration}\left(C_{x\cdot c},\ \text{mg}\cdot L^{-1}\right):\ C_{x\cdot c} = \frac{1000_{A470} - 2.05C_a - 114.8C_b}{245}$$

Data processing

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

Results and analysis

Comparison of light response curve parameters

The photosynthetic rate (P_n) of *P. emersonii* plants in the original habitat was significantly higher than that of the returning plants (*Fig. 1A*). When the PFD was 0-200 μmol·m^{-2}·s^{-1}, the P_n of *P. emersonii* in the original habitat and the returning land increased with the increase of PFD, and the P_n increased significantly. When the light intensity reached 400 μmol·m^{-2}·s^{-1}, the P_n of the regression plant leaves decreased with the increase of light intensity. When the light intensity reached 600 μmol·m^{-2}·s^{-1}, the P_n of the original habitat plant leaves also decreased with the increase of light intensity, indicating that the photosynthesis of *P. emersonii* in the original habitat and the regression two places under strong light showed obvious photoinhibition. As shown in *Fig. 1B and C*, the stomatal conductance (*gsw*) and transpiration rate (*Emm*) of in situ plants were higher than those of regression plants, and *gsw* and *Emm* showed an upward trend as a whole. From *Figure 1D*, it can be seen that with the increase of light intensity, C_i shows a trend of ' down-up-down-up ' as a whole. The C_i change trend of *P. emersonii* in the two places is roughly similar and there is no significant difference.

It can be seen from *Table 1* that the maximum photosynthetic rate (P_{max}), light saturation point (LSP) and apparent quantum efficiency (AQY) of the plants in the original habitat were higher than those of the regression plants, and the maximum photosynthetic rate (P_{max}), light saturation point (LSP) and apparent quantum efficiency (AQY) of the original habitat and the regression plants showed significant differences ($P < 0.05$). The maximum photosynthetic rate (P_{max}) was 3.1931 μmol·m^{-2}·s^{-1}, 2.1371 μmol·m^{-2}·s^{-1}, respectively, while there was no significant difference in light compensation point (LCP) and dark respiration rate (R_d) ($P > 0.05$).

Comparison of chlorophyll content

The determination results of chlorophyll content in the original habitat and regression plants of *P. emersonii* are shown in *Table 2*. Except for Car / Chl (a + b) content, the contents of Chl a, Chl b, Car, Chl (a + b) and Chl a / Chl b in the regression plants were higher than those in the original habitat plants. There were significant differences in Chl a, Chl b, Car, Chl (a + b), Chl a / Chl b, Car / Chl (a + b) and Car / Chl (a + b) between the original habitat and the regression plants ($P < 0.05$).

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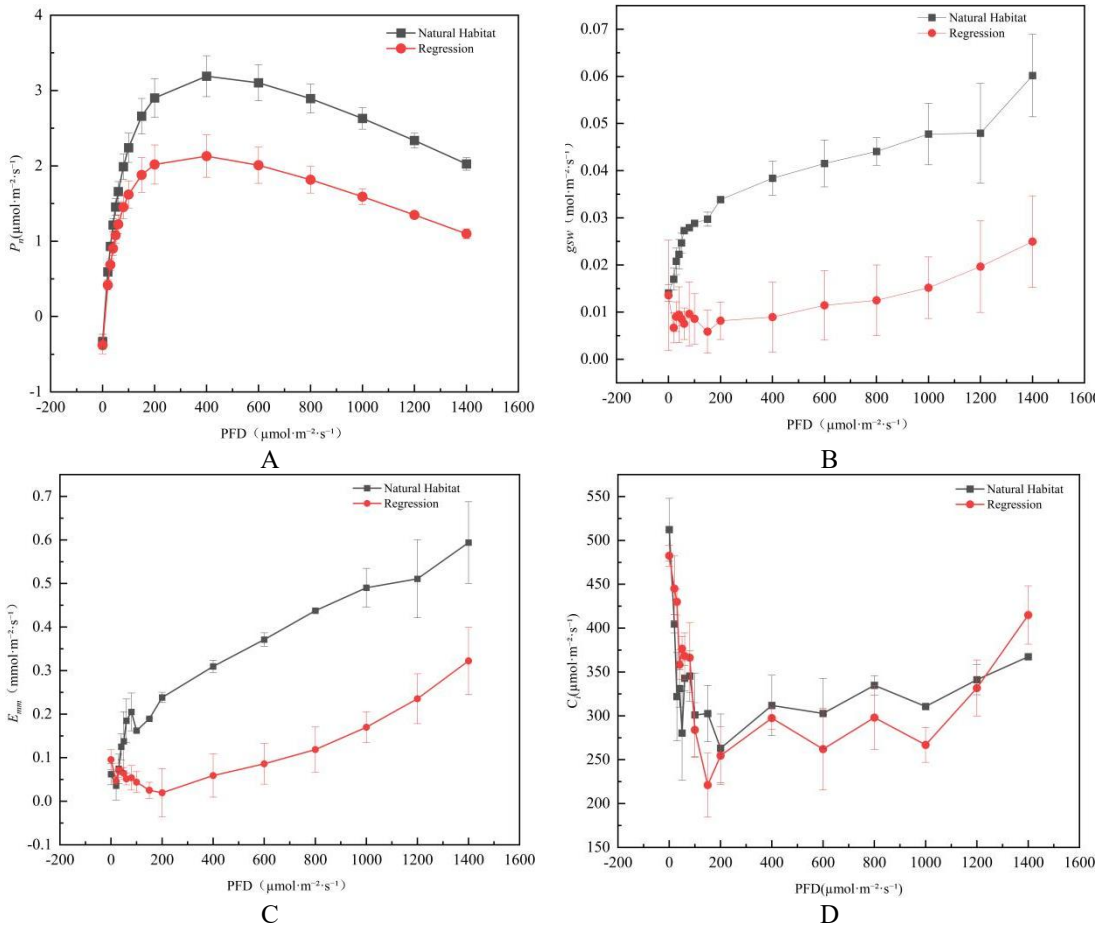

Figure 1. *Light response curve of original habitat and regression plant of P. emersonii (Note: PFD stands for photosynthetic photon flux density; P_n represents net photosynthetic rate; C_i denotes intercellular CO_2 concentration; E_{mm} refers to transpiration rate; gsw indicates stomatal conductance to water vapor.)*

Table 1. *Comparison of light response parameters between original habitat and regression plants of P. emersonii*

Plant	AQY/ (mol·mol^{-1})	P_{max}/ (μmol·m^{-2}·s^{-1})	LSP/ (μmol·m^{-2}·s^{-1})	LCP/ (μmol·m^{-2}·s^{-1})	R_d/ (μmol·m^{-2}·s^{-1})
Natural habitat	0.0159±0.0021a	3.1931±0.2688a	418.3641±27.8662a	6.1356±1.2820a	0.3320±0.1020a
Regression	0.0096±0.0011b	2.1371±0.2845b	352.8553±10.5240b	8.0493±2.0044a	0.3782±0.1159a

Note: Different letters in the same industry indicate significant differences ($P<0.05$); P_{max} denotes the maximum photosynthetic rate; LSP represents the light saturation point; LCP indicates the light compensation point; R_d refers to the dark respiration rate; AQY stands for the apparent quantum yield

Comparison of leaf structure

It can be seen from the cross-sectional structure of the leaves of the original habitat and the returning plants (*Fig. 2*) that the leaves are composed of upper epidermis, palisade tissue, spongy tissue and lower epidermis. The upper epidermal cells were significantly larger than the lower epidermal cells. In the cross-sectional structure of the leaves of the

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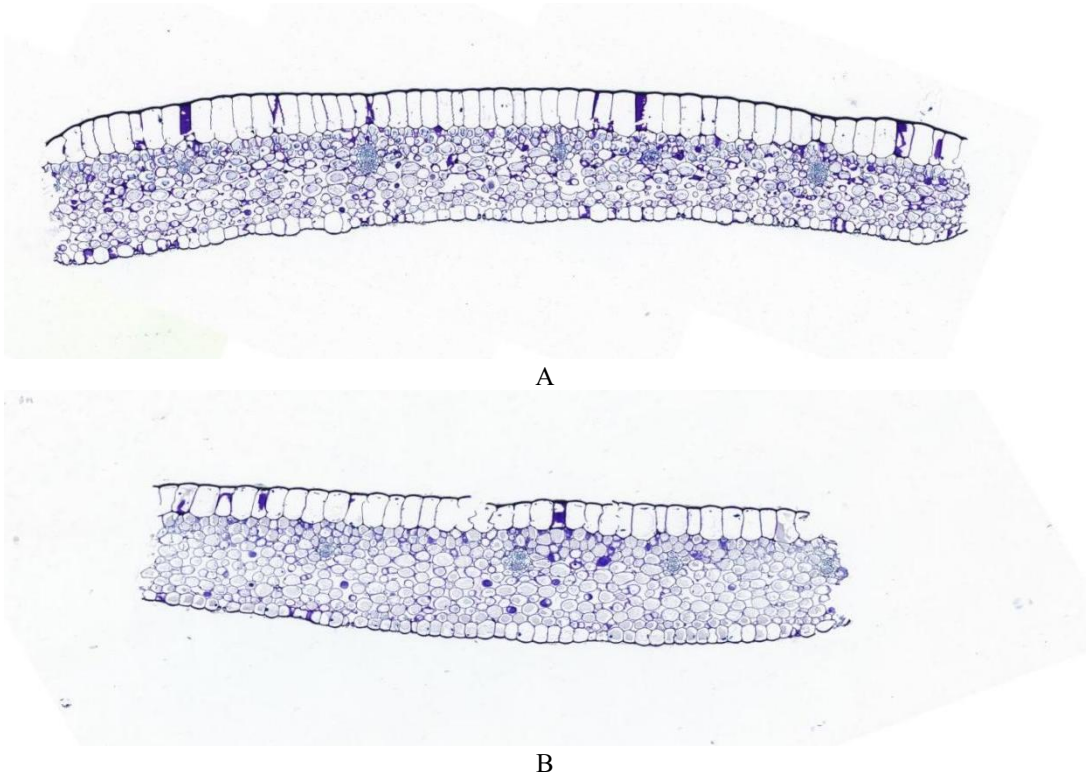
two plants, the palisade tissue was relatively closely arranged, and the sponge tissue cells were mostly irregular and loosely arranged, but the thickness was significantly larger than the palisade tissue.

Table 2. *Chlorophyll content in leaves of P. emersonii in situ and returning plants*

Plant	Chl a ($mg \cdot g^{-1}$)	Chl b ($mg \cdot g^{-1}$)	Car ($mg \cdot g^{-1}$)	Chl (a+b) ($mg \cdot g^{-1}$)	Chl a/ Chl b	Car/ Chl (a + b)
Natural habitat	3.5835± 0.0024b	2.2557± 0.0024b	0.7066± 0.0008b	5.8393± 0.0007b	1.5886± 0.0027b	0.1210± 0.0001a
Regression	4.9498± 0.0076a	3.0318± 0.0087a	0.8861± 0.0065a	7.9817± 0.0024a	1.6326± 0.0072a	0.1110± 0.0008b

Note: Different letters in the same industry indicate significant differences (P<0.05); Chl a stands for chlorophyll a content; Chl b denotes chlorophyll b content; Chl (a+b) represents total chlorophyll content; Car refers to carotenoid content; Chl a/Chl b indicates the ratio of chlorophyll a to chlorophyll b; Car/Chl (a+b) represents the ratio of carotenoids to total chlorophyll



A

B

Figure 2. *Leaf cross section of P. emersonii in situ and regression plants (A is the cross section of the leaves in the original habitat; B is the cross section of regression plant leaves.)*

It can be seen from *Table 3* that there were significant differences in the thickness of the upper epidermis, the thickness of the lower epidermis, the thickness of the palisade tissue, the thickness of the sea cotton tissue, and the thickness of the leaves between the original habitat and the returning plants ($P < 0.05$),There was no significant difference in the thickness ratio of palisade tissue to sponge tissue (PPT / SPT) ($P > 0.05$).The lower epidermis thickness, leaf thickness, palisade tissue and sponge tissue thickness of the regression plants were higher than those of the original plants.

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Table 3. *Comparison of anatomical structure parameters of leaf cross section between original habitat and regression plants of P. emersonii*

Plant	Leaf thickness (LT,μm)	Upper epidermal thickness (UET,μm)	Lower epidermal thickness (LET,μm)	Palisade parenchymathickness (PPT,μm)	Spongy parenchyma thickness (SPT,μm)	PPT/SPT
Natural habitat	866.04± 38.28b	258.70± 15.99a	83.83± 9.49b	121.93± 9.44b	400.17± 31.91b	0.31± 0.04a
Regression	985.18± 19.97a	221.68± 14.86b	107.13± 9.01a	160.89± 19.01a	485.78± 36.46a	0.33± 0.04a

Note: Different letters in the same industry indicate significant differences (P<0.05); LT represents leaf thickness; UET denotes upper epidermis thickness; LET indicates lower epidermis thickness; PPT refers to palisade tissue thickness; SPT stands for spongy tissue thickness; PPT/SPT represents the ratio of palisade tissue thickness to spongy tissue thickness

Comparison of leaf stomatal parameters

By observing the epidermis of the leaves of the two plants (*Fig. 3*), it was found that there was no stomatal distribution in the upper epidermis, and the stomata were distributed in the lower epidermis. The upper epidermal cells were significantly larger than the lower epidermal cells, and the morphology of the upper and lower epidermal cells was different. The upper epidermal cells were regular honeycomb polygons and arranged relatively neatly, while the lower epidermal cells were irregular polygons and arranged relatively irregularly.

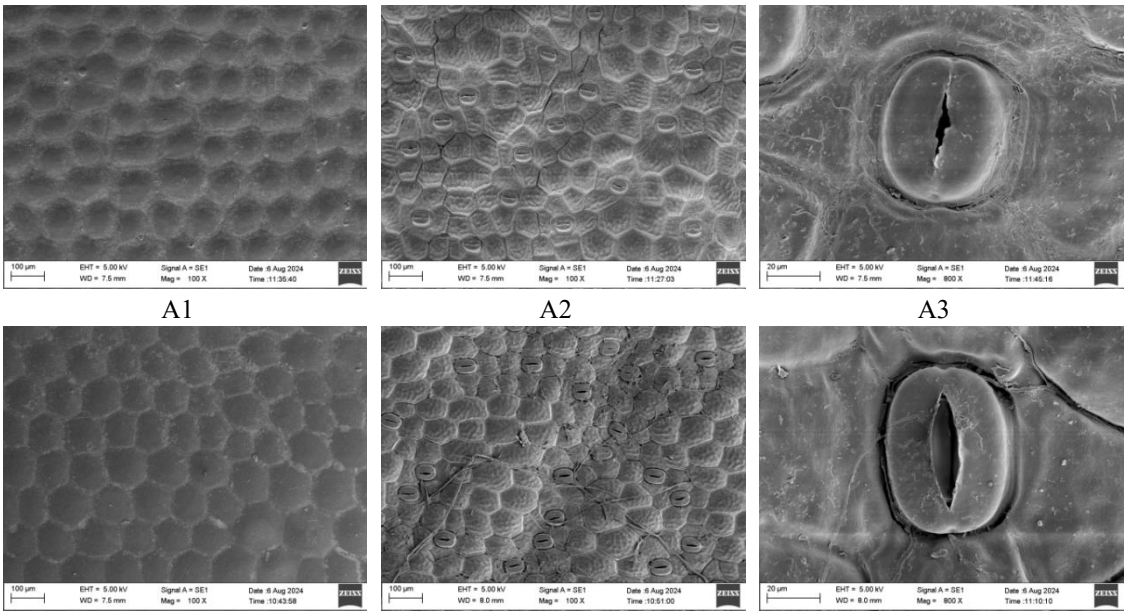

Figure 3. *Leaf epidermal characteristics of original habitat and regression plants. (A1, A2 and A3 were the upper epidermis, lower epidermis and stomata of the plants in the original habitat, respectively. B1, B2 and B3 are the upper epidermis, lower epidermis and stomata of the regression plants, respectively.)*

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It can be seen from *Table 4* that there were significant differences in the long axis (SL), short axis (SW) and single stomatal area (SA) between the original habitat and the regressed plants ($P < 0.05$), but there was no significant difference in stomatal density. The long axis (SL), short axis (SW) and single stomatal area (SA) of the regressed plants were higher than those of the original habitat.

Table 4. *Stomatal index of original habitat and regression plants of P. emersonii*

Plant	SL (μm)	SW (μm)	SD (mm^{-2})	SA ($μm^2$)
Natural habitat	26.8666±1.6765b	4.3966±0.2871b	22.6934±2.8087a	92.7913±9.5899b
Regression	40.7233±5.5025a	6.9100±1.4835a	21.2053±1.9330a	217.1930±27.7990a

Note: Different letters in the same industry indicate significant differences (P<0.05);SL denotes stomatal length; SW represents stomatal width; SD stands for stomatal density; SA refers to the individual stomatal apparatus area

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

It can be seen from *Fig. 4* that there is a certain correlation between the leaf structure parameters and the photosynthetic physiological and ecological parameters of the original habitat plants and the regression plants. There was a significant correlation between photosynthetic parameters P_{max}, LSP, LCP, AQY and leaf pigment content parameters Chl a, Chl b, Car, Chl (a + b), Chl a / Chl b, Car / Chl (a + b), and leaf structure parameters UET, LET, LT, PPT, SPT. In addition, P_{max} was significantly correlated with SL, SW, SA ; AQY was significantly correlated with SW and SA. Leaf pigment content Chl a, Chl b, Chl (a + b), Chl a / Chl b, Car / Chl (a + b) were significantly correlated with leaf structural parameters UET, LET, LT, PPT, SPT, SL, SW and SA.

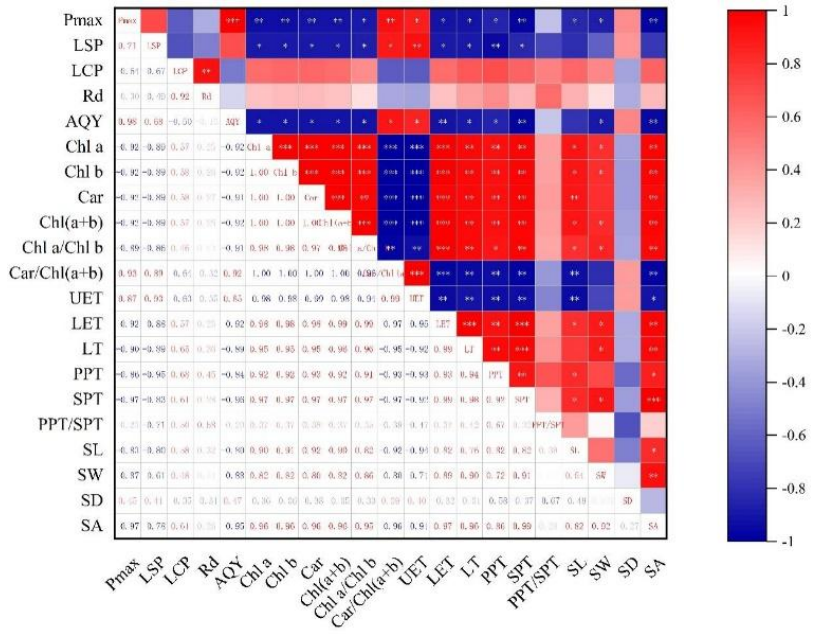

Figure 4. *Correlation analysis heat map of leaf structure and photosynthetic physiology of original habitat plants and regression plants. (Note: * means P < 0.05; ** means P < 0.01; *** means P < 0.001.)*

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Discussion

Photosynthesis is one of the important factors that determine the accumulation of plant organic matter and energy absorption (Xiong et al., 2022). Net photosynthetic rate (P_n) reflects the photosynthetic potential of plants (Zhou et al., 2019); in the photosynthetic-light response curve, under different light intensities, the P_n of the original habitat plants was significantly higher than that of the regression plants, indicating that the original habitat plants had stronger material accumulation ability and stronger adaptability to light. With the increase of light intensity, the P_n of the original habitat and the regression plants showed a downward trend, indicating that the plants were more sensitive to light intensity when the light intensity increased, and photoinhibition occurred. The stomatal conductance (*gsw*) of the original habitat plants and the regression plants showed an overall upward trend, and the change trend of transpiration rate (E_{mm}) was similar to that of *gsw*, and the *gsw* and E_{mm} of the original habitat plants were significantly higher than those of the regression plants. It indicates that the plants in the original habitat may have stronger gas exchange capacity and thus have higher photosynthetic rate (Chai et al., 2015).

The maximum net photosynthetic rate ($P_{\max}$), dark respiration rate (R_d), light saturation point (LSP), light compensation point (LCP) and apparent quantum efficiency (AQY) of the light response parameters of the original and returning plants of *P. emersonii* were significantly different. The light saturation point and light compensation point of plant leaves reflect the requirements of plant light conditions and are an important index to judge the shade tolerance of plants (Yue and Shi, 2010). It is generally believed that plants with low LCP and LSP are typical shade plants, while plants with low LCP and high LSP have strong adaptability to light environment (Leng et al., 2000). Compared with the regression plants, the in situ plants had higher light saturation point and lower light compensation point, indicating that the in situ plants had stronger tolerance to high light intensity and low light intensity than the regression plants, and had stronger adaptability to the light environment. AQY can reflect the ability of plants to use weak light. The larger the AQY value, the better the ability to use weak light. The AQY value of the original habitat plants is slightly higher than that of the regression plants, indicating that the original habitat plants have better utilization of weak light. The dark respiration rate (R_d) reflects the ability of plants to consume organic matter. There is no significant difference in Rd between the original habitat plants and the returning plants, indicating that the two have little difference in the ability to consume organic matter under dark conditions.

Chlorophyll is the main pigment involved in photosynthesis in plant cells. For different plant species, the level of chlorophyll content cannot be used as an indicator to measure the level of photosynthetic rate (El-Sharkawy and Hesketh, 1965). The contents of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and Chl a / b in the leaves of the returning plants were higher than those of the wild species, only Car / Chl (a + b) was slightly lower than that of the original habitat plants, but the photosynthetic capacity was significantly lower than that of the original habitat plants. The difference of visible light combining ability was not completely caused by the difference of chlorophyll content in leaves. It can also be speculated that this phenomenon is related to the adaptation of the two to the ecological environment of the origin. The high content of photosynthetic pigments in the regression plants is suitable for the regression sites with sufficient water and fertilizer and low radiation, while the low chlorophyll content and high carotenoid content in the original habitat plants can enhance the leaf transmittance and prevent

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sunburn caused by strong light. The field regression site is in the shade of the forest, and the light intensity is low. It is speculated that the regression plants may adapt to the weak light environment by increasing the chlorophyll content of the leaves.

The morphological and anatomical structure of plant leaves can reflect the physiological adaptability of plants to a certain extent (Hu et al., 2025). The leaf tissue structure of *P. emersonii* has obvious xerophytic characteristics, such as thick leaves, huge upper epidermal cells, thick stratum corneum, substomatal type, etc., which is consistent with the main performance results of xerophytic leaves found by Yang (2007). The thickening of leaf thickness can increase the water retention capacity of plants. The stratum corneum is developed, which can reduce water loss and reflect the irradiation of strong light. This study showed that the lower epidermis thickness, leaf thickness, palisade tissue and sponge tissue thickness of the leaves of the regression plants were higher than those of the original habitat plants, and the upper epidermis thickness of the original habitat plants was significantly higher than that of the regression plants. According to Zhang et al. (2017), the most sensitive indicators of leaf drought resistance were upper epidermis thickness / leaf thickness, upper epidermis thickness and leaf thickness, indicating that the drought resistance of the original habitat plants was higher than that of the regression plants. Stomatal distribution, size, density, morphology and behavior are closely related to plant transpiration (Brett and Waldron, 1996). Larger stomatal closure is slower, and hydraulic dysfunction is more likely to occur under drought conditions (Aasamaa et al., 2001; Guan et al., 2011). The stomatal long axis (SL), stomatal short axis (SW) and single stomatal area (SA) of the regression plants were higher than those of the original plants, and there was no significant difference in stomatal density, which further indicated that the original plants were more tolerant to drought environment than the regression plants.In the correlation analysis, there was a strong correlation between leaf pigment content and leaf structure, and there was a positive correlation with leaf structure parameters LET, LT, PPT and SPT at different levels. It can be seen that the difference in leaf tissue thickness may be the main cause of chlorophyll content. The net photosynthetic rate was positively correlated with Car / Chl (a + b) and UET at different levels. There were significant effects between photosynthetic parameters P_{max}, LSP, LCP, AQY and leaf pigment content parameters Chl a, Chl b, Car, Chl (a + b), Chl a / Chl b, and leaf structure parameters LET, LT, PPT, SPT. At the same time, the pigment content also has a certain correlation with the length, width and area of stomata. Thus, from the perspective of ecological adaptation, the morphology of reintroduced plants (increased LT, larger SA, and higher pigment content) demonstrates an acclimation response to stable environments with sufficient water and nutrient availability. In contrast, the morphological traits of native habitat plants (thicker UET, smaller SA, and higher Car/Chl ratio) reflect long-term evolutionary adaptations to the fluctuating conditions of their original habitat, which may include drought stress and patchy high-light exposure.

Conclusion

There were significant differences in photosynthetic physiological characteristics and leaf structure between the original habitat and the returning plants of *P. emersonii*. Through the comparison of photosynthetic parameters, it was found that the plants in the original habitat had higher net photosynthetic rate, light saturation point and lower light compensation point, and the adaptation range of light intensity was wider than that of the

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regression plants, and the absorption was better under low light. Both of them will produce photoinhibition when the light intensity is high. Therefore, it is suggested that the regression should be set as far as possible in the environment with weak light, such as the shade under the forest. The chlorophyll content of the original habitat plants was low, but the photosynthetic capacity was significantly higher than that of the regression plants. It can be seen that the chlorophyll content cannot be completely used as an indicator to measure the photosynthetic capacity. In terms of leaf structure, the in situ plants had thicker cuticle, upper epidermal cells, tighter palisade tissue and smaller stomatal area, which made them more adaptable to drought than the recurrent plants. The conditions around the original habitat of *P. emersonii* were complex and harsh. Based on the results of this experimental study, it was suggested that in the early stage of natural regression, the regression point should be selected as far as possible in the wet and shaded field habitat, so as to provide sufficient living environment for the initial growth of regression and increase its survival rate. In addition, for the later management monitoring, the survival rate and growth potential should also be investigated regularly.

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