Comparative Study on Photosynthetic Characteristics and Leaf Microstructure of Seedlings and Saplings of *Craigia yunnanensis*, a Species with Extremely Small Populations (Postprint)

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Abstract

Craigia yunnanensis is an endemic species with extremely small populations in China, listed as a national second-class key protected wild plant. Addressing the sapling gap phenomenon in the natural population regeneration of C. yunnanensis, this study used introduced and cultivated individuals as experimental material to investigate differences in photosynthetic characteristics, photosynthetic pigment content, leaf epidermal traits, leaf anatomical structure, and leaf functional traits between seedlings (6-month-old) and saplings (8-year-old). The results showed: (1) The sapling’s maximum net photosynthetic rate Pmax (12.00 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$) and light saturation point LSP (1 360.40 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$) were highly significantly ($P<0.01$) higher than those of seedlings (5.69 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$ and 839.6 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$, respectively), while the seedling’ s light compensation point LCP (11.37 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$) was lower, indicating that seedlings are shade-tolerant while saplings are light-demanding. (2) Saplings had significantly ($P<0.05$) higher chlorophyll (Chl) and carotenoid (Car) contents than seedlings, and their leaves were thicker with more developed palisade tissue, larger midrib vessel diameters, and also had greater leaf area, leaf mass per area, and leaf dry matter content. (3) Correlation analysis revealed that Pmax was significantly ($P<0.05$) or highly significantly ($P<0.01$) positively correlated with leaf thickness, chlorophyll content, leaf mass per area, and leaf dry matter content. In summary, seedlings adapt to low-light understory environments, while saplings require stronger light to support their high photosynthetic capacity. Insufficient understory light in natural populations hinders the transition from seedlings to saplings in C. yunnanensis, which may be an important reason for its endangered status. This study provides a scientific basis for the conservation, introduction, and domestication of this species. It is recommended that conservation efforts employ manual thinning to improve light conditions and promote population regeneration.

Full Text

Comparative Study on Photosynthetic Characteristics and Leaf Microstructure of Seedlings and Young Trees of *Craigia yunnanensis*, a Species with Extremely Small Populations

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Abstract: *Craigia yunnanensis*, a plant species with extremely small populations (PSESP) endemic to China, is listed as a National Class II protected wild plant. To clarify the causes of regeneration failure—particularly the lack of sapling recruitment in natural populations—this study compared the photosynthetic characteristics, photosynthetic pigment contents, leaf epidermal traits, leaf anatomical structures, and leaf functional traits between seedlings (6-month-old) and young trees (8-year-old) cultivated in an experimental plantation. The results were as follows: (1) The maximum net photosynthetic rate (Pmax) (12.00 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and light saturation point (LSP) (1,360.40 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) of young trees were extremely significantly higher ($P<0.01$) than those of seedlings (5.69 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and 839.60 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively), whereas the light compensation point (LCP) of seedlings was lower (11.37 $\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), indicating a shade-tolerant strategy in seedlings and a light-demanding strategy in young trees. (2) Chlorophyll (Chl) and carotenoid (Car) contents were significantly higher in young trees ($P<0.05$), and their leaves were thicker, with more developed palisade tissues, larger midrib vessel diameters, and higher leaf area, specific leaf weight (SLW), and leaf dry matter content (LDMC). (3) Correlation analysis revealed significant or highly significant positive correlations ($P<0.05$ or $P<0.01$) between Pmax and leaf thickness, chlorophyll content, SLW, and LDMC. In conclusion, seedlings adapt to low-light understory environments, whereas young trees require higher light availability to sustain their elevated photosynthetic capacity. Insufficient understory light in natural habitats likely hinders the transition from seedlings to young trees, contributing to the species' endangered status. These findings provide essential scientific support for conservation and cultivation practices. It is recommended that thinning or canopy-opening measures be implemented to improve understory light conditions and promote population regeneration.

Keywords: *Craigia yunnanensis*, extremely small population, photosynthetic characteristics, leaf anatomy, chlorophyll

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Species with extremely small populations represent the highest priority for biodiversity conservation, as their survival status directly affects ecosystem integrity and stability. Research on the conservation of such species holds major strategic significance for preventing extinction and protecting China' s unique biological genetic resources (Zang et al., 2016; Xu et al., 2022). *Craigia yunnanensis*, a wild plant species with extremely small populations endemic to China, is listed as a National Class II protected wild plant and assessed as Endangered by the International Union for Conservation of Nature (IUCN, 2008; National Forestry and Grassland Administration & Ministry of Agriculture and Rural Affairs, 2021). The species is primarily distributed in the south subtropical evergreen broad-leaved forests of Yunnan, Guangxi, and Guizhou provinces, commonly found on lower mountain slopes, foothills, and valley areas at elevations of 1,000–1,500 m (Tang et al., 2007). *C. yunnanensis* features tall, straight trunks with attractive wood coloration. Its timber is hard, heavy, wear-resistant, and exhibits good processing properties, making it a valuable timber species with important economic value. Additionally, the species demonstrates special adaptability to limestone karst habitats, serving as an excellent tree species for ecological restoration in rocky desertification areas (Gong et al., 2006). As the type species of the genus *Craigia*, it occupies a key position in plant systematic evolution research and holds important scientific value (Ying & Zhang, 1994). However, due to its narrow distribution range, severe habitat fragmentation, and human disturbance, wild populations of *C. yunnanensis* continue to decline, showing an obvious recession trend. Field surveys indicate that extant populations are mostly sporadically and discontinuously distributed, and commonly exhibit a regeneration gap phenomenon characterized by "presence of large trees, observation of seedlings, but absence of young trees" (Ye et al., 2022), suggesting severe obstacles in its natural regeneration process. This regeneration barrier not only directly threatens the sustainable survival of populations but also reflects the possible existence of certain key limiting factors in its life history. Therefore, in-depth research on the physiological and ecological characteristics of *C. yunnanensis* seedlings and young trees to reveal the key influencing factors of its regeneration obstacles is of great significance for developing effective conservation strategies.

Photosynthesis is the foundation of plant growth and development and constitutes an important physiological process in plant growth. Plant photosynthetic characteristics can be used to determine their most suitable growth environments and ecological conditions (Adamec, 1997), and light conditions are also important environmental factors determining their status in plant communities (Zhang et al., 2016). Photosynthesis changes throughout the plant life cycle with tree age (Cheng et al., 2018; Xiong et al., 2020; Liu et al., 2021). Studying differences in photosynthetic characteristics of endangered plants at different tree ages is of great significance for evaluating and selecting suitable habitats. Research has shown that many endangered tree species [such as *Paranephelium hainanense* and *Vatica guangxiensis*] exhibit significant differences in photosynthetic capacity, light adaptation characteristics, and leaf structure between seedlings and adult trees (Hong et al., 2020; Pan et al., 2023). Such differences often lead to different light environment requirements at different developmental stages, thereby affecting regeneration success in natural communities. Particularly in different light environments between canopy and understory layers, seedlings and adult trees may evolve different light adaptation strategies. Therefore, comparative studies on photosynthetic physiological characteristics of endangered plants at different developmental stages not only help elucidate their endangerment mechanisms but also provide an important basis for implementing appropriate conservation measures.

Currently, research on *C. yunnanensis* has mainly focused on potential suitable area prediction (Xu et al., 2024), community characteristics (Ye et al., 2022), seed characteristics and germination (Chen et al., 2021), breeding system and pollination biology (Gao et al., 2012), conservation genetics (Gao et al., 2010), and chloroplast whole genome (Wariss et al., 2019). However, research in the field of photosynthetic physiological ecology remains blank. Addressing the prominent problems in *C. yunnanensis* population regeneration, this study used *C. yunnanensis* introduced to the Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences as the research subject to explore differences in leaf photosynthetic characteristics and leaf microstructure between seedlings and young trees of this species and their interrelationships. The study aimed to address the following questions: (1) What are the differences in leaf photosynthetic capacity between *C. yunnanensis* seedlings and young trees? (2) If differences in photosynthetic capacity exist between seedlings and young trees, are they associated with leaf structure, chlorophyll content, and leaf phenotypic traits? The research results can provide a scientific basis for the conservation, introduction, and domestication of this endangered species.

1.1 Study Site Overview

The seed source of *C. yunnanensis* is located in Wenshan City, Yunnan Province, which belongs to the south subtropical monsoon climate zone at an elevation of approximately 1,000–1,500 m, with an average annual temperature of 16–18 °C and average annual precipitation of 1,150 mm. The soil is primarily calcareous soil developed from limestone. The planting site (Guangxi Institute of Botany) is located in the middle subtropical monsoon climate zone at an elevation of 178 m, with an average annual temperature of 19.12 °C. The average temperature of the hottest month (July) is 28.2 °C, and the coldest month (January) is 8.1 °C. The average annual precipitation is 1,854.8 mm, concentrated mainly from April to August, with an annual relative humidity of 78%, distinct dry and wet seasons, and approximately 1,550 annual sunshine hours. The soil is acidic soil developed from sandstone shale and Quaternary red soil, with a pH of 4.7-6.0 and clay texture. Although there are some differences in natural environmental conditions between the two locations, *C. yunnanensis* grows well at the planting site.

1.2 Materials

The *C. yunnanensis* seedlings used in this study were 6-month-old seedlings cultivated from seeds collected in 2023 from wild populations in Wenshan City, Yunnan Province, and sown in Guilin. The young trees were 8-year-old seedlings introduced from Kunming, Yunnan to the Guangxi Institute of Botany in 2022 for cultivation and maintenance, also originating from Wenshan City, Yunnan Province. Basic information for both types of nursery stock is shown in Table 1 . Seedlings were cultivated in an understory environment with 15% canopy transmittance, similar to the understory light environment where seedlings occur in most natural populations. Young trees were planted in forest edge areas with 50% canopy transmittance, similar to the light environment of a few natural populations where young trees occur. Regular watering, weeding, and other artificial tending management were implemented during cultivation. Five healthy seedlings and five healthy young trees without pests or diseases were selected as experimental materials for this study.

1.3 Methods

All experimental indices were measured in late September 2024. For seedlings, the third mature leaf growing downward from the top was selected; for young trees, the third mature leaf at the terminal end of sun-exposed branches in the middle of the canopy was selected for photosynthetic parameter measurement and tagged, after which corresponding leaves were collected for leaf microstructure observation and chlorophyll content determination, with five replicates per treatment.

1.3.1 Photosynthetic Parameter Measurement Measurement of photosynthetic light-response curves and photosynthetic CO_2-response curves followed the methods of Ye (2021) and Wang et al. (2024) using a Li-6400 portable photosynthesis system (Li-Cor, USA). Measurements were conducted on clear days between 8:30 and 12:00. Prior to measurement, selected leaves were induced with light for 30 minutes, with light intensities of 800 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$ for seedlings and 1,200 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$ for young trees. An open gas circuit was used during measurement with an air flow rate of 0.5 $\mathrm{L \cdot min^{-1}}$, leaf chamber temperature of 28 °C, and CO_2 concentration of 400 $\mathrm{mol \cdot mol^{-1}}$. The light intensity gradient for light-response curves was 1,800, 1,500, 1,200, 1,000, 800, 600, 400, 200, 150, 100, 50, 20, 10, and 0 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$. The CO_2 concentration gradient for CO_2-response curves was 400, 300, 200, 150, 100, 50, 100, 150, 200, 300, 400, 600, 800, 1,000, 1,200, 1,500, and 1,800 $\mathrm{mol \cdot mol^{-1}}$. Net photosynthetic rate (Pn) was recorded after 3 minutes of equilibration at each gradient. Based on the measurement results, a rectangular hyperbola model was used to fit the light-response curves, and a modified model was used to fit the CO_2-response curves to calculate relevant photosynthetic parameters. Stomatal conductance (Gs) and transpiration rate (Tr) were also recorded, and water use efficiency (WUE) was calculated using the formula WUE = Pn / Tr.

1.3.2 Photosynthetic Pigment Content Measurement Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were determined following the method of Li (2000). After photosynthetic measurement, leaf samples were collected, and 20 leaf discs of 1 cm^2 were obtained using a hole punch. Pigments were extracted with 95% ethanol in the dark for 24 hours, and absorbance at 665, 649, and 470 nm was measured using a UV spectrophotometer (Perkin Elmer, USA) to calculate pigment contents and the chlorophyll a/b ratio.

1.3.3 Leaf Microstructure Observation of leaf epidermal characteristics followed standard electron microscopy sample preparation methods. After sampling, fixation, dehydration, drying, and gold spraying, scanning electron microscopy (ZEISS EVO18) was used for observation. An image analysis system (Axio Vision SE64) was used to measure stomatal morphological parameters (vertical/horizontal diameter, area) and density (individuals/mm^2). Leaf anatomical structure was prepared using the improved paraffin section method. After fixation, embedding, sectioning, and staining, an automatic digital slide scanning system was used for imaging, and CaseViewer software was used to measure leaf thickness (LT), upper and lower epidermal thickness (UET, LET), and mesophyll tissue thickness, with 10 fields of view observed per sample.

1.3.4 Leaf Phenotypic Trait Measurement For each treatment, 30 healthy, pest-free, fully expanded leaves with consistent light exposure were randomly collected from the upper-middle canopy. Leaf length and width were measured using a digital caliper (Mahr, Germany), and leaf area (LA) was measured using a Li-3000 leaf area meter (Li-cor, USA). After fresh weight measurement, leaves were dried at 80 °C to constant weight to obtain dry weight, and specific leaf weight (SLW = dry weight/leaf area) and leaf dry matter content (LDMC = dry weight/fresh weight) were calculated.

1.4 Data Analysis

This study used Microsoft Excel 2017 and Origin 2015 software for statistical analysis and graph preparation. The rectangular hyperbola modified model in Photosynthesis Calculation Software 4.1.1 was used to fit and calculate relevant parameters (Ye, 2010). SPSS 25.0 software was used for data processing and t-tests, where $P<0.01$ indicated extremely significant differences, $P<0.05$ indicated significant differences, and P$ $0.05 indicated no significant differences. Correlation analysis was performed between leaf structural characteristics, chlorophyll content, leaf phenotypic traits, and photosynthetic parameters. All experimental data are expressed as mean ± standard deviation.

2.1.1 Response of Gas Exchange Parameters to Light Intensity in *C. yunnanensis* Seedlings and Young Trees

The Pn of both seedlings and young trees increased with increasing photosynthetic photon flux density (PPFD) (Figure 1 [Figure 1: see original paper]A). Within the range of 0-100 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$, Pn increased linearly with PPFD. After PPFD reached 400 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$ for seedlings and 800 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$ for young trees, Pn increased slowly and stabilized after reaching the light saturation point. The changing trends of Gs and Tr in seedlings and young trees were similar, both increasing with PPFD (Figure 1B, C). The WUE of seedlings and young trees first increased and then decreased with increasing PPFD, reaching peak values at 400 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$ (Figure 1D). Within the range of 50-1,800 $\mathrm{mol \cdot m^{-2} \cdot s^{-1}}$, the Pn, Gs, Tr, and WUE of seedlings were all lower than those of young trees.

2.1.2 Photosynthetic Light-Response Parameters of *C. yunnanensis* Seedlings and Young Trees

Photosynthetic light-response parameter results showed that *C. yunnanensis* seedlings and young trees exhibited significant or extremely significant differences in photosynthetic light-response parameters (Table 2). Compared with seedlings, the maximum net photosynthetic rate (Pmax) of young trees increased by 110.9% (extremely significant). Meanwhile, the light compensation point (LCP) and light saturation point (LSP) of young trees also increased by 36.5% and 62.0%, respectively (both extremely significant). In addition, the apparent quantum yield (AQY) of young trees increased by 24.7% compared with seedlings (extremely significant), while dark respiration rate (Rd) also significantly increased by 12.1%.

2.1.3 Photosynthetic CO_2-Response Curves of *C. yunnanensis* Seedlings and Young Trees

The Pn of both seedlings and young trees increased with increasing CO_2 concentration (Ca) (Figure 2 [Figure 2: see original paper]). Within the Ca range of 0-800 $\mathrm{mol \cdot mol^{-1}}$, Pn increased rapidly; when Ca was between 800-1,600 $\mathrm{mol \cdot mol^{-1}}$, the increase in Pn slowed; after Ca exceeded 1,600 $\mathrm{mol \cdot mol^{-1}}$, Pn gradually stabilized. Under the same Ca conditions, Pn of young trees was higher than that of seedlings, especially during the CO_2 saturation stage (Ca > 1,600 $\mathrm{mol \cdot mol^{-1}}$), where Pn of young trees was approximately 1.5 times that of seedlings.

2.1.4 Photosynthetic CO_2-Response Parameters of *C. yunnanensis* Seedlings and Young Trees

Photosynthetic CO_2-response parameter results showed (Table 3) that compared with seedlings, the potential maximum net photosynthetic rate (Amax) and CO_2 saturation point (CSP) of young trees significantly increased by 49.6% and 10.1%, respectively. Meanwhile, the initial carboxylation efficiency (α) and photorespiration rate (Rd) of young trees increased by 121.7% and 88.9%, respectively (both extremely significant). No significant difference was observed in CO_2 compensation point (CCP) between seedlings and young trees.

2.2 Photosynthetic Pigment Content of *C. yunnanensis* Seedlings and Young Trees

Significant or extremely significant differences existed in photosynthetic pigment content between *C. yunnanensis* seedlings and young trees (Figure 3 [Figure 3: see original paper]). Compared with seedlings, chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll (Chl) contents of young trees increased by 48.7%, 56.5%, and 51.0%, respectively; carotenoid (Car) content increased by 45.2%; while the chlorophyll a/b ratio (Chl a/b) showed no significant difference between different growth stages.

2.3.1 Leaf Epidermal Characteristics

Observation of upper and lower epidermis of seedling and young tree leaves showed (Figure 4 [Figure 4: see original paper]) that stomata were distributed only on the lower epidermis, and epidermal hairs were distributed on both upper and lower epidermis, indicating dorsiventral leaves. The vertical axis and area of stomata in young trees were significantly smaller than those in seedlings, while the horizontal axis of stomata showed no significant difference; however, stomatal density in young trees was extremely significantly higher than that in seedlings (Table 4).

2.3.2 Leaf Anatomical Structure Characteristics of *C. yunnanensis* Seedlings and Young Trees

Both seedling and young tree leaf anatomical structures showed clear stratification, mainly including upper epidermal cells, palisade tissue, spongy tissue, and lower epidermal cells (Figure 5 [Figure 5: see original paper]). Compared with seedlings, leaf thickness (LT) of young trees increased by 31.2% (extremely significant); upper epidermal thickness (UET) and lower epidermal thickness (LET) increased by 11.2% and 42.9%, respectively (both extremely significant); palisade tissue thickness (PTT) and spongy tissue thickness (STT) increased by 46.2% and 34.7%, respectively (both extremely significant); and midrib vessel diameter (VD) increased by 44.5% (extremely significant). No significant difference was observed in palisade/spongy tissue ratio (PTT/STT) between young trees and seedlings.

2.4 Leaf Phenotypic Traits of *C. yunnanensis* Seedlings and Young Trees

Extremely significant differences existed in leaf phenotypic traits between *C. yunnanensis* seedlings and young trees (Table 6). Leaf length (LL) of young trees was extremely significantly higher than that of seedlings, with an increase of 31.2%; leaf width (LW) increased by 58.0%; and leaf area (LA) showed the most obvious difference, with young trees showing an extremely significant increase of 431.7%. In terms of leaf structural traits, specific leaf weight (SLW) of young trees increased by 256.8% (extremely significant); leaf dry matter content (LDMC) also increased by 80.0% (extremely significant).

2.5 Correlation Between Leaf Microstructure Characteristics, Chlorophyll Content, Leaf Phenotypic Traits, and Photosynthetic Characteristic Parameters in *C. yunnanensis*

As shown in Figure 6 [Figure 6: see original paper], leaf thickness (LT) and midrib vessel diameter (VD) in *C. yunnanensis* showed significant or extremely significant positive correlations with maximum net photosynthetic rate (Pmax), light saturation point (LSP), and light compensation point (LCP), and extremely significant negative correlations with apparent quantum yield (AQY). Total chlorophyll content (Chl) showed extremely significant positive correlations with Pmax, LCP, and LSP, and an extremely significant negative correlation with AQY. Leaf area (LA), specific leaf weight (SLW), and leaf dry matter content (LDMC) showed significant or extremely significant positive correlations with Pmax, LCP, and LSP, and significant negative correlations with AQY.

3.1 Photosynthetic Characteristics

This study comprehensively compared differences in photosynthetic characteristics between *C. yunnanensis* seedlings and young trees, systematically revealing the photosynthetic adaptation strategies that accompany developmental stage transitions. The results indicate that the development from seedling to young tree in *C. yunnanensis* is accompanied by a profound transformation of the photosynthetic system from a conservative shade-tolerant strategy to an active high-light investment strategy. Compared with the generally observed trend of steadily increasing photosynthetic capacity with development in tree species such as *Vatica guangxiensis* and *Larix gmelinii* (Luo, 2023; Pan et al., 2024), the increases in key parameters such as Pmax, LSP, and Amax in *C. yunnanensis* young trees are particularly significant, possibly stemming from its unique photosynthetic apparatus reconstruction and carbon assimilation pathway optimization mechanisms. The driving force behind this leap in photosynthetic capacity lies in the co-evolution of leaf structural traits and photosynthetic function (Li, 2012). Correlation analysis showed that Pmax was extremely significantly positively correlated with LA, LT, VD, SLW, and LDMC. Increased leaf thickness (LT) is usually accompanied by more developed palisade tissue, thereby accommodating more chloroplasts and enhancing light capture capacity

(Leng et al., 2023). Meanwhile, increased midrib vessel diameter (VD) directly improves water transport efficiency, effectively alleviating stomatal limitations on photosynthesis (Gong et al., 2018), collectively providing a structural basis for high net photosynthetic rates. Compared with tree species such as *Vatica guangxiensis* and *Ulmus szechuanica* (Jin & Li, 2023; Pan et al., 2024), the relatively high Pmax exhibited by *C. yunnanensis* young trees suggests its special adaptability to high-light environments and carbon acquisition advantages (Cai et al., 2003). This is not simply quantitative growth but rather a physiological marker of its successful niche expansion from understory low-light environments to forest gap or canopy high-light environments. In contrast, the low LCP and AQY of *C. yunnanensis* seedlings are not optimized strategies for adapting to shaded environments but rather more likely represent manifestations of underdeveloped and activity-limited photosynthetic apparatus under comprehensive environmental stress in the understory (Li et al., 2016). Combined with their lower dark respiration rate (Rd), this low-activity photosynthetic state directly leads to slow accumulation of carbon assimilation products, leaving seedlings lacking sufficient material and energy support during the transition from understory low-light to high-light environments. Therefore, insufficient carbon acquisition capacity at the seedling stage likely constitutes a key physiological bottleneck in the transition from seedlings to young trees during natural population regeneration.

Based on these stage-specific strategic differences, light environment management in conservation and cultivation must be precise. In conservation and cultivation practices, we recommend implementing precise light environment management strategies by developmental stage. During the seedling stage (1–2 years), 10%–20% transmittance should be maintained through understory planting or shade nets to ensure survival and initial establishment. After entering the young tree transition stage (>3 years), light availability should be gradually increased through artificial thinning. We recommend thinning competing trees in stages before the spring growing season to gradually increase transmittance to approximately 50% natural light intensity, thereby promoting photosynthetic apparatus development and carbon accumulation. Thinning intensity should follow the principle of “small amounts, multiple times” to avoid photosynthetic system stress caused by sudden environmental changes. Future research could further combine photosynthetic enzyme activity, mesophyll conductance, and molecular regulatory mechanisms to deeply analyze the physiological and genetic basis of photosynthetic development in *C. yunnanensis*. Plant photosynthesis is a very complex process, and differences in photosynthetic characteristics of woody plants are part of the interaction among various physiological and ecological processes and adaptation mechanisms (Chen et al., 2023), influenced by many physiological and biochemical factors. A comprehensive understanding of these differences requires correlation analysis with physiological and biochemical indicators.

3.2 Photosynthetic Pigments

The content and ratio of photosynthetic pigments directly reflect the investment trade-off between light energy capture and consumption in plants (Zhou et al., 2011; Zhao et al., 2022). In this study, chlorophyll and carotenoid contents in young trees were significantly higher than those in seedlings and showed extremely significant positive correlations with Pmax. This finding contrasts with the common strategy of many plants that increase pigment content to enhance light capture under low light (Zheng & Feng, 2005; Deng et al., 2025). This phenomenon indicates that *C. yunnanensis* seedlings adopt a conservative strategy centered on risk avoidance in the fluctuating understory light environment. Their lower pigment content represents a trade-off between "carbon acquisition opportunity" and "photodamage risk," with the core strategy being to improve the utilization efficiency of existing pigments rather than blind resource expansion (Chen et al., 2025). This aligns with the discussion by Sun et al. (2010) that plants adjust the ratio of photosystems and antenna pigment complex structures under shading conditions to achieve efficient light energy conversion and dissipation. Conversely, young trees in stable high-light environments tend to increase pigment investment scale, which not only provides the basis for high carbon assimilation but also gives the increased carotenoid content dual significance: on the one hand, as auxiliary photosynthetic pigments broadening the light absorption spectrum; on the other hand, more importantly, enhancing photoprotection mechanisms (Wang & Feng, 2005; Gao et al., 2015). This transformation of pigment allocation strategy represents a precise differential adaptation of *C. yunnanensis* to the heterogeneity of light environments faced at different developmental stages. Simultaneously, this adaptation process may involve physiological remodeling at multiple levels, from photosynthetic pigment synthesis and photosystem structural adjustment to energy metabolism regulation, representing the result of long-term interaction and co-evolution between its growth and development strategies and habitat light resource conditions.

3.3 Leaf Microstructure and Functional Traits

The leaves of *C. yunnanensis* demonstrate strong support for their ecological strategies at the epidermal characteristic level. The leaves are typical dorsiventral leaves with stomata distributed only on the lower epidermis, which is an effective structural adaptation to reduce unnecessary transpiration caused by direct light on the upper epidermis, consistent with typical adaptation characteristics of many light-demanding tree species to high-light environments (Liu et al., 2018; Cheng et al., 2021). More importantly, compared with seedlings, young tree stomata exhibit typical "small aperture-high density" characteristics. The advantage of this structural combination lies in conferring more sensitive regulatory capacity to the stomatal population, where high-density stomata ensure the "basic capacity" for gas exchange, while the small aperture characteristic enables faster opening and closing responses in each stomatal unit (Wang et al., 2023). Research has shown that this stomatal configuration enables plants to achieve finer dynamic balance between water use efficiency and CO_2 assimilation, particularly suitable for coping with habitat conditions with large light intensity fluctuations such as forest gaps (He, 2018; Pan et al., 2024).

In terms of leaf anatomical structure, the transition from seedlings to young trees featured particularly significant palisade tissue development. The palisade tissue in young tree leaves not only increased in thickness but also showed more elongated cell morphology and more compact and orderly arrangement. This structural change not only increased chloroplast capacity per unit leaf area but, more importantly, optimized the distribution path of light energy within mesophyll tissue and improved light use efficiency (Sui et al., 2009; Zhao, 2016). Meanwhile, the arrangement pattern of spongy tissue cells also changed, with more developed intercellular spaces, which may facilitate CO_2 diffusion among mesophyll cells and indirectly support higher photosynthetic rates.

From seedlings to young trees, key functional traits including leaf area (LA), leaf thickness (LT), specific leaf weight (SLW), and leaf dry matter content (LDMC) all showed coordinated and significant increases. This coordinated enhancement of traits is not an isolated phenomenon but collectively points to a "stronger" and "more efficient" leaf growth pattern. Larger leaf area directly expands the light-receiving area for photosynthesis; thicker leaves and higher specific leaf weight usually indicate more developed palisade tissue, more cell layers, and more compact arrangement, which directly leads to increased density of photosynthetic apparatus (such as chloroplasts) per unit leaf area, laying a solid foundation for high photosynthetic rates (Chen et al., 2022; Leng et al., 2023). Higher dry matter content (LDMC) further reflects a higher investment proportion in structural substances (such as cell walls and fiber components), which not only enhances mechanical support capacity to cope with physical stresses such as stronger winds but also extends leaf functional lifespan, representing a prerequisite for achieving long-term efficient carbon accumulation (Zhou et al., 2022; Wang et al., 2024).

These coordinated transformations in microstructure and functional traits collectively constitute the structural basis for *C. yunnanensis* to transition from a "shade-tolerant conservative" to a "high-light investment" ecological strategy. Compared with tree species such as *Vatica guangxiensis* and *Larix gmelinii*, the transformation in leaf structure of *C. yunnanensis* is more significant, which may be related to its specific ecological niche and evolutionary history (Luo, 2023; Pan et al., 2024). This profound structural change not only supports its enhanced photosynthetic capacity but also reflects the precise regulation of plant adaptation strategies during individual development.

In summary, this study systematically reveals significant differences in photosynthetic characteristics and leaf microstructure between seedling and young tree stages of *C. yunnanensis*, collectively constituting its light adaptation strategy that shifts with developmental stage. Young trees exhibit typical light-demanding tree species characteristics, with significantly enhanced maximum net photosynthetic rate, light saturation point, and water use efficiency, accompanied by optimized leaf anatomical structure (such as thickened palisade tissue and expanded midrib vessels) and adjusted dry matter allocation strategies (increased specific leaf weight and leaf dry matter content), demonstrating strong adaptability to high-light environments and carbon assimilation advantages. While seedlings possess some shade tolerance capacity (such as lower light compensation point), their low photosynthetic pigment content and underdeveloped photosynthetic apparatus limit light use efficiency. Moreover, seedlings do not follow common low-light adaptation strategies (such as increased pigment investment) but likely maintain low pigment content and conservative resource allocation to avoid photoinhibition risks caused by sudden high light in forest gap environments. Limited carbon accumulation due to insufficient understory light in the field represents the key physiological bottleneck for the difficult transition from seedlings to young trees in *C. yunnanensis*, which may also be an important internal mechanism leading to its endangered status and hindered population regeneration. Therefore, in conservation and breeding practices, ecological requirements by developmental stage should be followed: maintaining moderate shading during the seedling stage (10%-20% transmittance) to ensure survival and establishment, while gradually increasing to approximately 50% natural light intensity through progressive artificial thinning during the young tree transition stage to promote photosynthetic apparatus development and carbon accumulation, providing physiological and ecological basis for natural population recovery of *C. yunnanensis*.

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Note: Figure translations are in progress. See original paper for figures.