

Article

Plasticity of Leaf Traits of *Juglans regia* L. f. *luodianense* Liu et Xu Seedlings Under Different Light Conditions in Karst Habitats

Deng Wang, XiaoLong Huang, JingZhong Chen, LiXia Li, Jing Cheng, Shu Wang and JiMing Liu *

Forest Ecology Research Center, Forestry of Guizhou University, Guiyang 550025, China; wangdengyaya@163.com (D.W.); guidah365@126.com (X.H.); HerbCHenJZ1@163.com (J.C.); 18798006671@163.com (L.L.); 15804470360@163.com (J.C.); lnbx625@163.com (S.W.)

* Correspondence: jmlu@gzu.edu.cn; Tel.: +86-139-850-153-98

Abstract: This study examined the effects of light intensity on the plasticity of the leaves of *Juglans regia* f. *luodianense* seedlings in karst habitat and how they respond to changes in light intensity. The light intensity of 1-year-old seedlings of *J. regia* f. *luodianense* in different niches in a karst area was set as 100% (bare land), 75% (forest margin), 50% (forest gap), and 25% (under forest) of natural light. The material harvested after four months was compared to analyze the differences in various morphological characteristics, biomass allocation, and physiological characteristics of the leaves of seedlings of *J. regia* f. *luodianense*, and a comprehensive evaluation of the plasticity indexes was conducted. The results showed that under moderate (50%) full light intensity, the leaf area, specific leaf area, leaf biomass, and chlorophyll content increased, and improved photosynthesis and promoted the accumulation of free proline content and peroxidase (POD) activity. The accumulation of malondialdehyde was also the lowest in this treatment, indicating that the plants had the strongest adaptability under this light intensity. Moreover, under high (75%) full light intensity, the above functional characteristics of plants showed good performance. Under low (25%) full light intensity, plants also had higher specific leaf area, leaf biomass, and photosynthetic parameters. However, under full light, the cell membrane permeability decreased, the chlorophyll accumulation was the lowest, and the photosynthetic index was seriously inhibited. Our results showed that the plasticity of morphological characters was greater than that of biomass allocation and physiological characters; POD activity and stomatal conductance were the highest, followed by leaf area and chlorophyll b, whereas the plasticity of palisade tissue/sponge tissue thickness and lower-epidermis thickness were the lowest. In summary, there are evident differences in the sensitivity and regulation mechanisms of morphological characteristics, biomass allocation, and physiological indices of the seedling leaves of *J. regia* f. *luodianense* in response to light intensity. During the stage of seedling establishment, only the plants in the bare ground under full light can be induced to show obvious inhibition of phenotypic traits. In contrast, the plants in the forest margins and gaps and under the forest habitats under light intensity can regulate their own characteristics to maintain their growth and development. The wide light range and strong plasticity of the species might be two of the important reasons for its existence in a highly heterogeneous karst habitat.

Keywords: anatomical structure; illumination range; morphological and biomass; physiological indices; unique species of the karst region



Citation: Wang, D.; Huang, X.; Chen, J.; Li, L.; Cheng, J.; Wang, S.; Liu, J. Plasticity of Leaf Traits of *Juglans regia* L. f. *luodianense* Liu et Xu Seedlings Under Different Light Conditions in Karst Habitats. *Forests* **2021**, *12*, 81. <https://doi.org/10.3390/f12010081>

Received: 4 December 2020

Accepted: 8 January 2021

Published: 13 January 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Seedling growth and development are critical to population dynamics, and they include complex processes of detecting, acceptance, and interpreting endogenous and environmental signals [1]. Plants respond to endogenous and environmental signals in many different ways [2]. Many of their responses stem from phenotypic plasticity, which is the ability of specific genotypes to respond to environmental changes [3]. The expression

of plasticity depends on the perception and transduction of environmental cues that trigger different developmental pathways [4]. Among the many environmental conditions that plants experience, light plays an important role in the growth and development of species [5]. Thus, changes in available light can lead to the differentiation of the morphological, anatomical, and physiological characteristics of plants [6].

Leaves are the primary site of photosynthesis and material and energy exchange with the external environment, and they are highly plastic [7]. The plasticity level of young plants is higher than that of adult plants [8], because they have less reserves and a limited ability to obtain external resources, making plasticity an essential process in species establishment. Leaf characteristics are essential in affecting the functioning of plants [9] and their adaptive strategies, which rely on their internal physiology and external morphology formed by the response of leaves to environmental changes [10]. Using leaf characteristics to study the adaptation mechanisms of plants to the environment has gradually advanced the field of physiological ecology. Studies have shown that the leaf morphology and physiological characteristics of plants change in the long-term adaptation response of plants to different light environments to maintain the maximum photosynthetic capacity for different light environments [11]. Under low-light intensity, there are increases in the leaf area, specific leaf area, dry matter weight of leaves [12,13], and the contents of chlorophyll a, chlorophyll b, and total chlorophyll [14,15]. At high light intensities, plants have thicker leaves and mesophyll cells, longer palisade cells, and more sponge cells [16,17]. Variation in light intensities also affects the soluble matter, cell membrane permeability, antioxidant enzyme activity, and photosynthetic parameters of plant leaves [18–21].

J. regia f. *luodianense* is a unique species in the karst region in Southwest China, which is only distributed in the northern karst forest community area with an altitude of 800–1000 m in Luodian County, Guizhou Province [22]. In a previous survey including eight plots, we observed that the *J. regia* f. *luodianense* communities were distributed in the southwest slope of the karst forest. The primary associated tree species were *Toona sinensis*, *Eucommia ulmoides*, *Paulownia fortunei*, *Broussonetia papyrifera*, and *Cunninghamia lanceolata*. The predominant soil is primarily limestone soil, followed by yellow–red soil. In the karst area, due to the combined effects of climate and soil, the habitat is highly heterogeneous in horizontal and vertical space [23–25]. Furthermore, owing to the unreasonable social and economic activities of human beings in this area, the fragmentation degree of primary forest has intensified, and the probability of species appearing in the four habitats (i.e., bare land, forest margin, forest gap, and under the forest) has increased in the micro scale. For plants to establish and gain competitive advantages over other species in this unique habitat, the plants in these four habitats must have effective characteristics to capture sunlight [26] and reduce the negative impact of the environment on plant growth. However, previous research only focused on traditional cultivation techniques, fruit quality, and plant physiological response to drought stress [27–30]. It grows well in the karst habitat with high light heterogeneity, indicating that *J. regia* f. *luodianense* has evolved into a unique ecological adaptation strategy. In the stage that seedlings establish, this paper discusses whether the seedlings of *J. regia* f. *luodianense* can be normally established in the four light environments, namely bare land, forest margin, forest gap, and under the forest, as well as plants, which are widely distributed in the karst area. There are no reports regarding how it adjusts the plasticity of its own characters to respond to this light heterogeneous environment, which is a problem worthy of discussion. Therefore, we examined the effects of four light intensities on the leaf characteristics of 1-year-old *J. regia* f. *luodianense* seedlings. The study shows how the plasticity of *J. regia* f. *luodianense* seedlings responds to different light intensities in the early stage of seedling establishment, providing information on the ecological adaptation strategies of the species.

2. Materials and Methods

2.1. Study Site

The study was conducted at a nursery experiment site (106°42' E, 26°34' N), on the South Campus of Guizhou University, Huaxi District, Guiyang City, which has an altitude of about 1020 m, annual average temperature of 15.3 °C, annual average relative humidity of 77%, annual total precipitation of 1129.5 mm, annual average rainy time of 235.1 days, annual average sunshine hours of 1148.3 h, and annual average snowfall time of 11.3 days.

2.2. Experimental Design

In autumn and winter of 2017, 200 seeds were collected from the same mother plant. First, they were bagged and brought to the Laboratory of Ecology, College of Forestry, Guizhou University, and the impurities and peel were then removed. Next, the seeds were washed using distilled water and dried indoors, and then rewashed in early January of the following year. Then, they were disinfected with 0.4% potassium permanganate solution for 30 min and stored at a low temperature. In mid-March, the seeds were seeded into trays and placed in an artificial climate incubator with the temperature controlled at 25 ± 3 °C, humidity controlled at $60 \pm 2\%$, light and dark conditions for 12 h. Then, seedlings were transplanted into flowerpots with an inner diameter of 22 cm and a depth of 20 cm at the 3–4 leaf stage. Limestone soil, which was slightly alkaline and of medium fertility, was initially dried, crushed, disinfected, and sterilized and then used as the substrate. After planting for 1 month, the seedlings that showed the same growth and no disease were selected for light treatment. There were four different shading treatments, five replicates per treatment and four seedlings per replicate prepared to simulate the light intensity of four niches of the karst area [31,32]: bare land, forest margin, forest gap, and under forest, which has the same light intensity. Among the four treatments, one treatment was 100% natural light photon irradiance (bare land), and different shades were then adjusted by covering three different densities of specifically shaded cloth on three iron frames (Meshel Netting Co., Ltd, Changzhou, Jiangsu, CN; customized one needle, two needle, and four needle three specifications of shading cloth by the company, it spectrally provides rather uniform shade at those levels of shade presently marketed) with a height of 2.0 m. For the photon radiation gradient, the shading cloth was 20 cm away from the ground to ensure ventilation [1,33]. The seedlings were randomly divided into four groups and placed in the aforementioned regions with four light treatments. The seedlings of annual *J. regia* f. *luodianense* were divided irregularly into four groups and then placed in the preceding four light treatment regions, respectively. In the course of the experiment, the fertilizer and water management was strengthened, the compound fertilizer was applied once a month, disease and pest control was executed at any time, and the pots were placed randomly every week to guarantee that the same light was acquired in different light treatment basins. In early June 2018, the measurement period was 8:00–18:00 every day during three consecutive days of sunny weather. The photon irradiance of bare land was $1134.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (solar photon irradiance or full light, CK) by MQ500 handheld optical quantum sensor (Apogee Instruments Inc, Logan, UT, USA), and the average photon irradiance in three sheds were 850.5 (75% full light, HL), 567.0 (50% full light, ML), and $283.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (25% full light, LL), respectively. There was no significant difference in air temperature and humidity among the four light environments.

2.3. Measurement and Calculation of Leaf Properties

2.3.1. Anatomical Structure of Leaves

After four months of light control, five plants were selected from each treatment, and then selected from each plant one mature leaf with the same light direction, light time and growth trend. A double-sided blade was used to cut a $5 \text{ mm} \times 5 \text{ mm}$ leaf along the main vein at the base $1/3$ of the leaf, fixed using a Formalin-Aceto-Alcohol fixative on site. The slides were prepared using the paraffin section method, ethanol, xylene gradient dehydration, wax immersion, and embedding. The section thickness was 6–8 μm ,

and safranin–fast green double stain was used along with a neutral gum-sealing piece. The slices were observed and photographed using the Olympus-Bx 51 optical microscope (Olympus Corporation, Tokyo, Japan). The thickness of the upper epidermis, lower epidermis, palisade tissue, spongy tissue, and leaf were measured using Image-Proplus 6.0 (Media Cybernetics, Rockville, MD, USA), and the ratio of palisade tissue thickness to spongy tissue thickness was calculated. Each treatment was selected using a 15-field observation, and the average was calculated [34].

2.3.2. Morphological and Biomass Indicators

After four months of light control, five plants were selected from each treatment, then the maximum leaf in each plant was selected and the maximum leaf area was measured by scanning with a Yaxin-1241 leaf area meter. Then, all the leaves of each plant were placed on an electronic balance with an accuracy of 0.001 g to measure the fresh weight. Each envelope was placed in an oven; the leaves were dried at 110 °C for 1 h, and then dried to constant weight at 70 °C to determine leaf biomass. Finally, the fleshy degree of leaves of the five plants from each treatment was calculated as the fresh weight divided by the dry weight of leaves, and the maximum specific leaf area was calculated as the single maximum leaf area divided by its dry weight.

2.3.3. Physiological and Biochemical Indices

After four months of light control, five plants were selected from each treatment, and then selected from each plant one mature leaf with the same light direction, light time and growth trend. A sunny morning was selected, and measurements were made of the net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration, and transpiration rate at 09:00–12:00 using a Li-6400 photosynthesis instrument. The temperature was 25 °C, the concentration of CO₂ was 400 µmol m^{−1}, the relative humidity of air was 50–70%, and the photoactive radiation (PAR) was 500 µmol m^{−2} s^{−1}.

Biochemical analysis of the leaf samples was conducted using a variety of approaches: an extract pigment with 80% acetone was used to calculate the total, chlorophyll a, and chlorophyll b contents [35], the free proline content was determined using the method of ninhydrin [36], the soluble sugar content was determined using anthrone colorimetry [37], malondialdehyde (MDA) content was determined using the thiobarbituric acid method [38], peroxidase (POD) activity was estimated using the callus xylan oxidation method [39], and catalase (CAT) activity was determined using the Aebi method [40].

Data analysis: Excel 2007 was used to conduct preliminary statistical analysis. Then, SPSS 18.0 statistical software (IBM Corporation, Somers, NY, USA) was used to process the experimental data further. One-way analysis of variance was used to analyze the significance of different light intensity treatments on the leaf characteristics of recipient plants, and Duncan's multiple comparison method was used to test differences between the means of treatments using a significance level of $p < 0.05$. Origin 9.1 (OriginLab Corporation, Northampton, MA, USA) was used to plot the data.

According to Ashton et al. [41], the measures of size class plasticity (P) of the various leaf characteristics were calculated for each species using the smallest and largest values for a given measure, $p = (X - x)/X$, where x is the smallest value and X is the largest.

3. Results

3.1. Anatomical Structure of Leaves

Palisade tissue, spongy tissue, upper epidermis, lower epidermis and leaf thickness decreased with a decrease in light intensity (Table 1, Figure S1). There was no significant difference in the palisade tissue thickness between CK and HL ($p > 0.05$), but it was significantly higher in these two treatments than the LL treatments ($p < 0.05$). There was no significant difference in sponge tissue thickness between ML and LL ($p > 0.05$), but it was significantly lower than the other treatments ($p < 0.05$). There was no significant difference in the thickness ratio of the palisade tissue to sponge tissue ($p > 0.05$). The thickness of

the upper epidermis was significantly higher with CK than with ML and LL treatments ($p > 0.05$). The thickness of lower epidermis under CK treatment was significantly higher than that of the other three groups ($p > 0.05$). The leaf thickness was significantly higher with CK and HL than with MS and LS treatments ($p > 0.05$).

Table 1. The anatomical structure of *J. regia* f. *luodianense* seedlings leaves under different light intensities.

Treatments	Thickness of Palisade Tissue (μm)	Thickness of Spongy Tissue (μm)	Palisade Tissue/Spongy Tissue	Thickness of Upper Epidermis (μm)	Thickness of Lower Epidermis (μm)	Thickness of Leaf (μm)
CK	47.46 \pm 0.3 a	59.96 \pm 0.92 a	0.82 \pm 0.01 a	11.64 \pm 0.17 a	5.25 \pm 0.02 a	124.31 \pm 1.95 a
HL	47.04 \pm 0.84 ab	57.43 \pm 0.72 b	0.81 \pm 0.01 a	11.35 \pm 0.06 ab	5.11 \pm 0.02 b	120.93 \pm 1.54 a
ML	45.28 \pm 0.58 b	54.18 \pm 0.36 c	0.81 \pm 0.01 a	11.20 \pm 0.05 b	5.07 \pm 0.02 b	115.73 \pm 2.68 ab
LL	43.28 \pm 0.31 c	52.78 \pm 0.73 c	0.83 \pm 0.01 a	10.89 \pm 0.05 c	5.06 \pm 0.03 b	112.01 \pm 2.37 b

Different lowercase letters represent significant differences between treatment groups ($p < 0.05$). CK: 100% full light treatment; HL: 75% full light treatment; ML: 50% full light treatment; LL: 25% full light treatment.

3.2. Leaf Morphology and Biomass

The maximum leaf area and specific leaf area of *J. regia* f. *luodianense* increased gradually with a decrease in light intensity (Table 2), and there were significant differences in the leaf area indexes of the four light intensity treatments ($p < 0.05$). There was no significant difference in the specific leaf area between the CK and HL treatments ($p > 0.05$), which were significantly lower than the other treatments with lower light intensity ($p < 0.05$). Leaf succulence in the HL treatment showed no significant difference between the CK and ML treatments ($p > 0.05$), which were significantly higher than the LL treatment with the lowest light intensity ($p < 0.05$). The leaf biomass was highest under the ML treatment, which was similar to that under the LL treatment ($p > 0.05$), and there was no significant difference between the CK and HL treatments ($p > 0.05$), but they were significantly lower than the ML and LL treatments ($p < 0.05$).

Table 2. The leaf area, specific leaf area, leaf succulence and leaf biomass of *J. regia* f. *luodianense* seedlings treated with different light intensities.

Treatments	Maximum Leaf Area (cm^2)	Specific Leaf Area	Leaf Biomass (g)	Leaf Succulence
CK	2705.54 \pm 46.93 d	3.52 \pm 0.05 a	9.36 \pm 0.11 b	251.99 \pm 6.72 c
HL	3069.02 \pm 52.39 c	3.63 \pm 0.07 a	9.90 \pm 0.04 b	254.54 \pm 4.33 c
ML	4690.26 \pm 66.02 b	3.46 \pm 0.03 a	13.03 \pm 0.30 a	299.17 \pm 4.25 b
LL	4994.78 \pm 55.40 a	2.24 \pm 0.06 b	12.81 \pm 0.41 a	321.99 \pm 4.73 a

Different lowercase letters represent significant differences between treatment groups ($p < 0.05$). CK: 100% full light treatment; HL: 75% full light treatment; ML: 50% full light treatment; LL: 25% full light treatment.

3.3. Soluble Matter Content of Leaves

The soluble sugar content of the leaves of *J. regia* f. *luodianense* was not significantly different between the HS, MS and LS treatments ($p > 0.05$, Figure 1), and was significantly lower than the CK treatment ($p < 0.05$). The free proline content was the highest under MS treatment, which was not significantly different from that under CK ($p > 0.05$), but it was significantly higher than that under HS and LS treatments ($p < 0.05$).

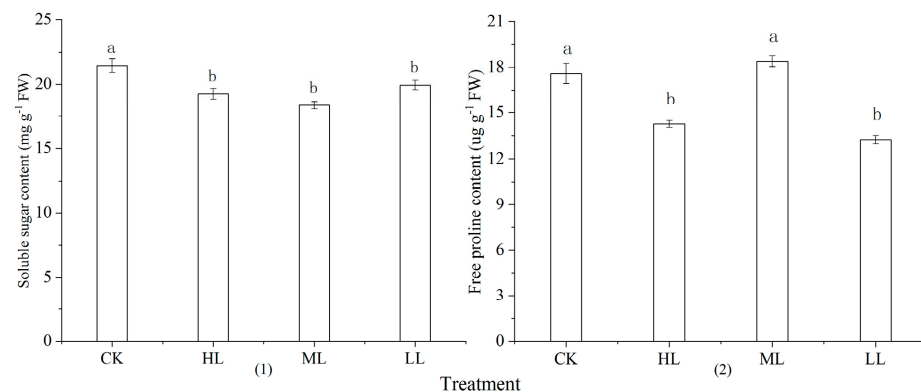


Figure 1. The soluble sugar content (1) and free proline content (2) of *J. regia f. luodianense* seedlings treated with various light intensities. Different lowercase letters depict significant differences between treatment groups ($p < 0.05$). CK: 100% full light treatment; HL: 75% full light treatment; ML: 50% full light treatment; LL: 25% full light treatment.

3.4. Chlorophyll Content

The chlorophyll a, chlorophyll b, and total chlorophyll contents in *J. regia f. luodianense* seedlings increased with the decrease in light intensity (Figure 2). The chlorophyll a and chlorophyll b contents of leaves in the ML and LL treatments were not significantly different ($p > 0.05$), but they were significantly higher than that in the high light intensity treatments ($p < 0.05$); there were no significant differences in the total chlorophyll content of leaves in each light intensity treatment ($p > 0.05$). The amount of chlorophyll a/b was the highest under the MS treatment and lowest under the LL treatment, but there were no significant differences among the four treatments ($p > 0.05$).

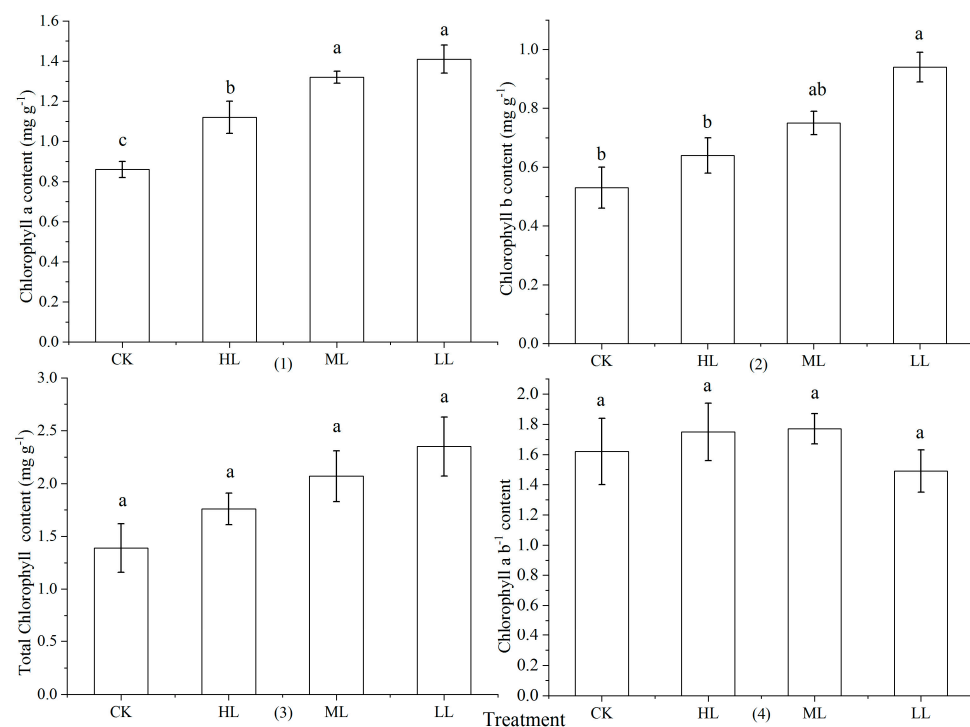


Figure 2. The chlorophyll a content (1), chlorophyll b content (2), total chlorophyll content (3), and chlorophyll a/b content (4) of *J. regia f. luodianense* seedlings treated with various light intensities. Different lowercase letters represent significant differences between treatment groups ($p < 0.05$). CK: 100% full light treatment; HL: 75% full light treatment; ML: 50% full light treatment; LL: 25% full light treatment.

3.5. Photosynthetic Parameters

The net photosynthetic rate, intercellular CO₂ concentration, transpiration rate, and stomatal conductance of *J. regia* f. *luodianense* seedlings were the highest under the MS treatment and the lowest under the CK treatment (Figure 3); there were all significantly higher than that under the CK treatment ($p < 0.05$) and the differences were not significant between the HS treatment ($p > 0.05$). The intercellular CO₂ concentration was not significantly different from that under the MS and LS treatments ($p > 0.05$).

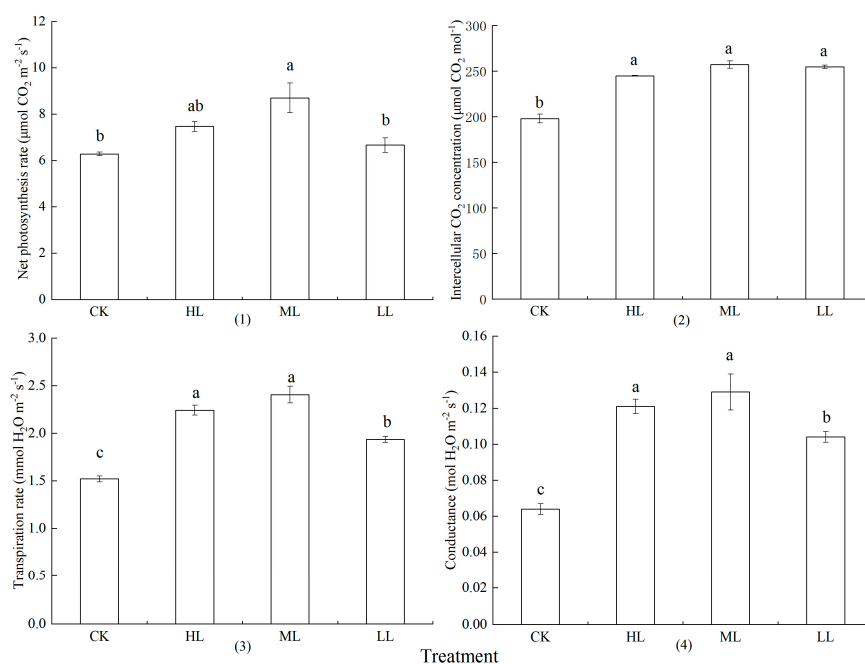


Figure 3. The photosynthesis rate (1), intercellular CO₂ concentration (2), transpiration rate (3), and conductance (4) of *J. regia* f. *luodianense* seedlings treated with different light intensities. Different lowercase letters represent significant differences between treatment groups ($p < 0.05$). CK: 100% full light treatment; HL: 75% full light treatment; ML: 50% full light treatment; LL: 25% full light treatment.

3.6. Malondialdehyde Content and Related Enzyme Activity

The MDA content of the *J. regia* f. *luodianense* seedlings under CK treatment was significantly higher than that under the other treatments ($p < 0.05$), and there were no significant differences between the other treatments ($p > 0.05$; Figure 4). The activity of CAT was highest under the LL treatment and was significantly higher than under the other treatments ($p < 0.05$), and the activity of CAT under the MS treatment was the lowest. The activity of POD under the MS treatment was significantly higher than that under the other groups ($p < 0.05$), and the activity under the CK treatment was the lowest, which was significantly lower than that under the other treatments ($p < 0.05$).

3.7. Evaluation of Leaf Plasticity

The plasticities of anatomical structure, external morphology, soluble matter, photosynthetic parameters, and other characteristics of *J. regia* f. *luodianense* seedlings to different light intensities were significantly different (Figure 5). For anatomical structure, the plasticity index of sponge tissue thickness was the largest, and the thickness of palisade/sponge tissue and lower epidermis was the smallest, which was almost unchanged. Among the morphological characteristics, the plasticity index of the leaf area was the highest (0.48), and the specific leaf area was the lowest. The plasticity index of free proline content was the highest of the soluble matter content of the leaves. Among the indices of chlorophyll and photosynthetic parameters, the plasticity of stomatal conductance was the highest (0.50),

followed by chlorophyll b, and the lowest was total chlorophyll. The plasticity index of POD activity was the highest (0.51), and MDA content was the lowest. Overall, the results showed that POD activity had the highest plasticity index, followed by stomatal conductance, whereas palisade tissue/sponge tissue thickness and lower epidermis thickness had the lowest plasticity indexes.

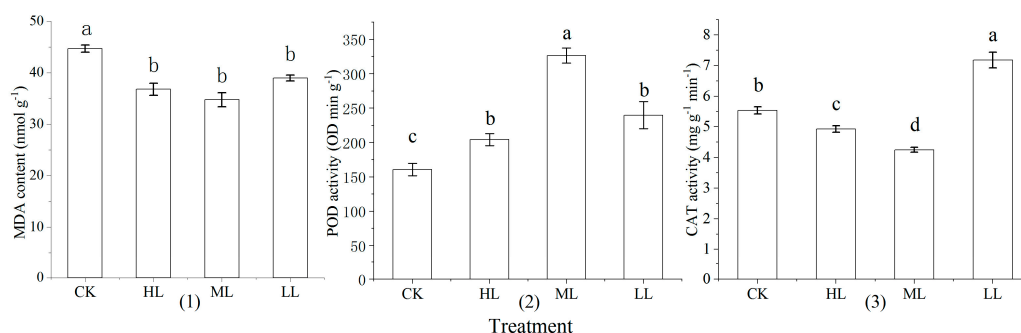


Figure 4. The MDA content (1), POD enzyme activity (2), and CAT enzyme activity (3) of *J. regia* f. *luodianense* seedlings treated with various light intensities. Different lowercase letters represent significant differences between treatment groups ($p < 0.05$). CK: 100% full light treatment; HL: 75% full light treatment; ML: 50% full light treatment; LL: 25% full light treatment.

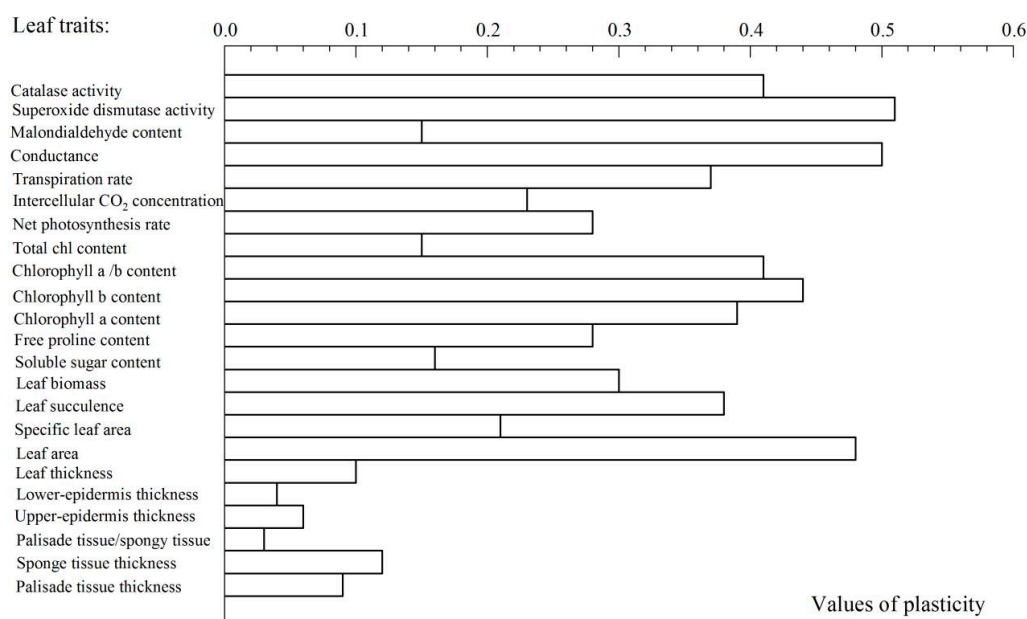


Figure 5. Plasticity values of different characteristics.

4. Discussion

4.1. Effect of Different Light Intensities on the Leaf Characteristics of *J. Regia* f. *Luodianense* Seedlings

Our finding that the thickness of the palisade tissue, spongy tissue, upper epidermis, lower epidermis and leaves decreased with a decrease in light intensity. This is consistent with the previous research results. Under full light, the thickness of the plant leaf increases, epidermis structure is developed, and palisade tissue is developed [1,42], whereas leaf thinning, spongy tissue porosity, and ventilation are adaptive characteristics under low light conditions [43]. Under full light, plants retain their water contents by having thick mesophyll tissue, thick epidermis, and strong refraction, which effectively regulates the mesophyll tissue protecting it from damage from strong light [44,45]. However,

thick mesophyll tissue can affect CO₂ transportation from the lower stomatal chamber to the photosynthetic site, reducing the photosynthetic rate of leaves [46]. This was consistent with the results that the net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and transpiration rate of the seedlings were significantly decreased under full light compared with other treatments. Under 50% full light intensity, the photosynthetic parameters of *J. regia* f. *luodianense* seedlings reached the highest, and there was no significant difference with 75% full light intensity. This finding shows that this light intensity range effectively promotes the adenosine triphosphate activity of *J. regia* f. *luodianense* seedlings, affects the enzyme system of chlorophyll synthesis, enhances photosynthetic energy, and promotes the growth of *J. regia* f. *luodianense* seedlings.

The results showed that the leaf area and specific leaf area of *J. regia* f. *luodianense* seedlings increased significantly with the decrease of light intensity. Generally, reducing the leaf area exposed to high light is a strategy to avoid damage [47–49]; as light intensity decreases, the leaf area and specific leaf area of plants increases to improve their ability to capture light energy [50,51]. The leaf biomass of *J. regia* f. *luodianense* seedlings was the highest under 50% full light intensity, followed by that under low light intensity. In low-light environments, plants often adapt by increasing the proportion of leaf biomass to optimize their ability to capture light energy and the efficiency of their leaves [52]. Similar results were also obtained by former scholars in response to light in three karst habitat plants, i.e., *Illicium difengpi*, *Ardisia corymbifera* var. *tuberifera*, *Pittosporum pulchrum*, and *Juglans mandshurica*, ‘LYuling’ *Juglans* of the same genus [32,53,54]. The leaf succulence of *J. regia* f. *luodianense* seedlings was the highest under moderate light. In general, the higher the degree of succulence of plant leaves, the higher the relative water content, the smaller the water saturation deficit value and the higher photosynthetic efficiency.

Chlorophyll absorbs, transfers, and transforms light energy, and its content directly affects the utilization of light energy. In this study, with the decrease of light intensity, the contents of chlorophyll a, chlorophyll b and total chlorophyll of *J. regia* f. *luodianense* seedlings increased with the decrease of light intensity. The results of previous studies on plants in the karst habitat were also the same [32]. This finding is in agreement with Taiz et al. and Guo et al. [15,55], who suggested that reduced light intensity causes plants to increase their chlorophyll to use light energy better to maintain growth. Thus, when levels of photosynthetic effective radiation are low, the seedlings of *J. regia* f. *luodianense* can capture more light energy by increasing chlorophyll b and chlorophyll a to fix and store captured light energy [56]. Therefore, the seedlings of *J. regia* f. *luodianense* can maximize photosynthesis by increasing their leaf area and chlorophyll content.

Being able to osmoregulate effectively is essential to ensure the survival of plants in adversity. Under full light conditions, the soluble sugar content of the leaves of *J. regia* f. *luodianense* seedlings was significantly higher than at lower light intensities, followed by the 25% full light intensity treatment, indicating that the plants adjusted their soluble sugar content to respond to different degrees of external stress and make corresponding osmotic regulation responses [57]. Free proline is essential in stabilizing the integrity of biomembranes and maintaining the advanced structure of protein [58]. The free proline content of the leaves of *J. regia* f. *luodianense* seedlings was highest under 50% full light intensity, indicating that the plants had a strong ability to osmoregulate under 50% full light intensity.

There is a stable, dynamic balance between the active oxygen and the antioxidant systems of plants. When they are exposed to external stress, membrane lipid peroxidation occurs, which is reflected by the level of MDA content [59]. However, plants often reduce the damage of MDA and other secondary metabolites to plants by changing the activities of POD and CAT [60]. The MDA content in the leaves of *J. regia* f. *luodianense* seedlings was highest under full light. The results showed that the plasma membrane of *J. regia* f. *luodianense* seedlings was damaged substantially, but they did not show the mechanism of damage [61]. We found that *J. regia* f. *luodianense* seedlings could effectively regulate themselves using POD and CAT or other antioxidant enzymes. However, MDA content was

the lowest under the 50% full light intensity, but there was no significant difference between the 75% and 25% full light intensity, indicating that the degree of damage to the plasma membrane of plant cells under this light intensity range was relatively small. Peroxidase activity increased first and then decreased with decreasing light intensity, whereas CAT activity decreased first and then increased with decreasing light intensity. The reverse activity of POD and CAT of *J. regia* f. *luodianense* seedlings is more conducive to the precise and efficient cooperation of plants to remove excess H_2O_2 after each exposure to stress.

4.2. Evaluation of the Plasticity of the Seedling Leaves of *J. Regia* f. *Luodianense* Under Different Light Intensities

We found significant differences in the plasticity of leaf anatomical structure, external morphology, photosynthetic parameters, osmoregulation substance content, and antioxidant enzyme activity to different light intensities. Generally, plants with wide light amplitude have higher biomass distribution plasticity [62], but leaf structure and physiological plasticity play a more important role in plant adaptation to changing light environments [63]. The plasticity of the leaf physiological index and biomass allocation of *J. regia* f. *luodianense* seedlings was second only to the plasticity of leaf external form, and the plasticity of leaf anatomical structure index was the lowest of the plasticity indexes; however, the plasticity of the leaf physiological index and biomass distribution of *J. regia* f. *luodianense* seedlings were just below those of the external shape of the leaves, the plasticity of the leaf anatomical structure index was the lowest, and the plasticity of leaf area also reached a very high level, which shows that *J. regia* f. *luodianense* seedlings are not completely dependent on biomass distribution and morphological changes strategy to adapt to the changing light environment, stomatal conductance, chlorophyll b, and POD activity. Sexual physiological characters and leaf area plasticity also play an important role in adapting to the changing light environment of *J. regia* f. *luodianense* seedlings, which can improve the efficiency of light energy capture and utilization, and maintain its normal physiological metabolism.

In this paper, the effects of light intensity conditions on the leaf morphology, biomass distribution, and physiological characteristics of *J. regia* f. *luodianense* seedlings were simulated in four habitats: out of forest or at the top of mountain, at the edge of forest, forest gap, and under forest. The results showed that under the simulated 75% and 50% full light intensity in the edge of forest and the window of forest, the leaves had larger thickness, larger stomatal opening, and increased photosynthetic pigment content. Furthermore, more light energy was captured and fixed, more biomass was available for leaves, and there was higher free proline content and POD activity. At the same time, the seedlings also had the lowest content of soluble sugar and MDA. Under 25% full light intensity, seedlings also have higher specific leaf area, leaf biomass, photosynthetic parameters, and other characteristics. Only under the full light outside the forest or in the mountaintop habitat, the cell membrane permeability of *J. regia* f. *luodianense* seedling decreased and its growth was inhibited. Following a thorough evaluation of the seedling's plasticity, its highest plasticity index in the process of adapting to the change of light intensity was screened out. From this point of view, it was additionally shown that during the period of species establishment, *J. regia* f. *luodianense* seedling principally altered the thickness structure of both leaf palisade tissue and sponge tissue and the morphological characteristics of the leaf area and adjusted the stomatal conductance, chlorophyll b, and POD. The ecological strategy of the active physiological characteristics can adapt to the 25–75% full light intensity. Previous studies show that most appropriate light intensity for growth of seedlings of *Celtis tetrandra* and *Pteroceltis tatarinowii* is 100% and 40–100% of full light intensity, respectively [64]. This difference in light adaptation may be because these species are pioneer tree species in the karst area, with high light compensation and saturation points and substantial ability to utilize light of high intensity. The previous study also noted that the seedlings of 2-year-old karst plants, for instance, *Ardisia corymbifera* Mez var. *tuberifera* and *Illicium difengpi.*, were more appropriate to grow under 25–50% of full light intensity, whereas *Pittosporum pulchrum* seedlings were not sensitive to the light intensity change [32], meaning the seedlings of *J. regia* f. *luodianense* have a wider light range than that of the maples and nuggets. In com-

parison with the normal landform, *A. crenata* Sims and *Baccaurea ramiflora* seedlings [56,65] also adapt better to light of high intensity and have a more obvious weak light competitive advantage than that of *P. tatarinowii* Maxim seedlings.

5. Conclusions

During species establishment, long-term growth in bare land obviously inhibited the phenotypic traits of *J. regia* f. *luodianense* seedlings. However, in the forest margins, forest gaps, and under forests habitats (25–75% full light intensity), the plant can regulate its own characteristics to maintain its growth and development. Therefore, the seedlings of *J. regia* f. *luodianense* have the characteristics of wide light range and strong plasticity, which help them shape their own characteristics flexibly to actively respond to light heterogeneous habitats of karst forests.

Supplementary Materials: The following are available online at <https://www.mdpi.com/1999-4907/12/1/81/s1>, Figure S1: The anatomical structure of *J. regia* f. *luodianense* seedlings leaves under different light intensities.

Author Contributions: D.W. and J.L. designed the experiments. X.H., J.C. (Jingzhong Chen), L.L., J.C. (Jing Cheng) and S.W. participated in the experiment, helped to perform the experiments, and analyzed the data. D.W. wrote the first draft of manuscript. D.W. and J.C. (Jingzhong Chen) revised the article. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundation of China, “Plasticity and its variability of plants in response to the heterogeneity of key environmental factors in karst habitats” (NSFC 31800335), priority support project of cultivation project of National Natural Science Fund for young teachers (qkh platform talent (2017) 5788), Guizhou Science and Technology Program “Source Screening and Mycorrhizal Seedling Breeding Technology of *Cinnamomum migao*” (Qiankehe Supporting (2019) 2774), and Ecology Domestic First-Class Discipline Construction Program (GNYL2017–007 to SW).

Institutional Review Board Statement: The study did not require ethical approval.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is contained within the article or supplementary material.

Acknowledgments: The authors would like to thank Forest Resources and Environment Research Center in Guizhou Province for its support.

Conflicts of Interest: There are no conflicts of interest.

References

1. Kariny, C.N.; Lindamir, H.P.; Mariza, B.R.; de Luiz, A.S. Do eugenia hiemalis seedling leaves under different light conditions develop phenotypic plasticity? *Plant Ecol.* **2015**, *216*, 1571–1581.
2. Sultan, S.E. Promising directions in plant phenotypic plasticity. *Perspect. Plant Ecol. Evol. Syst.* **2004**, *6*, 227–233. [CrossRef]
3. Gianoli, E.; Valladares, F. Studying phenotypic plasticity: The advantages of a broad approach. *Biol. J. Linn. Soc.* **2012**, *105*, 1–7. [CrossRef]
4. Aubin-Horth, N.; Renn, S.C.P. Genomic reaction norms: Using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.* **2009**, *18*, 3763–3780. [CrossRef]
5. Lambers, H.; Chapin, F.S.; Pons, T.L. *Plant Physiological Ecology*, 2nd ed.; Springer: New York, NY, USA, 2008; pp. 320–374.
6. Smith, H. Phytochromes and light signal perception by plants—an emerging synthesis. *Nature* **2000**, *407*, 585–591. [CrossRef]
7. Chen, F.S.; Zeng, D.H.; Fahey, T.J.; Yao, C.Y.; Yu, Z.Y. Response of leaf anatomy of *Chenopodium acuminatum* to soil resource availability in a semi-arid grassland. *Plant Ecol.* **2010**, *209*, 375–382. [CrossRef]
8. Valladares, F.; Arrieta, S.; Aranda, I.; Lorenzo, D.; Sánchez-Gómez, D.; Tena, D.; Suárez, F.; Alberto Pardos, J.A. Shade tolerance, photoinhibition sensitivity and phenotypic plasticity of *Ilex aquifolium* in continental Mediterranean sites. *Tree Physiol.* **2005**, *25*, 1041–1052. [CrossRef]
9. Cornelissen, J.H.C.; Lavorel, S.; Garnier, E.; Díaz, S.; Buchmann, N.; Gurvich, D.E.; Reich, P.B.; Steege, H.; Morgan, H.D.; Heijden, M.G.; et al. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* **2003**, *51*, 335–380. [CrossRef]
10. Vendramini, F.; Díaz, S.; Gurvich, D.E.; Wilson, P.J.; Thompson, K.; Hodgson, J.G. Leaf traits as indicators of resource-use strategy in floras with succulent species. *New Phytol.* **2002**, *154*, 147–157. [CrossRef]

11. Pandey, S.K.; Singh, H.; Singh, J.S. Species and site effects on leaf traits of woody vegetation in a dry tropical environment. *Curr. Sci.* **2009**, *96*, 1109–1114.
12. Poorter, H.; Nagel, O.W. The role of biomass allocation in the growth response of plants to different levels of light, CO₂, nutrients and water: A quantitative review. *Aust. J. Plant Physiol.* **2000**, *27*, 595–607.
13. Feng, Y.L.; Wang, J.F.; Sang, W.G. Biomass allocation, morphology and photosynthesis of invasive and noninvasive exotic species grown at four irradiance levels. *Acta Oecol.* **2007**, *31*, 40–47. [[CrossRef](#)]
14. Rego, G.M.; Possamai, E. Efeito do sombreamento sobre o teor de clorofila e crescimento inicial do Jequitibá-Rosa. *Bol. Pesqui. Florestal.* **2006**, *53*, 179–194.
15. Taiz, L.; Zeiger, E. *Fisiologia Vegetal*, 5th ed.; Artmed Porto Alegre: Rio Grande do Sul, Brazil, 2013.
16. Tazoe, Y.; Noguchi, K.; Terashima, I. Effects of growth light and nitrogen nutrition on the organization of the photosynthetic apparatus in leaves of a C4 plant, *Amaranthus cruentus*. *Plant Cell Environ.* **2006**, *29*, 691–700. [[CrossRef](#)]
17. Osada, N.; Onoda, Y.; Hikosaka, K. Effects of atmospheric CO₂ concentration, irradiance, and soil nitrogen availability on leaf photosynthetic traits of *Polygonum sachalinense* around natural CO₂ springs in northern Japan. *Oecologia* **2010**, *164*, 41–52. [[CrossRef](#)]
18. Crabtree, R.C.; Bazzaz, F.A. Seedling response of four birch species to simulated nitrogen deposition: Ammonium vs. nitrate. *Ecol. Appl. Publ. Ecol. Soc. Am.* **1993**, *3*, 315–321. [[CrossRef](#)]
19. Streb, P.; Tel-or, E.; Feierabend, J. Light stress effects and antioxidative protection in two desert plants. *Funct. Ecol.* **1997**, *11*, 416–424. [[CrossRef](#)]
20. Valladares, F.; Wright, S.J.; Lasso, E.; Kitajima, K.; Pearcy, R.W. Plastic phenotypic response to light of 16 congeneric shrubs from a panamanina rainforest. *Ecology* **2000**, *81*, 1925–1936. [[CrossRef](#)]
21. Tang, G.; Li, X.; Lin, L.; Li, L.; Lu, J. Change of different shading on moisture conditions and the physiological response in *Alhagi sparsifolia*. *Chin. J. Plant Ecol.* **2013**, *37*, 354–364. [[CrossRef](#)]
22. Liu, J.M.; Xu, G.R. A New Forma of *Juglans regia* L. *Guizhou Sci.* **2011**, *29*, 95–96.
23. Zhu, S.Q.; He, J.X.; Wei, L.M.; Zhang, C.G.; Chen, Z.R. *Karst Forest Ecology Research III*; Guizhou Science and Technology Press: Guiyang, China, 2003.
24. Li, A.D.; Jia, S.; Yu, L.F. Microclimates of different microhabitats in Huajiang karst area. *J. Zhejiang For. Coll.* **2010**, *27*, 374–378.
25. Du, X.L.; Wang, S.J. Micro-habitat characteristics in the karst desertification area: A case study of the wangjiashai catchment in Guizhou Province. *Earth Environ.* **2010**, *38*, 255–261.
26. Klich, M.G. Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. *Environ. Exp. Bot.* **2000**, *44*, 171–183. [[CrossRef](#)]
27. Xu, G.R.; Liu, J.M.; Yan, G.H.; Li, M.J.; He, X.; Xu, X.J. Quantification methods of chlorophyll from *Juglans regia* L. f. *luodianense*. *J. Mount. Agric. Biol.* **2010**, *29*, 419–423.
28. Liu, J.M.; Zhao, X.P.; Liao, X.F.; Zhang, D.K.; Yan, G.H.; Wang, M.; Wen, P. Study on protective enzyme system and MDA content of *Juglans regia* L. f. *luodianense* Liu et Xu under dry stress. *J. Henan Agric. Sci.* **2012**, *41*, 122–126.
29. Wen, P.; Liu, J.M.; Xu, G.R.; Liao, X.F.; Zhang, D.K.; Yan, G.H.; Wang, M.; Zhao, X.P. Effect of water stress on photosynthesis and transpiration of *Juglans regia* L. f. *luodianense* Liu et Xu. *Guizhou Agric. Sci.* **2013**, *41*, 57–60.
30. Wang, D.; Liu, J.M.; Xu, G.R.; Chen, J.Z. Main nutritional ingredients and morphological characters of *Juglans regia* L. f. *luodianense* Liu et Xu. *Chin. Oils Fats* **2019**, *44*, 95–98.
31. Lu, Y.F.; Shi, L.; Yan, S.C. Effects of different light intensities on activities of the primary defense proteins in needles of *Larix gmelinii*. *Acta Ecol. Sin.* **2012**, *32*, 3621–3627.
32. Wang, M.L.; Wei, X.; Tang, H.; Liang, H.L.; Zou, R. Effects of light intensity on growth and photosynthesis of three karst plant seedlings. *Chin. J. Ecol.* **2015**, *34*, 604–610.
33. Gaskin, T.A. Light quality under saran shade cloth. *Agron. J.* **1965**, *57*, 313–314. [[CrossRef](#)]
34. Zhou, Y. *Experiment of Plant Tissue Anatomy (Revised)*; Beijing Normal University Press: Beijing, China, 1993.
35. Lichtenthaler, H.K.; Wellburn, A.R. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* **1983**, *11*, 591–592. [[CrossRef](#)]
36. Zou, Q. *Experimental Guidance of Plant Physiology*; China Agriculture Press: Beijing, China, 2000.
37. Li, H.S. *Principles and Techniques of Plant Physiological and Biochemical Experiments*; Higher Education Press: Beijing, China, 2000.
38. Hodges, D.M.; DeLong, J.M.; Forney, C.F.; Prange, R.K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **1999**, *207*, 604–611. [[CrossRef](#)]
39. Shi, J.; Fu, X.Z.; Peng, T.; Huang, X.S.; Fan, Q.J.; Liu, J.H. Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidative capacity and stomatal response. *Tree Physiol.* **2010**, *30*, 914–922. [[CrossRef](#)] [[PubMed](#)]
40. Aebi, H. Catalase in vitro. *Methods Enzymol.* **1984**, *105*, 121–126. [[PubMed](#)]
41. Ashton, P.M.S.; Olander, L.P.; Berlyn, G.P.; Thadani, R.; Cameron, I.R. Changes in leaf structure in relation to crown position and tree size of *Betula papyrifera* within fire-origin stands of interior cedar-hemlock. *Can. J. Bot.* **1998**, *76*, 1180–1187.
42. Vogelmann, T.C.; Martin, G. The functional significance of palisade tissue: Penetration of directional versus diffuse light. *Plant Cell Environ.* **1993**, *16*, 65–72. [[CrossRef](#)]
43. James, S.A.; Bell, D.T. Influence of light availability on leaf structure and growth of two *Eucalyptus globulus* ssp. *globulus* provenances. *Tree Physiol.* **2000**, *20*, 1007–1018.

44. Gordon, D.C.; Percy, K.E.; Riding, R.T. Effects of uv-B radiation on epicuticular wax production and chemical composition of four *Picea* species. *New Phytol.* **1998**, *138*, 441–449. [[CrossRef](#)]
45. Dickson, W.C. *Integrative Plant Anatomy*; Academic Press: San Diego, CA, USA, 2000.
46. Li, F.L.; Bao, W.K. Response and adaptation of morphological and anatomical structures of plant leaves to environmental changes. *Bull. Bot.* **2005**, *22*, 118–127.
47. Parelle, J.; Roudaut, J.P.; Ducrey, M. Light acclimation and photosynthetic response of beech (*Fagus sylvatica*) saplings under artificial shading or natural Mediterranean conditions. *Ann. For. Sci.* **2006**, *63*, 257–266. [[CrossRef](#)]
48. Espíndola-Júnior, A.; Boeger, M.R.T.; Maccari-Júnior, A. Anatomia foliar de *Mikania glomerata* Spreng. (Asteraceae) sob diferentes condições de luminosidade. *Rev. Bras. Bot.* **2009**, *32*, 749–758. [[CrossRef](#)]
49. Guo, X.; Wang, R.Q.; Wang, C.D.; Xu, F.; Zhao, S.; Guo, W.H. *Acer truncatum* seedlings are more plastic than *Quercus variabilis* seedlings in response to different light regimes. *Dendrobiology* **2016**, *76*, 35–49. [[CrossRef](#)]
50. Saldaña-Acosta, A.; Meave, J.A.; Sánchez-Velásquez, L.R. Seedling biomass allocation and vital rates of cloud forest tree species: Responses to light in shade house conditions. *For. Ecol. Manag.* **2009**, *258*, 1650–1659. [[CrossRef](#)]
51. Xiao, H.; Wang, C.; Liu, J. Insights into the differences in leaf functional traits of heterophyllous *Syringa oblata* under different light intensities. *J. For. Res.* **2015**, *26*, 613–621. [[CrossRef](#)]
52. Gommers, C.M.; Visser, E.J.; Onge, K.R.S.; Voeselek, L.A.; Pierik, R. Shade tolerance: When growing tall is not an option. *Trends Plant Sci.* **2013**, *18*, 65–71. [[CrossRef](#)]
53. Zhang, D.L.; Zhang, L.; Ge, W.Z. Phenotypic plasticity and respond of *Juglans mandshurica* seedling under different light environments. *Bull. Bot. Res.* **2017**, *37*, 658–663.
54. Liang, M.M.; Gong, M.M.; Li, H.; Zhang, S.Y.; Wang, Z.S.; Zhang, X.M.; Guo, S.P.; Qi, G.H. Effects of different shading treatments on the growth and development of ‘LYuling’ *Juglans regia*. *J. Northwest Agric. For. Univ.* **2017**, *32*, 120–124.
55. Guo, Q.Q.; Li, H.E.; Gao, C.; Yang, R. Leaf traits and photosynthetic characteristics of endangered *Sinopodophyllum hexandrum* (Royle) Ying under different light regimes in Southeastern Tibet Plateau. *Photosynthetica* **2019**, *57*, 548–555.
56. Xiong, J.; Wang, C.; Xing, W.L.; Wu, M.K.; Cheng, X.R.; Zhang, C. Morphological and physiological responses of *Ardisia crenata* seedlings under different light intensities. *Plant Sci. J.* **2018**, *36*, 736–744.
57. Yu, S.W.; Tang, Z.C. *Plant Physiology and Molecular Biology*, 2nd ed.; Science Press: Beijing, China, 1998.
58. Verbruggen, N.; Hermans, C. Proline accumulation in plants: A review. *Amino Acids* **2008**, *35*, 753–759. [[CrossRef](#)]
59. Wang, D.; Chen, J.Z.; Xiong, X.; Wang, S.; Liu, J.M. Allelopathic effects of *Cinnamomum migao* on seed germination and seedling growth of its associated species *Liquidambar formosana*. *Forests* **2019**, *10*, 535. [[CrossRef](#)]
60. Fang, C.; Zhihui, C. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Front. Plant Sci.* **2015**, *6*, 1020.
61. Pintó-Marijuan, M.; Munné-Bosch, S. Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: Advantages and limitations. *J. Exp. Bot.* **2014**, *65*, 3845–3857. [[CrossRef](#)] [[PubMed](#)]
62. Wu, G.L.; Chen, M.; Du, G.Z. Response of biomass allocation and morphological characteristics to light and nutrient resources for seedlings of three alpine species. *Acta Ecol. Sin.* **2010**, *30*, 60–66.
63. Cheng, X.R.; Xing, W.L.; Yuan, H.J.; Xiong, J.; Yu, M.K. Phenotypic plasticity of *Illicium lanceolatum* in response to varied light environments. *Acta Ecol. Sin.* **2019**, *39*, 1935–1944.
64. Wang, Y.; Wei, X.L. The effect of different light intensity on seedling ecological adaptability of two karst tree species. *Guizhou Agric. Sci.* **2009**, *37*, 139–142.
65. Huang, H.T.; Huang, J.J.; Chen, J.; Chen, Y.J.; Guan, D.S. Growth, physiological and biochemical response of *Baccaurea ramiflora* Lour. seedlings to different shading environments. *Chin. J. Ecol.* **2020**, *39*, 1538–1547.