



# Article Physiological, Photosynthetic and Stomatal Ultrastructural Responses of *Quercus acutissima* Seedlings to Drought Stress and Rewatering

Dan Liu<sup>1,\*,†</sup>, Haili Guo<sup>1,†</sup>, Li-Ping Yan<sup>2</sup>, Lei Gao<sup>3</sup>, Shasha Zhai<sup>3</sup> and Yan Xu<sup>4</sup>

- <sup>1</sup> Shandong Provincial Center of Forest and Grass Germplasm Resources, Jinan 250102, China; ghaili0415@163.com
- <sup>2</sup> Shandong Provincial Academy of Forestry, Jinan 250014, China; ylp\_982@163.com
- <sup>3</sup> College of Agronomy and Agricultural Engineering, Liaocheng University, Liaocheng 252003, China; 17658258675@163.com (L.G.); 18563510688@163.com (S.Z.)
- <sup>4</sup> State-Owned Yishan Forest Farm of Yishui County, Linyi 276414, China; 15646700501@163.com
- \* Correspondence: 1821618@163.com
- <sup>†</sup> These authors contributed equally to this work.

Abstract: The physiological mechanisms of drought-stress response in *Quercus acutissima* were explored with the aim to develop potentially valuable drought-resistant species that are adapted to arid regions and barren mountains. Potting experiments of Q. acutissima that simulated droughtstress conditions, and morphological, physiological, photosynthetic, and ultrastructural changes were investigated at different stages of drought stress, including after rehydration and recovery. During drought stress and rewatering, the leaves exhibited yellowing and abscission, followed by the sprouting of new leaf buds. The relative water content (RWC) changed under the drought-rewatering treatment, with a decreasing and then increasing trend, while the relative electrical conductivity (REC) had a more gradual increasing and then decreasing trend. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), and the proline (Pro) and soluble sugar (SS) contents increased and then decreased. The change in soluble protein (SP) content showed an N-shaped trend of increasing, decreasing, and then increasing again. The malondialdehyde (MDA) content decreased and then slowly increased. From the drought to recovery phase of the experiment, the net photosynthesis (Pn), stomatal conductance (Gs), and transpiration rate (Tr) decreased gradually at first, and then the Pn increased significantly, while the Gs and Tr increased slowly. During this period, the internal CO<sub>2</sub> concentration (Ci) did not decrease significantly until the last stage of the drought treatment, and then it increased slowly thereafter. The open stomata count in the Q. acutissima leaves was reduced significantly as drought stress increased, but after rewatering, the stomata recovered rapidly, with their opening size increasing. The number of leaf epidermal trichomes gradually declined to a low count in response to drought stress, but it rapidly recovered and increased within a short period of time after rehydration. Q. acutissima was found to have a strong drought tolerance and recovery ability after exposure to drought stress, and it may be an effective pioneer species for reforestation in barren lands.

Keywords: Quercus acutissima; drought stress; photosynthetic response; stomatal ultrastructure

# 1. Introduction

In nature, abiotic stresses are the primary environmental conditions that reduce plant growth, yield, and quality. Drought stress is considered one of the most harmful abiotic stresses in agroforestry production. China's arid areas (including arid and semi-arid climatic zones) account for more than 50% of the country's land mass, and soil drought and desertification are becoming increasingly serious problems in these areas [1,2]. Statistically, the annual global economic loss due to drought is as high as  $6 \times 10^9$  to  $80 \times 10^9$  USD,



Citation: Liu, D.; Guo, H.; Yan, L.-P.; Gao, L.; Zhai, S.; Xu, Y. Physiological, Photosynthetic and Stomatal Ultrastructural Responses of *Quercus acutissima* Seedlings to Drought Stress and Rewatering. *Forests* **2024**, *15*, 71. https://doi.org/ 10.3390/f15010071

Academic Editor: Giovanbattista De Dato

Received: 24 November 2023 Revised: 23 December 2023 Accepted: 27 December 2023 Published: 29 December 2023



**Copyright:** © 2023 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/). which far exceeds that of other climatic disasters [3]. In addition, plant growth and agroforestry production use large, unsustainable amounts of freshwater resources. This depletion is expected to increase with global warming and the frequent occurrence of extreme conditions [4]. Forests are the world's largest carbon reservoir and source of nutrient water, and so they play a key role in slowing global warming and in improving arid ecosystems [5]. Many tree species, such as *Fagus sylvatica* and *Abies alba*, are at potential risk of habitat loss in arid regions [6,7]. Drought-tolerant species, on the other hand, such as *Quercus*, are less exposed to such risk [8]. *Quercus acutissima* is commonly used as an economic and ecological tree species for afforestation in arid and semi-arid regions. Q. acutissima is considered to be an excellent soil and water conservation species, which is important for maintaining the functional integrity and stability of forest ecosystems [9,10]. However, there are not many studies on the drought resistance of *Q. acutissima* [11,12]. Analyzing the physiological mechanisms of drought-stress response in Q. acutissima can assist in cultivating drought-resistant species for use in forestry production. Therefore, the mechanisms of drought resistance under different degrees of drought stress in Q. acutissima need to be further investigated.

How plants respond to environmental stress is a fundamental biological question. To withstand drought, plants have evolved a range of mechanisms, including a series of changes that occur at the morphological, physiological, and biochemical levels [13–15]. The cessation of growth is the basic response of plants under water deficit conditions. Gradual water loss and disorganized enzyme activity increase as the degree of stress increases or the duration of drought lengthens [16]. Photosynthesis is the basis for plant survival, and plant photosynthetic physiology is not only inextricably linked to the basic processes of the carbon and water cycles in terrestrial ecosystems, but it is also a basic indicator for assessing plant growth and physiological status [17]. However, drought stress leads to a reduction in energy transfer during photosynthesis, thus negatively affecting plant growth and development [16,18]. Therefore, during their long-term evolution, plants have developed complex regulatory mechanisms for drought tolerance and drought avoidance [19,20]. A protective mechanism during water deficit where the net photosynthetic rate and transpiration of plants are reduced is one such important strategy that commonly occurs in areas with high evaporative demand [21,22]. Stomata closure is an effective way for plants to circumvent the damage caused by water deficit when they are especially sensitive to the response of environmental (e.g., temperature,  $CO_2$  concentration, etc.) and plant physiological factors. The regulation of stomatal conductance enables the control and optimization of gas exchanges, such as CO<sub>2</sub> and water vapor, between plant leaves and the atmosphere under continuously changing environmental conditions [23]. The ability of a species to maintain its carbon balance depends largely on the stomatal response to drought-stress differences [24]. Additionally, in studies of Gibasis geniculata and Medicago sativa, it has been found that changes in leaf ultrastructure can provide clues at a deeper and more intuitive level to the extent of the damage in the leaf organelle structure due to abiotic stresses [25,26]. The increase in reactive oxygen species (ROS) in different cellular compartments is an unavoidable consequence of drought stress that is tightly controlled by plant enzymatic and non-enzymatic antioxidant systems such as the ROS scavenging enzymes of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), etc. The multifunctional collaboration of this system sets the redox state of the cell and regulates the ROS concentration because the ROS production that exceeds the load of the antioxidant system can lead to cell damage and death [27]. It has also been reported that antioxidant enzyme activities, such as SOD and CAT, tend to increase in plants under stress, with drought-tolerant varieties having higher protective enzyme activities than counterpart varieties [28]. ROS can also be used as an alarm signal in plants responding to stress. It triggers a stress response through stress signaling, and there is a large body of evidence showing that ROS signaling is correlated with ABA and sugar sensing in a process that involves  $H_2O_2$  as a secondary messenger [27]. Osmotic regulatory substance content and membrane lipid peroxidation metabolism are also closely related to drought tolerance in

plants, with proline (Pro), whose high concentration is considered an indicator of tolerance to water stress, being one of the most important cytosols [29]. Differences in antioxidant enzyme activities and osmotic regulatory substance contents are the physiological basis for

interspecific differences in drought-stress tolerance. Nonetheless, the combined effects of drought stress and rehydration on physiological, photosynthetic, and ultrastructural changes in *Q. acutissima* are not clear. In this study, we used a potting experiment to simulate drought-stress conditions for *Q. acutissima*, and we investigated the morphological, physiological, and photosynthetic changes at different drought-stress stages, including after rehydration and recovery. We used scanning electron microscopy to observe changes in leaf stomata and chloroplast ultrastructure under different levels of stress. We expect that these observations will elucidate the physiological mechanisms of the adaptation of *Q. acutissima* to drought adversity and will provide opportunities for the utilization of the species in arid areas and barren mountains.

#### 2. Materials and Methods

### 2.1. Plant Material and Drought Treatment

Materials used for the experimental treatments were live Q. acutissima seedlings from the Zaoyuan Conservation Bank of Shandong Provincial Center of Forest and Grass Germplasm Resources (117°27'49", 36°46'9"), China. The Q. acutissima seedlings were planted into plastic pots (21 cm  $\times$  21 cm and 60 cm  $\times$  48 cm) with soil consisting of Pindstrup substrate, organic matter, and perlite (4:2:1 v/v/v). The test plants were 12 3-year-old (3 a) Q. acutissima seedlings, with a control (CK) group and an experimental group of 6 plants each. These were subjected to morphological observation, and physiological and biochemical indexes were also measured. A second test group included 12 1-year-old (1 a) Q. acutissima seedlings, with 6 in the CK group and 6 in the experimental group, and these were subjected to morphological observation only. This experiment was conducted in a controlled climate chamber at the Shandong Provincial Center of Forest and Grass Germplasm Resources (117°7′35″, 36°39′23″), where a constant temperature and light could be maintained (25 °C, 16 h light/day, RH 70%–80%), and the experimental and control groups could experience the same experimental conditions. The soil moisture gradient of experimental potted plants was obtained using natural water depletion after artificial watering, while CK was watered every day from 30 August 2022, until reaching 80% relative soil water content (RSWC). The experimental group, where watering had been stopped, then underwent rewatering when RSWC was between 20 and 25%, and the rewatering criterion was RSWC > 80%. The growth data of 3A Q. acutissima were collected at five times: D1 (pre-treatment, RSWC = 78%-85% on 30 August 2022), D2 (moderate drought, RSWC = 30%-40% on 30 September 2022), D3 (severe drought, RSWC = 20%-25%on 22 October 2022), D4 (48 h of rewatering, RSWC > 80% on 24 October 2022), and D5 (10 d of rewatering, RSWC > 80% on 1 November 2022). Similar leaves were frozen in liquid nitrogen and stored at -80 °C prior to determination of physiological indices. We also collected fresh leaf samples to observe ultrastructural changes and to determine their relative water content and conductivity.

# 2.2. Measurement of Physiological Indexes

#### 2.2.1. Photosynthetic Parameters

The net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO<sub>2</sub> concentration (Ci), and transpiration rate (Tr) of seedlings during the drought treatment were measured between 8 and 10 a.m. using a Li-6400XT-portable photosynthesizer (Beijing ecotek Technology Co., Beijing, China). Flow was set to 500, and the light intensity was set to 1000 µmol photons·m<sup>-2</sup>·s<sup>-1</sup> according to the light response curve results (Figure S1). The temperature, CO<sub>2</sub> content, and relative humidity in the cuvette were 26~28 °C, 400 µmol·mol<sup>-1</sup>, and 55%~65%, respectively.

#### 2.2.2. Antioxidant Enzyme Activity, MDA, PRO Measurement

Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and proline (Pro), malondialdehyde (MDA) were extracted following the manufacturers protocol (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). For each index, 0.1 g of tissue samples and 1 mL of the corresponding extraction solution for ice bath homogenization were added. Then, the solution was centrifuged at  $8000 \times g 4$  °C for 10 min using a HERMLE Z326K benchtop freezer centrifuge, and the supernatant was recovered and placed on ice for measurement following the different manufacturers protocols. Finally, 200 µL was transferred to a micro glass cuvette. A spectrophotometer (Thermo Evolution 201) was used to determine the SOD (560 nm), POD (470 nm: absorbance at 30 s and 1 min 30 s), CAT (240 nm: absorbance at 0 s and 1 min), PRO (520 nm), and MDA (532 nm and 600 nm) concentrations.

#### 2.2.3. Determination of Soluble Sugar and Protein Contents

Soluble sugar contents were determined using the anthrone colorimetric method. The standard curve was first plotted, and then 0.1 g of *Q. acutissima* leaves were placed in a mortar. A total of 1 mL of distilled water was added, and the leaves were ground to homogenization. Next, the solution was poured into a centrifuge tube and placed in a 95 °C water bath for 10 min. After cooling, the solution was centrifuged at  $8000 \times g$  at 25 °C for 10 min. The supernatant was transferred to a 10 mL test tube and brought to volume with distilled water. After shaking well, the tube was placed in a 95 °C water bath for 10 min. After cooling to room temperature, 200 µL was taken and transferred to a trace glass colorimetric dish. The absorbance at 620 nm was measured, and the soluble sugar content was determined using the standard curve.

The soluble sugar calculation formula:

Soluble sugar 
$$(mg/g) = (y \times V_1)/(W \times V_2/V_1)$$
 (1)

where y is calculated from the standard curve, mg/mL;  $V_1$  is the volume of sample spiked during determination, mL; W is the fresh weight of sample, g; and  $V_2$  is the total volume of extract, mL.

The soluble protein content was determined according to the colorimetric method of Caulmers Brilliant Blue G-250. The standard curve was first plotted, and then 0.2 g of *Q. acutissima* leaves were weighed and placed in a mortar. A total of 8 mL of distilled water was added, and the leaves were ground to homogenization and then centrifuged at  $4000 \times g$  for 10 min. The supernatant was transferred to a 10 mL centrifugal tube and brought to volume with distilled water before being shaken well prior to measurement. A pipette with 1 mL of extract was placed in a test tube with 5 mL of kosmos blue reagent, shaken well, and left to stand for 2 min. Then, the absorbance at 595 nm was determined, and the standard curve was used to determine the protein content.

The soluble protein calculation formula:

Soluble protein 
$$(mg/g) = (C \times Vt)/(WF \times Vs \times 1000)$$
 (2)

where C is calculated from the standard curve, g; Vt is the total volume of extract, mL; WF is the fresh weight of sample, g; and Vs is the volume of sample spiked during determination, mL.

#### 2.2.4. Determination of Relative Water Content and Electrical Conductivity

The relative water content (RWC) of leaves was determined by the following procedure: (1) three fresh leaves under similar growth conditions from different 3 a *Q. acutissima* individuals with similar apical parts were collected and weighted to measure the original fresh weight; (2) immersing the fresh leaves in distilled water for 24 h and weighing them to measure the saturated fresh weight; (3) the fresh leaves were immersed in distilled water for 24 h and weighted to measure the saturated fresh weight; and (4) the results were

corrected using the standing rehydration technique [30]. The RWC (%) was determined as follows:

RWC (%) = (original fresh weight – dry weight)/(saturated fresh weight – dry weight)  $\times$  100% (3)

The relative electrolyte conductivity (REC) was determined using 0.1 g of leaves, rinsed twice with deionized water, that were then placed on filter paper to absorb the surface moisture, and then placed in a test tube with 8 mL deionized water and soaked at room temperature for 12 h. The conductivity (R1) was measured using a conductivity meter, and then all the tubes were heated in 95 °C boiling water for 12 h before conductivity was measured once more (R2).

$$REC = R1/R2 \times 100\% \tag{4}$$

#### 2.2.5. Ultrastructural Changes in *Q. acutissima* Leaves

The scanning electron microscopy sample preparation method was used to observe the ultrastructure [31] as follows: (1) *Q. acutissima* leaves from five different time points were immersed in 2.5% glutaraldehyde solution and fixed in a refrigerator at 4 °C for 12 h.; (2) the leaves were washed three times using 0.1 mol/L PBS buffer, and graded dehydration was performed using an ethanol solution (30, 50, 70, 80, 90, 95, and 100%), for 20 min at each level; (3) the leaves were washed three times using isoamyl acetate at 4 °C for 30 min, and then washed with ethanol solution for 20 min; (4) isoamyl acetate was used to displace the samples at 4 °C for 30 min, and then the dislocated samples were placed in the sample cage, and a CO<sub>2</sub> critical point dryer was used (Quorum K850) to dry them; and (5) the dried samples were cut into appropriate sizes with a razor blade, pasted onto the sample stage, with the lower epidermis of the blade facing upwards, using conductive adhesive, sprayed with gold coating using an ion sputtering apparatus for 40 s, and placed under a ZEISS EVOLS10 scanning electron microscope for observation. Three replications were set for each treatment, and representative parts were selected for photos.

### 2.3. Statistical Analysis

Excel 2021, IBM SPSS Statistics 26, and Origin 2021 softwares were used to organize, analyze, and graph the data. A one-way ANOVA test and the least significant difference (LSD) method were used to test for significant differences among treatment groups at the 5% level (p = 0.05). The results are shown as mean  $\pm$  SD, and different asterisks indicate significant differences at different levels.

#### 3. Results

# 3.1. Effect of Drought Stress and Rewatering on Leaf Morphology, Relative Water Content and Relative Electrical Conductivity

As the main organ of photosynthesis in *Q. acutissima*, leaves can effectively reflect the drought adaptation of an individual. In this experiment, we observed and recorded seedling and leaf morphology of 1A and 3A individuals at different time points during drought and rewatering (Figure 1, Table S1), and we measured the relative water content (RWC) and relative electrical conductivity (REC) of leaves of 3A (Figure 2). The healthy leaves of 1A were mostly lanceolate, with a bright green color. As stress increased, the seedling morphology did not change significantly in the CK group, and those in the experimental group gradually weakened (Figure 1A,B). Beginning on the 27th day, the leaf blades drooped due to the water deficit, and the leaf margins slowly curled. Under further persistence of stress, the degree of leaf drooping intensified, the leaf margins of almost all leaf blades were severely curled, and the leaf color changed from bright green to yellowish green (Figure 1D,E). Although the drooping state of the leaf blades improved after rewatering, the damaged blades gradually fell off within 10 days. In addition, this process was accompanied by the sprouting of new leaf buds, and the seedlings produced new tender leaves from the top (Figure 1B6,B7). The 3A individuals showed a better drought-tolerance phenotype than the 1A. Throughout the entirety of the stress treatments, 3A did not have an obvious growth inhibition during the first and middle stages of the process, the leaf color changes were not obvious, and the leaf surface only exhibited mild crumpling. When the RSWC was reduced to 25.4%, the leaves started to droop under the severe water deficit, and this continued for 10 days (Figure S2). The leaves began to droop as the leaf margins and the front part of the leaf blade withered and turned yellow, and some of the leaf blades appeared to be curled, but the symptoms were much better in 3A than in 1A, with a considerable portion of the healthy green leaves persisting. After rewatering, the senescent yellowed leaves were successively shed according to the degree of yellowing, and many new leaf buds rapidly sprouted. Compared with the CK group, leaf yellowing and abscission were the most obvious changes that appeared in response to stress.



**Figure 1.** Plant and leaf morphology of *Q. acutissima* in response to drought stress and recovery treatments. (**A**) 1A control with RSWC  $\geq$  80% for (**A1–A5**); (**B**) 1A treatments with RSWC 82.6%, 35.4%, and 23.7% for (**B1–B3**), respectively, and RSWC  $\geq$  80% for (**B4–B7**); (**C**) 3A treatments, with RSWC 81.1%, 29.4%, 23.7%, and 80.5% for (**C1–C4**), respectively; (**D**) 1A *Q. acutissma* control with RSWC  $\geq$  80% for (**D1–D5**); (**E**) 1A treatments with RSWC 82.6%, 35.4%, 23.7%, 83.1%, and 81.5% for (**E1–E5**), respectively.

The RWC reflects the degree of water deficit in plants. Drought stress affects the RWC while damaging the plant cell membranes, which leads to electrolyte extravasation. The RWC and REC in the CK group leaves showed some dynamic changes but tended to stabilize. Meanwhile, in the experimental group, the leaf RWC showed a decreasing and then increasing trend. In addition, we found that the measured leaf RWC was reduced after 4 h of standing rehydration (Figure S3), while, in contrast, the RWC was reduced by about 11% after 24 h of submerged rehydration; so, we corrected our results accordingly (Figure 2A). As shown in Figure 2B, the leaf REC changed with the drought-rewatering treatment, first increasing and then decreasing, though slowly. The REC is an important index for measuring the permeability of cell membranes, and its increase predicts the membrane permeability damage. The results indicated that under extreme drought conditions,



the leaf cell membranes showed relatively less damage than under less extreme drought conditions. In other words, *Q. acutissima* is extremely drought tolerant.

**Figure 2.** Physiological responses of *Q. acutissima* to drought stress and recovery of (Drought: D1, D2, D3, Rewater: D4 and Recovery: D5). (**A**) Relative water content (%). (**B**) Relative electrolyte conductivity (%). (\* significance level of 0.05, \*\*\* significance level of 0.001, \*\*\*\* significance level of 0.001).

# 3.2. Effect of Drought Stress and Rewatering on Antioxidant Enzyme Activities in *Q. acutissima Leaves*

At the beginning of the drought treatment (Figure 3), there was no significant difference in the different antioxidant enzyme activities between the experimental group and CK. As the degree of drought stress increased, however, the total activities of plant antioxidant enzymes in the experimental group seedlings exceeded that of the CK, with the SOD, POD, and CAT activities showing a tendency to increase progressively until peaking at severe drought (D3). At D2 and D3, antioxidant enzyme activity levels differed significantly (p < 0.05) between the experimental group and CK. In addition, the SOD, POD and CAT activities increased by 68.14%, 87.5%, and 80.19%, respectively, from the CK levels, indicating that Q. acutissima scavenges free radicals produced in the body and reduces oxidative damage, thereby increasing the activities of different phytoprotective enzymes to adapt to the unfavorable environment under drought stress. This indirectly shows that Q. acutissima has strong drought adaptability. After rewatering, the SOD, POD and CAT activities declined, with POD and CAT recovering to the CK levels in a relatively short period. The SOD activity, however, was still higher than that of the CK after rewatering. Thus, we surmised that even when drought stress disappears, Q. acutissima continues to suffer some damage from drought and requires a longer time period than the experiment to recover.



**Figure 3.** Variation in antioxidant enzyme activity in *Q. acutissima* leaves during drought stress and recovery. (**A**) Superoxide dismutase (SOD), (**B**) Peroxidase (POD), (**C**) Catalase (CAT). (\* significance level of 0.05, \*\* significance level of 0.01, \*\*\* significance level of 0.001), ns no significance.

# 3.3. Effect of Drought Stress and Rewatering on MDA, Proline, Soluble Sugar and Soluble Protein in *Q. acutissima Leaves*

The osmotic adjustment substance content can directly reflect the degree of plant leaves subjected to stress adversity. The contents of Pro and SS contents in Q. acutissima leaves in the experimental group increased as the drought-stress time increased, peaking at severe drought, and gradually recovering to the level of the CK after rewatering (Figure 4). The Pro and SS contents increased significantly (p < 0.0001) at the D3 stage, up to 5.19 and 1.84 times higher than in the CK, respectively. This indicates that the increase of free Pro and SS had a significant effect on the regulation of the osmotic capacity of Q. acutissima cell membranes. The Pro content decreased rapidly after 48 h of rewatering and returned to normal on the 10th day, while the SS content decreased but still maintained a high level. The change in the SP content showed an N-shaped trend of increasing, then decreasing, and then increasing again. At the initial stage, Q. acutissima leaves synthesized more proteins in response to drought stress. With the accumulation of stress time, however, the plant cells sustained damage, and the rate of protein synthesis decreased. The rate of protein degradation remained unchanged, thus resulting in a decrease in the SP content. Contents in Q. acutissima seedlings began to return to the CK levels as the stress was relieved via rewatering. Normally, drought leads to an imbalance between the production and scavenging state of ROS in plants, as well as to an increase in the degree of lipid peroxidation. This leads to the production and accumulation of the peroxidation product MDA. In *Q. acutissima*, however, the opposite change was observed. As drought stress increased, the MDA content in the experimental group decreased from 312.80 nmol/g (D1) to 142.80 nmol/g (D3), which is a decrease of 54.35%. The MDA content slowly increased after rewatering, possibly due to the high antioxidant enzyme activity. This further emphasizes the excellent drought tolerance of Q. acutissima. The MDA content was lower in the experimental group than in the CK at all stages.



**Figure 4.** Variation in MDA, proline, soluble sugar and soluble protein contents in *Q. acutissima* leaves during drought stress and recovery. (**A**) Proline (Pro), (**B**) Malondialdehyde (MDA), (**C**) Soluble sugar (SS), (**D**) Soluble protein (SP). (\* significance level of 0.05, \*\* significance level of 0.01, \*\*\* significance level of 0.001, ns no significance).

### 3.4. Effect of Drought Stress and Rewatering on Photosynthesis in Q. acutissima Leaves

Photosynthesis is one of the physiological processes in plants most sensitive to environmental factors, and drought stress in particular is known to affect physiological parameters related to photosynthesis, including net photosynthesis (Pn), stomatal conductance (Gs), internal CO<sub>2</sub> concentration (Ci), and transpiration rate (Tr). We determined the gas exchange parameters in the experimental and CK plants at least twice a week throughout all phases (Figure 5). During this period, the Pn and Gs gradually decreased with drought duration in the experimental group, reaching a minimum at about 40 days (Pn: 1.626~2.545 µmol  $CO_2 \cdot m^{-2} \cdot s^{-1}$ , Gs: 0.010~0.025 mol·m<sup>-2</sup>·s<sup>-1</sup>), and this state persisted until rewatering. We noted that the strength of photosynthesis in Q. acutissima was correlated with stomatal closure, and indeed there was a significant correlation between these two parameters. The Tr was significantly higher in the CK than in the experimental group, except on days 3 and 6, and the CK levels remained significantly higher after day 15, even when both the experimental group and CK declined to some extent. During the first and middle stages of drought stress, the Ci did not change significantly, ranging between 208.67 and 351.3  $\mu$ mol CO<sub>2</sub>·m<sup>-2</sup>·s<sup>-1</sup>. The Ci did not decrease significantly until the last stage of the drought treatment, which could be attributed to the insufficient  $CO_2$  supply due to the stomatal closure caused by drought stress. After 48 h of rehydration, the Pn increased significantly and the Gs, Ci, and Tr increased slowly. Still, all these physiological parameters improved significantly during the recovery period.



**Figure 5.** Measurements of photosynthetic parameters in *Q. acutissima* seedlings. (**A**) Net photosynthetic rate (Pn), (**B**) Stomatal conductance (Gs). (**C**) Internal CO<sub>2</sub> concentration (Ci), and (**D**) Transpiration rate (Tr). All parameters mentioned were measured using a portable photosynthesis system. (Different colors represent irrigation and drought conditions, Blue: watering, Light Orange: drought and Dark Blue: recovery; \* significance level of 0.05).

Stomatal closure is a common plant response to drought stress, and stomatal opening is more sensitive than stomatal density to changes in the soil water content. Additionally, the lower epidermis is much more sensitive than the upper epidermis. Drought stress greatly affected the stomatal opening of Q. acutissima leaves, with the number of open stomata decreasing significantly as drought stress increased (Figure 6 and Figure S4, Table S2). Additionally, fewer stomata were closed under moderate levels of drought stress compared with D1 (Figure 6). Under severe drought stress, the majority of stomatal openings became smaller or closed completely. Compared with D1, the leaves were obviously dried out, so they reduced water loss by closing stomata in the face of drought stress. After rewatering, the stomata recovered rapidly and their opening size increased, which indicates that Q. acutissima has strong resilience after drought stress. Notably, we also found that the trichomes in the epidermis of Q. acutissima leaves showed significant differences at different drought and recovery stages. The density of trichomes gradually became sparser as the drought was prolonged, and the density gradually increased after rewetting (D4, D5) to an extent that far exceeded the density of the D1 period. This might be an adaptive feature and survival strategy developed by Q. acutissima during the long-term evolution of its adaptation to drought.



**Figure 6.** Ultrastructural changes in the stomatal structure of the lower epidermis of *Q. acutissima* leaves during drought stress and recovery. (**A**,**B**) ultrastructure of subepidermal stomata of leaves pre-treatment (D1); (**C**,**D**) ultrastructure of subepidermal stomata of leaves under severe drought stress (D3); (**E**,**F**) ultrastructure of subepidermal stomata of leaves 10 d after rewatering (D5).

# 4. Discussion

Seedling Leaves

In this study, the physiological response mechanisms of *Q. acutissima* under drought stress were thoroughly investigated. Drought stress induces many plant responses, including morphological and physiological changes [14,15]. Important tools for determining the drought tolerance and drought adaptation in plants are plant water relation traits such as water potential at turgor loss point, osmotic potential, and RWC [30,32–35]. Among them, the leaf RWC is a key controller of plant physiological processes, and a reduced leaf RWC is a common consequence of drought stress in crop plants and forest trees [36,37]. Considering phenotypic and RWC changes, 3A *Q. acutissima* showed no water deficit symptoms before

and during the middle of the stress period, and developmental inhibition was not evident until 40 d. It has been reported that a 20% decrease in the RWC inhibits PSI reaction center electron transfer and reduces PSII electron transport, thus resulting in increased electrolyte leakage. However, the RWC of *Q. acutissima* decreased by 32.67% in the late stage of stress, but the REC did not increase. In other words, severe drought did not cause the alteration or loss of cell membrane selective permeability, so damage in Q. acutissima was limited, and its strong drought tolerance was apparent. During drought, leaf wilting and abscission led to reduced water loss due to transpiration. During the rewatering and recovery period, the Q. acutissima leaf condition improved, and the RWC and REC gradually recovered. Q. acutissima shed its leaves after rewatering, but it regenerated new leaves in a short period of time. This is a way for the plant to both reduce transpiration and retain water during rewatering and to stimulate the shedding of unhealthy and senescent leaves and counteract the negative effects of drought. The ability of plants to recover from post-drought rewatering is related to drought tolerance, and species with high drought tolerance also have a higher resilience and a greater ability to recover from rehydration [38,39]. We also found that the resistance of Q. acutissima to drought environments increased with age, but the rate of recovery decreased, according to observations of phenotypic changes in Q. acutissima at different ages. However, some studies have also reported that delayed senescence reduces plant resistance, which implies that different species, or the same species responding to different environments, may adopt different coping strategies. For example, in one study, adult Oryza sativa was more resistant under flooding stress than were juvenile plants, but juvenile plants of Arabidopsis thaliana were more resistant to flooding stress than were adults [40]. The root system is an important organ for plants to absorb water and nutrients, and under drought stress, it can be used to absorb more water by increasing the number and length of roots extending horizontally and vertically, increasing the absorbing area, and improving the ability to utilize soil moisture [41]. It has been shown that Q. acutissima seedlings absorb more water to mitigate drought damage through continuous rooting by a well-developed root system [42].

On the other hand, Q. acutissima achieves drought tolerance through physiological and biochemical mechanisms, including osmoregulation and the production of antioxidants and scavengers. Antioxidant enzymes in Q. acutissima responded positively to drought stress during the stress period. SOD, POD, and CAT showed an increasing trend under moderate (D2) and severe drought (D3) stress (Figure 3), and the free radical scavenging ability was enhanced, thus playing a role in protecting the photosynthesis apparatus. After rewatering, POD and CAT recovered to the control level, and SOD remained highly active, effectively removing excess  $H_2O_2$  and  $O_2$  and reducing stress damage to plants. The sensitivity of Pro synthase to Pro feedback inhibition in Q. acutissima was reduced under stress, which resulted in a surge of free Pro content in plants under extreme drought conditions (D3) (Figure 4A). In order to enhance its water retention capacity, Q. acutissima cells accumulated a large amount of SS and SP to stabilize osmotic pressure balance in vivo during the stress period (Figure 4B,D), thus allowing them to maximally resist the negative effects of drought. SS did not show a clear increasing or decreasing trend after rewatering, and Ji et al. also found in their study that rewatering affected the SS content of Robinia pseudoacacia seedlings in relation to the drought stress conditions and growing season before rewatering [43]. MDA generation is a result of membrane oxidation, and thus MDA reflects membrane damage. *Hippophae rhamnoides* is commonly used for ecological restoration on the Loess Plateau due to its strong stress tolerance. When the species is subjected to mild stress, PSII light energy conversion and reactive oxygen species metabolism act synergistically to jointly stabilize the function of the photosynthetic apparatus. In soil drought or under severe stress, the significantly elevated MDA in *H. rhamnoides* leaves disrupts the biofilm structure and function [37]. A similar pattern has been observed in studies on Quercus mongolica, Quercus *fabri* and other *Quercus* [44,45], where water stress enhanced the activity of protective enzymes and effectively reduced the degree of lipid peroxidation in plant cell membranes. Unlike H. rhamnoides and several Quercus species, MDA followed a continuously decreasing

trend in *Q. acutissima* during the stress period (Figure 4B) and gradually increased during the recovery period, indicating that *Q. acutissima* has strong resilience and was able to mitigate the damage of water deficit on leaf physiological activity under conditions of long-term stress. *Q. acutissima* through the changes in the morphology and physiological indicators showed that its strong drought resistance, which was of great significance to windbreaks and sand fixation, deserted mountain afforestation and vegetation restoration in water-scarce areas. Therefore, selecting *Q. acutissima* for afforestation in water-scarce areas is a feasible way to increase the local greening rate.

Changes in plant photosynthesis under water stress have been the focus of research for some time, and stomatal and non-stomatal restrictions are two important concepts in plant photosynthesis and water movement [46]. Stomatal restriction refers to the control of transpiration and photosynthesis via the regulation of the opening and closing of stomata, while the latter refers to the obstruction of water conductivity or uptake in plants. Nonstomatal restriction is characterized by the reduction of chloroplast activity, Rubisco activity, and the regeneration of Ribulose-1,5-bisphosphate (Ru BP), etc., all of which affect the water-conducting or water-absorbing properties of cellular structures such as cell walls, protoplasmic layers, and vesicles, and can thereby limit water movement in plants [47]. Gas exchange data combined with changes in leaves ultrastructure showed that the Pn and Tr decreased with the GS at the early period of water stress. During the moderate and severe drought periods, the Tr maintained its lower level, but the Pn continued to decrease with the GS. Stomata in the lower epidermis of Q. acutissima remained open throughout the moderate drought stress, and stomatal opening even increased in some leaves. The open stomata continued to close until severe water deficit, which indicates that stomatal restriction may not be a major factor affecting photosynthesis in the middle and late stages of drought stress. Stomatal conductance decreased to its minimum under 40 days of prolonged water deficit, which is consistent with the observation shown in Figure 5C. We speculate that this phenomenon was due to the inability of *Q. acutissima* to uptake water from the soil to meet the transpiration demand, to the translocation of ABA synthesized in the roots to the guard cells, and to the simultaneous activation of ABA biosynthesis in the leaves via stress-dependent mechanisms that promoted stomatal closure [48]. Compared with the "passive regulation" of stomata by hydraulic forces under water stress in ferns, the "active regulation" mechanism of stomata in seed plants such as *Q. acutissima* is more competitive for water utilization [49]. The Gs is also an important indicator of water and CO<sub>2</sub> balance and cycling between plants and the environment. The Ci of *Q. acutissima* leaves fluctuated with the duration of drought stress, and it declined rapidly during the severe drought period. The recovery of the Ci after rewatering, however, was significantly faster than that of the Gs. Farquhar and Sharkey considered changes in the Ci to be a key factor in determining the cause of the decrease in photosynthetic rate (stomatal and non-stomatal limiting factors), which suggests that the decrease in photosynthesis in *Q. acutissima* was not only caused by stomatal closure but may also be the result of non-stomatal limitation factors resulting from a decrease in the photosynthetic activity of the chloroplasts. In addition, many studies have shown that plants recover quickly after mild stress, but the maximum photosynthetic rate does not always recover after severe water stress [50,51]. The Pn and Tr of Q. acutissima continued to rise during the rewatering period, and the Gs had already reached the control level after one week of rewatering, which suggests that the photosynthetic capacity of *Q. acutissima* leaves made a smooth recovery during the transition stage.

It is interesting to note the changes in *Q. acutissima* leaf epidermal trichomes in response to drought stress. Trichomes can be regarded as one of the surface structures of plants that evolved by facing harsh environments [52,53]. In order to maintain hydration, plants have evolved dense patches of trichomes on leaves and aerial roots to absorb water from the air and to reduce water loss [54]. Up until now, many studies have focused on the hydraulic response of plants to drought during dehydration, and the ability of trichomes to mitigate drought-stressed atmospheric water via their influence on leaf hydraulics in

plant species inhabiting arid environments has been largely overlooked. In Q. acutissima, the number of leaf epidermal trichomes gradually declined in response to drought stress, but the trichomes rapidly recovered and increased in number within a short period of time after rehydration, even far exceeding the number of trichomes prior to the stress. Some scholars have speculated that the scar tissue left behind by the shedding of trichomes may be one of the pathways for water absorption by leaves [55]. Schwerbrock and Leuschner (2016) also found that the density of trichomes of Polystichum braunii increased as air humidity increased, significantly elevating the density of the boundary layer on the leaf surface [56]. This suggests that trichomes play a role in the water balance of the leaf. In our opinion, drought stress might also be an adaptation process for Q. acutissima, where Q. acutissima responds to water deficit by shrinking and shedding its trichomes while under stress; then, after rewatering, the trichomes return to further improve drought tolerance. Overall, the dense trichomes are to some extent beneficial to Q. acutissima in water-deficient environments. Maintaining leaf water content and photosynthetic systems helps plants tolerate drought as well. However, the questions of whether the trichomes on Q. acutissima leaves led to the increased leaf water uptake content, and for what proportion of the water uptake content of the whole leaf they are responsible, still need to be investigated further.

#### 5. Conclusions

We found that the resistance of Q. acutissima to drought environments increased with age, but the rate of recovery decreased with age, according to observations in the phenotypic changes of Q. acutissima at different ages during drought stress and rewatering. The RWC changed throughout the drought-rewatering treatment, showing a decreasing and then increasing trend, while the REC showed a slower increasing and then decreasing trend. Severe drought did not cause the alteration or loss of cell membrane selective permeability, meaning that Q. acutissima suffered little damage. The SOD, POD and CAT activities, and the Pro and SS contents increased and then decreased. The change in SP content showed an N-shaped trend of increasing, then decreasing, and then increasing again, while the MDA content decreased and then slowly increased. From the drought to recovery phase, the Pn, Gs and Tr started off with a gradual decrease, and then the Pn increased significantly while the Gs and Tr increased slowly. The Ci did not decrease significantly until the last stage of drought treatment, and then it increased slowly. Q. acutissima was able to mitigate the damage of water deficit on leaf physiological activity under conditions of long-term stress. The stomatal opening of Q. acutissima leaves decreased significantly as drought stress increased, and after rewatering, the stomata recovered rapidly, with many reopening. Notably, the number of leaf epidermal trichomes became gradually sparser in response to drought stress, but then the count rapidly recovered and even surpassed the initial count within a short period of time after rehydration. This indicates that trichomes play a role in the water balance of the leaf. Considering the morphological, physiological, and ultrastructural aspects of this study on the dynamic changes in Q. acutissima in response to drought stress, Q. acutissima has strong drought tolerance and recovery ability after drought stress. The next step will be to combine modern genetics, genomics, proteomics, and metabolomics to study the mechanism of drought resistance of Q. acutissima, to discover the factors and genes related to drought tolerance, and to carry out a more comprehensive and in-depth genetic improvement of drought tolerance at the molecular level.

**Supplementary Materials:** The following supporting information can be downloaded at: https:// www.mdpi.com/article/10.3390/f15010071/s1, Figure S1: Light response curve of *Q. acutissima* leaves. Figure S2: Differences in plant morphology in 3a *Q. acutissima* under severe drought-stress treatments. A, treatment; B, control. Figure S3: The effect of standing rehydration time on leaf RWC in *Q. acutissima*. Figure S4: Ultrastructural changes in the stomata of the lower epidermis of *Q. acutissima* leaves during drought stress and recovery. A and B: ultrastructural changes in the stomata of the lower epidermis of leaves in moderate drought (D2); C and D: ultrastructural changes in the stomata of the lower epidermis of leaves after 8 h of rewatering (D4). Table S1: Morphological characteristics of leaf in *Q. acutissima*. Table S2. Leaf stomatal characterization parameters of *Q. acutissima*. **Author Contributions:** D.L. designed and conceived the experiments. L.G., S.Z., and Y.X. collected the materials. H.G. and L.-P.Y. processed the data. D.L. and H.G. drafted the manuscript and contributed to the revision of the manuscript. D.L. supervised this whole process and revised this paper. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Biosafety and Genetic Resources Management Project of State Forestry and Grassland Administration "Collection and arrangement of genetic resources and genetic diversity evaluation of *Q. acutissima* (No. KJZXSA202111)", the National Forest Germplasm Resources Sharing Service Platform Construction and Operation Project (2005-DKA21003), the Subject of Key R & D Plan of Shandong Province (Major Scientific and Technological Innovation Project) "Mining and Accurate Identification of Forest Tree Germplasm Resources (No. 2021LZGC023)", and the Taishan Scholars Program.

**Data Availability Statement:** All data generated or analyzed during this study are included in the published article.

Conflicts of Interest: The authors declare no conflicts of interest.

### References

- Ciais, P.; Reichstein, M.; Viovy, N.; Granier, A.; Ogée, J.; Allard, V.; Aubinet, M.; Buchmann, N.; Bernhofer, C.; Carrara, A.; et al. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 2005, 437, 529–533. [CrossRef] [PubMed]
- Cheng, L.L.; Yin, C.B.; Lu, Q.; Wu, B.; Que, X.E. Economic analysis of desertification causes and irrational human activities. *Chin. J. Agric. Resour. Reg. Plan.* 2016, *37*, 123–129. [CrossRef]
- 3. Xu, L. Effects of drought on agriculture in China and countermeasures. *Guangdong Agric. Sci.* 2011, 38, 201–203. [CrossRef]
- 4. Gong, Z.Z.; Xiong, L.M.; Shi, H.Z.; Yang, S.H.; Herrera-Estrella, L.R.; Xu, G.H.; Chao, D.Y.; Li, J.R.; Wang, P.Y.; Qin, F.; et al. Plant abiotic stress response and nutrient use efficiency. *Sci. China Life Sci.* **2020**, *63*, 635–674. [CrossRef] [PubMed]
- 5. Wang, T.B.; Lu, J. The Advance on Foreign Biomass Mode. Chin. Agric. Sci. Bull. 2012, 28, 6–11. [CrossRef]
- Geßler, A.; Keitel, C.; Kreuzwieser, J.; Matyssek, R.; Seiler, W.; Rennenberg, H. Potential risks for European beech (*Fagus sylvatica* L.) in a changing climate. *Trees* 2006, 21, 1–11. [CrossRef]
- Ohlemuller, R.; Gritti, E.S.; Sykes, M.T.; Thomas, C.D. Quantifying components of risk for European woody species under climate change. *Glob. Chang. Biol.* 2006, 12, 1788–1799. [CrossRef]
- 8. Zimmermann, N.E.; Bolliger, J.; Gehrig-Fasel, J.; Guisan, A.; Kienast, F.; Lischke, H.; Rickebusch, S.; Wohlgemuth, T. Wo wachsen die Bäume in 100 Jahren? *Forum. Für. Wissen* **2006**, 2006, 63–71.
- 9. Yang, C.; Dang, K.L.; Liu, J.J. Study on water conservation efficiency of *Quercus* aculeatus plantation. *J. Northwest For. Univ.* **1997**, 12, 16–20.
- 10. You, L.X.; Li, Y.; Yin, Z.F.; Fang, Y.M. A Study of Sawtooth Oak in China: Provenance Test, Afforestation and Forest Management. *World For. Res.* 2017, *30*, 75–79. [CrossRef]
- 11. Hao, P.L.; Zhang, J.P.; Han, X.Y.; Dong, Y.F. Effect on transpiration among *Quercus acutissima* clones under drought stress. *J. For. Eng.* **2012**, *26*, 62–65.
- 12. Dong, Y.F.; Jiang, Y.Z.; Wang, H.T.; Kong, L.G.; Ge, Z.Q. Study on resistance of *Quercus acutissima* clones to different soil drought conditions. *J. Shandong For. Sci. Technol.* **2010**, *40*, 28–31 + 42.
- 13. Farooq, M.; Hussain, M.; Wahid, A.; Siddique, K.H.M. Drought Stress in Plants: An Overview. In *Plant Responses to Drought Stress*; Aroca, R., Ed.; Springer: Berlin/Heidelberg, Germany, 2012; pp. 1–33. [CrossRef]
- 14. Wu, J.S.; Zhang, J.H.; Li, R.J.; Li, Y.Y.; Gao, H.J.; Zhao, Q.J. The Plant's physiological mechanism and adaptability to drought stress. *J. Shanxi Agric. Univ.* 2017, *37*, 452–456. [CrossRef]
- 15. Tian, X.R.; Lei, Y.B. Physiological responses of wheat seedlings to drought and UV-B radiation. Effect of exogenous sodium nitroprusside application. *Russ. J. Plant Physiol.* **2007**, *54*, 676–682. [CrossRef]
- 16. Ayub, M.; Ashraf, M.Y.; Kausar, A.; Saleem, S.; Anwar, S.; Altay, V.; Ozturk, M. Growth and physio-biochemical responses of maize (*Zea mays* L.) to drought and heat stresses. *Plant Biosyst.* **2021**, *155*, 535–542. [CrossRef]
- Smith, S.D.; Huxman, T.E.; Zitzer, S.F.; Charlet, T.N.; Housman, D.C.; Coleman, J.S.; Fenstermaker, L.K.; Seemann, J.R.; Nowak, R.S. Elevated CO<sub>2</sub> increases productivity and invasive species success in an arid ecosystem. *Nature* 2000, 408, 79–82. [CrossRef] [PubMed]
- 18. Osakabe, Y.; Osakabe, K.; Shinozaki, K.; Tran, L.-S.P. Response of plants to water stress. Front. Plant Sci. 2014, 5, 5–86. [CrossRef]
- 19. Bressana, R.; Bohnertb, H.; Zhu, J.-K. Abiotic Stress Tolerance: From Gene Discovery in Model Organisms to Crop Improvement. *Mol. Plant* **2009**, *2*, 1–2. [CrossRef]
- 20. Chaves, M.M.; Maroco, J.P.; Pereira, J.S. Understanding plant responses to drought—From genes to the whole plant. *Funct. Plant Biol.* **2003**, *30*, 239–264. [CrossRef]
- Chaves, M.M.; Flexas, J.; Pinheiro, C. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Ann. Bot. 2009, 103, 551–560. [CrossRef]

- 22. Feng, Y.L.; Cao, K.F. Photosynthesis and photoinhibition after night chilling in seedlings of two tropical tree species grown under three irradiances. *Photosynthetica* 2005, *43*, 567–574. [CrossRef]
- 23. Sperry, J.S.; Venturas, M.D.; Anderegg, W.R.L.; Mencuccini, M.; Mackay, D.S.; Wang, Y.J.; Love, D.M. Predicting stomatal responses to the environment from the optimization of photosynthetic gain and hydraulic cost. *Plant Cell Environ.* **2016**, *40*, 816–830. [CrossRef] [PubMed]
- Chaves, M.M.; Zarrouk, O.; Francisco, R.; Costa, J.M.; Santos, T.; Regalado, A.P.; Rodrigues, M.L.; Lopes, C.M. Grapevine under deficit irrigation: Hints from physiological and molecular data. *Ann. Bot.* 2010, 105, 661–676. [CrossRef] [PubMed]
- 25. Chen, B.; Li, H.Y.; Liu, X.W.; Xia, B.; Sun, S.W.; Sun, Y.; He, M. Effects of different light intensities on morphogenesis and ultrastructure of *Gibasis pellucida* leaf. *Acta Prataculturae Sin.* **2019**, *28*, 175–185. [CrossRef]
- Li, L.; Li, D.N.; Yang, Y.Y.; Pan, X.Y.; Wan, Y.Q.; Zhang, G.Y.; Xi, J.J.; Wang, Y.F.; Yang, P.Z. Analysis of Metabolomics and Screening of Stomatal Regulating Substances in *Alfalfa* Leaves under Drought Stress. *Acta Agrestia Sin.* 2023, *31*, 2671–2683.
- 27. Carvalho, M.H.C.D. Drought stress and reactive oxygen species. Plant Signal. Behav. 2008, 3, 156–165. [CrossRef] [PubMed]
- 28. Du, C.Y.; Duan, Z.Y.; Pan, Y.H.; Lei, B.K.; Hu, W.L.; Fu, B.; Chen, A.Q.; Chen, S.H.; Yang, Y.X.; Jin, G.M. Effect of drought stress on growth and activities of antioxidant enzymes of maize seedling. *Agric. Res. Arid. Areas.* **2015**, *33*, 124–129. [CrossRef]
- Liu, C.; Wang, Y.; Pan, K.; Li, W.; Zhang, L.; Shen, X.; Liu, L.; Deng, M. Responses of the antioxidant defense system to drought stress in the leaves of *Fargesia denudata* seedlings, the staple food of the giant panda. *Russ. J. Plant Physiol.* 2014, 61, 374–383. [CrossRef]
- 30. Arndt, S.K.; Irawan, A.; Sanders, G.J. Apoplastic water fraction and rehydration techniques introduce significant errors in measurements of relative water content and osmotic potential in plant leaves. *Physiol. Plant.* **2015**, *155*, 355–368. [CrossRef]
- 31. Zhang, M.J.; Han, B.; Zhu, Q.; Cui, C.C.; Xian, Y.; Yang, K.Q.; Tong, B.Q. Effects of Osmotic Treatment on the Biological Properties of *Quercus acutissima* and *Quercus variabilis'* Seeds. *Genom. Appl. Biol.* **2023**, Accepted.
- Farrell, C.; Szota, C.; Arndt, S.K. Does the turgor loss point characterize drought response in dryland plants? *Plant Cell Environ*. 2017, 40, 1500–1511. [CrossRef] [PubMed]
- 33. Zhu, S.D.; Chen, Y.J.; Ye, Q.; He, P.C.; Liu, H.; Li, R.H.; Fu, P.L.; Jiang, G.F.; Cao, K.F. Leaf turgor loss point is correlated with drought tolerance and leaf carbon economics traits. *Tree Physiol.* **2018**, *38*, 658–663. [CrossRef] [PubMed]
- Kaproth, M.A.; Fredericksen, B.W.; Antonio González-Rodríguez, A.; Hipp, A.L.; Cavender-Bares, J. Drought response strategies are coupled with leaf habit in 35 evergreen and deciduous oak (*Quercus*) species across a climatic gradient in the Americas. *New Phytol.* 2023, 239, 888–904. [CrossRef] [PubMed]
- 35. Kunert, N.; Tomaskova, I. Leaf turgor loss point at full hydration for 41 native and introduced tree and shrub species from Central Europe. *J. Plant Ecol.* **2020**, *13*, 754–756. [CrossRef]
- 36. Zhang, H.L.; Qi, J.C.; Wang, X.J. Effects of Water Stress on Epicuticular Wax Content and Main Physiological Parameters of Barley. *J. Triticeae Crops* **2012**, *32*, 280–283. [CrossRef]
- 37. Pei, B.; Zhang, G.C.; Zhang, S.Y.; Wu, Q.; Xu, Z.Q.; Xu, P. Effects of soil drought stress on photosynthetic characteristics and antioxidant enzyme activities in *Hippophae rhamnoides* Linn. seedings. *Acta Ecol. Sin.* **2013**, *33*, 1386–1396. [CrossRef]
- Guo, Z.F.; Gong, D.Z.; Hao, W.P.; Mei, X.R.; Li, Y.Q.; Liu, B.H. Study on Compensation of Maize Varieties under Drought Stress and Re-watering in Different Growth Stages. J. Maize Sci. 2011, 19, 84–88. [CrossRef]
- 39. Du, J.X.; Hou, X.Y.; Liu, J.R. A study on physiological response to drought and re-watering treatments in Kentucky bluegrass. *Acta Prataculturae Sin.* **2010**, *19*, 31–38. [CrossRef]
- 40. Rankenberg, T.; Geldhof, B.; Veen, H.V.; Holsteens, K.; Poel, B.V.D.; Sasidharan, R. Age-Dependent Abiotic Stress Resilience in Plants. *Trends Plant Sci.* 2021, 26, 692–705. [CrossRef]
- 41. Du, M.F. Molecular Mechanisms of Drought-Resistant Germplasm in Response to Drought Stress in Masson Pine (*Pinus massoniana*). Ph.D. Thesis, Guizhou University, Guizhou, China, 2018.
- 42. Liu, X.; Luo, G.J. Effects of water stress on growth and physiological characteristics in *Quercus Acutissima*. J. North. Agric. 2019, 47, 11–14.
- Ji, Z.B.; Wang, J.X.; Li, J.W.; Xue, S.; Zhang, M.L. Dynamic Changes of Soluble Sugar in the Seedlings of *Robinia pseudoacacia* under Drought Stress and Rewatering in Different Seasons. *Acta Bot. Boreal.* 2009, 29, 1358–1363.
- Xiong, S.F.; Wu, L.W.; Chen, Y.C.; Gao, M.; Jiang, X.G.; Li, Q.Y.; Huang, S.H.; Wang, Y.D. Response of leaf of *Quercus fabri* seedlings from different provenances to drought stress and drought resistance evaluation. *Chin. J. Ecol.* 2020, 39, 3924–3933. [CrossRef]
- 45. He, B.R. Effects of Drought Stress on Physiological Characteristics and Anatomical Structure of *Quercus mongolica*. Master's Thesis, Shenyang Agricultural University, Shenyang, China, 2019.
- 46. Liu, M.Y.; Chen, P.Y. Effects of stomatal and nonstomatal factors on wheat photosynthesis under water stress. *Plant Physiol. Commun.* **1990**, *4*, 24–27. [CrossRef]
- 47. Yao, Q.Q.; Xie, G.S. The Photosynthetic Stomatal and Nonstomatal Limitation under Drought Stress. *Chin. J. Trop. Agric.* 2005, 25, 84–89. [CrossRef]
- 48. Mukherjee, A.; Dwivedi, S.; Bhagavatula, L.; Datta, S. Integration of light and ABA signaling pathways to combat drought stress in plants. *Plant Cell Rep.* **2023**, *42*, 829–841. [CrossRef] [PubMed]
- 49. Yang, Y.J.; Bi, M.H.; Nie, Z.F.; Jiang, H.; Liu, X.D.; Fang, X.W.; Brodribb, T.J. Evolution of stomatal closure to optimize water-use efficiency in response to dehydration in ferns and seed plants. *New Phytol.* **2021**, *230*, 2001–2010. [CrossRef]

- 50. Gallé, A.; Haldimann, P.; Feller, U. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytol.* **2007**, 174, 799–810. [CrossRef]
- 51. Grzesiak, M.T.; Grzesiak, S.; Skoczowski, A. Changes of leaf water potential and gas exchange during and after drought in triticale and maize genotypes differing in drought tolerance. *Photosynthetica* **2006**, *44*, 561–568. [CrossRef]
- Waseem, M.; Nie, Z.F.; Yao, G.Q.; Hasan, M.; Xiang, Y.; Fang, X.W. Dew absorption by leaf trichomes in *Caragana korshinskii*: An alternative water acquisition strategy for withstanding drought in arid environments. *Physiol. Plant.* 2021, 172, 528–539. [CrossRef]
- 53. Pan, Z.L.; Guo, W.; Wang, T.; Li, Y.P.; Yang, S.J. Research progress on foliar water uptake. *Plant Physiol. J.* **2021**, *57*, 19–32. [CrossRef]
- 54. Liu, W.Y.; Ma, W.Z.; Yang, L.P. Advances in ecological studies on epiphytes in forest canopies. *Chin. J. Plant Ecol.* **2006**, *30*, 522–533. [CrossRef]
- Fernández, V.; Sancho-Knapik, D.; Guzmán, P.; Peguero-Pina, J.J.; Gil, L.; Karabourniotis, G.; Khayet, M.; Fasseas, C.; Heredia-Guerrero, J.A.; Heredia, A.; et al. Wettability, Polarity, and Water Absorption of *Holm oak* Leaves: Effect of Leaf Side and Age. *Plant Physiol.* 2014, 166, 168–180. [CrossRef] [PubMed]
- 56. Schwerbrock, R.; Leuschner, C. Air humidity as key determinant of morphogenesis and productivity of the rare temperate woodland fern *Polystichum braunii*. *Plant Biol.* **2016**, *18*, 649–657. [CrossRef] [PubMed]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.