Effects of drought stress and rehydration on physiological and biochemical of four oak species in China

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Research

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Abstract

Background: *Quercus fabri* Hance, *Quercus serrata* Thunb, *Quercus acutissima* Carruth, and *Quercus variabilis* BL are four Chinese oak species commonly used for forestation. In recent years, with the global warming caused by the greenhouse effect, seedlings in mountainous areas after afforestation often suffer seasonal drought stress, which seriously affects their survival and growth. In order to ensure the survival of seedlings, we need to select oak species with strong drought resistance. Therefore, we first need to understand the differences in drought resistance of the four oak tree species at the seedling stage, and comprehensively evaluate their drought resistance capabilities by studying the changes in the physiological and biochemical characteristics of the seedlings under continuous drought and rehydration conditions.

Methods: The four oak seedlings were divided into drought-rewatering treatment group and normal watering control group. For the seedlings of the drought-rewatering treatment group, drought stress lasting 31 days was used, and then re-watering and recovering for 5 days. The water parameters, osmotic adjustment substance content, antioxidant enzyme activity and photosynthesis parameters of the seedlings in the two groups were measured every 5 days. Principal component analysis, correlation analysis and membership function were used to analyze the physiological and biochemical characteristics of the seedlings of the four oak in two groups.

Results and conclusions: Compared with the control group, the relative water content, water potential, net photosynthetic rate, transpiration rate, and stomatal conductance levels of the four oaks all showed a downward trend under continuous drought stress, and showed an upward trend after rehydration. The soluble protein, soluble sugar, proline, peroxidase, superoxide dismutase and catalase content of the four oaks increased first and then decreased under drought stress, and then increased after rehydration. The content of glycine betaine and malondialdehyde continued to increase, and gradually decreased after rehydration. The weight of each index was calculated by PCA, and then the comprehensive evaluation of each index was carried out through the membership function method. The drought resistance levels of the four oak species were as follows: *Q. serrata* > *Q. fabri* > *Q. variabilis* > *Q. acutissima*.

1. Introduction

With rising global temperatures and frequent extreme phenomena, 64% of the world's land is already under drought stress, which has seriously affected the normal growth and development of many plants and crops (Li and Geng 2013). Half of China's land is arid or semiarid, and drought is a serious problem in northwest and southwest China (Piao et al. 2010). Moreover, even southern regions with abundant rainfall are often affected by seasonal drought, which is a major factor that limits the normal growth and development of plants (Zhai et al. 2005).

When plants are under drought stress, the plant water balance is destroyed, and normal physiological processes are affected (Zhu 2002). The relative water content (RWC) and water potential of plant leaves
decrease (Farooq et al. 2009). In addition, plants close their stomata to reduce water loss, which can limit the supply of CO$_2$ and lead to reduced photosynthesis Reactive oxygen species (ROS) metabolism is the primary response of plants to stress (Ben-Gal et al. 2009). Drought stress can break the dynamic balance of production and elimination of ROS in plants, and excessive accumulation of ROS causes oxidative stress (Gechev et al. 2006). To protect themselves from oxidative stress, plants produce antioxidant enzymes and nonenzymatic substances that scavenge ROS (Huang et al. 2019). The antioxidant enzymes mainly include peroxidase (POD), superoxide dismutase (SOD), ascorbate oxidase (APX) and catalase (CAT), and the nonenzymatic antioxidant substances mainly include reduced glutathione (GSH), reduced ascorbic acid (AsA) and carotenoids (Ahmad et al. 2010). Many studies have shown that the responses of different protective enzymes to drought stress vary (Ashraf 2009, Anjum et al. 2011). The protective enzymes that play a dominant role in plants may also differ between periods of stress (Seki et al. 2007). In addition, plants also reduce their osmotic potential by accumulating osmoregulatory substances, such as soluble sugars (SS), proline (Pro) and glycine betaine (GB), to obtain the water needed to maintain normal growth under drought stress, thus alleviating the damage caused by drought stress (Chaves et al. 2009).

The leaf is an important organ that is in close contact with the external environment and is the main organ for photosynthesis and transpiration (Koyro 2006). The leaf tissue structure and physiological characteristics are susceptible to environmental factors and are the best reflection of a plant's adaptability to its environment (Xu et al. 2015). The morphological structure (leaf area, leaf thickness and stomatal density) and physiological properties (RWC, antioxidant system and osmoregulatory substances) of leaves are often used as important indicators in drought studies (Medina et al. 1990, Kruskopf and Flynn 2006). At present, many studies have been carried out on the physiological and biochemical responses and drought resistance mechanisms of plants under drought stress (Toscano et al. 2016, Bandeppa et al. 2019). A pot experiment with artificially controlled water was performed to simulate the natural drought process in the soil and to observe the plant resistance response to different levels of drought stress (Rivest et al. 2013). Pot experiments more objectively reflect the response characteristics of plants to natural drought stress. There are many methods to evaluate the drought resistance of plants, such as the affiliation function method, hierarchical evaluation, grey correlation analysis and principal component analysis (Zhang et al. 2018, Du et al. 2020). Among them, the affiliation function method can make a more comprehensive and integrated evaluation based on the determination of multiple indicators, avoiding the limitations and inaccuracies of a single evaluation indicator, and is the most widely used in drought resistance evaluation (Sio-Se Mardeh et al. 2006). In recent years, there has been a preference for combining multiple evaluation methods to improve the accuracy of the results (Lu et al. 2011). Seedlings are sensitive to drought stress at the beginning of their development, which not only threatens their survival but also affects their later growth, biomass formation and ability to overwinter (Ditmarová et al. 2010). Therefore, by studying the physiological and biochemical characteristics of oak seedling leaves under drought stress and combining various evaluation methods, we can evaluate drought resistance with greater reliability.
The area of *Quercus* accounts for 13.7% of the total area of natural forests in China and is the main component of natural forests in China (Liu et al. 2016). The *Quercus* plant genus plays an important role in water conservation, soil and water conservation and ecological stability (Liu et al. 2019). *Quercus fabri* Hance, *Quercus serrata* Thunb, *Quercus acutissima* Carruth and *Quercus variabilis* Bl are the dominant species in deciduous broad-leaved forests and mixed coniferous forests in temperate and warm temperate regions of China and are also the forest building blocks in most parts of the country, with northern China as the centre of distribution (Wu et al. 2012, Yang et al. 2018, Xiong et al. 2020). Oak tree wood is dense and beautifully textured, resistant to wear and decay and is often used as a high-quality material for building and household purposes (Blanchet et al. 2008). Oak tree bark can be used for extracting tannins, young leaves can be used to raise sericulture, and dead branches can also be used to cultivate valuable medicinal herbs such as edible mushrooms and ganoderma lucidum (Silva et al. 2016). Acorns are an important food resource for wildlife and have become an internationally popular green food in recent years (Park et al. 2016). At present, most research on oak trees has focused on the nutritional and mineral contents of the fruit, the distribution patterns of populations in individual areas and genetic diversity (Yang et al. 2016, Chen et al. 2021, Szabłowska and Tańska 2021). However, in the process of artificial afforestation, seedlings transplanted to mountains often suffer from seasonal drought, which seriously affects growth and development. This leads to a low survival rate and poor afforestation effects. Understanding how drought resistance varies across different oak species is helpful for reasonable cultivation and extension. In this study, four common oak species were selected, and the photosynthetic capacity, osmotic regulation substance content and antioxidant enzyme activity of the leaves were studied under short-term natural drought stress and rehydration processes. The differences in the response mechanisms of the four oak species to drought stress were preliminarily investigated, and drought resistance was comprehensively evaluated to provide a theoretical basis for extensive cultivation and promotion.

2. Materials And Methods

2.1 Plant materials

The seeds of *Q. fabri*, *Q. serrata*, *Q. acutissima* and *Q. variabilis* were collected from Wanzhou, Chongqing, China, in October 2018 (30°38′N, 108°37′E). Then, the seeds were germinated in a sand bed, and the seedlings were transplanted to a light net bag container after rooting. The substrate of choice was a mixture of perlite and peat moss at a 1:3 ratio. The seedlings were cultivated in experimental greenhouses with automatic spray watering and natural light. Robust, disease-free seedlings were selected and transferred to a 21 cm×16 cm×21 cm (upper diameter × lower diameter × height) container in August 2019. One seedling was planted in each pot, and the soil weight was 5 kg. The cultivated substrate was nursery soil, the organic matter content was 54.07 g/kg, the fast nitrogen was 0.16 g/kg, the fast phosphorus was 0.04 g/kg, and the fast potassium was 0.16 g/kg. Routine field management of the planted seedlings was conducted without fertiliser. After one month of recovery, seedlings with similar growth were selected for natural soil drought stress and rehydration tests. Seasonal drought occurred
frequently in Chongqing, China, and the choice of local oak species could better reflect the difference in response to seasonal drought stress.

2.2 Experimental design

There were three replicates for each treatment, with 10 pots for each replicate, for a total of 120 pots, which were randomly divided into a drought-rewatering treatment group (D-RW) and a normal watering control group (CK). All pots were fully watered one day before the start of the treatment. The D-RW group was not watered again, lost water naturally and dried-out, while the CK group was watered normally. The D-RW group was rehydrated after one month. Leaf samples were taken at approximately 9:00 AM on days 1, 6, 11, 16, 21, 26, 31 and 36 of treatment, with consistent sampling sites each time (3rd-5th from the tip of the branch down). The leaves were removed from the petioles, placed in self-sealing bags, numbered, and transported back to the laboratory in ice boxes at 0–4°C. Some of the fresh samples were taken for water potential measurement, and the remaining parts were quickly ground with liquid nitrogen and stored in an ultralow temperature refrigerator at -80°C for the determination of relevant physiological and biochemical parameters. Each indicator was repeated three times for each oak seedling species. Soil moisture was monitored using a TDR200 soil moisture sensor.

2.3 Leaf relative water content and water potential

Three leaves at the same position in each treatment group were selected and weighed, and the weight was recorded as the fresh weight (FW). Then, the leaves were placed in the dark in pure water for 24 h. The leaves were then removed to dry the water on the surface of the leaves and then were weighed, and the weight was recorded as the saturated fresh weight (TW). Finally, the leaves were placed in an oven at 85°C and dried for 48 h to obtain a constant weight. Then, the leaves were removed and weighed, and the weight was recorded as the dry weight (DW). The RWC of the leaves was calculated according to the formula: \( \text{RWC(\%)} = \frac{(\text{FW-dW}/\text{TW-dW}) \times 100\%}{1} \). A WP4C dew point water potential metre (Psypro, Wescor Company, USA) was used to measure the leaf water potential. The water potential probe was clamped on each test sample, and rubber cement was added around the probe to form a closed space, which was the measurement chamber. The data were read every 5 minutes, and when the value was stable, the value was recorded 3 times to determine the average value, which was the leaf water potential.

2.4 Measurements of osmotic regulatory substances

The Pro content was measured using the ninhydrin colorimetric method (G0111W Assay Kit, Suzhou Geruisi, China). The SS content was measured by the anthrone sulphate method (G0501W Assay Kit, Suzhou Geruisi, China). The Soluble protein (SP) content was measured using the Komas Brilliant Blue G250 staining method (G0417W Assay Kit, Suzhou Geruisi, China). The GB content was measured using the cycle colorimetric method (G0122W Assay Kit, Suzhou Geruisi, China).

2.5 Measurements of antioxidant enzyme activity and malondialdehyde (MDA) content
The SOD activity was determined by the nitrogen blue tetrazole (NBT) method (G0101W Assay Kit, Suzhou Geruisi, China). The CAT activity was determined by sodium thiosulfate titration (G0105W Assay Kit, Suzhou Geruisi, China). The POD activity was determined by the peroxidase chromatographic method (G0107W Assay Kit, Suzhou Geruisi, China). The MDA content was determined by thiobarbituric acid colorimetry (G0109W Assay Kit, Suzhou Geruisi, China).

2.6 Photosynthetic parameters

After the experiment, the photosynthetic physiological parameters of the leaves were measured at 9:30 – 11:30 AM every five days. The upper, healthy, sunny-side leaves of the seedlings were observed using a LI-COR 6400 system (Li-COR, Lincoln, NE, USA). The air flow rate was set at 500 µmol/s, and the data were read after 2 min of stabilisation to record the net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs).

2.7 Statistical analyses

All the data were tested for normality and Leneve’s test. The data were recorded and processed using Excel, and statistical analysis was carried out using SPSS 19.0. One-way analysis of variance (ANOVA) was used, and differences between treatments were analysed using Duncan's multiple comparisons (p < 0.05). GraphPad Prism 9 and JASP 0.14.1 were used for plotting data. For principal component analysis (PCA), the number of principal components is usually selected as the variable containing more than 80% information; that is, the cumulative contribution rate of characteristic roots should be greater than 80%. The drought tolerance of four oak species was evaluated by the membership function method. When the indicator was positively correlated with drought resistance, the formula was $U(X_{ij}) = (X_{ij} - X_{j\text{min}})/(X_{j\text{max}} - X_{j\text{min}})$. When the indicator was negatively correlated with drought resistance, the formula was $U(X_{ij}) = 1 - (X_{ij} - X_{j\text{min}})/(X_{j\text{max}} - X_{j\text{min}})$. The overall evaluation value was calculated as $X_i = \Sigma U(p_mX_{ij})/n$. In the formula, $X_{ij}$ is the measured value of an index of a certain oak tree. $X_{j\text{max}}$ and $X_{j\text{min}}$ are the minimum and maximum values of the index. $p_m$ is the weight coefficient of the m-th principal component, and n is the number of indicators (Xiu et al. 2019). These values were expressed as the mean ± standard error (SE) of three replicate samples.

3. Results

3.1 Soil moisture content

The soil moisture content of the four oak control groups ranged from 28.0–31.9%. With continuous drought stress, the soil moisture content of the treatment group decreased gradually, and the range of the decrease was similar. The soil moisture content reached the lowest point of 3.1% ~ 3.5% at day 31 of drought stress and returned to the control level after rewatering (Fig. 1).

3.2 Water potential and RWC
The RWC and water potential of the leaves of all four oak species tended to decrease under sustained drought stress compared to those of the control group. The values of the treated groups were not significantly different from those of the control group on the first day, but all decreased and reached the lowest values, which were significantly lower than those of the control group, on day 31 ($P<0.05$). After rewatering, the leaf water potential increased rapidly, and the recovery rate of the leaf water potential was faster than that of RWC on day 36. The RWC and water potential of *Q. fabri* leaves decreased from 81.13% and $-1.52$ MPa (day 1) to 41.10% and $-2.84$ MPa (day 31), with decrease rates of 49.47% and 87.25%, respectively. After rewatering, the RWC and water potential recovered to 73.02% and $-1.67$ MPa (day 36), respectively, but did not recover to the normal level. The RWC and water potential of *Q. serrata* leaves decreased from 81.67% and $-1.48$ MPa (day 1) to 48.67% and $-2.45$ MPa (day 31), with decrease rates of 40.41% and 66.14%, respectively. After rewatering, the RWC and water potential recovered to 76.02% and $-1.52$ MPa (day 36), respectively. The RWC and water potential of *Q. acutissima* leaves decreased from 81.72% and $-1.43$ MPa (day 1) to 43.23% and $-2.46$ MPa (day 31), with decrease rates of 47.10% and 72.03%, respectively. After rewatering, the RWC and water potential recovered to 72.72% and $-1.54$ MPa (day 36), respectively. The RWC and water potential of *Q. variabilis* leaves decreased from 81.02% and $-1.52$ MPa (day 1) to 38.07% and $-2.48$ MPa (day 31), with a decrease rate of 53.10% and 63.16%, respectively. After rewatering, the RWC and water potential recovered to 73.67% and $-1.57$ MPa (day 36), respectively.

The leaf water potential of *Q. serrata*, *Q. acutissima* and *Q. variabilis* returned to normal levels, but the RWC remained lower than the normal level (Fig. 2). Fig. 2: Effects of drought stress and rewatering on relative water content and water potential of four oak species (a, relative water content; b, water potential). DR-F: drought-rewatering group of *Q. fabri*, CK-F: normal watering group of *Q. fabri*, DR-S: drought-rewatering group of *Q. serrata*, CK-S: normal watering group of *Q. serrata*, DR-A: drought-rewatering group of *Q. acutissima*, CK-A: normal watering group of *Q. acutissima*, DR-V: drought-rewatering group of *Q. variabilis*, CK-V: normal watering group of *Q. variabilis*. Different lowercase letters indicate significant ($P<0.05$) differences among the four oak species subjected to the same treatment time.

### 3.3 Osmotic regulating substances

Under continuous drought stress, the levels of soluble protein, SS and Pro in the four oak species first increased, then decreased, and then increased after rehydration. However, GB level increased continuously and decreased after rehydration. The SS levels of *Q. fabri*, *Q. acutissima* and *Q. variabilis* (Day 6) peaked earlier than that of *Q. serrata* (day 11). Compared with the control group, the SS level of the four oak species increased by 1.50, 1.28, 1.15 and 1.11 times, respectively. Then, the SS levels of the four oak species were the lowest on day 31 and were significantly lower ($P<0.05$) than that of the control group, decreasing by 43.74%, 48.75%, 42.52% and 51.60%, respectively. After rewatering, only *Q. fabri* returned to a normal level, while the levels in the other three oak species were significantly lower than normal ($P<0.05$) (Fig. 3a). There were no significant differences in the SS levels when comparing between the four oak species and the control group at the early stage (day 1 and day 6) ($P>0.05$). On day 26, the SS level in each of the four oak species reached a peak, which was 1.46, 1.48, 1.35 and 1.37 times higher than that of the control group, respectively. Then, the levels decreased and were the lowest on day
31, decreasing by 15.44%, 12.79%, 10.62% and 14.22%, respectively. After rewatering, the SS level of *Q. serrata* was significantly lower than the normal level (*P* < 0.05), but the other three oak species returned to normal levels (Fig. 3b). The Pro level in the four oak species reached a peak on day 26 and was significantly higher than that in the control group (*P* < 0.05), increasing by 1.89, 1.66, 1.98 and 1.87 times, respectively. Then, the Pro level decreased but was still significantly increased compared with the level in the control group (day 31) (*P* < 0.05) and returned to the normal level after rehydration (Fig. 3c). The GB level in the four oak species showed a continuous increasing trend, which was significantly higher than that in the control group (*P* < 0.05), increasing by 1.56, 2.05, 1.96 and 2.29 times, respectively. After rehydration, the GB level decreased sharply but was still significantly higher than that in the control group (*P* < 0.05), increasing by 1.28, 1.50, 1.65 and 1.98 times, respectively (Fig. 3d).

**Figure 3** Effects of drought stress and rewatering on osmotic regulating substances content of four oak species (**a**, soluble protein; **b**, soluble sugar; **c**, proline; **d**, glycline betaine). DR-F: drought-rewatering group of *Q. fabri*; CK-F: normal watering group of *Q. fabri*; DR-S: drought-rewatering group of *Q. serrata*; CK-S: normal watering group of *Q. serrata*; DR-A: drought-rewatering group of *Q. acutissima*; CK-A: normal watering group of *Q. acutissima*; DR-V: drought-rewatering group of *Q. variabilis*; CK-V: normal watering group of *Q. variabilis*. Different lowercase letters indicate significant (*P* < 0.05) differences among the four oak species subjected to the same treatment time.

### 3.4 Antioxidant enzyme activities and MDA

Under continuous drought stress, the activities of POD, SOD and CAT of the four oak species first increased, then decreased, and then increased after rehydration. The MDA level increased continuously and decreased after rehydration. The POD activity of *Q. fabri*, *Q. acutissima* and *Q. variabilis* (day 16) reached a peak later than that of *Q. serrata* (day 11), which was 1.36, 1.11, 1.34 and 1.18 times higher than that of the control group, respectively. The POD activity levels of the four oak species were the lowest on day 31 and were significantly lower than that of the control group (*P* < 0.05). The POD activity levels were decreased by 18.34%, 32.21%, 34.74% and 36.65%, respectively, and did not return to normal levels after rehydration (Fig. 4a). The SOD activity level of the four oak species was significantly different from that of the control group at day 5 (*P* < 0.05) and peaked at day 21. The SOD activity levels were 2.14, 2.33, 1.93 and 2.28 times higher than that of the control group, respectively. Then, the SOD activity levels were the lowest at day 31 and were significantly lower than that in the control group (*P* < 0.05). The SOD activity levels were reduced by 32.55%, 29.26%, 37.10% and 20.73%, respectively, and did not recover to normal levels after rehydration (Fig. 4b). The CAT activity levels of *Q. fabri*, *Q. acutissima* and *Q. variabilis* (day 21) reached a peak later than that of *Q. serrata* (day 16). The CAT activity levels were 1.54, 1.48, 1.52 and 1.39 times higher than that of the control group, respectively. The CAT activity levels of the four oak species were the lowest on day 31 and were significantly lower than that of the control group (*P* < 0.05). The CAT activity levels decreased by 25.13%, 20.61%, 8.12% and 11.43%, respectively. After rehydration, only *Q. fabri* returned to the normal CAT activity level, but the levels of the other three oaks were significantly lower than normal (*P* < 0.05) (Fig. 4c). The MDA levels of the four oak species showed a continuous growth trend, and there were no significant differences in the levels at the early stage (day 1
and day 6) when comparing between the four oak species and the control group ($P > 0.05$). The MDA levels peaked on day 31 and were significantly higher than that in the control group ($P < 0.05$), increasing by 2.71, 2.15, 2.23 and 2.06 times, respectively. The MDA levels decreased sharply after rehydration but were still significantly higher than that in the control group ($P < 0.05$), increasing by 1.38, 1.41, 1.36 and 1.45 times, respectively (Fig. 4d).

### 3.5 Photosynthetic parameters

Compared with the control group, the $P_n$, $G_s$ and $T_r$ values for the four oak species showed a decreasing trend under continuous drought stress. The values were the lowest on day 31 and rose rapidly after rehydration. As drought stress was prolonged, the $P_n$ values of the four oak species decreased continuously. The $P_n$ of *Q. serrata* at day 6 was significantly different from that of the control group ($P < 0.05$). The $P_n$ values of the four oak species decreased to the lowest level on day 31 and were significantly lower than that of the control group ($P < 0.05$). The $P_n$ values decreased by 72.10%, 63.29%, 63.67% and 60.16%, respectively. After rehydrating, the $P_n$ of *Q. serrata* was significantly lower than normal ($P < 0.05$), but the $P_n$ values of the other three oak species returned to normal (Fig. 4a). There were no significant differences in the $G_s$ values when comparing between the four oak species and the control group at the early stage (day 1 and day 6) ($P > 0.05$). The $G_s$ values of the four oak species were the lowest on day 31 and were significantly lower than that of the control group ($P < 0.05$). The $G_s$ values decreased by 71.14%, 57.45%, 50.03% and 45.24%, respectively, but returned to normal after rehydration (Fig. 4b). There were significant differences in the $T_r$ values when comparing between the four oak species and the control group at day 5 ($P < 0.05$). The $T_r$ values were the lowest on day 31 and were significantly lower than that of the control group ($P < 0.05$). The $T_r$ values decreased by 62.37%, 60.08%, 61.28% and 60.72%, respectively, and did not return to normal after rehydration (Fig. 4c).

### 3.6 Multivariate statistical analysis

The eigenvalues and contribution rates of principal components were the basis for selecting principal components. The 13 physiological and biochemical indexes of the leaves from the 4 oak species were analysed by PCA. Two principal components with eigenvalues greater than 1 were obtained, and their contribution rates were 64.24% and 19.70%, respectively. The cumulative contribution rate was 83.94%, and most of the information on the original characteristics was retained (Table 1). Therefore, the first two principal components could be selected as the important principal components of the drought resistance of the four oak species. The factors with higher loading capacity in the first principal component were $\Psi_w$, $RWC$, $Pro$, $SP$, $GB$, $MDA$, $P_n$, $GS$ and $Tr$, which were mainly related to leaf water status, osmoregulatory substances and photosynthesis. The second principal component was mainly related to antioxidant enzymes. The treatments of the 4 oaks under drought stress and during rehydration were completely separated. In addition, *Q. acutissima*, *Q. serrata* and *Q. variabilis* highly overlapped under drought stress and during rehydration. In contrast, *Q. fabri* varied greatly with the other three species (Fig. 5). To further understand the relationship between leaf water status, osmotic regulatory substances, photosynthesis and antioxidant enzymes, Pearson correlation analysis was used to analyse the data. The results showed
that Pn, GS and Tr positively correlated with each other, negatively correlated with MDA, GB and Pro levels and positively correlated with SP, Ψw and RWC. Ψw and RWC levels were significantly negatively correlated with Pro, GB and MDA levels. MDA levels were significantly positively correlated with GB and Pro levels and negatively correlated with SP levels and POD activities. Pro levels were significantly negatively correlated with SP levels and positively correlated with SS and GB levels. In addition, SOD, POD and CAT activities were significantly positively correlated (Fig. 6). To comprehensively evaluate the drought resistance of the four oak species, 13 physiological and biochemical indexes of the four oak species under continuous drought stress and rehydration conditions were analysed by membership functions (Table 2). The membership function value of each index was calculated according to a formula. The degree of correlation between different indexes and drought resistance was different. The arithmetic mean of the membership value of each index did not fully reflect the level of drought resistance in the four oak species. Therefore, the proportion of eigenvalues corresponding to each principal component to the sum of the total eigenvalues of the extracted principal components was taken as the weight. The comprehensive evaluation value of the four oak species was calculated. Higher comprehensive evaluation values were positively correlated with stronger drought-resistance ability. The results showed that the order of drought resistance of the four oak species were as follows: Q. serrata > Q. fabri > Q. variabilis > Q. acutissima.
Table 1
Eigenvalue and cumulative contribution rate of each index of four oak species

| Measured index       | Principal component |
|----------------------|---------------------|
|                      | PC1     | PC2     |
| Relative water content| 0.335   | -0.014  |
| Water potential      | 0.326   | 0.018   |
| Soluble sugar        | -0.174  | 0.365   |
| Soluble protein      | 0.276   | 0.084   |
| Glycine betaine      | -0.309  | -0.134  |
| Pro                  | -0.322  | 0.099   |
| SOD                  | -0.083  | 0.551   |
| POD                  | 0.150   | 0.447   |
| CAT                  | -0.142  | 0.540   |
| MDA                  | -0.311  | -0.176  |
| Pn                   | 0.331   | 0.041   |
| Gs                   | 0.334   | 0.045   |
| Tr                   | 0.328   | -0.024  |
| Eigenvalue           | 8.352   | 2.562   |
| Cumulative contribution rate (%) | 64.24   | 83.94   |
Table 2
Membership function values and evaluation index of the drought resistance of four oak species

| Item                | Q. fabri | Q. serrata | Q. acutissima | Q. variabilis |
|---------------------|----------|------------|---------------|---------------|
| Relative water content | 0.3340   | 0.2399     | 0.2314        | 0.2325        |
| Water potential     | 0.4529   | 0.5773     | 0.4193        | 0.3608        |
| Soluble sugar       | 0.3002   | 0.2288     | 0.3272        | 0.2844        |
| Soluble protein     | 0.0900   | 0.0883     | 0.0863        | 0.0823        |
| Glycine betaine     | 0.3613   | 0.3867     | 0.1349        | 0.1464        |
| Pro                 | 0.1980   | 0.2321     | 0.3348        | 0.3919        |
| SOD                 | 0.0777   | 0.1053     | 0.0780        | 0.1094        |
| POD                 | 0.1384   | 0.0909     | 0.1013        | 0.0920        |
| CAT                 | 0.1125   | 0.1110     | 0.1236        | 0.1305        |
| MDA                 | 0.4770   | 0.5235     | 0.4702        | 0.5055        |
| Pn                  | 0.4322   | 0.4225     | 0.4932        | 0.5193        |
| Gs                  | 0.4354   | 0.4725     | 0.5329        | 0.4984        |
| Tr                  | 0.3608   | 0.3990     | 0.3285        | 0.3118        |
| Comprehensive evaluation | 0.2900   | 0.2983     | 0.2817        | 0.2819        |
| Sequencing          | 2        | 1          | 4             | 3             |

4. Discussion

4.1 Leaf moisture status

Drought stress can lead to a decrease in the RWC and water potential of plant leaves (Ritchie et al. 1990). It is generally believed that smaller decreases in the RWC and water potential of plant leaves correlate with higher water-retaining ability and stronger adaptability of the leaves to drought stress (Schonfeld et al. 1988). In our research, under continuous drought stress, the relative water content and water potential of the four oak species decreased to different degrees, which indicated that the four oak species could absorb more water by reducing $\Psi_w$ to resist drought stress. Among them, the RWC of *Q. serrata* declined the least and recovered the fastest after rehydration. *Q. variabilis* showed the fastest decrease in RWC but the smallest decrease in $\Psi_w$. The $\Psi_w$ of *Q. fabri* decreased the most and recovered the slowest after rehydration. These results indicate that *Q. serrata* can maintain a higher water balance than the other three oak species. *Q. serrata* leaves may be more leathery than the leaves from the other species, which would be beneficial for reducing water transpiration during drought stress. The results herein were similar
to those from studies of soybean (Du et al. 2020) and *Cyamopsis tetragonoloba* (L.) Taub. (Upreti et al. 2021). This study also found that $\Psi_w$ and RWC were highly significantly positively correlated with Pn, Gs and Tr. This indicates that under continuous drought stress, the water potential of plant leaves decreases with a constant decrease in water content, leading to a decrease in photosynthesis.

### 4.2 Osmotic regulating substances

Plants under drought stress are often damaged by osmotic stress. When cells lose water, it can decrease turgor pressure and lead to death (Xiong and Zhu 2002). Under a certain degree of drought stress, plants can maintain turgor pressure by accumulating osmotic substances, such as SS, Pro, GB and SP (Gao et al. 2020). In our research, the SS, Pro, GB and SP levels of the four oak species all showed an increasing trend in the early stages of sustained drought stress. The results showed that the four oak species reduced their osmotic potential by accumulating osmotic adjustment substances during early stages of drought stress to ensure that water could be absorbed from the external environment and to resist drought-induced damage. Soluble proteins respond faster than other osmotic regulators, similar to the results of Cheng et al (Cheng et al. 2018). However, osmotic regulation cannot unlimitedly regulate the osmotic pressure of cells. When drought stress reached the limit of plant self-regulation, we found that the levels of Pro and SP of the four oak species began to decline at day 26. It is possible that the stress damage exceeded the tolerance limit, which blocked normal plant metabolism and then affected the production of osmotic adjustment substances. Similar results were observed in *Capsicum spp.* (Okunlola et al. 2017), in which the contents of SP and SS decreased under severe drought stress. At present, a large number of studies have shown that GB has multiple functions in resisting drought stress, including scavenging ROS, maintaining the stability of biofilms, and protecting the photosynthetic apparatus (Quan et al. 2004, Räsänen et al. 2004). In this study, we found that GB increased continuously in the four oak species, indicating that they could stimulate their own GB synthesis under drought stress to resist the damage caused by stress. After rehydration, the Pro levels of the four oak species all returned to normal, while the other three osmotic regulatory substances recovered to different extents in the different tree species. Among them, in *Q. fabri*, the levels of SP, SS and Pro returned to normal. In *Q. acutissima* and *Q. variabilis*, the levels of SS and Pro returned to normal. However, in *Q. serrata*, only the Pro level returned to the normal level.

### 4.3 Antioxidant enzyme activities and MDA

Under normal conditions, there is a dynamic equilibrium between the production and clearance of ROS in plants, but when plants are under drought stress, the dynamic equilibrium is disrupted (Zhang et al. 2018). The excessive accumulation of ROS can damage cells and cause oxidative deterioration of cell membranes, which may lead to plant death in severe cases. There is an active oxygen scavenging system in plants, in which SOD, POD and CAT are important antioxidant enzymes for scavenging ROS (Shawon et al. 2020). In our research, the activities of POD, SOD and CAT enzymes in the four oak species showed a trend of increasing first and then decreasing. The results showed that all four oak species could resist the damage caused by drought stress by enhancing the activity of antioxidant enzymes. However, as the drought stress was intensified, the ROS production levels exceeded the scavenging abilities of the plants,
and the activities of the three protective enzymes all decreased to varying degrees. Similar results were observed in *Handeliodendron bodinieri* (Levl.) (Leng et al. 2020), *Olea europaea* L (Denaxa et al. 2020). We also found that the SOD activity increased significantly at day 6. The results showed that SOD was the first to respond to the early stages of drought stress, and then POD and CAT enzymes responded. Compared with the control group, there were no significant differences in the MDA levels of the four oak species at day 6. The results indicated that the three enzymes could effectively eliminate the production of reactive oxygen species in the early stages of drought stress. However, in the late stages of drought stress, the MDA levels in the four oak species were significantly higher than that of the control group ($P < 0.05$). It is possible that the four oak species suffered from long-term drought stress, which damaged the antioxidant enzyme system to different degrees. ROS overaccumulation may have destroyed the cell membranes, which may have caused MDA to accumulate in large quantities.

4.4 Photosynthetic parameters

Drought stress can also affect the photosynthetic physiology of plant leaves, which is mainly reflected by the chlorophyll content, Pn and Tr (Xiong et al. 2020). In this study, the Pn, Gs and Tr values of the four oak species showed a continuous decreasing trend as drought stress was prolonged and were significantly lower than those of the control group at day 31 ($P < 0.05$). This result shows that photosynthesis was inhibited in the four oak species under continuous drought stress, which might be due to the production and accumulation of a large number of reactive oxygen species in the leaves. Accumulation of ROS results in damage to the mesophyll cell membrane, destruction of photosynthetic enzyme activity and a decrease in photosynthesis. In addition, at day 31, the Pn, Gs and Tr values of *Q. fabri* were the lowest among the four oak species, the Pn and Tr values of *Q. serrata* were the highest, and the Gs value of *Q. variabilis* was the highest. The results showed that the resistance of *Q. fabri* photosystems was the worst, and *Q. serrata* was the strongest. It may be that the osmoregulation (especially the GB content) of *Q. serrata* is stronger than that of the other three species, and the decrease in RWC is the smallest, which greatly reduces the damage to the photosystem. After rehydration, there was no significant difference in Gs, but the Tr value was still significantly lower than that of the control group. With the exception of *Q. fabr*, there were no significant differences in the Gs values when comparing the four oak species with the control group. The results showed that the recovery ability of the *Q. serrata* photosystem was weaker than that of the other three oak species. *Q. serrata* may have weak repair ability after severe drought, which was indicated by the osmotic regulatory substance recovery results.

4.5 Multivariate statistical analysis

The four oak species are distributed in different regions in China. In the long-term adaptation and evolution of the geographical environment, each species has a different level of drought resistance. The main differences are in the water parameters, osmotic regulation, antioxidant enzyme activity and photosynthesis. The changes in the physiological and biochemical indexes of the four oak species were complex and varied under drought stress, which indicated that different oak species have different ways to adapt to drought stress. The PCA results showed that the boundary between the drought rehydration
group and the control group was clear, and the PC1 components of the four oak species showed the same trend during the drought rehydration period, indicating similar adaptation to drought stress. Correlation analysis showed that there were complex and close relationships between the leaf water status, osmotic regulatory substances, antioxidant enzyme activities and photosynthesis, and they cooperated with each other to resist drought stress. Our results showed that there were significant correlations between osmotic regulatory substances and photosynthesis and between water parameters and antioxidant enzyme activities. In addition, there were significant correlations between photosynthesis and water parameters and between antioxidant enzyme activities and MDA levels. The sensitivity of different oak species to drought stress is different, so the response time is also different, and the trees may be in different response stages simultaneously (Fang and Xiong 2015). During continuous drought stress and rehydration, plants experience stress, adaptation, injury and repair. The comprehensive adjustment of different response mechanisms in different stress stages constitutes the overall drought resistance of plants (Bhusal et al. 2020). The drought resistance of plants is the result of multiple factors. It is difficult to accurately and comprehensively reflect the drought resistance of plants with only one drought resistance index. The ranking results of a specific oak tree under different drought resistance indexes can vary greatly, so it is difficult to reasonably evaluate drought resistance according to a single index. Therefore, the weights of each indicator are calculated by PCA and then through the membership function method to comprehensively evaluate each index. The evaluation result can approximate the actual result. The drought resistance levels of the four oak species were as follows: *Q. serrata* > *Q. fabri* > *Q. variabilis* > *Q. acutissima*.

5. Conclusion

This study simulated continuous drought stress that occurs under natural conditions. Changes in water parameters, osmotic regulation substance content, antioxidant enzyme activity and photosynthesis were observed dynamically. It was discovered that four oak species showed adaptive changes to drought stress and resisted early stages of drought stress by increasing the level of osmotic regulation substances and regulating the activity of antioxidant enzymes. However, with prolonged drought stress, all four oak species reached a tolerance limit. Water content, osmotic regulatory substance content, antioxidant enzyme activity and photosynthetic parameters all decreased to different degrees. The recovery ability of the four oak species also differed after rehydration. Multivariate statistical analysis showed that there were complex and close relationships between the leaf water status, osmotic regulatory substances, antioxidant enzyme activities and photosynthesis, and they cooperated with each other to resist drought stress. PCA and the membership function method were used to analyse 13 physiological and biochemical indexes under continuous drought stress and rehydration. The drought resistance levels of the four oak species were as follows: *Q. serrata* > *Q. fabri* > *Q. variabilis* > *Q. acutissima*.

Abbreviations
RWC: Relative water content; \( \Psi_w \): Water potential; Pn: Net photosynthetic rate; Tr: Transpiration rate; Gs: Stomatal conductance; ROS: Reactive oxygen species; Pro: Proline; SP: Soluble protein; SS: Soluble sugar; GB: Glycine betaine; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; MDA: Malondialdehyde; PCA: Principal component analysis

Declarations

Authors’ contributions

Shifa Xiong, Yangdong Wang and Liwen Wu contributed to the conception and design of the study; Yicun Chen, Ming Gao and Yunxiao Zhao supervised the experiments and organized the data and performed the statistical analysis. Shifa Xiong and Liwen Wu wrote the manuscript. All authors approved the submitted version.

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Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S (2010) Roles of enzymatic and nonenzymatic antioxidants in plants during abiotic stress. Crit Rev Biotechnol 30:161–175. https://10.3109/07388550903524243

2. Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. Afr J Agr Res 6(9):2026–2032. https://10.5897/AJAR10.027
3. Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnol Adv 27:84–93. https://10.1016/j.biotechadv.2008.09.003

4. Bandeppa S, Paul S, Thakur JK, Chandrashekar N, Umesh DK, Aggarwal C, Asha AD (2019) Antioxidant, physiological and biochemical responses of drought susceptible and drought tolerant mustard (Brassica juncea L) genotypes to rhizobacterial inoculation under water deficit stress. Plant Physiol Bioch 143:19–28. https://10.1016/j.plaphy.2019.08.018

5. Ben-Gal A, Agam N, Alchanatis V, Cohen Y, Yermiyahu U, Zipori I, Presnov E, Sprintsin M, Dag A (2009) Evaluating water stress in irrigated olives: correlation of soil water status, tree water status, and thermal imagery. Irrigation Sci 27:367–376. https://10.1007/s00271-009-0150-7

6. Blanchet P, Beauregard R, Belleville B (2008) Prefinished oak flooring surface checking and physical properties. Forest Prod J 58:25–28. https://10.1007/s10570-007-9194-0

7. Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot-london 103:551–560. https://10.1093/aob/mcn125

8. Chen X, Yang J, Guo Y, Zhao Y, Zhou T, Zhang X, Ju M, Li Z, Zhao G (2021) Spatial Genetic Structure and Demographic History of the Dominant Forest Oak Quercus fabri Hance in Subtropical China. Front Plant Sci 11:583284. https://10.3389/fpls.2020.583284

9. Cheng L, Han M, Yang LM, Yang L, Sun Z, Zhang T (2018) Changes in the physiological characteristics and baicalin biosynthesis metabolism of Scutellaria baicalensis Georgi under drought stress. Ind Crop Prod 122:473–482. https://10.1016/j.indcrop.2018.06.030

10. Denaxa N, Damvakaris T, Roussos PA (2020) Antioxidant defense system in young olive plants against drought stress and mitigation of adverse effects through external application of alleviating products. Sci Hortic-amsterdam 259:108812. https://10.1016/j.scienta.2019.108812

11. Ditmarová Ł, Kurjak D, Palmroth S, Kmet J (2010) Physiological responses of Norway spruce (Picea abies) seedlings to drought stress. Tree Physiol 30(2):205–213. https://10.1093/treephys/tpp116

12. Du Z, Hu Y, Buttar NA (2020) Analysis of mechanical properties for tea stem using grey relational analysis coupled with multiple linear regression. Sci Hortic-amsterdam 260:108886. https://10.1016/j.scienta.2019.108886

13. Fang Y, Xiong L (2015) General mechanisms of drought response and their application in drought resistance improvement in plants. Cell Mol Life Sci 72:673–689. https://10.1007/s00018-014-1767-0

14. Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agron Sustain Dev 29:185–212. https://10.1051/agro:2008021

15. Gao S, Wang Y, Yu S, Huang Y, Liu H, Chen W, He X (2020) Effects of drought stress on growth, physiology and secondary metabolites of Two Adonis species in Northeast China. Sci Hortic-amsterdam 259:108795. https://10.1016/j.scienta.2019.108795

16. Gechev TS, Van Breusegem F, Stone JM, Denev I, Laloí C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bioessays 28:1091–1101. https://10.1002/bies.20493
17. Huang H, Ullah F, Zhou D, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci 10:800. https://10.3389/fpls.2019.00800

18. Koyro HW (2006) Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte Plantago coronopus (L.). Environ Exp Bot 56:136–146. https://10.1016/j.envexpbot.2005.02.001

19. Kruskopf M, Flynn KJ (2006) Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. New Phytol 169(3):525–536. https://10.2307/3694692

20. Leng X, Xue L, Wang J, Li S, Yang Z, Ren H, Yao X, Wu Z, Li J (2020) Physiological responses of Handeliodendron bodinieri (Levl.) Rehd. to exogenous calcium supply under drought stress. Forests 11:69. https://10.3930/f11010069

21. Li R, Geng S (2013) Impacts of climate change on agriculture and adaptive strategies in China. J Integr Agr 12:1402–1408. https://10.1016/S2095-3119(13)60552-3

22. Liu Y, Liu S, Miao R, Liu Y, Wang D, Zhao C (2019) Seasonal variations in the response of soil CO$_2$ efflux to precipitation pulse under mild drought in a temperate oak (Quercus variabilis) forest. Agr Forest Meteorol 271:240–250. https://10.1016/j.agrformet.2019.03.009

23. Liu Y, Liu S, Wan S, Wang J, Luan J, Wang H (2016) Differential responses of soil respiration to soil warming and experimental throughfall reduction in a transitional oak forest in central China. Agr Forest Meteorol 226:186–198. https://10.1016/j.agrformet.2016.06.003

24. Lu Y, Hao Z, Xie C, Crossa J, Araus J, Gao S, Vivek BS, Magorokosho C, Mugo S, Makumbi D, Taba S, Pan G, Li X, Rong T, Zhang S, Xu Y (2011) Large-scale screening for maize drought resistance using multiple selection criteria evaluated under water-stressed and well-watered environments. Field Crop Res 124:37–45. https://10.1016/j.fcr.2011.06.003

25. Medina E, Garcia V, Cuevas E (1990) Sclerophyllly and oligotrophic environments: relationships between leaf structure, mineral nutrient content, and drought resistance in tropical rain forests of the upper Rio Negro region. Biotropica 22:51–64. https://10.2307/2388719

26. Bhusal N, Lee M, Han AR, Han A, Kim HS (2020) Responses to drought stress in Prunus sargentii and Larix kaempferi seedlings using morphological and physiological parameters. For Ecol Manag 465:118099. https://doi.org/10.1016/j.foreco.2020.118099

27. Okunlola GO, Olatunji OA, Akinwale RO, Tariq A, Adelusi AA (2017) Physiological response of the three most cultivated pepper species (Capsicum spp.) in Africa to drought stress imposed at three stages of growth and development. Sci Hortic-amsterdam 224:198–205. https://10.1016/j.scienta.2017.06.020

28. Park S, Hongu N, Daily JW (2016) Native American foods: History, culture, and influence on modern diets. Journal of Ethnic Foods 3:171–177. https://10.1016/j.jef.2016.08.001

29. Piao S, Ciais P, Huang Y, Shen Z, Peng S, Li J, Zhou L, Liu H, Ma Y, Ding Y, Friedlingstein P, Liu C, Tan K, Yu Y, Zhang T, Fang J (2010) The impacts of climate change on water resources and agriculture in China. Nature 467:43–51. https://10.1038/nature09364
30. Quan R, Shang M, Zhang H, Zhao Y, Zhang J (2004) Engineering of enhanced glycine betaine synthesis improves drought tolerance in maize. Plant Biotechnol J 2:477–486. https://10.1111/j.1467-7652.2004.00093.x

31. Räsänen LA, Saijets S, Jokinen K, Lindström K (2004) Evaluation of the roles of two compatible solutes, glycine betaine and trehalose, for the Acacia senegal-Sinorhizobium symbiosis exposed to drought stress. Plant Soil 260:237–251. https://10.1023/B:PLSO.0000030181.03575.e1

32. Ritchie SW, Nguyen HT, Holaday AS (1990) Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. Crop Sci 30:105–111. https://10.2135/cropsci1990.0011183X0030000010025x

33. Rivest D, Lorente M, Olivier A, Messier C (2013) Soil biochemical properties and microbial resilience in agroforestry systems: Effects on wheat growth under controlled drought and flooding conditions. Sci Total Environ 463:51–60. https://10.1016/j.scitotenv.2013.05.071

34. Shawon RA, Kang BS, Lee SG, Kim SK, Ju Lee H, Katrich E, Gorinstein S, Ku YG (2020) Influence of drought stress on bioactive compounds, antioxidant enzymes and glucosinolate contents of Chinese cabbage (Brassica rapa). Food Chem 308:125657. https://10.1016/j.foodchem.2019.125657

35. Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW (1988) Water relations in winter wheat as drought resistance indicators. Crop Sci 28:526–531. https://10.2135/cropsci1988.0011183X0028000030021x

36. Seki M, Umezawa T, Urano K, Shinozaki K (2007) Regulatory metabolic networks in drought stress responses. Curr Opin Plant Biol 10:296–302. https://10.1016/j.pbi.2007.04.014

37. Silva S, Costa EM, Borges A, Carvalho AP, Monteiro MJ, Pintado MME (2016) Nutritional characterization of acorn flour (a traditional component of the Mediterranean gastronomical folklore). J Food Meas Charact 10:584–588. https://10.1007/s11694-016-9340-1

38. Sio-Se Mardeh A, Ahmadi A, Poustini K, Mohammadi V (2006) Evaluation of drought resistance indices under various environmental conditions. Field Crop Res 98:222–229. https://10.1016/j.fcr.2006.02.001

39. Szablowska E, Tańska M (2021) Acorn flour properties depending on the production method and laboratory baking test results: A review. Compr Rev Food Sci F 20:980–1008. https://10.1111/1541-4337.12683

40. Toscano S, Farieri E, Ferrante A, Romano D (2016) Physiological and biochemical responses in two ornamental shrubs to drought stress. Front Plant Sci 7:645. https://10.3389/fpls.2016.00645

41. Upreti P, Narayan S, Khan F, Tewari LM, Shirke PA (2021) Drought-induced responses on physiological performance in cluster bean (Cyamopsis tetragonoloba (L.) Taub.). Plant Physiology Reports 26(1):49–63. https://10.1007/s40502-021-00574-4

42. Wu T, Dong Y, Yu M, Geoff Wang G, Zeng D (2012) Leaf nitrogen and phosphorus stoichiometry of Quercus species across China. Forest Ecol Manag 284:116–123. https://10.1016/j.foreco.2012.07.025
43. Xiong C, Zhao S, Yu X, Sun Y, Li H, Ruan C, Li J (2020) Yellowhorn drought-induced transcription factor XsWRKY20 acts as a positive regulator in drought stress through ROS homeostasis and ABA signaling pathway. Plant Physiol Bioch 155:187–195. https://10.1016/j.plaphy.2020.06.037
44. Xiong L, Zhu JK (2002) Molecular and genetic aspects of plant responses to osmotic stress. Plant cell environment 25:131–139. https://10.1046/j.1365-3040.2002.00782.x
45. Xiong S, Zhao Y, Chen Y, Gao M, Wu L, Wang Y (2020) Genetic diversity and population structure of Quercus fabri Hance in China revealed by genotyping-by-sequencing. Ecol Evol 10:8949–8958. https://10.1002/ece3.6598
46. Xiu WY, Zhu Y, Chen B, Hu Y, Dawuda MM (2019) Effects of paclobutrazol on the physiological characteristics of Malus halliana Koehne Seedlings under drought stress via principal component analysis and membership function analysis. Arid Land Res Manag 33:97–113. https://10.1080/15324982.2018.1488300
47. Xu Z, Jiang Y, Zhou G (2015) Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO₂ with environmental stress in plants. Front Plant Sci 6:701. https://10.3389/fpls.2015.00701
48. Yang J, Di X, Meng X, Feng L, Liu Z, Zhao G (2016) Phylogeography and evolution of two closely related oak species (Quercus) from north and northeast China. Tree Genet Genomes 12(5):1–14. https://10.1007/s11295-016-1044-5
49. Yang J, Vázquez L, Feng L, Liu Z, Zhao G (2018) Climatic and soil factors shape the demographical history and genetic diversity of a deciduous oak (Quercus liaotungensis) in Northern China. Front Plant Sci 9:1534. https://10.3389/fpls.2018.01534
50. Zhai P, Zhang X, Hui W, Pan X (2005) Trends in total precipitation and frequency of daily precipitation extremes over China. J Climate 18:1096–1108. https://10.1175/JCLI-3318.1
51. Zhang W, Yu Y, Zhou X, Yang S, Li C (2018) Evaluating water consumption based on water hierarchy structure for sustainable development using grey relational analysis: Case study in Chongqing, China. Sustainability-basel 10:1538. https://10.3390/su10051538
52. Zhang X, Lei L, Lai J, Zhao H, Song W (2018) Effects of drought stress and water recovery on physiological responses and gene expression in maize seedlings. Bmc Plant Biol 18(1):1–16. https://10.1186/s12870-018-1281-x
53. Zhu J (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53(1):247–273. https://10.1146/annurev.arplant.53.091401.143329

Figures
Figure 1

Effects of drought stress and rewatering on soil moisture content of four oak species (DR-F: drought-rewatering group of Q. fabri; CK-F: normal watering group of Q. fabri; DR-S: drought-rewatering group of Q. serrata; CK-S: normal watering group of Q. serrata; DR-A: drought-rewatering group of Q. acutissima; CK-A: normal watering group of Q. acutissima; DR-V: drought-rewatering group of Q. variabilis; CK-V: normal watering group of Q. variabilis).
Figure 2

Effects of drought stress and rewatering on relative water content and water potential of four oak species (a, relative water content; b, water potential). DR-F: drought-rewatering group of Q. fabri; CK-F: normal watering group of Q. fabri; DR-S: drought-rewatering group of Q. serrata; CK-S: normal watering group of Q. serrata; DR-A: drought-rewatering group of Q. acutissima; CK-A: normal watering group of Q. acutissima; DR-V: drought-rewatering group of Q. variabilis; CK-V: normal watering group of Q. variabilis. Different lowercase letters indicate significant (P < 0.05) differences among the four oak species subjected to the same treatment time.

Figure 3

Effects of drought stress and rewatering on osmotic regulating substances content of four oak species (a, soluble protein; b, soluble sugar; c, proline; d, glycine betaine). DR-F: drought-rewatering group of Q. fabri; CK-F: normal watering group of Q. fabri; DR-S: drought-rewatering group of Q. serrata; CK-S: normal watering group of Q. serrata; DR-A: drought-rewatering group of Q. acutissima; CK-A: normal watering group of Q. acutissima; DR-V: drought-rewatering group of Q. variabilis; CK-V: normal watering group of Q. variabilis. Different lowercase letters indicate significant (P < 0.05) differences among the four oak species subjected to the same treatment time.
Figure 4

Effects of drought stress and rewatering on antioxidant enzyme activities and malondialdehyde of four oak species (a, peroxidase activity; b, superoxide dismutase activity; c, catalase activity; d, malondialdehyde content). DR-F: drought-rewatering group of Q. fabri; CK-F: normal watering group of Q. fabri; DR-S: drought-rewatering group of Q. serrata; CK-S: normal watering group of Q. serrata; DR-A: drought-rewatering group of Q. acutissima; CK-A: normal watering group of Q. acutissima; DR-V: drought-rewatering group of Q. variabilis; CK-V: normal watering group of Q. variabilis. Different lowercase letters indicate significant (P < 0.05) differences among the four oak species subjected to the same treatment time.
Figure 5

Effects of drought stress and rewatering on photosynthetic parameters of four oak species (a, net photosynthetic rate; b, transpiration rate; c, stomatal conductance). DR-F: drought-rewatering group of Q. fabri; CK-F: normal watering group of Q. fabri; DR-S: drought-rewatering group of Q. serrata; CK-S: normal watering group of Q. serrata; DR-A: drought-rewatering group of Q. acutissima; CK-A: normal watering group of Q. acutissima; DR-V: drought-rewatering group of Q. variabilis; CK-V: normal watering group of Q.
variabilis. Different lowercase letters indicate significant (P < 0.05) differences among the four oak species subjected to the same treatment time.

![Figure 6](image)

**Figure 6**

Principal component analysis of four oak species under continuous drought stress and rehydration conditions. DR-F: drought-rewatering group of Q. fabri; CK-F: normal watering group of Q. fabri; DR-S: drought-rewatering group of Q. serrata; CK-S: normal watering group of Q. serrata; DR-A: drought-rewatering group of Q. acutissima; CK-A: normal watering group of Q. acutissima; DR-V: drought-rewatering group of Q. variabilis; CK-V: normal watering group of Q. variabilis.
Figure 7

Correlation analysis among 13 physiological and biochemical indexes of four oak species under continuous drought stress and rehydration conditions. $\Psi_w$: Water potential; RWC: Relative water content; Pro: proline; SS: soluble sugar; SP: soluble protein; GB: glycine betaine; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; MDA: malondialdehyde; Pn: net photosynthetic rate; Tr: transpiration rate; Gs: stomatal conductance. * P < 0.05, ** P < 0.01, and *** P < 0.001.