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Physiological response and drought resistance evaluation of Gleditsia sinensis seedlings under drought- rehydration state

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Abstract

G. sinensis is an economically and ecologically important tree species with a wide distribution in China. Nearly two thirds of China's landmass is an area of arid or semi-arid regions. *G. sinensis* seedlings are very susceptible to drought-rehydration-drought cyclic pattern during the growth process, the physiological mechanisms of drought resistance and rehydration of *G. sinensis* lack of quantitative systematic research, the lack of good drought-resistant family in production practice, and the lack of reasonable evaluation of drought-resistant ability of the method, which seriously impeded the promotion of the selection and breeding of good family lines of *G. sinensis* and the development of the industry. Therefore, in this study, we conducted simulated natural drought and rewatering experiments on seedlings of 58 *G. sinensis* families to compare the differences between morphological, photosynthetic and physiological indexes of seedlings of different families with different degrees of drought and rewatering, with the aim of searching for suitable methods of evaluating the drought resistance of *G. sinensis* seedlings and screening drought-resistant families, elaborating on the differences in the effects of drought stress on *G. sinensis*, and revealing the mechanisms of *G. sinensis* growth and physiological response in different periods of drought. The drought resistance index (DRI) was used as the main index, combined with growth, leaf morphology and photosynthetic physiological indexes to comprehensively grade out five different drought tolerance taxa and screen out five representative family lines. The contents of proline (Pro), soluble protein (SP) and malondialdehyde (MDA), and the activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) of the seedlings of the five families in successive droughts increased with the degree of drought, and the highest values basically appeared in the period of severe drought, and then gradually declined after the rehydration. Pro, SP and MDA contents, CAT, POD and SOD activities of the high resistance (HR) family were 1.70, 0.61, 2.24, 3.75, 1.28 and 1.16 times higher than those of the natural control treatments during the severe drought period. Principal component analysis (PCA) revealed MDA and soluble sugars (SS) as the main indicators of response to drought and rehydration in *G. sinensis*. Increased antioxidant enzyme activity and changes in the content of osmoregulatory substances may be important factors causing drought tolerance mechanisms. The synthesis of growth, leaf morphology and photosynthetic indexes of *G. sinensis* seedlings under arid environments can be used as a method for its evaluation of drought resistance, and provide a reference for the screening of good drought- resistant family lines. *G. sinensis* are less drought tolerant in the seedling stage, so drought tolerant family lines should be selected for production applications and adequate moisture should be ensured for the seedlings.

Introduction

Dramatic changes in global climate and precipitation patterns have exacerbated the existing unequal distribution of water resources, leading to drought conditions for many plants. Drought is an important environmental factor limiting the development of agroforestry, and the reduction in crop yields due to drought is higher than that of other environmental factors combined [1]. Drought is an important environmental factor limiting the development of agroforestry, and the reduction in crop yields due to

drought is higher than the sum of other environmental factors[2]. Guizhou Province is one of the regions with the most severe karst landscapes in China [3], where water infiltration is severe and the special geographical environment leads to frequent temporary droughts, which to a certain extent limits the development of agroforestry. Drought stress has important effects on plant growth, development and physiology, mainly in terms of water redistribution in various parts, damage to membranes and nuclei, inhibition of leaf and root growth, reduced photosynthesis, excessive production of reactive oxygen species (ROS) and reduced osmotic potential. When plants are subjected to drought stress it accelerates the abnormal yellowing and shedding of leaves [4]. Previous researchers assessed the drought resistance of *Brassica napu*s L and five tree species using plant leaves affected by disasters and screened for HR and highly susceptible (HS) materials [5, 6].Drought resistance of plants can also be evaluated using photosynthetic parameters [7]. *Aronia melanocarpa*[8] has increased MDA content and antioxidant enzyme activity with increased drought, and changes in its physiological indicators can also be used to evaluate drought resistance. Therefore, when evaluating the drought resistance of plants and screening for drought-resistant lines, a comprehensive evaluation and analysis can be carried out in conjunction with plant morphology, photosynthesis and physiological and biochemical characteristics.

G. sinensis is an important ecological and economic tree species, widely distributed in China, and is one of the characteristic forestry species that Guizhou Province focuses on developing. The growth of *G. sinensis* is susceptible to drought due to its special geographical environment, and in its natural state is prone to a recurring pattern of drought-rehydration-drought cycles. Drought has a definite effect on the growth of *G. sinensis* seedlings, but differences in the effects of different degrees of drought and rehydration on different families have not been studied. Current research on *G. sinensis* has focused on seedling propagation techniques and seedling physiological resistance [9, 10].

Although drought stress in *G. sinensis* has been studied, there is a lack of quantitative and systematic studies on the physiological mechanisms of drought tolerance and rehydration in *G. sinensis*. In order to explore the methods and criteria for screening excellent drought-resistant lines, and to study the growth and physiological differences between drought-highly resistant and drought-sensitive lines, we conducted natural drought and rehydration experiments on *G. sinensis* seedlings, comparing the growth indexes, leaf morphology, photosynthesis and physiological indexes among different drought-resistant *G. sinensis* lines to elucidate the effects of drought stress on *G. sinensis* The differences in growth, leaf morphology and physiological indexes were compared among different drought-resistant *G. sinensis* lines to illustrate the effects of drought stress on *G. sinensis*, to provide a reference basis for the screening of drought-resistant lines and methods, and to reveal the growth and physiological response mechanism of *G. sinensis* during drought, so as to lay a physiological foundation for further elucidation of the molecular mechanism of drought resistance in *G. sinensis*.

Materials and methods

Test materials

The test materials were 58 seeds of semi-sibling lineages of *G. sinensis* from 22 counties and cities collected during the previous research work on germplasm resources of wild *G. sinensis* in Guizhou Province[11]. They were harvested and dried and stored in a refrigerator at 4°C. Seed treatment and seedling sowing were carried out in spring. Soak in 98% concentrated sulfuric acid for 50 min, rinse with water and soak in warm water at 38°C until the seeds have completely water absorption and expansion. Seedling soil treated with 0.1% Potassium permanganate. The soil ratio is *Pinus massoniana* L under the forest soil of Guizhou University: nutrient soil = 1:1, the volume of soil in its pots is 80% of the pot volume, and the potted seedlings are managed routinely in a rain shelter.

The seeds of *G. sinensis* collected in this study were collected with permission to collect, accompanied by relevant staff led by the Guizhou Forestry and Grassland Bureau, and some of the wild plants were harvested for seeds with friendly communication and consultation with the people to whom the plants belonged, and permission to collect was obtained, without any conflict of interest. Experimental and field studies on plants, including the collection of plant material and the conduct of plant trials, are conducted in accordance with relevant institutional, national and international guidelines and legislation.

Test method

Seedlings were selected for natural drought treatment after 5 months of growth, and the test materials were fully irrigated every day for 3 d before the treatment, which was used as the soil saturated water content (soil water content of 30%~35%). Referring to the research method on the physiological effects of rewatering after drought on *Platycladus orientalis* seedlings and improving it as appropriate[12], the relative soil water content of 75%~80% was used as the control group, and three drought gradients were set, namely, mild drought (relative soil water content (percentage of the maximum water holding capacity of the soil) of 55%~60%); severe drought (relative soil water content of 30%~35%). The natural drought time was determined by the moisture reduction to reach the drought interval, and the soil moisture content to reach mild drought (7 d of continuous drought) and severe drought (14 d of continuous drought) was determined by the soil moisture meter using the control (with the relative soil moisture content of 75–80%) as the 0 d. The rehydration test was conducted immediately after the drought test, during which the relative soil moisture content of the test seedlings was restored using a soil moisture meter (Delta-T, UK), with three replications for each treatment.

Methods for determining morphological and photosynthetic indicators

(1) For each family line, thirty plants were randomly selected from the control and heavy drought treatments to measure the diameter of the seedling heights. (2) Thirty plants were randomly selected from the control and drought treatments, and the soil and plant analyzer development (SPAD) values of the third leaf below the top of the plant were determined using the SPAD apparatus. (3) Thirty plants were randomly selected from the control and treatments, and net photosynthetic rate (Pn) was measured on the 3rd, 4th and 5th leaves under the top of the plants using a LI-6400 Photosynthesis measurement system (LI-COR company, USA) from 9:00 to 11:00 on a sunny day, and Pn, stomatal conductance (Gs),

intercellular CO_2 concentrations (Ci), and transpiration rate (Tr) were counted. (4) The leaves of *G. sinensis* were counted for 14 d of drought, and the DRI was calculated and graded [6] with the following grading criteria (Table 1).

Measurement of physiological indicators

The middle part of the plant stem, intact and pest-free leaves were selected from 0 d of drought, 7 d of drought, 14 d of drought, 1 d of rehydration, and 3 d of rehydration, and were washed with distilled water, then frozen in liquid nitrogen after removing the water on the surface of the leaves, and stored in a refrigerator at -80 ℃. Referring to Li Hesheng's method [13], SS content was determined by anthrone colourimetry; SP content was determined by Bradford method; Pro content was determined by acidic ninhydrin colourimetric method; MDA content was determined by thiobarbituric acid method; CAT enzyme activity was determined by UV spectrophotometry; SOD enzyme activity was determined by nitroblue tetrazolium method; and POD enzyme activity was determined by guaiacol method.

Data analysis

The data obtained were statistically analysed using Excel 2019 and IBM SPSS Statistics 26 and plotted using Origin 2022b. The significance of the difference between two-by-two data was analysed using the t-test and multiple comparisons of the experimental data from different families at different drought times were made using the LSD method at the level(P<0. 05), where the main formulas applied were as follows:

$$\varepsilon i=(\min_i\min_m|A_m-B_{im}|+\rho\max_i\max_m|A_m-B_{im}|)/\ |A_m-B_{im}|+\rho\max_i\max_m|A_m-B_{im}|)$$

where εi is the correlation degree, A is the DRI, B is the evaluation vector, miniminm|Am-Bim| is the second-level minimum difference, and maximaxm|Am-Bim| is the second-level maximum difference; ρis the resolution coefficient, with the value range of 0–1, and ρ = 0. 5 was taken in this experiment.

(2) $\mathrm{DRI}(\%)=\frac{\mathrm{Measuredvaluesofeachindicatorinthetreatmentgroup}}{\mathrm{Measuredvaluesofindicatorsinthecontrolgroup}}*100\%$

(3)Affiliation value

$$u(xi)=\frac{xi_j-xi_{\min}}{xi_{\max}-xi_{\min}}$$

When the indicator drought tolerance coefficient is positively correlated with drought resistance,

resistance, $u(xi) = 1 - \frac{xi_j - xi_{\min}}{xi_{\max} - xi_{\min}}$

Drought Composite Score $D = \frac{1}{n}\sum_{i=1}^{n} u(xi)$

Results

DRI Grading for G. sinensis

The drought resistance indices of the 58 *G. sinensis* families ranged from 0.44 to 1. The drought resistance indices of the 58 *G. sinensis* families were clustered according to the Euclidean distance, and at a Euclidean distance of 0.2, they could be classified into five clusters, namely, 8 HS families, 13 low susceptibility (LS) families, 12 low resistance (LR) families, 14 medium resistance (MR) families, and 11 HR families type families (Table 2).

Drought affiliation values and drought tolerance indices of G. sinensis families

The DRI of *G. sinensis* was used as a basis for screening representative plants of *G. sinensis* with HS, LS, LR, MR and HR in conjunction with growth indices and photosynthesis physiological indices of *G. sinensis* during drought (appendix 1). Five drought-resistant groups were analysed by combining the DRI and affiliation function analysis of each *G. sinensis* family line, and the HR representative family line 17, MR representative family line 49, LR representative family line 23, LS representative family line 28 and HS representative family line 50 were comprehensively screened out to be used as representative families for the study of drought-resistant physiological indexes in *G. sinensis*.

Correlation analysis of growth indicators with photosynthetic physiology and drought tolerance in G. sinensis

The drought resistance of *G. sinensis* has a high correlation with the growth index and photosynthetic physiological index of *G. sinensis* (Table 3), in which the seedling height has the highest correlation with drought resistance, reaching 0. 80, followed by SPAD value, diameter, Pn, with correlation coefficients of 0.79, 0. 60, and 0.66. The correlation between Gs and Tr and the DRI of *G. sinensis* was 0.63, while the correlation between Ci and the DRI was the lowest at 0.58. The correlation between drought tolerance and growth index of *G. sinensis* was high, and it was more accurate to judge the drought tolerance of *G. sinensis* by growth index.

Analysis of changes in physiological indicators of G. sinensis under drought stress

Changes in SS and SP content of G. sinensis under drought stress.

SS and SP are important osmoregulators. The SS content in the leaves of *G. sinensis* of all families showed less change under mild drought stress compared to the control, while the SS in the leaves of *G. sinensis* of HS, LS and MR families was significantly reduced under severe drought stress. In contrast to the respective severe drought, SS content increased significantly at 1 d of rehydration and decreased significantly after 3 d of rehydration (Fig. 1). There were no significant changes in SP in the HS, LR and HR lines and a significant increase in SP in the LS and MR lines under mild drought compared to their respective controls; however, SP in the leaves of the *G. sinensis* were significantly increased under severe drought; and a significant decrease in SP was observed at 1 d of rehydration (except for LR familiy), as compared to their respective severe droughts.

Changes in Pro and MDA content of G. sinensis under drought stress. The Pro in the leaves of *G. sinensis* s was significantly reduced in the HS families during mild drought stress compared to the control, while the rest of the families showed insignificant changes (Fig. 2). At severe drought, Pro content was significantly increased in all the families. Leaf Pro content of HS, LS, LR and MR lines was significantly reduced after rehydration compared to severe drought, while there was no significant change in leaf Pro content of HR lines. When plants are subjected to drought stress, reactive ROS are produced in the cells, and excessive ROS can damage plant cells and membrane lipid peroxidation occurs, while the end product of its decomposition is MDA, and the end product of its decomposition will be MDA, so the content of MDA can often respond to the degree of ROS damage in plants, and a certain amount of ROS can be used as a signaling factor to stimulate the activation of drought-resistant mechanisms in plants. MDA content in *G. sinensis* leaves increased during mild drought compared to their respective controls. The MDA content in all the *G. sinensis* leaves was significantly increased during severe drought (Fiureg. 2), indicating elevated ROS damage in *G. sinensis* leaves during severe drought. MDA in rehydration *G. sinensis* leaves was significantly reduced in highly susceptible, LS and HR lines, and insignificantly changed in LR and MR lines as compared to their respective severe drought.

Changes in antioxidant enzyme activities of G. sinensis under drought stress. CAT and SOD can be used as scavengers to remove ROS in plant stress situations and reduce the damage of reactive ROS to plant cells. Both CAT and SOD activities within the leaves of *G. sinensis* were significantly increased under mild drought stress compared to their respective controls (Fig. 3). CAT activity within the *G. sinensis* leaves was significantly lower after rehydration compared to the respective severe drought stress, indicating that *G. sinensis* leaves need to maintain a certain level of CAT activity after rehydration to scavenge excessive ROS. SOD enzyme activity was significantly higher in both LR and HR lines during severe drought compared to mild drought. After rehydration, SOD activity was basically decreased in all families compared to severe drought.

POD activity was elevated but not significant under mild drought stress compared to their respective controls (except for the LS family). POD activity was significantly elevated in all the *G. sinensis* leaves under severe drought stress (Fig. 4). POD activity in *G. sinensis* leaves was significantly lower after 1 d of

rehydration when compared to their respective severe drought stresses. The POD activity of the HR lines was lower than the other four treatments throughout the drought-rehydration process.

PCA of physiological indicators of drought tolerance in G. sinensis

PCA is the process of taking multiple variables and downscaling them into several principal components that can respond to the majority of the information in the original data, as shown in Fig. 5, for the physiological indicators of drought tolerance in *G. sinensis*; the first four principal components had a cumulative contribution rate of 85%, which can be used to represent the original data, and thus four principal components can be used to represent the seven physiological indicators of drought tolerance in *G. sinensis*. MDA, SS, Pro and CAT activity were the largest factor loadings in principal components 1, 2, 3 and 4, respectively, so these four indicators can be used as the main drought assessment indicators when *G. sinensis* are subjected to drought stress.

Discussion

Plants may experience frequent droughts during their growth cycle, and drought is one of the most severe abiotic stresses limiting plant survival, growth and distribution, affecting and physiological and metabolic functions [14, 15]. To survive, most plants have evolved various adaptive mechanisms and strategies to cope with drought stress [16]. Plants can adapt to drought by altering various morphological, physiological, biochemical and gene expression changes [17]. Plant drought tolerance is an adaptive response of plants, a characteristic of plants to tolerate drought with minimal damage and yield reduction. Drought tolerance varies widely among plants. The study of the ability of plants to maintain physiological functions under drought conditions and to recover rapidly after removal of the stress is essential to ensure growth and development under intermittent drought.

Plant resistance evaluation is an important way of material source for plant tolerance research and an indication method for screening plant resources under various adversities, which has an important application value for the ecological and economic development of environmentally hostile areas, and there is basically no specific index and method for selecting plant resistance materials, so the method of selecting plant resistance materials is complicated. Commonly used selection indexes for resistant materials are mainly growth, physiology, biochemistry and other aspects, combined with a number of indicators to select resistant materials [18]. Numerous studies have shown that when plants are subjected to adversity stress, it will be directly reacted to the morphology of the plant, so the appearance of the plant morphology is an important and most intuitive indicator for evaluating the resistance to adversity[19], therefore, in this study, we used the appearance and morphology of *G. sinensis* under drought stress, and calculated DRI as a main indicator for evaluating the drought resistance of *G. sinensis* to evaluate the drought resistance of the *G. sinensis*. It was found that the DRI varied in different family lines of *G. sinensis*, which is similar to the study of watermelon on drought tolerance evaluation in arid environments [20], suggesting that morphological indices play an important role in the study of plant tolerance and can be used for screening of stress tolerant materials.

When plants are subjected to drought stress, plant leaves close their stomata in order to secure water within the plant, thus affecting Pn and Tr. When *G. sinensis* were subjected to drought stress in this study, the Pn, Gs and Tr of leaves were reduced, while the changes in Ci were not homogeneous, suggesting that Pn is affected when *G. sinensis* are subjected to drought stress. *Broussonetia papyrifera*[21] showed some reduction in Ci, Pn and Gs in response to drought stress, but in this study, we found that some *G. sinensis* family lines showed an increase in their Ci under drought, suggesting that under successive droughts, the changes in Ci of *G. sinensis* do not change in a single direction of decrease or reduction, there may be some ups and downs. Ci was found to increase and then decrease with increasing drought [22] in plants such as *Schima superba*, *Liquidambar formosana*, and *Choerospondias axillaris*, and the same change may exist in *G. sinensis*, but the specifics of this need to be further investigated over a long period of time. Chlorophyll is also an important indicator in case of water deficit in plants, and a certain degree of drought plants will lead to a decrease in Cha content [23], which is the same as the findings of this study, indicating that under drought stress, *G. sinensis* close their stomata and reduce Tr in order to ensure that water is excessively dissipated from the leaves. Drought conditions inhibited plant height, basal diameter and biomass accumulation of *Artemisia selengensis*, but root to stem ratio (R/S) increased. Drought stress also reduced total Chlorophyll, chlorophyll a, chlorophyll b and carotenoids, while SS and SP accumulated dramatically with severe drought [24] Drought was also found to inhibit the growth of seedling height, ground diameter, and biomass accumulation of *G. sinensis* seedlings in this study, which may be related to the severe inhibition of photosynthesis.

The ability of plants to withstand harsh environments is closely linked to their ability to resist oxidation and regulate cellular osmotic content [25]. Under drought stress, plants produce large amounts of ROS, which activates the ROS scavenging mechanism in plants, and POD, CAT and SOD are important ROS scavenging enzymes in plants. Plant stress is often accompanied by the production of ROS, the rate of accumulation of which correlates with the degree of drought to which the plant is subjected and increases dramatically during severe drought [26]. High concentration of ROS produces strong membrane lipid peroxidation, which makes proteins and cell membranes damaged by oxidation, and thus a large amount of MDA is produced [27], MDA can react with macromolecules such as proteins and nucleic acids in cells, leading to denaturation of proteins and nucleic acids and weakening the covalent bonds between cellulose molecules [28]. Therefore, MDA content represents the degree of stress damage to plant cells, and can be used as an important indicator for evaluating the drought tolerance of plants. In the present study, a small increase in MDA was observed during mild drought, indicating that *G. sinensis* seedlings suffered less drought damage. During severe drought, MDA of *G. sinensis* increased sharply in five families. MDA decreased after rehydration, indicating that rehydration reduced membrane lipid peroxidation and membrane permeability, thus allowing plants to recover from drought stress. Under drought stress, plant cells produce ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-), single linear oxygen (O_1^-) and superoxide radicals (O_2^-) [29]. In order to minimise the damage caused by ROS, the cells stimulate their antioxidant enzyme system, so the antioxidant enzyme reaction system is closely related to the plant's resilience. A dramatic increase in ROS will inevitably stimulate the activity of the

antioxidant enzyme system, which firstly reacts with SOD to produce low oxidative H_2O_2, and then H_2O_2 reacts with POD and CAT to produce further catalytic reactions, which is decomposed into H_2O and O_2[30]. In mild drought, *G. sinensis* were less damaged and the activities of SOD and CAT were significantly increased, suggesting that under mild drought, SOD and CAT enzymes scavenged excess ROS [26], which is important for the resistance of *G. sinensis* seedlings to mild drought, whereas the accumulation of ROS was increased in severe drought, which was associated with the drastic increase in ROS during severe drought. In this study, CAT, POD and SOD all increased to some extent during drought, but their increases were different, so the mechanism of reactive ROS scavenging during drought was not exactly the same in different family lines of *G. sinensis*, with a certain degree of specificity, and the performances of their three enzymes varied among different family lines, and among different drought and rehydration stages, all antioxidant enzyme activities decreased after rehydration, indicating that the damage of drought stress was attenuated after rehydration.

Osmoregulation is an important mechanism for drought adaptation in plants. Drought-resistant plants have better osmotic adjustment, although this adjustment is reduced or even lost under severe drought [31]. Plants actively regulate the osmotic pressure of their cells in order to absorb more water under conditions of drought stress, and thus accumulate large amounts of osmoregulatory substances in their cells. SP, SS and Pro are often considered important for osmoregulation. Plants under arid conditions maintain proper Gs for photosynthesis through osmotic substances. These osmotic substances include organic solutes synthesised in cells, mainly Pro, SP and SS. SS plays an important role in maintaining the structure and growth of plants and is highly sensitive to environmental stress, where environmental changes affect the supply of carbohydrates from source to sink organs [32]. SS acts as an osmoprotectant, regulating osmotic pressure, providing membrane protection, scavenging toxic ROS, and protecting the stability of plant enzymes or proteins [33]. Pro regulates cellular osmotic pressure, stabilises the structure of proteins and cell membranes, is an enzyme protector, free radical scavenger and antioxidant in many plants in response to biotic and abiotic stresses, enhanced hydration between proteins [34]. In addition to being a permeable substance, SP is a key nutrient [35]. The accumulation of SP content under drought stress promotes osmotic adjustment and declines after stress relief. Changes in SP content under drought stress are often accompanied by changes in protein composition [36]. The increase in SP content under drought stress suggests that these proteins play a role in drought tolerance. Whether the increase in SP content is related to the synthesis of new osmoregulatory proteins needs to be further investigated. Studies on *Hemerocallis*[37] and *Carex breviculmis*[38] showed a significant increase in SP content under moderate drought, and a decrease in SS content under severe and moderate drought. Drought stress was also found to reduce SS content of leaves in studies on rice seedlings [39]. Pro also improves drought tolerance in *fennel*[40]. Accumulation of Pro and sugar in strawberry leaves under drought stress was significantly higher than that of the control [41]. Therefore, SP, Pro and SS are used as important indicators for the selection of drought tolerant varieties [42]. In the present study, it was found that with increasing drought, SP and Pro of *G. sinensis* leaves also increased to improve osmotic potential, but SS decreased sharply, which is similar to the results of the above study. The decrease in SS

content may be due to the inhibition of SS synthesis as drought stress reduced photosynthesis in the plant. After rehydration, the SS content was still lower than that of the control group, indicating that the damage caused by drought was difficult to eliminate completely. It has been suggested that drought inhibits protein synthesis, leading to lower SP levels in plants [43]. The Pro content of *G. sinensis* increased dramatically in all five families during the severe drought in this study and decreased to some extent after rehydration, which was attributed to the conversion of Pro to glutamate via Pro dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) [44]. Therefore, both Pro and SP are osmotic regulators of drought stress in *G. sinensis*, with Pro being the main osmotic regulator under severe drought in *G. sinensis*[45].

Conclusion

In the present study, drought-rehydration treatment of seedlings of 58 *G. sinensis* families revealed that DRI was highest in family 17 and 48 and lowest in family 50. The DRI was used to classify the *G. sinensis* seedling lines into five groups: HS lines, LS lines, LR lines, MR lines and HR lines. The DRI was used as the main index, combined with growth, leaf morphology and photosynthesis physiological indexes to comprehensively screen five representative families of different drought tolerance taxa. The contents of Pro, SP and MDA, and the activities of CAT, POD and SOD of the seedlings of the five families in successive droughts increased with the degree of drought, and the highest values basically appeared in the period of severe drought, and then gradually declined after the rehydration. The SS content of the seedlings of the five families decreased with drought duration as the drought intensified, with the highest content at 1 d of rehydration. PCA shows MDA and SS are the main indicators of *G. sinensis* response to drought and rehydration. Increased antioxidant enzyme activity and changes in the content of osmoregulatory substances may be important factors causing drought tolerance mechanisms. The synthesis of growth, leaf morphology and photosynthetic indexes of *G. sinensis* seedlings under arid environment can be used as a method for its evaluation of drought resistance and provide a reference for the selection of good drought-resistant family lines. *G. sinensis* are less drought tolerant in the seedling stage, so drought tolerant family lines should be selected for production applications and adequate moisture should be ensured for the seedlings.

Declarations

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Data availability

All data are presented in the article, and can be requested from the corresponding author if required.

Author Contributions

L.F.H., and Z.Y., conceived and designed the study. L.F.H., W.B., and H.K.Q., collected the data. X F., Z.Y., and W X R., analysed and interpreted the data. L.F.H., and W.B., wrote and translated the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

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Tables

Table 1 Grading criteria for drought damage survey of *G. sinensis* leaves.

Grade	Drought symptoms
0	No obvious drought stress symptoms
1	Lightly affected, few leaves wilt and turn yellow
2	Moderate damage, about 1/2 of the leaves turning yellow and drying out
3	Severe damage, total leaf yellowing, massive defoliation

Table 2 Classification of DRI of *G. sinensis*

Grading	Family Number	Number of families	DRI
HS	3, 8, 12, 31, 35, 46, 50, 52	8	0. 52
LS	1, 5, 9, 10, 16,18, 25, 28, 29, 30, 32, 38, 42	13	0. 7
LR	4, 6, 14, 19, 20, 22, 23, 26, 34, 43, 47, 55	12	0. 78
MR	7, 21, 24, 27, 36, 37, 39, 41, 45, 49, 54, 56, 57, 58	14	0. 86
HR	2, 11, 13, 15, 17, 33, 40, 44, 48, 51, 53	11	0. 95

Table 3 Correlation coefficient, correlation degree and correlation order between drought resistance of *G. sinensis* and various indexes

	HR	MR	LR	LS	HS	Degree	Order
Seedling height	0. 65	1. 00	0. 69	0. 81	0. 88	0. 80	1
Diameter	0. 82	0. 51	0. 57	0. 83	0. 61	0. 67	3
SPAD	0. 88	0. 84	0. 98	0. 61	0. 64	0. 79	2
P_n	0. 75	0. 61	0. 97	0. 61	0. 38	0. 66	4
G_s	0. 56	0. 55	0. 83	0. 85	0. 33	0. 63	6
C_i	0. 40	0. 78	0. 68	0. 65	0. 37	0. 58	7
T_r	0. 57	0. 56	0. 91	0. 76	0. 34	0. 63	5

Figures

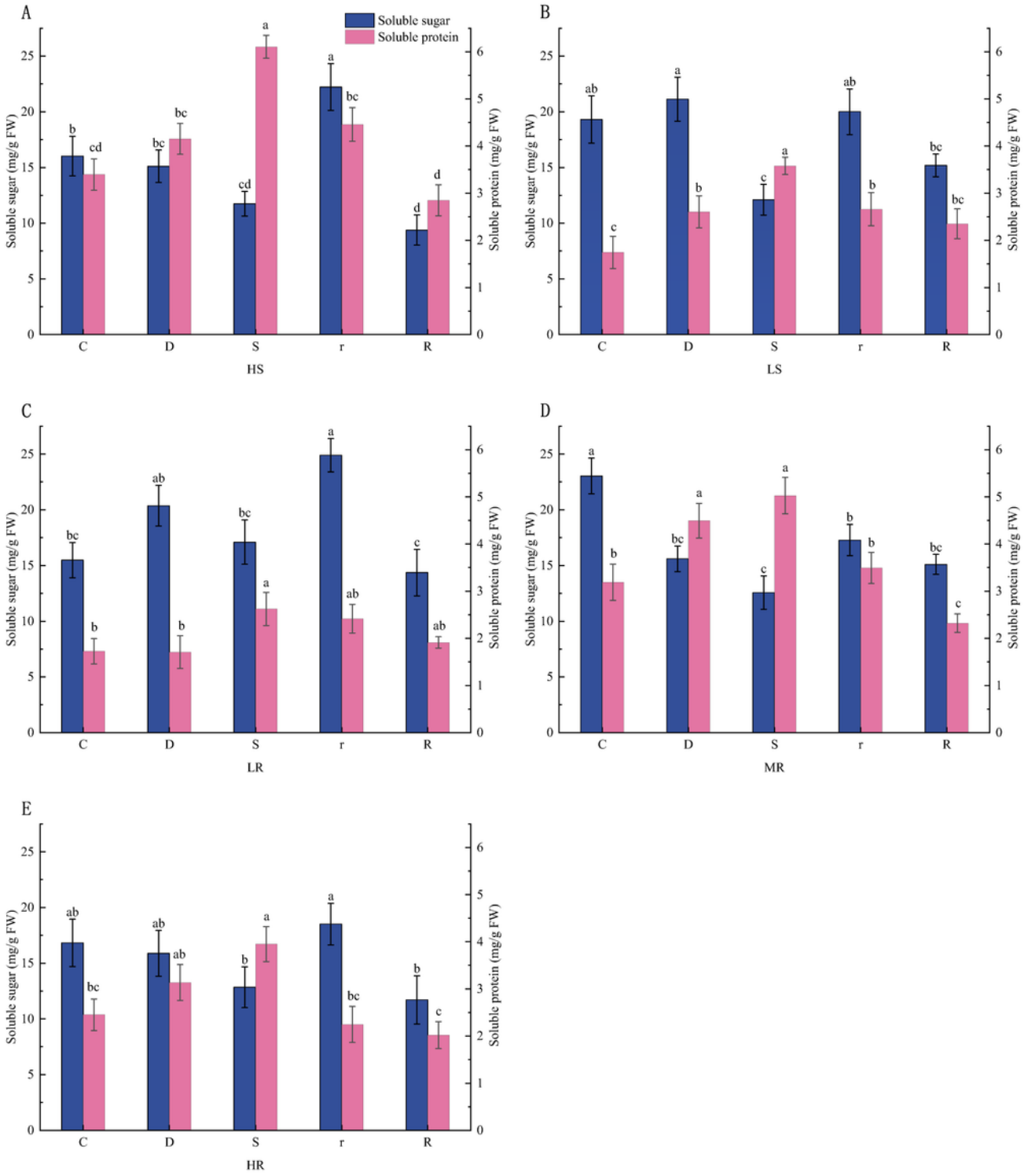


Figure 1

Changes of SS and SP contents in leaves of *G. sinensis* under drought stress and rehydration.

Note: C, D, S, R and R represent control, natural drought for 7 d, natural drought for 14 d, rehydration for 1 d and rehydration for 3 d respectively. Different letters indicate significant differences at the $P < 0.05$ level, the same as below.

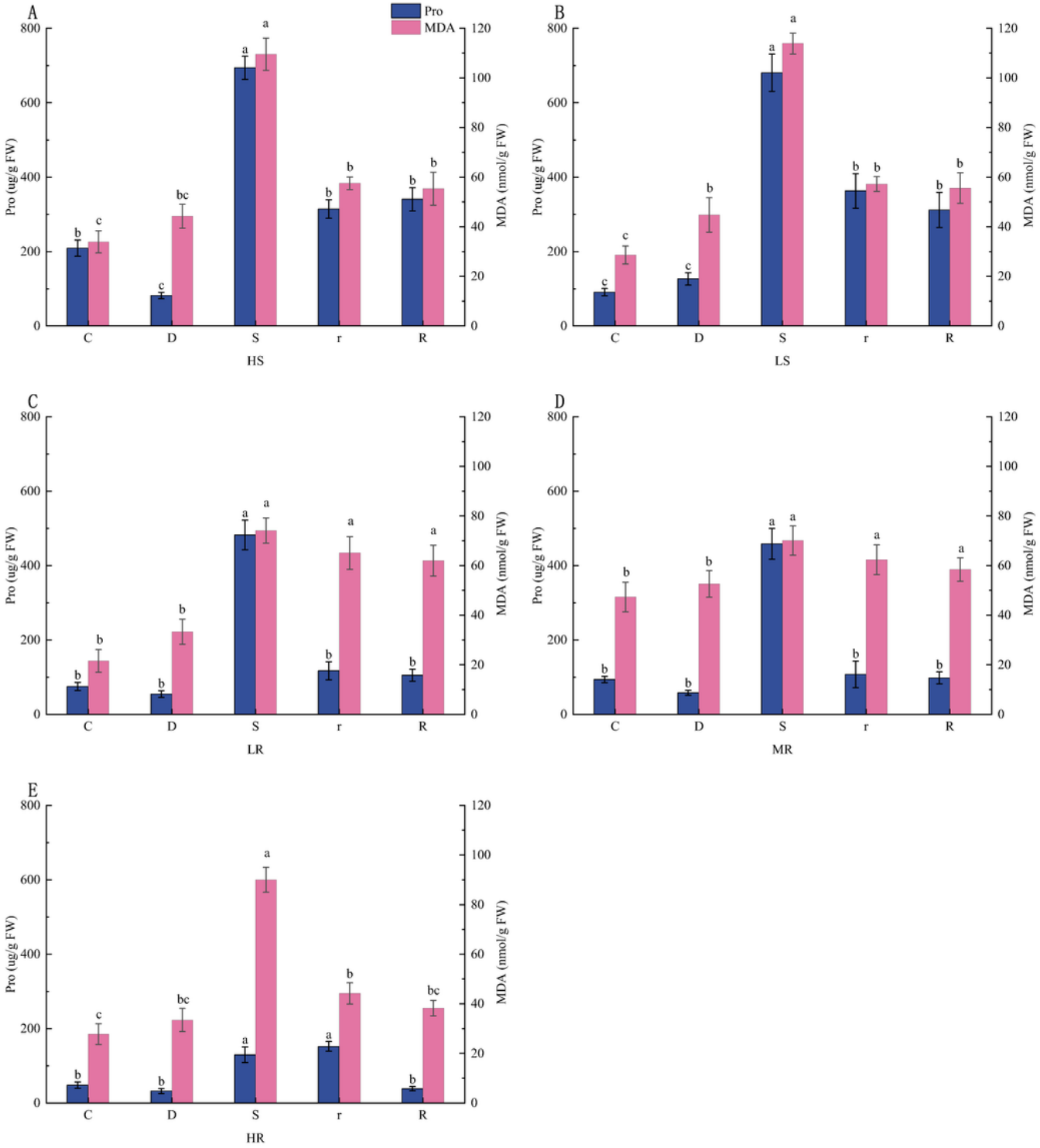


Figure 2

Changes of Pro and MDA contents in leaves of *G. sinensis* under drought stress and rehydration.

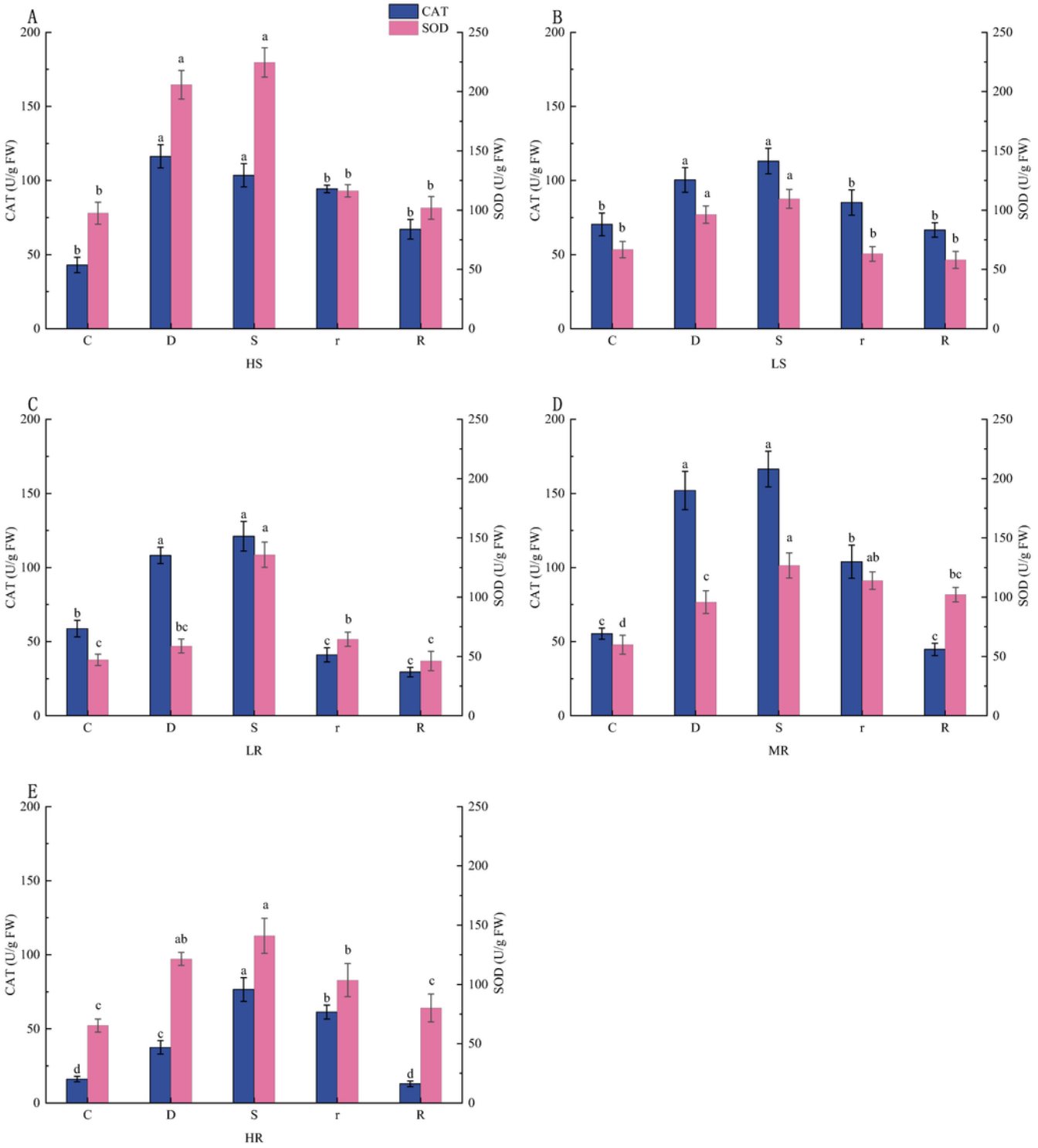


Figure 3

Changes of CAT and SOD enzyme activities in *G. sinensis* leaves under drought stress and rehydration.

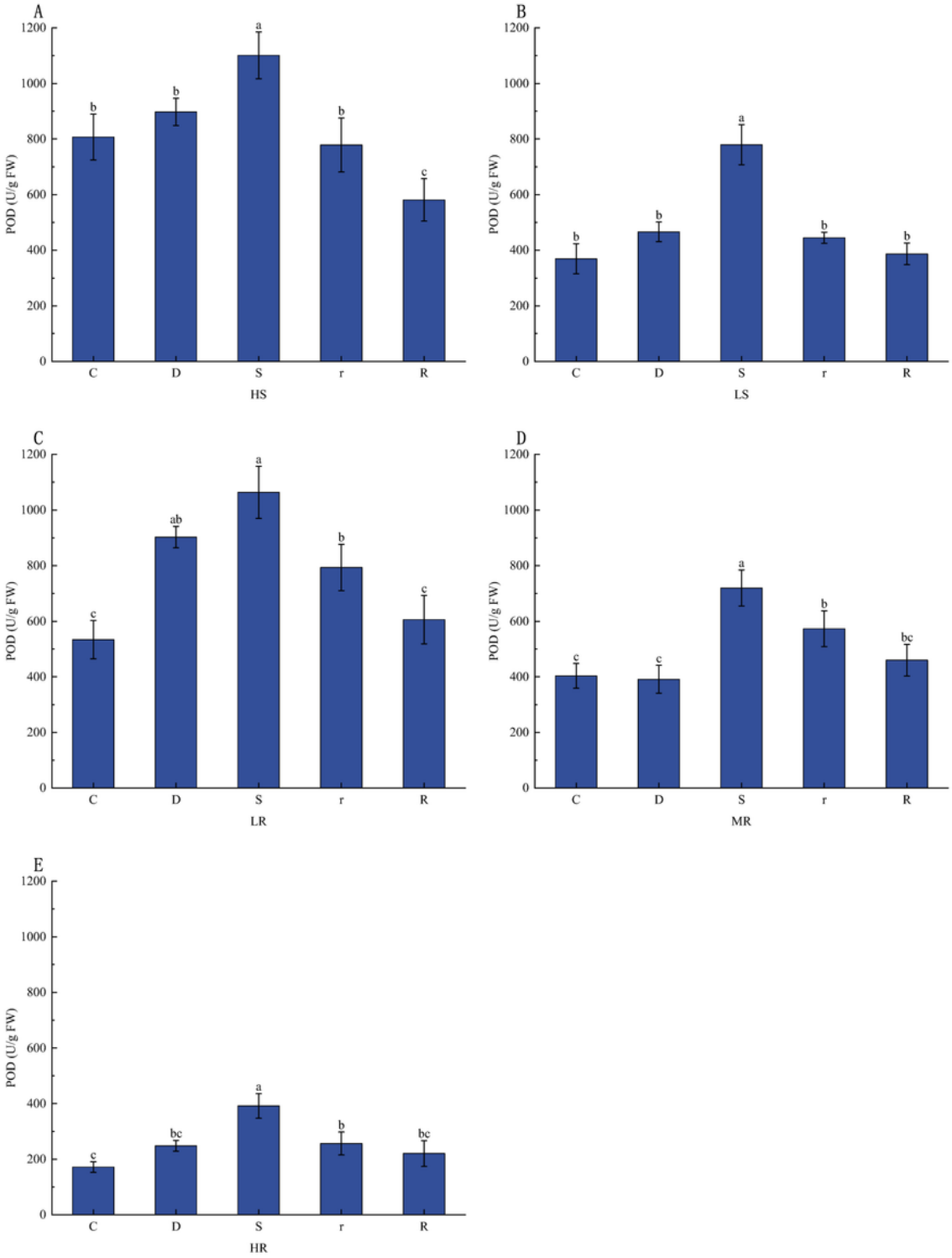


Figure 4

Changes of POD enzyme activity in *G. sinensis* leaves under drought stress and repeated rehydration.

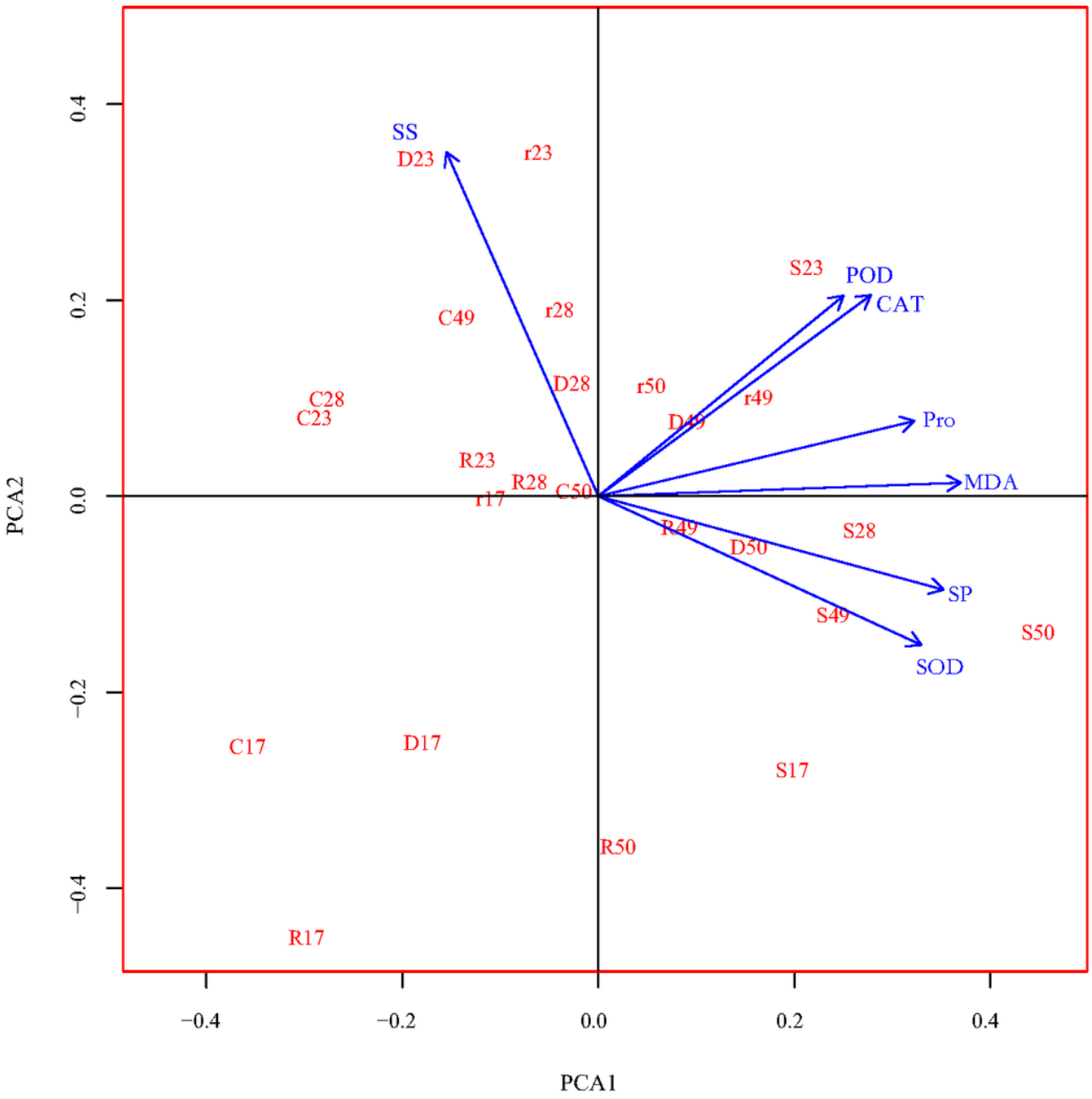


Figure 5

PCA of physiological characteristics of *G. sinensis* under drought stress and rehydration.

Supplementary Files

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- Appendix1.docx
- supplementarymaterial.zip