Effects of Drought-Rewatering on the Physiological Characteristics of Pinus sylvestris var. mongolica Seedlings (Postprint)

Authors: Wang Zixiang, Ren Yue, Lu Ying, Gao Guanglei, Ding Guodong, Zhang Ying.

Date: 2024-12-16T00:00:00+00:00

Abstract

To reveal the response characteristics of physiological indices of Mongolian pine (Pinus sylvestris var. mongolica) seedlings to drought stress and re-watering, a pot experiment was conducted using 2-year-old Mongolian pine seedlings as experimental materials. Five water treatments were established: control (80%), mild drought (40%), moderate drought (20%), severe drought (10%), and extremely severe drought (5%). Changes in water status, photosynthetic parameters, chlorophyll fluorescence, osmotic adjustment substances, and antioxidant enzyme activities were measured and analyzed during drought and after re-watering. The results showed that: (1) Mild drought stress had minor effects on the physiological status of Mongolian pine seedlings. Under moderate and severe drought stress, the activities of superoxide dismutase and peroxidase in seedlings reached their maximum values, increasing by 25.26% and 38.8% compared with the control, respectively. Under extremely severe drought stress, the net photosynthetic rate, transpiration rate, and steady-state light-adapted quenching coefficient of seedling leaves decreased by 94.76%, 87.19%, and 72.35% compared with the control, respectively, while leaf malondialdehyde content reached its maximum. (2) After re-watering, chlorophyll fluorescence and leaf proline content of Mongolian pine seedlings basically recovered to control levels, but photosynthetic indices of leaves after re-watering from extremely severe drought stress only recovered to an average of 28.51% of the control. Leaf water use efficiency, soluble sugar content, and antioxidant enzyme activities were all significantly higher than the control after re-watering ($P<0.05$). In summary, extremely severe drought stress mainly reduced the photosynthetic capacity and destroyed cell membrane stability of Mongolian pine seedlings, while re-watering after moderate drought could improve plant water utilization and reactive oxygen species scavenging capacity, thereby enhancing plant drought resistance. The research results can provide theoretical references for efficient cultivation,

tending management, and drought resistance evaluation of Mongolian pine plantations.

Full Text

Effects of Drought and Rehydration on Physiological Characteristics of *Pinus sylvestris* var. *mongolica* Seedlings

WANG Zixiang[1], REN Yue[2], LU Ying[1], GAO Guanglei[1,3,4,5,6], DING Guodong[1,4,5,6], ZHANG Ying[1,4,5,6]

[1]School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China
[2]College of Desert Control Science and Engineering, Inner Mongolia Agricultural University, Hohhot 010018, Inner Mongolia, China
[3]State Key Laboratory for Efficient Production of Forest Resources, Beijing 100083, China
[4]Yanchi Ecology Research Station of the Mu Us Desert, Yanchi 751500, Ningxia, China
[5]Engineering Research Center of Forestry Ecological Engineering, Ministry of Education, Beijing 100083, China
[6]Key Laboratory of State Forestry and Grassland Administration on Soil and Water Conservation, Beijing 100083, China

Abstract

To reveal the response characteristics of physiological indicators of *Pinus sylvestris* var. *mongolica* seedlings to drought stress and rehydration, we conducted a pot experiment using 2-year-old seedlings subjected to five water treatments: control (80%), light drought (40%), moderate drought (20%), severe drought (10%), and extremely severe drought (5%). We measured and analyzed changes in water relations, photosynthesis, chlorophyll fluorescence, osmotic adjustment substances, and antioxidant enzyme activity during drought and after rehydration. The results showed that: (1) Light drought stress had minimal impact on the physiological state of Mongolian pine seedlings. Under moderate and severe drought stress, the activities of superoxide dismutase and peroxidase reached their maximum values, increasing by 25.26% and 38.8% compared to the control, respectively. Under extremely severe drought stress, the net photosynthetic rate, transpiration rate, and steady-state photochemical quenching coefficient of seedling leaves decreased by 94.76%, 87.19%, and 72.35% compared to the control, respectively, while leaf malondialdehyde content reached its highest level. (2) After rehydration, chlorophyll fluorescence and leaf proline content of the seedlings were restored to control levels, but the average photosynthetic index of leaves after rehydration following extremely severe drought stress only recovered to 28.51% of the control. Leaf water use efficiency, soluble sugar content, and antioxidant enzyme activity after rehydration were all significantly higher than the control ($P<0.05$). In conclusion, extremely severe drought stress primarily reduces the photosynthetic capacity and disrupts cell membrane stability of *Pinus sylvestris* var. *mongolica* seedlings, while rehydration after moderate drought can improve plant water utilization and reactive oxygen species scavenging capacity, thereby enhancing drought resistance. These findings provide theoretical support for efficient cultivation, management, and drought resistance evaluation of Mongolian pine plantations.

Keywords: *Pinus sylvestris* var. *mongolica*; drought stress; rehydration; physiological characteristics

Introduction

Since the Industrial Revolution, extensive burning of fossil fuels and other unsustainable human activities have intensified global warming. The Sixth Assessment Report of the IPCC indicates that global temperature increases are projected to exceed 1.5°C. Persistent climate change has led to increased drought frequency in many regions, particularly in the Northern Hemisphere. Drought is considered one of the primary factors limiting normal plant growth and development, causing numerous negative effects including reduced leaf area, damaged hydraulic architecture, decreased photosynthetic rates, reduced photosynthetic carbon assimilation, and hormonal imbalances. Currently, large-scale tree mortality caused by drought has been observed worldwide. As global climate change intensifies, improving plant adaptability under drought stress is crucial for ecological restoration projects.

Plant adaptation to drought is manifested not only through self-regulation during drought but also through the ability to rapidly recover after rehydration. Studies have shown that after drought and rehydration, plants exhibit enhanced transcription of tolerance-related genes, improved osmotic adjustment, and increased antioxidant capacity, demonstrating the positive effects of rehydration on plant physiological status. Therefore, investigating plant responses to drought and rehydration is essential for elucidating adaptation strategies and recovery mechanisms.

Pinus sylvestris var. *mongolica* is characterized by drought tolerance, cold resistance, barren tolerance, and sand adaptation. Its natural distribution in China is primarily in the northern Greater Khingan Mountains and Hulunbuir Sandy Land, and it serves as one of the main afforestation tree species in the "Three-North" Shelterbelt Program. However, due to drought stress and other factors, the survival rate of young trees in Mongolian pine forests is not high, severely limiting the sustainable development of shelterbelts. The physiological characteristics of Mongolian pine are important indicators for evaluating its ecological benefits and growth performance. Previous studies on drought adaptation mechanisms in Mongolian pine have mainly focused on changes in non-structural carbohydrates or leaf water potential and net photosynthetic rate, while neglecting the effects of alternating "drought-rehydration" cycles on drought resistance. Meanwhile, osmotic adjustment substances and antioxidant enzymes constitute important components of plant biochemical regulation under drought stress. Clarifying the changing patterns of these indicators helps improve and deepen our understanding of physiological response characteristics of Mongolian pine seedlings under drought-rehydration conditions.

Given this context, we conducted a pot experiment using 2-year-old Mongolian pine seedlings to measure leaf water potential, water use efficiency, transpiration rate, net photosynthetic rate, maximum photochemical efficiency, steady-state photochemical quenching coefficient, malondialdehyde, soluble sugar, and antioxidant enzyme activity parameters during drought and after rehydration. Our objective was to explore the physiological response characteristics of Mongolian pine seedlings under drought-rehydration conditions, providing a theoretical basis for nursery cultivation and afforestation promotion of Mongolian pine in arid and sandy regions of northern China.

Materials and Methods

1.1 Experimental Materials and Design

The drought stress and rehydration experiment was conducted at the Sanqingyuan Nursery of Beijing Forestry University (40°0 28 N, 116°20 19 E) from May to October 2023. The Mongolian pine seedlings and sandy soil used in the experiment were both obtained from Zhanggutai Town, Zhangwu County, Fuxin City, Liaoning Province. Healthy and uniformly growing 2-year-old Mongolian pine seedlings were selected, with an average height of 16.7±0.7 cm and ground diameter of 3.9±0.1 mm. The seedlings were transplanted into uniform pots (upper diame… 40 cm soil layer of a Mongolian pine forest. Gravel and litter were removed before transplanting. The soil substrate h… pH 7.59, organic matter content 7.59 $g \cdot kg^{-1}$, total nitrogen content 0.52 $g \cdot kg^{-1}$, total phosphorus content 0.47 $g \cdot kg^{-1}$, ammonium nitrogen content 0.98 $mg \cdot kg^{-1}$, and available phosphorus content 1.95 $mg \cdot kg^{-1}$. During the recovery period, soil water content was maintained at 80% of saturated water content to ensure seedling health.

The experiment used soil saturated water content (80%) as the control and established five drought stress gradients: light drought (40%), moderate drought (20%), severe drought (10%), and extremely severe drought (5%). These corresponded to approximately 80%, 40%, 20%, 10%, and 5% of soil saturated water content, respectively. There were 15 replicates per group. The experimental groups reached target soil water content through natural drying, while the control group was watered every 2-3 days between 17:00-20:00, with soil water content controlled by weighing. When reaching the drought endpoint, physiological indicators of Mongolian pine seedlings were measured, followed immediately by rehydration to saturated water content. Measurements were then taken again after rehydration.

1.2 Measurement Methods

1.2.1 Soil Property Determination Soil pH was measured using a pH analyzer (Shanghai Leici, China). Soil saturated water content was determined by the drying method. Soil organic matter content was measured by the potassium dichromate dilution-heat method. Total nitrogen content was determined by the indophenol blue colorimetric method. Total phosphorus content was measured by the molybdenum-antimony anti-colorimetric method. Available nitrogen content was determined by the alkali hydrolysis diffusion method. Available phosphorus content was measured by the sodium bicarbonate method.

1.2.2 Leaf Photosynthetic Parameter Measurement A Li-6800 photosynthesis analyzer (COR, Lincoln, USA) was used to measure photosynthetic parameters of upper, middle, and lower leaves of Mongolian pine seedlings between 9:00-11:00 on clear days. Parameters included transpiration rate (Tr), net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs). The transparent chamber was exposed to direct sunlight with flow rate set at 500 $\text{mol} \cdot \text{s}^{-1}$. Each leaf was measured three times. After measurement, the measured leaves were collected for leaf area calculation.

1.2.3 Leaf Water Parameter Measurement Leaf relative water content (RWC) was measured using the saturated water weight method. Collected needles were quickly brought to the laboratory to measure fresh weight, then soaked in deionized water for 24 h to measure saturated weight, and finally oven-dried at 105°C to measure dry weight. RWC was calculated as: RWC = (fresh weight - dry weight) / (saturated weight - dry weight) × 100%. Leaf water potential (l) and root water potential (r) were measured using a PSYPRO dew point water potential meter (USA), with three replicates per leaf and root system. Leaf water use efficiency (WUE) was calculated as: WUE = Pn / Tr, where Pn is net photosynthetic rate ($\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and Tr is transpiration rate ($\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$).

1.2.4 Chlorophyll Fluorescence Parameter Measurement A FluorCam closed chlorophyll fluorescence imaging system (FluorCam, Czech Republic) was used to measure chlorophyll fluorescence parameters of Mongolian pine seedlings. Plants were dark-adapted for 20 min between 18:00-22:00, after which initial fluorescence (Fo), maximum photochemical efficiency [Fv/Fm, the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm)], non-photochemical quenching coefficient (NPQ), and photochemical quenching coefficient (qP) were measured, with three replicates per leaf.

1.2.5 Leaf Osmotic Regulator Content Measurement The thiobarbituric acid method, ninhydrin colorimetric method, and anthrone colorimetric method were used to determine leaf malondialdehyde (MDA), proline (Pro), and soluble sugar (SS) contents, respectively.

1.2.6 Leaf Antioxidant Enzyme Activity Measurement The nitroblue tetrazolium photoreduction method, ultraviolet spectrophotometry, and guaiacol colorimetric method were used to determine leaf superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities, respectively.

1.3 Data Processing and Analysis

SPSS 22.0, OriginPro 2021, and Excel 2021 software were used for data processing and graphing. One-way ANOVA was used for statistical analysis of differences in physiological parameters among different groups, with Duncan' s method for multiple comparisons. Pearson correlation coefficients were used to analyze correlations among physiological indicators of Mongolian pine seedlings during drought and after rehydration. Principal component analysis (PCA) was used to explain the response characteristics of Mongolian pine seedlings to drought and rehydration.

Results

2.1 Changes in Leaf Water Parameters Under Drought-Rehydration Conditions

With increasing drought stress, leaf relative water content (RWC), leaf water potential (l), and root water potential (r) of Mongolian pine seedlings showed decreasing trends, while leaf water use efficiency (WUE) showed an initial increase followed by a decrease [Figure 1: see original paper]. Compared with the control group, leaf water potential decreased by 23.71%, 45.18%, 61.53%, and 72.35% from light to extremely severe drought stress, while root water potential decreased by 12.22%, 31.94%, 45.18%, and 61.53%, respectively. The decline in leaf water potential was greater than that in root water potential, indicating that drought stress affected transpiration organs more than water absorption organs. WUE reached its maximum under moderate drought stress, significantly higher than the control ($P<0.05$). After rehydration, RWC, WUE, and leaf water potential of Mongolian pine seedlings all increased significantly. WUE showed overcompensation after rehydration, as did RWC after light drought stress rehydration. Except for extremely severe drought stress, leaf water potential recovered to control levels after rehydration, while root water potential was significantly lower than the control except after moderate drought stress ($P<0.05$).

2.2 Changes in Photosynthetic Parameters Under Drought-Rehydration Conditions

With increasing drought stress, net photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO_2 concentration (Ci), and stomatal conductance (Gs) of Mongolian pine seedling leaves all showed decreasing trends [Figure 2: see original paper]. Under extremely severe drought stress, Pn was 1.22 $\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, a decrease of 94.76% compared with the control. Under the same conditions, Gs decreased by 92.18% and Tr decreased by 87.19% ($P<0.05$). After rehydration, Pn, Tr, Ci, and Gs all increased significantly ($P<0.05$). Pn was higher than the control after light and moderate drought stress rehydration, with significant differences between them, indicating a compensation effect. However, after extremely severe drought stress rehydration, Pn, Tr, and Gs only recovered to 23.71%, 21.45%, and 12.73% of the control levels, respectively, showing that the compensation effect was not obvious. Notably, Tr only recovered to the control level after light drought stress rehydration.

2.3 Changes in Chlorophyll Fluorescence Parameters Under Drought-Rehydration Conditions

With increasing drought stress, initial fluorescence (Fo), maximum photochemical efficiency (Fv/Fm), non-photochemical quenching coefficient (NPQ), and photochemical quenching coefficient (qP) of Mongolian pine seedling leaves all showed decreasing trends [Figure 3: see original paper]. Except for Fo under moderate drought stress, all parameters were significantly different from the control under various drought stresses ($P<0.05$). Compared with the control, Fo, Fv/Fm, NPQ, and qP decreased by an average of 31.94%, 45.18%, 38.8%, and 28.51%, respectively. After rehydration, Fo, Fv/Fm, NPQ, and qP all increased significantly and showed overcompensation. Fv/Fm and NPQ recovered to control levels after severe drought stress rehydration, while Fo and qP recovered to control levels after moderate drought stress rehydration, indicating that drought stress had a relatively small impact on Fo.

2.4 Changes in Leaf Osmotic Regulators Under Drought-Rehydration Conditions

With increasing drought stress, leaf malondialdehyde (MDA) content and leaf proline (Pro) content of Mongolian pine seedlings showed significant increasing trends, while leaf soluble sugar (SS) content showed an initial increase followed by a decrease [Figure 4: see original paper]. Soluble sugar content reached its maximum under moderate drought stress at 47.41 $g \cdot mL^{-1}$, an increase of 25.26% compared with the control. MDA content and proline content reached their maximum under extremely severe drought stress at 4.25 $g \cdot g^{-1}$ and 1046.89 $U \cdot g^{-1}$, respectively, both increasing by 38.8% compared with the control. After rehydration, proline content basically recovered to control levels, while soluble sugar content was significantly higher than during drought ($P<0.05$). Additionally, MDA content under extremely severe drought stress remained significantly higher than the control after rehydration ($P<0.05$).

2.5 Changes in Leaf Antioxidant Enzyme Activity Under Drought-Rehydration Conditions

With increasing drought stress, leaf superoxide dismutase (SOD) and peroxidase (POD) activities of Mongolian pine seedlings showed initial increases followed by decreasing trends, while leaf catalase (CAT) activity showed an increasing trend

[Figure 5: see original paper]. SOD and POD activities reached their maximum values under moderate and severe drought stress at 1046.89 $U \cdot g^{-1}$ and 6.03 $U \cdot g^{-1}$, respectively. After rehydration, different antioxidant enzyme activities showed different patterns but were all higher than the control. SOD and POD activities decreased slightly after rehydration, while CAT activity increased significantly. Compared with drought conditions, SOD and POD activities decreased by 5.14%, 7.40%, 19.95%, and 38.74% from light to severe drought stress rehydration, respectively, while CAT activity under extremely severe drought stress rehydration was significantly higher than the control (P<0.05).

2.6 Principal Component and Correlation Analysis of Physiological Characteristics Under Drought-Rehydration Conditions

The first two principal components under drought stress explained 94.2% of the cumulative variance, effectively explaining the physiological characteristics of Mongolian pine seedlings under drought stress [Figure 6: see original paper]. Parameters with large contributions to PC1 included Pn, Gs, Fv/Fm, and Ci, while those with large contributions to PC2 included WUE, Tr, and l. Under drought stress, Tr, Pn, Ci, and Gs were all extremely significantly positively correlated with each other (P<0.001), and all were significantly positively correlated with RWC, l, and r (P<0.05). However, MDA content was significantly negatively correlated with most photosynthetic parameters, chlorophyll fluorescence parameters, and water parameters (P<0.05). SOD activity was extremely significantly positively correlated with leaf MDA content (P<0.001), and POD activity was extremely significantly positively correlated with CAT activity (P<0.001).

After rehydration, the first two principal components explained 94.2% of the cumulative variance, effectively explaining the physiological characteristics of Mongolian pine seedlings after rehydration [Figure 6: see original paper]. Parameters with large contributions to PC1 included Gs, l, NPQ, and qP, while those with large contributions to PC2 included Fo and Fv/Fm. After rehydration, the correlations and significance among parameters were weakened compared with drought conditions. Gs, Fv/Fm, and NPQ were extremely significantly positively correlated (P<0.001), while MDA content was extremely significantly negatively correlated with Tr, Pn, Ci, and Gs (P<0.001).

Discussion

3.1 Effects of Drought Stress on Physiological Characteristics of Mongolian Pine Seedlings

Water content greatly influences plant life activities. When facing drought or water deficit conditions, plants regulate through morphological or physiological mechanisms. Physiological indicators such as stomatal conductance, transpiration rate, and leaf relative water content are commonly used as drought resistance evaluation criteria, while proline and soluble sugar are used as drought tolerance evaluation indicators. Plant water potential values follow the general pattern of water transport from high to low water potential and decrease with intensifying water stress. In this experiment, as RWC of Mongolian pine seedlings decreased under drought stress, leaf and root water potential also decreased, with leaf water potential showing greater decline than root water potential at all drought gradients, indicating that drought stress affected transpiration organs more than water absorption organs.

Leaf water use efficiency (WUE) represents the amount of assimilates produced per unit of water consumed, with higher WUE indicating that plants can fix more carbon with less water. In this experiment, WUE showed an initial increase followed by a decrease with intensifying drought stress, consistent with many previous studies. When drought stress was mild, stomatal closure caused transpiration rate to decrease more than net photosynthetic rate, leading to increased WUE as Mongolian pine seedlings exhibited self-regulation capacity. The subsequent WUE decrease may be related to reduced soluble sugar content, dehydration of mesophyll cells, and affected physiological metabolism under severe drought stress.

Drought stress reduces plant photosynthetic rate through stomatal and non-stomatal factors. The specific cause can be determined by Ci values: if Gs and Ci both decrease significantly, it represents typical stomatal limitation; otherwise, it represents non-stomatal limitation. In this experiment, as drought stress intensified, Pn, Ci, and Gs showed consistent trends, indicating that Pn reduction was caused by stomatal limitation. This result aligns with previous studies showing that stomatal closure leads to decreased CO_2 concentration and insufficient photosynthetic raw materials. Under mild drought stress, Mongolian pine seedlings reduced water loss by closing stomata and decreasing transpiration, thereby increasing leaf water retention capacity and preventing withering—a drought avoidance strategy. However, severe drought stress caused irreversible damage to stomatal conductance that was difficult to recover from in a short time, reducing CO_2 exchange efficiency, decreasing photosynthetic capacity, and ultimately causing severe physiological impacts.

Photosystem II (PS II) is highly sensitive to abiotic stress, and chlorophyll fluorescence parameters can accurately analyze plant processes of light energy absorption, utilization, and conversion, reflecting plant photosynthetic performance and responses to adversity. In this experiment, as drought stress intensified, Fv/Fm and qP both decreased, indicating reduced PS II reaction center electron transport rate and inhibited primary reactions, ultimately decreasing photosynthetic capacity. Meanwhile, NPQ showed a decreasing trend, which differs from most plants, suggesting that Mongolian pine seedlings reduced heat dissipation proportion to maximize light energy utilization for carbon fixation and maintain basic metabolism under severe drought stress.

In addition to non-enzymatic antioxidants like proline, enzyme substances are primarily responsible for reactive oxygen species (ROS) scavenging, with superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) being important antioxidant enzymes in plants. Under mild drought stress, Mongolian pine seedlings mainly relied on SOD and POD for scavenging, while under severe drought stress, CAT played a key role, indicating that Mongolian pine seedlings adopt different ROS scavenging strategies facing different drought stress levels. Malondialdehyde (MDA) is a product of membrane lipid peroxidation, and its content directly reflects the degree of cell membrane damage. In this experiment, leaf MDA content increased significantly with intensifying drought stress, reflecting deepening cell membrane damage.

Soluble sugars and proline function not only as osmotic adjustment substances to reduce cell osmotic potential and minimize water loss but also as important antioxidants that effectively scavenge ROS. In this experiment, soluble sugar content showed an initial increase followed by a decrease, indicating that plant osmotic adjustment capacity has certain limits. Under mild drought stress, Mongolian pine seedlings could accumulate soluble sugars and proline to improve water absorption capacity and maintain normal metabolism. Under severe drought stress, excessive water loss reduced the leaf' s ability to synthesize soluble sugars. Proline content continued to increase with intensifying drought stress, reaching its maximum under extremely severe drought stress, suggesting that Mongolian pine relies more on proline regulation to maintain cell water potential under extremely severe drought conditions.

3.2 Effects of Rehydration After Drought Stress on Physiological Characteristics of Mongolian Pine Seedlings

Drought-rehydration cycles can restore plant physiological functions and compensate for drought-induced damage. In this experiment, all physiological parameters of Mongolian pine seedlings recovered to some extent after rehydration, but the recovery capacity and degree differed depending on drought stress intensity. After rehydration, leaf water status improved significantly, with RWC, WUE, and leaf water potential all significantly higher than during drought, consistent with previous studies on other species. However, after rehydration following severe and extremely severe drought stress, the recovery degree of RWC and root water potential was lower than after light and moderate drought stress rehydration, with leaf water potential failing to recover to control levels only after extremely severe drought stress rehydration.

After rehydration, Pn, Ci, Gs, and Tr of Mongolian pine seedlings all recovered to varying degrees, but Pn only showed overcompensation after light and moderate drought stress rehydration. Combined with changes in water status, this indicates that Mongolian pine seedlings have strong post-drought recovery ability under mild drought conditions, which weakens under severe drought stress. Chlorophyll fluorescence parameters recovered well after rehydration, with Fo, Fv/Fm, NPQ, and qP all restoring to control levels, and overcompensation occurring in Fo, Fv/Fm, and NPQ. This suggests that Mongolian pine seedlings experienced reversible damage to the photosynthetic system during drought stress and recovered rapidly after rehydration, likely due to their strong drought tolerance.

After rehydration, leaf MDA and proline content of Mongolian pine seedlings decreased, while soluble sugar content increased significantly. MDA content remained significantly higher than the control only after extremely severe drought stress rehydration, indicating that cell membranes suffered severe damage at this level. Soluble sugar content increased significantly after rehydration compared with the control, possibly because Mongolian pine seedlings actively increased cell solute content to rapidly absorb water from the environment and further replenish water for normal physiological activity recovery.

After rehydration, most antioxidant enzyme activities of Mongolian pine seedlings were significantly higher than the control, with CAT activity even higher than during drought stress, indicating that rehydration can effectively improve antioxidant enzyme activity. These enzymes continued to scavenge ROS accumulated during the drought period, promoting rapid plant recovery after drought stress. Under light and moderate drought stress rehydration, Pn, WUE, soluble sugar content, and antioxidant enzyme activity of Mongolian pine seedlings were all significantly higher than the control, indicating that moderate drought stress followed by rehydration can improve drought resistance. However, under extremely severe drought stress, MDA content still accumulated, indicating limited regulation capacity of Mongolian pine seedlings.

Conclusion

Based on the drought-rehydration experiment on 2-year-old *Pinus sylvestris* var. *mongolica* seedlings, the main conclusions are as follows:

1) Drought stress significantly negatively affected physiological indicators including water relations, photosynthesis, chlorophyll fluorescence, osmotic adjustment substances, and antioxidant enzyme activity of Mongolian pine seedlings. All physiological indicators recovered to some extent after rehydration, but the recovery capacity and degree differed depending on drought stress intensity.

2) The reduction in leaf Pn of Mongolian pine seedlings under drought stress was mainly caused by stomatal limitation. Under mild drought stress, photosynthesis and water status were significantly affected, and seedlings primarily adopted stomatal closure and reduced transpiration to resist drought, while synthesizing large amounts of soluble sugars for osmotic adjustment. Under severe drought stress, cell membranes were severely damaged, and seedlings mainly relied on proline and CAT for resistance, while reducing heat dissipation to fix carbon and maintain basic metabolism.

3) Rehydration can effectively improve leaf CAT activity and WUE of Mongolian pine seedlings, thereby promoting rapid recovery after drought stress. After light and moderate drought stress rehydration, osmotic adjustment

capacity and antioxidant enzyme activity of Mongolian pine seedlings were significantly enhanced, indicating that moderate drought can improve drought resistance of Mongolian pine seedlings. However, under extremely severe drought stress, MDA content still accumulated, indicating limited regulation capacity of Mongolian pine seedlings.

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